Chapter 12

Monographs of ^{99m}Tc Pharmaceuticals

12

12.1 ^{99m}Tc-Pertechnetate

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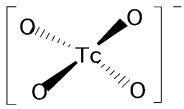
Chemical name	**Chemical structure**
Sodium pertechnetate Sodium pertechnetate ^{99m}Tc injection (fission) (*Ph. Eur.*) Technetium Tc 99m pertechnetate injection (*USP*) ^{99m}Tc(VII)-Na-pertechnetate	 Pertechnetate anion ($^{99m}TcO_4^-$)
Physical characteristics	**Commercial products**
$E\gamma = 140.5$ keV (IT) $T_{1/2} = 6.02$ h	^{99}Mo/^{99m}Tc generator: GE Healthcare Bristol-Myers Squibb Mallinckrodt/Tyco

Preparation

Sodium pertechnetate ^{99m}Tc is eluted from an approved ^{99}Mo/^{99m}Tc generator with sterile, isotonic saline. Generator systems differ; therefore, elution should be performed according to the manual provided by the manufacturer. Aseptic conditions have to be maintained throughout the operation, keeping the elution needle sterile. The total eluted activity and volume are recorded at the time of elution. The resulting ^{99m}Tc activity concentration depends on the elution volume.

Sodium pertechnetate ^{99m}Tc is a clear, colorless solution for intravenous injection. The pH value is 4.0–8.0 (*Ph. Eur.*).

Description of Eluate

^{99m}Tc eluate is described in the *European Pharmacopeia* in two specific monographs depending on the method of preparation of the parent radionuclide ^{99}Mo, which is generally isolated from fission products (Monograph 124) (Council of Europe 2005 a), or produced by neutron activation of metallic ^{98}Mo-oxide (Monograph 283) (Council of Europe 2005 b). Sodium pertechnetate ^{99m}Tc injection solution satisfies the general requirements of parenteral preparations stated in the *European Pharmacopeia* (Council of Europe 2004).

The specific activity of ^{99m}Tc-pertechnetate is not stated in the *Pharmacopeia*; however, it is recommended that the eluate is obtained from a generator that is eluted regularly,

every 24 h. Details for obtaining carrier-free technetium from the ^{99}Mo/^{99m}Tc generator and the resulting radionuclidic purity of eluates are discussed in Chap. 5, Sect. 5.1.1.

Pertechnetate anion ($^{99m}TcO_4^-$) is stable in aqueous solutions. It is chemically not reactive; its ability to form ligand complexes depends on the reduction to lower valence states (Steigman and Eckelman 1992; Steigman and Richards 1974; Steigman et al. 1975). The major use of this short-lived radionuclide in nuclear medicine to date is for the ad hoc preparation of ^{99m}Tc pharmaceuticals, which is performed with kits containing stannous ion as a reducing agent (Johannsen and Narasimhan 1992; Lin and Winchell 1972; Lin et al. 1971).

Clinical Applications

^{99m}Tc(VII)-pertechnetate is used after intravenous injection:

- Thyroid scintigraphy
  - Determination of technetium uptake and morphology
  - Diagnosis and localization of hot/cold nodules
- Salivary gland scintigraphy
  - To asses salivary gland function and duct status
- Imaging of gastric mucosa
  - To diagnose ectopic gastric mucosa (Meckel's diverticulum)
- Brain scintigraphy
  - Visualization of brain lesions when the blood-brain barrier (BBB) is defective
- Lachrimal duct scintigraphy
  - To evaluate nasolachrimal drainage
- In vivo labeling of RBC
  - Regional blood pool imaging
  - First-pass cardiac radionuclide angiography (ejection fraction, wall motion)
  - Detection of occult gastrointestinal bleeding

Sodium pertechnetate Tc-99m was introduced for scanning the thyroid (Harper 1964) and for brain scanning (McAfee et al. 1964; Quinn 1965), primarily because of its physical properties; other applications followed (Harper et al. 1966). The striking similarity of the heptavalent anion with iodide, however, has made pertechnetate an excellent radionuclide for thyroid scanning and for the study of thyroid physiology (Andros et al. 1965; Kusic et al. 1990).

In vivo labeling of red blood cells (RBC) with sodium ^{99m}Tc-pertechnetate (Callahan et al. 1982) is performed subsequent to pretreatment of RBC in vivo with a stannous reducing agent, using stannous pyrophosphate cold kits (TechneScan PYP, AngioCis) as well as Amerscan Stannous Agent.

Time of Examinations

- Thyroid scintigraphy is performed 20 min after intravenous injection.
- Salivary gland scintigraphy should begin immediately after intravenous injection and at regular intervals up to 60 min.
- Meckel's diverticulum scintigraphy should commence immediately after intravenous injection and at regular intervals up to 30 min.
- For brain scintigraphy, sequential images are taken immediately within the first minute after intravenous injection; static imaging is performed 1-h later.
- Lachrimal duct scintigraphy's dynamic imaging should begin immediately after tracer application for 10 min.
- For in vivo RBC labeling, the blood pool scintigraphy should start 10 min after intravenous injection of a bolus of ^{99m}Tc-pertechnetate: cardiac dynamic imaging should begin immediately, and abdominal imaging should also begin immediately and at various times up to 24 h.

Recommended Activities for Indications. The activity range for intravenous administration in patients (70 kg) is:

- Thyroid scintigraphy: 75 MBq
- Salivary gland scintigraphy: 40 MBq
- Meckel's diverticulum: 185 MBq
- Brain scintigraphy: 550 MBq, after blocking thyroid and choroid plexus to avoid nonspecific uptake of ^{99m}Tc-pertechnetate
- Lachrimal duct scintigraphy: 2–4 MBq instilled into each eye
- In vivo RBC labeling: 740 MBq, after pretreatment with a stannous agent

Pediatric Dose. The amount of radioactivity for infants and children administered for myocardial scintigraphy is based on body weight, using the scaling factors given in Appendix 1 (Table A1.2). The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

Iodinated contrast agents or iodine-containing medication interfere with ^{99m}Tc-pertechnetate thyroid imaging.

Persistent vascular activity of ^{99m}Tc-pertechnetate has been observed when scintigraphy has been performed following a bone scan, due to radiolabeled RBC that had retained stannous ions (Ancri et al. 1977; Montelibano et al. 1979).

Several drugs interfere with the normal biodistribution of ^{99m}Tc-pertechnetate (Hladik et al. 1987). Thus, cancer chemotherapeutic agents (methotrexate) can affect brain scintigraphy; atropine, isoprenaline, and analgesics interfere in abdominal imaging; iodine and other blockers (perchlorate, perrhenate) can modify thyroid uptake.

Quality Control

Radiochemical Purity. Sodium pertechnetate [^{99m}Tc] must satisfy the requirements stated in the *European Pharmacopeia* (Council of Europe 2004 a). More than 95% of ^{99m}Tc activity must be present as pertechnetate anion.

Paper Chromatography. The *European Pharmacopeia* describes descending paper chromatography using methanol/water (80 : 20 v/v) as solvent; developing time is 2 h. The ^{99m}Tc-pertechnetate anion migrates with an R_f value of 0.6. More than 95% of the measured radioactivity corresponds to an R_f of 0.6; less than 5% are detected at the start.

Recommended Methods by the Manufacturer

Thin-layer chromatography		
Stationary phase:	Gelman ITLC-SG (fiberglass), 2×9.5 cm	
Solvent:	Saline (0.9% NaCl)	
Developing time:	10 min	
R_f values:	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-Na-pertechnetate:	0.9–1.0 (>99%)

Thin-layer chromatography on silica gel plates offers reliable results faster; ^{99m}Tc-pertechnetate migrates with the solvent (saline) front; reduced, hydrolized activity remains at the start. The results of radiochemical purity generally exceed 99%.

Radionuclidic Purity. The *European Pharmacopeia* and national regulatory agencies recommend determination of ^{99}Mo in the primary eluate to assure high quality of generator eluates (DIN 6854).

Generators are eluted after shipment, before administration of eluates to patients. The primary eluate contains the highest concentration of chemical impurities and of carrier ^{99}Tc (decay product). Also, parent ^{99}Mo is highest in the first eluate (Hammermayer et al. 1986).

Less than 0.1% of the total ^{99m}Tc activity is due to parent ^{99}Mo, and not more than 0.01% is due to other radionuclidic impurities. These limits are however, never observed with the available generator systems.

Determination of ^{99}Mo Impurity. Determination of ^{99}Mo should be performed with a sample of the fresh eluate (37 MBq) by γ-spectrometry. A lead absorber 6-mm thick is placed between the sample and the NaI-detector. The fraction of γ-radiation measured at 740 keV (^{99}Mo) should not exceed the reading obtained with a reference (^{99}Mo) of 37 kBq.

The purity of generator eluates is directly related to the performance of a generator system (see Chap. 5, Sect. 5.1.1).

Pharmacokinetic Data

The monovalent anion pertechnetate is actively transported into the thyroid in a manner similar to iodide; however, it is not metabolized and is released from the thyroid unchanged. It has been demonstrated that the active transport of iodide and pertechnetate share the same carrier mechanism (Wolff and Maurey 1962). Uptake in the thyroid gland is between 1.5 and 2% of the injected activity within 20 min (Andros et al. 1965).

Assuming an activity of 74 MBq for thyroid scintigraphy, the concentration of pertechnetate in blood has been calculated as 2.5×10^{-12} *M*, based on a volume of distribution of 20 l. This concentration is far below the value required to saturate the carrier-mediated uptake mechanism. However, iodinated contrast agents or iodine-containing medication do affect serum levels of iodide (up to 3×10^{-4} *M*) and thus would interfere with ^{99m}Tc-pertechnetate thyroid imaging (Loberg 1979).

In blood, 70–80% of ^{99m}Tc-pertechnetate is bound to proteins. Perchlorate has been shown to displace pertechnetate from plasma protein-binding sites (Oldendorf et al. 1970). The unbound fraction is preferentially concentrating in the thyroid gland and other related structures, such as salivary glands, gastric mucosa, choroid plexus, and mammary tissue. ^{99m}Tc-pertechnetate is selectively excluded from the cerebrospinal fluid (Andros et al. 1965).

Elimination of ^{99m}Tc-pertechnetate from plasma after intravenous or oral administration (same subject) showed disappearance curves with an initial fast elimination of radioactivity; 50–60% are cleared with a half-time of 15 min; the remainder is eliminated more slowly, with half-times of approximately 3 h. After oral administration, the highest value of ^{99m}Tc activity in blood was reached within 30 min; the activity level was approximately one half of the radioactivity measured at the same and subsequent times after intravenous injection (Andros et al. 1965). The rates of disappearance for both whole blood and plasma were shown to be the same (Prince et al. 1980).

Pertechnetate is excreted by the kidneys, but other pathways may be relevant in specific circumstances, such as saliva, gastric juice, milk, sweat, etc. (Hays 1973). Lactating women secrete 10% of pertechnetate in milk (Ahlgren et al. 1985). Pertechnetate crosses the placental barrier.

A major difference between the kinetics of pertechnetate and iodide in humans is the excretion pattern; only pertechnetate is excreted in the feces (Andros et al. 1965; Hays and Berman 1977). Renal excretion by glomerular filtration is observed in the first 24 h after administration; 25–30% of the injected activity is recovered in the urine. During the following 48–72 h, fecal excretion predominates and may amount to 35% of the injected radioactivity. A total of approximately 60% of the administered radioactivity is recovered in urine and feces in 72 h; approximately 40% is retained in the body, mainly in the digestive tract. The whole-body biological half-time is estimated to be 53 h (Andros et al. 1965). Tubular reabsorption of pertechnetate was determined as 86.5% of the filtered amount. ^{99m}Tc-pertechnetate is excreted unchanged (Dayton et al. 1969).

In certain clinical situations, when thyroidal uptake of ^{99m}Tc-pertechnetate should be avoided, pretreatment with an oral dose of potassium perchlorate is used to inhibit uptake. Perchlorate anion shows greater affinity for the transporter than does iodide and is, therefore, a competitive inhibitor of the thyroid iodide trap (Wolff and Maurey 1962). Like pertechnetate, perchlorate is concentrated in the thyroid and is not metabolized. Assuming 100% resorption of an oral dose of 300 mg of $KClO_4$ and an initial distribution volume of 20 l, the resulting concentration of perchlorate in blood is approximately 10^{-4} *M*, sufficient for saturation of the thyroid trapping mechanism (Loberg 1979).

The effect of pretreatment with perchlorate and iodide on pertechnetate pharmacokinetics has been studied (Oldendorf et al. 1970; Prince et al. 1980; Welch et al. 1969).

^{99m}Tc-pertechnetate cannot pass through the intact BBB, but in areas of the brain where structural defects permit diffusion, uptake has been used as an indicator of vascular and neoplastic brain lesions (Jhingram and Johnson 1973).

^{99m}Tc-pertechnetate has an affinity for RBC that have been treated (in vivo) with a reducing agent, causing "stannous loading". Approximately 95% of the administered ^{99m}Tc activity is taken up by RBC. Unbound pertechnetate is excreted by the kidneys. Approximately 15% of the activity is excreted in the urine during the first day (Porter et al. 1983). Radioactivity is removed from blood with a half-time of 60 h by renal excretion (International Commission on Radiological Protection 1987).

Radiation Dose

The radiation exposure after the intravenous administration of ^{99m}Tc-pertechnetate depends on the thyroid status, and whether a blocking agent has been administered. The thyroid gland, stomach wall, small intestine, upper and lower intestinal wall, and urinary bladder wall are the most exposed organs.

The effective (whole body) dose equivalent for pertechnetate ^{99m}Tc is 0.013 mSv/MBq (International Commission on Radiological Protection 1987). The effective dose in adults (70 kg) resulting from 75 MBq of intravenously injected ^{99m}Tc-pertechnetate for thyroid scintigraphy is approximately 1 mSv. The absorbed radiation dose to the thyroid (without a blocking agent) resulting from an intravenous injection of 75 MBq of ^{99m}Tc-pertechnetate corresponds to 1.7 mGy.

The effective dose in adults (70 kg) resulting from 40 MBq of intravenously injected ^{99m}Tc-pertechnetate for salivary gland scintigraphy is approximately 0.5 mSv.

Lachrimal duct scintigraphy using 4 MBq of ^{99m}Tc-pertechnetate corresponds to an effective dose of 0.05 mSv. The absorbed radiation dose to the optical lens is given as 0.038 mGy/MBq. Using 4 MBq of ^{99m}Tc-pertechnetate, the absorbed radiation dose to the lens corresponds to 0.15 mGy.

Diagnosis of ectopic gastric mucosa using 185 MBq of intravenously injected ^{99m}Tc-pertechnetate corresponds to an effective dose of approximately 2.4 mSv.

The effective dose in adults (70 kg) resulting from 550 MBq of intravenously injected ^{99m}Tc-pertechnetate for brain scintigraphy (after blocking thyroid and choroid plexus) is approximately 2.9 mSv. The effective (whole body) dose equivalent (with blocking agent) is 0.0053 mSv/MBq.

^{99m}Tc-Labeled Erythrocytes. The effective (whole body) dose equivalent for ^{99m}Tc-labeled erythrocytes is 0.0085 mSv/MBq (International Commission on Radiological Protection 1987). The effective dose in adults (70 kg) resulting from 740 MBq of intravenously injected ^{99m}Tc-pertechnetate for angioscintigraphy is approximately 6.3 mSv. The absorbed radiation dose to the heart resulting from an intravenous injection of 740 MBq of ^{99m}Tc-pertechnetate corresponds to 17.0 mGy. The absorbed radiation dose to the kidneys is corresponding to 7.4 mGy.

Storage and Stability

Storage. Sodium pertechnetate ^{99m}Tc injection is stored at room temperature with shielding.

Stability. Sodium pertechnetate ^{99m}Tc injection may be used up to 6 h after elution if the generator had been eluted within 24 h.

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12.2 ^{99m}Tc-Labeled Human Serum Albumin

12.2.1 ^{99m}Tc-Albumin (HSA)

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Chemical name

Human serum albumin (HSA)

Technetium ^{99m}Tc albumin injection (*Ph. Eur.*)

Technetium Tc 99m albumin injection (*USP*)

^{99m}Tc-HSA

Kit components		**Commercial products**	
Human serum albumin	10 mg	Albumoscint	Nordion
Stannous chloride dihydrate	0.02 mg	TechneScan HSA	Mallinckrodt/Tyco
Sodium chloride q.s.		VasculoCis (TCK-2)	CIS Bio

Preparation

Commercial kits contain the freeze-dried, sterile formulation in a multidose vial, sealed under a nitrogen atmosphere. The lyophilized preparation is readily soluble in ^{99m}Tc-pertechnetate injection and in saline. For labeling, the vial is placed into a lead-shielded container. Aseptically sterile ^{99m}Tc-pertechnetate should be injected into the vial in a volume of 1–8 ml, with an activity up to 2.22 GBq (60 mCi). Before removing the syringe, 2–5 ml of gas should be withdrawn from the space above the solution to normalize the pressure inside the vial. The shielded vial should be agitated gently to dissolve the lyophilized material. The reaction should proceed at room temperature for about 20 min, with occasional agitation.

^{99m}Tc-human serum albumin (HSA) is a clear, pale yellow solution for intravenous injection. The pH value is 2.5–3.5 (2.0–6.5, *European Pharmacopeia*).

Description of the Kit

^{99m}Tc-labeled albumin is a product derived from HSA, which is a natural constituent of blood. HSA is isolated from donor blood and complies with the purity standards stated in the *European Pharmacopeia* (monographs 255 and 853) (Council of Europe 2004a, b) in accordance with EU and WHO requirements for biological substances (Council of Europe 1982; World Health Organization 1994).

Several methods for labeling HSA with technetium-99m in the reduced state (Steigman et al. 1975) have been described. Originally, ferric ion and ascorbic acid were used as a reducing system; labeling of HSA was performed at acidic pH (Stern et al. 1965; Persson and Liden 1969). Adjustment of the pH to 7.8 was essential in order to produce

ferrous ion, the active principle for the reduction of Tc(VII)-pertechnetate (Zolle et al. 1973). An alternate approach used reduction of pertechnetate with concentrated HCl to produce Tc(V) in the dry state and subsequent addition of albumin for labeling at acidic pH (Williams and Deegan 1971). Additional insight into the labeling mechanism was obtained by the electrolytic reduction of pertechnetate using a zirconium crucible as the anode and a platinum wire as the cathode (Benjamin 1969; Benjamin et al. 1970). Zirconium as the anode was eventually replaced by tin (Narasimhan and Mani 1975). Anodic dissolution of Zr resp. Sn ions was made responsible for the reduction of pertechnetate and labeling of HSA at acidic pH. A sterile electrolytic kit procedure was introduced for the preparation of ^{99m}Tc-HSA by Dworkin and Gutkowski (1971). Further studies have demonstrated that high labeling yields were obtained by reduction with either Fe(II) or Sn(II) alone (Lin et al. 1971). The advantages of using stannous ion in the production of ^{99m}Tc-pharmaceuticals had been demonstrated by the one-step kit preparation of ^{99m}Tc-HSA (Eckelman et al. 1971). Freeze-drying of the nonradioactive components has considerably enhanced the stability of kits containing stannous ion (Deutsch and Redmond 1972).

Clinical Applications

Intravenous injection: Cardiac blood pool imaging (static)
First-pass ventriculography
Gated equilibrium ventriculography
Regional circulatory imaging

Angiocardiography by first-pass imaging of right ventricular (RV) and left ventricular (LV) function is performed in patients with coronary artery disease. A bolus of ^{99m}Tc-HSA or ^{99m}Tc-red blood cells (RBC) is injected intravenously in a small volume (Berger et al. 1979; Philippe et al. 1988).

Gated radionuclide ventriculography or equilibrium (gated) radionuclide angiocardiography is performed to evaluate LV function after the radiotracer has become distributed throughout the vascular space (Strauss et al. 1971).

Time of Examination. Immediately or shortly after intravenous injection, depending on the type of examination.

Recommended Activities for Indications

Blood pool imaging:	111–185 MBq (3–5 mCi)
Angiocardiography:	370–740 MBq (10–20 mCi) as a bolus of 1–2 ml
Gated ventriculography:	185–925 MBq (5–25 mCi) (or ^{99m}Tc-RBC)
Circulation and blood flow:	18.5–185 MBq (0.5–5 mCi)

Additional Information

^{99m}Tc-HSA is administered by intravenous injection, intrathecal application is contraindicated. ^{99m}Tc-HSA has originally been used to image the placenta; however, this indi-

cation is no longer accepted. The preparation of ^{99m}Tc-HSA must not be injected into individuals hypersensitive to protein.

Quality Control

Radiochemical Purity. The *European Pharmacopeia* requires thin-layer chromatography (TLC) (distance 10–15 cm) for the identification of impurities using methylethylketone (MEK) as solvent. The radioactivity corresponding to ^{99m}Tc-HSA must not be less than 95% (Council of Europe 2005).

Here, a similar procedure was used for analysis. A sample of approximately 1,000 counts/s is applied to silica gel fiberglass sheets and developed in acetone. Parallel to the labeled product, sodium ^{99m}Tc-pertechnetate is also analyzed. After development, the dried strips are cut into 1-cm pieces and measured in an NK-350 type scintillation well counter coupled with an automatic sample sorter. ^{99m}Tc-HSA remained at the start (R_f = 0.0), ^{99m}Tc-pertechnetate moved with the solvent front (R_f = 0.9–1.0).

Thin-layer chromatography		
Stationary phase:	Gelman ITLC-SG (fiberglass), 2×9.5 cm	
Solvent:	Acetone	
Developing time:	5 min	
R_f values:	^{99m}Tc-HSA:	0.0–0.1
	^{99m}Tc reduced, colloidal:	0.0–0.1
	^{99m}Tc-Na-pertechnetate:	0.9–1.0 (< 5%)

A quick test for determination of unbound ^{99m}Tc-pertechnetate in labeled albumin preparations has been described using precipitation with trichloroacetic acid (TCA) and separation by membrane filtration. Free ^{99m}Tc-pertechnetate is efficiently separated from ^{99m}Tc-HSA (Lamson et al. 1974). ^{99m}Tc-HSA shows high in vitro stability (Benjamin 1969; Stern et al. 1965).

Pharmacokinetic Data

Following intravenous injection, ^{99m}Tc-HSA is distributed homogenously in the vascular compartment. It does not concentrate in the thyroid, salivary, and gastric glands. In pregnant women receiving a placental scan, 50–75% of the radioactivity was measured in the blood 30 min after injection. The elimination from blood has been described by two half-times, namely 6 h and 3 days (McAfee et al. 1964; Stern et al. 1966).

A comparison with ^{131}I-albumin (^{131}I-IHSA) in experimental animals showed that ^{99m}Tc-HSA parallels the disappearance of ^{131}I-albumin, being eliminated slightly faster. The tissue distribution in pregnant rabbits was also similar, showing slightly lower blood levels and a much lower concentration within the fetus. ^{99m}Tc-HSA accumulates in the kidneys, which showed the highest tissue concentration. Though at low levels, an increase of radioactivity was seen also in the stomach and gut (McAfee et al. 1964; Stern et al. 1966).

In another extensive study (184 samples of maternal plasma and 98 samples of fetal plasma), the disappearance curve indicated an initial rapid decrease with a half-time of 2 h and a slow elimination corresponding to a half-time of 35 h. Initial plasma clearance was 16% in the first hour and 51% in 6 h, the incremental loss being 38% per day. The major loss from the albumin pool resulted from urinary excretion (32% in the first 24 h). Fecal excretion was low, with a maximum of 5.8% in 48 h. Free pertechnetate in maternal plasma (17 samples) was 2–21.2%, with a mean value of 13.6% (Herbert et al. 1969).

Transplacental passage of activity was evident a few minutes after maternal injection; after 2.5 h, fetal plasma activity was in equilibrium with maternal plasma, showing 4.3% of the maternal plasma concentration, totally as free ^{99m}Tc-pertechnetate. However, the total plasma content in the fetus never exceeded 0.15% of the maternal administered activity. Urine immediately obtained after birth showed high concentrations of free ^{99m}Tc-pertechnetate. Passage of ^{99m}Tc-HSA across the placenta is minimal (Herbert et al. 1969).

The fetal plasma equilibrium value of 4.3% of maternal plasma concentration was approximately twice that of IHSA reported by Hibbard and Herbert (1960); also, accumulation in the liquor was high (no liquor activity was detected with IHSA).

^{99m}Tc-HSA was labeled by the method of Stern et al. (1965). Preparations showed high in vitro stability and contained virtually no free radionuclide when injected. Yet, no explanation has been given for the rapid liberation of technetium-99m from albumin after injection reflected in the rapid initial plasma clearance, the excretion pattern, and the evidence of fetal uptake of pertechnetate.

The results of clinical studies performed by McAfee et al. (1964) and by Herbert et al. (1969) are well documented; they are, however, at variance with respect to the excretion of activity in urine and uptake in the fetus. McAfee et al. (1964) recovered less than 0.5% of the injected radioactivity in urine or feces, and 0.4% of the maternal radioactivity in two infants delivered approximately 1 and 4 h after intravenous injection to the mother.

Evidence that ^{99m}Tc-HSA prepared by electrolytic kit labeling shows high in vivo stability has been provided by measuring the whole-blood disappearance in mice over a period of 90 min (Dworkin and Gutkowski 1971) and in patients for 1 h (Callahan et al. 1976).

Radiation Dose

In analogy with iodine-labeled albumin, absorbed dose calculations are based on the elimination of radioactivity from blood by three half-times, namely 6.8 h (0.40), 1.29 days (0.22), and 19.4 days (0.38, Takeda and Reeve 1963). Uniform distribution of ^{99m}Tc-HSA outside the blood pool and rapid renal excretion of the released radionuclide is assumed (International Commission on Radiological Protection 1987).

The effective (whole body) dose equivalent for ^{99m}Tc-labeled albumin is 0.0079 mSv/MBq (International Commission on Radiological Protection 1987). The effective dose in adults (70 kg) resulting from 740 MBq (20 mCi) of intravenously injected ^{99m}Tc-HSA for angioscintigraphy is approximately 5.8 mSv. Blood pool imaging with 185 MBq (5 mCi) of ^{99m}Tc-HSA is delivering 1.5 mSv.

The absorbed radiation dose to the heart resulting from an intravenous injection of 740 MBq of ^{99m}Tc-HSA corresponds to 14.8 mGy, and to the kidneys, 6.0 mGy.

The absorbed radiation dose per unit activity (GBq) of administered ^{99m}Tc-HSA is shown for selected organs in adults (70 kg) in Table 12.2.1.1. Calculations are based on a biological half-time of 6.8 h for the elimination from blood and whole body (expressed as mGy/GBq and rad/mCi).

Table 12.2.1.1. ^{99m}Tc-human serum albumin (HSA)-Absorbed organ dose

Organs	mGy/GBq	rad/mCi
Adrenals	8.3	0.022
Heart	20.0	0.054
Kidney	8.1	0.022
Liver	7.3	0.019
Spleen	14.0	0.038

Storage and Stability

Storage. Kits should be stored at 2–8 °C. ^{99m}Tc-HSA injection solution should be kept in the refrigerator.

Stability. ^{99m}Tc-HSA injection solution should be used within 6 h after labeling.

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12.2.2 ^{99m}Tc-Albumin Macroaggregates (MAA) (Size Range: 10–50 μm)

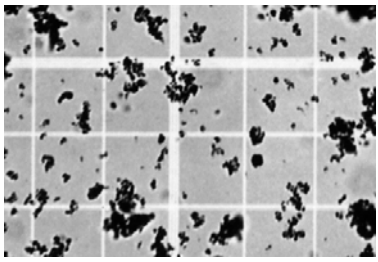
I. Zolle and Gy. Jánoki

Chemical name

Macroaggregated human serum albumin (MAA)

Technetium 99m Macrosalb injection (*Ph. Eur.*)

^{99m}Tc-MAA



Albumin Macroaggregates
(Small square 50×50 μm, 150-fold)

Kit components	
Macroaggregated albumin	0.5–2 mg
Human albumin (HSA)	0.5 mg
Stannous chloride (Dihydrate)	1.2–2.0 mg

Commercial products	
TechneScan MAA	Mallinckrodt/Tyco
MAAScint	Nordion
PulmoCis (TCK-8)	CIS Bio
Macrotec Sorin	GE Healthcare
MAASol Sorin	GE Healthcare

Preparation

Commercial kits contain the lyophilized, sterile components in a multidose vial, sealed under a nitrogen atmosphere. Labeling is carried out by adding aseptically a volume of 2–10 ml of sterile ^{99m}Tc-pertechnetate to the vial with an activity up to 3.7 GBq. The manufacturer's instructions should be followed. The lyophilized material will dissolve by agitating the reaction vial. The reaction should be allowed to proceed for 5–20 min, with occasional agitation. ^{99m}Tc-macroaggregated albumin (MAA) is a pale-white suspension ready for intravenous injection. The pH of the suspension is 3.5–7.5.

Description of the Kit

Macroaggregates are obtained by aggregation of human serum albumin (HSA). HSA is isolated from donor blood and complies with the purity standards stated in the *European Pharmacopeia,* monographs 255 and 853) (Council of Europe 2004a, b) in accordance with EU and WHO requirements for biological substances (Council of Europe 1992; World Health Organization 1994).

Aggregation of albumin is carried out at acidic pH under controlled conditions, yielding macroaggregates with a particle size distribution between 10 and 90 μm. Larger aggregates are separated by filtration of the crude product. Typically, kits contain a

suspension of MAA with a size distribution of 10–50 μm. Generally, 2 mg of macroaggregates correspond to 1.5–2 million albumin particles.

Lactose, glucose (anhydrous), HSA, succinic acid, sodium acetate, sodium phosphate, dibasic sodium phytate (anhydrous), as well as Tween-80 or PVP-40 may serve as stabilizers. The kit contains no preservatives.

The first suspensions of radioalbumin were introduced for photoscanning the liver, spleen, lung, and other organs, because they contained particles in the colloidal range (≤1 μm) and between 10 and 20 μm (Taplin et al. 1964a, b). At the time, solutions of iodinated albumin (0.1% radioalbumin, pH 5.5) with a specific activity of 1 mCi of ^{131}I/mg of HSA) had been provided by E.R. Squibb & Sons for investigative purposes, and were heat-aggregated in-house for 20 min, at different temperatures in a water bath (60–80 °C). At Johns Hopkins, these preparations (10–100 μm) were evaluated in dogs in which pulmonary embolism had been produced experimentally, extending lung perfusion scanning to the first human studies with ^{131}I-MAA (Wagner et al. 1964a,b). In the early seventies, one-step kit preparations with technetium-99m became available and have contributed to the worldwide application of ^{99m}Tc-MAA (Chandra et al. 1973; Monroe et al. 1974; Robbins et al. 1976; Subramanian et al. 1972; Taplin and MacDonald 1971).

Clinical Applications

Intravenous injection: Lung perfusion scintigraphy
Radionuclide venography

Lung perfusion imaging with labeled, biodegradable particles has become an important diagnostic tool for the diagnosis of regional perfusion defects observed in patients with pulmonary disease (i.e., emphysema, chronic obstructive disease, pulmonary hypertension, fibrosis, acute arterial obstruction) (Bell and Simon 1976; Saenger et al. 1985; Tow et al. 1966). The mechanism of lung retention of particles like MAA is known as capillary blockade.

Even though a perfusion scan will localize an obstructed pulmonary artery, accurate diagnosis of pulmonary embolism requires an additional ventilation study with a radioactive gas or aerosol (Agnew 1991; Gottschalk et al. 1993a,b; Taplin and Chopra 1978; Tow and Wagner 1967; Wagner 1995; Wagner et al. 1968).

^{99m}Tc-MAA has also been used for the evaluation of deep vein thrombosis by venous blood flow studies (Dibos 1995; Vlahos et al. 1976). In order to visualize the deep venous system, a special technique is used for injection into veins on the dorsum of each foot.

Time of Examination

- Lung perfusion scintigraphy: immediately after intravenous injection
- Scintigraphy of the lower extremities: shortly after bilateral intravenous injection

Recommended Activities for Indications

- Lung scintigraphy (adults): 37–185 MBq (1–5 mCi)
- Scintigraphy of the lower extremities: 130–150 MBq (3.5–4.0 mCi).

The lung scanning dose. The number of aggregates to be administered for a lung scan lies between 250,000 and 700,000. The *United States Pharmacopeia* (*USP*) limits the pro-

tein concentration to 1 mg of MAA per 37 MBq (1 mCi) of Tc-99m at the time of administration (United States Pharmacopeia Convention 2000). The number of macroaggregates in 1 mg has been calculated as approximately 700,000 particles, which is the upper limit for intravenous injection in adults. A sufficient number of particles need to be administered to avoid nonuniform spatial distribution of radioactivity in lung regions (Heck and Duley 1974).

Calculation of safety factors. Clinical safety factors for particulate injections are derived from lethal dose (LD_{50}) values or the minimum lethal dose (MLD) expressed in units of weight (mg/kg body weight [BW]) or as the number of particles per gram of BW. Accordingly, the amount of particles injected is given in milligrams or as the number of particles. BW is based on 70 kg (average adult).

$$\text{Safety factor (SF)} = \frac{LD_{50} \text{ or MLD (mg/kg)} \times 70 \text{ (kg) BW}}{\text{Injected dose (mg or number of particles)}}$$

Based on SF derived from toxicity studies, the minimum amount of MAA for a lung scan in adults (70 kg) has been derived as 10 μg/kg BW, resulting in a lung scanning dose (0.7 mg) at least a 1,000 times below the minimum toxic dose in dogs (20 mg/kg) (Taplin and MacDonald 1971). If 1 mg of MAA is injected, an SF of 1,400 would apply. This safety margin also applies to an intravenous dose of 700,000 aggregates.

Pediatric Dose. The amount of radioactivity for infants and children administered for lung scintigraphy is based on body weight, using the scaling factors given in Appendix 1 (Table A1.2). The number of macroaggregates in 1 ml of the injection solution should be considered. Not more than 165,000 particles should be injected in children up to 1 year of age, and not more than 50,000 aggregates in newborns (Davis and Taube 1979; Heyman 1979).

Additional Information

Macroaggregates of albumin must not be injected in patients with a history of hypersensitivity to human albumin.

Careful consideration should be given to limiting the number of aggregates injected when studying patients with known severe pulmonary hypertension (Vincent et al. 1968). Also, in patients with right-to-left cardiac shunts, the number of aggregated albumin particles administered for a lung scan should be reduced to the minimum, because shunted macroaggregates are distributed throughout the entire systemic circulation and may cause microembolism in the brain and kidneys (Taplin and MacDonald 1971).

^{99m}Tc-MAA is injected intravenously with the patient in supine position. Prior to injection, the syringe should be inverted repeatedly to resuspend sedimented macroaggregates; they should be injected slowly over a period of at least 30 s to normalize different phases of the respiratory cycle (Wagner 1995).

Aspiration of blood must be avoided; if blood is drawn into the syringe, formation of larger aggregates with coagulated blood occurs. The syringe should not be back flushed.

^{99m}Tc-MAA should not be injected through a lying catheter (butterfly) because of the occasional observation of "hot spots" in the lung.

Quality Control

Radiochemical Purity. The *European Pharmacopeia* (Council of Europe 2005) requires membrane filtration (3 μm) for the determination of radiochemical purity. Unbound radioactivity is in the filtrate; not less than 90% of the total radioactivity is measured on the filter.

The *USP* permits centrifugation of a sample obtained from a well mixed injection solution. Less than 10% of the total radioactivity is measured in the supernatant.

Paper chromatography (*USP*) is recommended for the identification of impurities, using 70% methanol as solvent. Free ^{99m}Tc-sodium pertechnetate is measured at $R_f = 0.6$, and ^{99m}Tc-MAA is identified at the origin. The radiochemical purity of ^{99m}Tc-MAA should not be less than 90%.

Paper chromatography (*USP*)		
Stationary phase:	Whatman No. 1 paper	
Solvent:	Methanol : water, 70 : 30 (v/v)	
R_f values:	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-MAA:	0.0–0.1 (>90%)
	^{99m}Tc-Na-pertechnetate:	0.6–0.7

Thin-layer chromatography (recommended by the manufacturer)		
Stationary phase:	Gelman ITLC-SG (fiberglass), 2×9.5 cm	
Solvent:	Acetone (resp. methylethylketone [MEK])	
R_f values:	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-MAA:	0.0–0.1
	^{99m}Tc-Na-pertechnetate:	0.9–1.0

Thin-layer chromatography offers the advantage of rapid development (5–10 min).

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter), and the regional radioactivities are expressed as a percentage of the total recovered counts:

$$^{99m}\text{Tc-MAA}\ (\%) = 100 - F$$

where F (%) = ^{99m}Tc-Na-pertechnetate (free).

Results of ^{99m}Tc-MAA analysis *(12 samples)*

Labeling and stability	20 min (%)	4 h (%)
Paper chromatography		
^{99m}Tc-macroaggregates	98.8±0.06	99.2±0.04
^{99m}Tc-Na-pertechnetate	1.2±0.05	0.8±0.06
Thin-layer chromatography		
^{99m}Tc-macroaggregates	99.4±0.01	99.6±0.01
^{99m}Tc-pertechnetate	0.6±0.02	0.4±0.01

Particle Size Distribution. The method described in the *European Pharmacopeia* is based on the microscopic examination of 5,000 albumin aggregates, using a suitable counting chamber such as a hemocytometer.

Macroaggregates show a size distribution of diameters between 10 and 90 μm. Typically, 90% of a suspension is within 10–50 microns. Less than ten aggregates are bigger than 75 μm; none is larger than 100 μm. The number of macroaggregates in 1 ml of the injection solution should also be determined.

Pharmacokinetic Data

Following intravenous injection, more than 90% of the technetium-99m MAA is extracted during the first pass and retained in lung capillaries (8.2 ± 1.5 μm) and arterioles (25 ± 10 μm) (Taplin and MacDonald 1971). Organ selectivity is directly related to particle size. Albumin aggregates smaller than 8 μm pass the pulmonary capillary bed and are taken up in the reticuloendothelial system. With a particle diameter above 15 μm, aggregates are retained in the lung capillaries by a purely mechanical process. Distribution of aggregated albumin in the lung is a function of regional pulmonary blood flow (Wagner et al. 1964 a).

Macroaggregates are sufficiently fragile for the capillary microocclusion to be temporary. Erosion and fragmentation reduce the particle size, facilitating removal of aggregates from the lung (Taplin and MacDonald 1971). Subsequently, the fragments are accumulated in the liver by phagocytosis (Chandra et al. 1973; Robbins et al. 1976).

The elimination of radioactivity from the lung is described by half-times between 4 and 6 h (Taplin and MacDonald 1971). Experimental data indicate an initial fast component, which has been interpreted as rapid release of unbound ^{99m}Tc activity (Malone et al. 1983).

Accumulation in the liver is assumed to amount to 25%, with an uptake half-time of 6 h and an elimination half-time of 5 days (International Commission on Radiological Protection 1987); considerable uptake in the liver has been reported (Chandra et al. 1973; Robbins et al. 1976).

Excretion of released pertechnetate in the urine is reported as 40 ± 14% in 24 h, and an additional 9.0 ± 3.8% up to 48 h. Intestinal activity has been shown to increase slowly up to 24 h (Malone et al. 1983).

Radiation Dose

Lung, liver, and the bladder wall are the most exposed organs. The effective (whole body) dose equivalent is 0.012 mSv/MBq (International Commission on Radiological Protection 1987). Elimination from the lung is assumed with half-times of 6 h (0.85) and 3 days (0.15). The liver takes up a fraction of 0.25, with an uptake half-time of 6 h and an elimination half-time of 5 days. Radionuclide released from the lung is primarily excreted by the kidneys (Malone et al. 1983).

The effective (whole body) dose in adults (70 kg) resulting from an intravenous injection of 185 MBq of ^{99m}Tc-MAA for lung scintigraphy is approximately 2.2 mSv. The absorbed radiation dose to the lung resulting from an intravenous injection of 185 MBq of ^{99m}Tc-MAA for a lung scan corresponds to 12.4 mGy, and to the liver, approximately 3.0 mGy.

The effective (whole body) dose resulting from bilateral venography injecting a total of approximately 185 MBq (5 mCi) of ^{99m}Tc-MAA was estimated as 1.35 mSv (Malone et al. 1983).

Storage and Stability

Storage. Kits should be stored at 2–8 °C, and ^{99m}Tc-MAA injection solution should be kept at 2–8 °C, with adequate shielding.

Stability. ^{99m}Tc-MAA injection solution should be used within 6 h after labeling.

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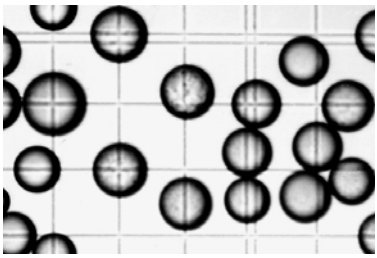
12.2.3 ^{99m}Tc-Albumin Microspheres (HAM) (Size Range: 10–50 μm)

I. Zolle

Chemical name	
Human serum albumin microspheres (HAM) HSA-microspheres Technetium ^{99m}Tc microspheres injection (*Ph. Eur.*) ^{99m}Tc-HSA microspheres ^{99m}Tc-HAM	Albumin microspheres (40 μm) (Small square 50×50 μm, 150-fold)

Kit components[1]		**Kit components**[2]	
HSA-microspheres	10.0 mg	HSA-microspheres	2.5 mg
Stannous chloride·2 H_2O	1.3 mg	Stannous chloride·2 H_2O	0.1 mg
Pluronic F68	0.25 mg	Tween-80	0.6 mg
Sodium chloride	9.0 mg		

Preparation

Commercial kits contain the lyophilized, sterile components including preformed albumin microspheres in a multidose vial, sealed under a nitrogen atmosphere. Labeling with ^{99m}Tc eluate is carried out under aseptic conditions by injecting a volume of 2–10 ml of eluate corresponding to a ^{99m}Tc activity of maximal of 5.5 GBq (5–150 mCi). The reaction is allowed to proceed at room temperature for 15 min. ^{99m}Tc-human albumin microspheres (HAM) is a sterile, pyrogen-free suspension suitable for intravenous injection. The pH of the injection solution is 4.0–9.0 (*European Pharmacopeia*).

Description of the Kit

Albumin microspheres are obtained by heat denaturation of HSA in vegetable oil (Zolle et al. 1970). HSA (25% solution) used for preparation of microspheres complies with the purity standards stated in the *European Pharmacopeia* (Council of Europe 2004 a, b) in accordance with EU and WHO requirements for biological substances (Council of Europe 1992; World Health Organization 1994).

The size distribution of albumin particles depends mainly on the degree of dispersion of HSA in oil; generally, a size distribution between 12 and 45 μm was produced.

Fractions with distinct size ranges were obtained by sieving (Rhodes et al. 1969; Zolle et al. 1970). This permits a close estimate of the number of microspheres in 1 mg.

Commercial kits may contain 10 mg of microspheres, corresponding to 800,000–1,600,000 microspheres per vial (kit formulation 1) or 2.5 mg of microspheres, corresponding to 300,000–500,000 microspheres per vial (kit formulation 2). Typically, kits contain albumin microspheres with a size distribution between 10 and 50 μm. Tween-80 or Pluronic F68 serve as surfactants to avoid aggregation of microspheres.

Originally, albumin microspheres containing iron (ferric hydroxide) were used for labeling with ^{113m}In or ^{99m}Tc; these labeling procedures included heating in a water bath (Rhodes et al. 1969). Later on, a kit preparation of ^{99m}Tc-HAM, using iron loaded microspheres in the presence of sodium thiosulfate at acidic pH (and heating) was introduced (Bolles et al. 1973; Krejcarek et al. 1974). A comparison of different labeling techniques has been reported (Mayron and Kaplan 1975).

Preformed albumin microspheres are labeled with high radiochemical yield either by using a commercial kit or by electrolytic reduction by using a tin electrode. No heating or pH adjustments are required. The specific activity of ^{99m}Tc-HAM should not be less than 185 MBq (5 mCi) per 1 million microspheres according to the *European Pharmacopeia* (Council of Europe 2005).

^{99m}Tc-eluate used for labeling the preformed microspheres should be obtained from a generator by daily elution in order to minimize ^{99}Tc carrier, and should comply with specifications stated in the *European Pharmacopeia*.

Clinical Applications

Intravenous injection:	Pulmonary perfusion scintigraphy
	Determination of right-to-left shunts
Arterial injection:	Regional perfusion in other organs

^{99m}Tc-albumin microspheres satisfy the requirements of particles for systemic application with the indicator fractionation technique, namely, uniform size and shape, available in desired sizes, extracted completely in a single passage through capillary beds, and metabolized within hours after injection (Rhodes et al. 1969, 1971; Wagner et al. 1968, 1969; Zolle and Kropf 1982).

When injected into a systemic artery, microspheres indicate regional blood flow in that organ. Myocardial blood flow was measured in dogs (Fortuin et al. 1971; Weller et al. 1972) . ^{99m}Tc-HAM has also been used for measuring regional blood perfusion in the heart (Martin et al. 1973) and the brain in monkeys (Alm 1975). This method has been used for the measurement of the shunted blood through patent arteriovenous connections in the leg after femoral artery injection (Rhodes et al. 1969, 1973).

The size and number of microspheres can be controlled, and labeling is performed with high specific activity; as a result, ^{99m}Tc-albumin microspheres of distinct particle size have been used to quantify the shunted blood flow reaching the lungs (Strauss et al. 1969; Wagner et al. 1969) and arteriovenous shunts in brain tumors (Bergmann et al. 1973).

Time of Examination

- Lung perfusion scintigraphy: immediately after intravenous injection
- Regional organ perfusion: immediately after arterial injection

Recommended Activities for Indications

Lung scintigraphy: 75–185 MBq, (2–5 mCi) injected intravenously
≤0.010 mg microspheres/kg body weight (BW)
≤250,000 microspheres (Food and Drug Administration [FDA])

Regional organ scintigraphy: 75–111 MBq, (2–3 mCi) injected intraarterially

The lung scanning dose. The number of microspheres to be administered for a lung scan lies between 100,000 and 250,000. The lung scanning dose normalized to BW is 10 μg/kg, keeping the patient dose generally below 1 mg of microspheres. The FDA has limited the number of microspheres used for lung scintigraphy to 250,000 particles. However, a sufficient number of particles need to be administered to avoid nonuniform spatial distribution of radioactivity in lung regions (Heck and Duley 1974).

Calculation of safety factors. Clinical safety factors for particulate injections are derived from LD_{50} values or the minimum lethal dose (MLD) expressed in units of weight (mg/kg BW) or as the number of particles per gram of BW. Accordingly, the amount of particles injected is given in mg or as the number of particles. BW is based on 70 kg (average adult).

$$\text{Safety factor (SF)} = \frac{LD_{50} \text{ or MLD (mg/kg)} \times 70 \text{ (kg) BW}}{\text{Injected dose (mg or number of particles)}}$$

The sublethal toxicity of microspheres (15–30 μm) in the lung was determined as 20 mg/kg BW in dogs (Bolles et al. 1973). Based on a patient dose of 0.7–1 mg of microspheres (15–30 μm) for lung perfusion scintigraphy, SFs between 1,400 and 2,000 are derived. Theoretically, 1 mg of microspheres with a diameter of 20 μm contains 238,732 spheres. A sample of microspheres ranging between 12 and 29 μm in diameter actually showed 234,800–246,000 spheres in 1 mg by Coulter measurements (Zolle et al. 1970).

Pediatric Dose. The amount of radioactivity for infants and children administered for lung scintigraphy is based on body weight, using the scaling factors given in Appendix 1 (Table A1.2). In addition, the number of microspheres in 1 ml of injection solution should also be considered. Only a fraction of the number of microspheres used in adults should be administered in children (Davis and Taube 1978; Heyman 1979).

Additional Information

The use of ^{99m}Tc-albumin microspheres in patients with a history of hypersensitivity to human albumin is contraindicated.

^{99m}Tc-albumin microspheres should not be injected together with other drugs or components to avoid aggregates.

For intravenous injection ^{99m}Tc-HAM microspheres should be homogenously suspended to avoid in vivo aggregates. For this reason, the aspiration of blood into the syringe must be avoided.

^{99m}Tc-HAM should not be injected through a lying catheter (butterfly) because of the occasional observation of "hot spots" in the lung.

Quality Control

Radiochemical Purity. ^{99m}Tc-albumin microspheres are described in the *European Pharmacopeia* (Council of Europe 2005). Membrane filtration (3-μm pore size) is used to determine unbound radioactivity in the filtrate. Not less than 95% of the total radioactivity is measured on the filter.

An alternate method for laboratory use is centrifugation of a homogenously suspended sample of ^{99m}Tc-albumin microspheres for 2 min at 2,000 rpm and separation of the supernatant. Both test tubes are measured in a gamma counter, and the radioactivity is expressed as a percentage of the sum of recovered counts. Less than 5% of the total radioactivity is measured in the supernatant.

Recommended Methods

Paper chromatography is recommended by the manufacturer, using 80% methanol as solvent. Free ^{99m}Tc-sodium pertechnetate is measured at an R_f of 0.6 and ^{99m}Tc-HAM at the origin ($R_f = 0.0$). The radiochemical purity of ^{99m}Tc-HAM should not be less than 95%.

Recommended methods for the determination of radiochemical purity

Paper chromatography		
Stationary phase:	Whatman No. 1 paper strips, 2×9.5 cm	
Solvent:	Methanol:water, 80:20 (v/v)	
Developing time:	10 min	
R_f values:	^{99m}Tc-HAM:	0.0–0.1 (>95%)
	^{99m}Tc-pertechnetate:	0.6–0.7
Thin-layer chromatography		
Stationary phase:	Gelman ITLC-SG (fiberglass), 2×9.5 cm	
Solvent:	Acetone	
Developing time:	5 min	
R_f values:	^{99m}Tc HAM:	0.0–0.1
	^{99m}Tc reduced, colloidal:	0.0–0.1
	^{99m}Tc-pertechnetate:	0.9–1.0 (<5%)

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter), and the regional radioactivities are expressed as a percentage of the total recovered counts:

$$^{99m}\text{Tc-HAM}\ (\%) = 100 - F$$

where F (%) = ^{99m}Tc-Na-pertechnetate (free).

Results of analysis *(12 samples)*
Results were obtained using paper chromatography and methanol-water as solvent.

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-HSA microspheres	99.1 ± 0.05	98.6 ± 0.20
Free ^{99m}Tc-pertechnetate	0.9 ± 0.05	1.4 ± 0.21

Particle Size Distribution. The method described in the *European Pharmacopeia* is based on the microscopic examination of 5,000 albumin microspheres, using a suitable counting chamber such as a hemocytometer.

Microspheres show a homogenous distribution of diameters between 10 and 50 μm. Less than ten microspheres are bigger than 75 μm, and none is larger than 100 μm.

The number of microspheres in 1 ml of the injection solution should also be determined.

Pharmacokinetic Data

^{99m}Tc-albumin microspheres are trapped in the first capillary bed they encounter. When injected intravenously, this is the lung (Zolle and Kropf 1982; Zolle et al. 1970). More than 95% of the injected radioactivity is extracted by the pulmonary capillaries and arterioles in a single passage and distributed in the lung according to regional pulmonary arterial blood flow. The size of the microspheres and the diameter of blood vessels affect the measurement of blood flow. Capillary sizes may vary from organ to organ, and a fraction of the blood may pass through arteriovenous shunts. Under these conditions, ^{99m}Tc-HAM bypass the pulmonary circulation when injected for lung scanning. Microspheres passing through transpulmonary shunts are distributed throughout the systemic circulation. The brain is selected for measuring the fractional radioactivity of microspheres lodged in the cerebral microcirculation (Strauss et al. 1969).

^{99m}Tc-albumin microspheres are metabolized in the capillary lumen (Zolle and Kropf 1982; Zolle et al. 1970). Lung removal rates depend on the size and density of microspheres. Larger microspheres are removed from the lung more slowly. Microspheres with a particle diameter of 12–44 μm are extracted from blood almost completely (≥98%); when prepared at 146 °C, HSA microspheres are eliminated from lung capillaries with a biological half-time of 7.2 h (Zolle and Kropf 1982; Zolle et al. 1970).

Toxicity and Safety Factors. Hemodynamic effects caused by capillary blockade show a direct relationship between the size and number of microspheres injected (Mishkin and Brashear 1971). To avoid an overdose of microspheres, the parameters causing an increase in pulmonary arterial pressure were systematically investigated (Allen et al. 1978; Davis and Taube 1978; Harding et al. 1973; Heyman 1979).

Calculations derived from microspheres with uniform size, having diameters of 20, 28, 40, and 60 μm, have indicated a similar proportion of pulmonary vessels blocked, namely 0.2–0.3%, when 1 mg of these microspheres was injected. However, when calculations were based on the same number of microspheres (i.e., 100,000), the percentage of blocked vessels increased considerably with the diameter of the microspheres (Harding et al. 1973).

Table 12.2.3.1 Influence of particle size on safety factors expressed for total number (100,000) and total mass (mg) of particles injected

Particle diameter (μm)	No. of particles per mg	Safety factors	
		Per 10^5 particles injected	Per mg total mass injected
13.5	776,247	62,380	8,094
15.0	565,884	33,600	5,938
15.8	484,206	33,600	6,939
25.7	112,513	16,630	14,781
28.0	87,002	16,630	19,114
45.4	20,410	4,010	19,647
90.7	2,560	360	14,062

HSA microspheres with a size distribution between 20 and 40 μm contain approximately 90,000 microspheres per milligram. Smaller HSA microspheres with a size distribution between 15 and 20 μm contain approximately 500,000 microspheres per milligram (Table 12.2.3.1).

^{99m}Tc-albumin microspheres (≤1 mg) are well tolerated without complications (Rhodes 1971; Stang et al. 1975). One case of adverse reaction to ^{99m}Tc-HAM has been reported (Littenberg 1975). Hypersensitivity reactions are rare and caused mainly by agents used for suspension of ^{99m}Tc-albumin microspheres. Intracoronary injection of up to 200,000 microspheres had no significant effect upon cardiac function and does not cause myocardial damage (Weller 1975).

Sublethal pulmonary toxicity. Albumin microspheres (15–30 μm) were repeatedly administered in dogs and monkeys showing transient symptoms such as diarrhea, emesis, or decreased activity with amounts of 20 mg/kg BW (Bolles et al. 1973). Single doses up to 40 mg/kg BW were tolerated in mice, rabbits, and dogs without complications (Rhodes et al. 1969).

Acute pulmonary toxicity. The first signs of pulmonary toxicity are an abrupt increase in the pulmonary artery pressure and a sharp fall in the femoral artery pressure (Bolles et al. 1973). The lethal dose (LD_{50}) of microspheres (15–30 μm) in mice is 72.2 ± 8.3 mg/kg BW and in rats, 43.8 ± 5.0 mg/kg BW.

Radiation Dose

The lung and bladder wall are the most exposed organs. The effective (whole body) dose equivalent is 0.011 mSv/MBq (International Commission on Radiological Protection 1987). Elimination from the lung is assumed with half-times of 1.8 h (0.60) and 1.5 days (0.40). Radionuclide released from the lung is primarily excreted by the kidneys (Blau et al. 1982).

The effective (whole body) dose in adults (70 kg) resulting from an intravenous injection of 185 MBq of ^{99m}Tc-HAM for lung scintigraphy is 2.0 mSv.

The absorbed radiation dose to the lung resulting from an intravenous injection of 185 MBq of ^{99m}Tc-HAM is 10.7 mGy.

Storage and Stability

Storage. The lyophilized kit is stored at 2–8 °C.

Stability. The ^{99m}Tc-HAM injection solution is stable for 6 h after preparation.

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12.3 ^{99m}Tc-Labeled Colloids

12.3.1 ^{99m}Tc-Labeled Microcolloids

12.3.1.1 ^{99m}Tc-Tin Colloid (Size Range: 0.2–0.8 μm)

I. Zolle

Chemical name		**Listed trade names**	
Colloidal tin hydroxide		Amerscan Hepatate II	GE Healthcare (1)
Technetium ^{99m}Tc tin colloid injection (*Ph. Eur.*)		Livoscint	Bristol-Myers Squibb (2)
^{99m}Tc-tin colloid			
Kit components[1]		**Kit components**[2]	
Tin(II)-fluoride	0.125 mg	Tin(II)-chloride dihydrate	0.3 mg
Sodium fluoride	1.0 mg	Sodium fluoride	0.9 mg
Poloxamer 188	0.5 mg	Sodium chloride	4.5 mg

Preparation

The kit contains the lyophilized, sterile ingredients in a multidose vial. Labeling with ^{99m}Tc-pertechnetate is carried out under aseptic conditions by adding a suitable volume of sterile ^{99m}Tc eluate, up to 3.7 GBq (100 mCi) to the reaction vial. The reaction is allowed to proceed at room temperature for 20 min. ^{99m}Tc-tin colloid is a sterile, pyrogen-free, opalescent solution suitable for intravenous injection. The pH is 4.0–6.0 on pH paper.

Description of the Kit

^{99m}Tc-tin colloid is a hydrolysis product and easily formed at pH > 3, with the reduced technetium-99m-oxide (Lin and Winchell 1972). Poloxamer 188 is added to stabilize the colloid. No heating or pH adjustments are required. Once formed, the particle size distribution is unaffected by time.

The ^{99m}Tc-tin colloid shows a particle size distribution between mainly 0.2 and 0.8 μm (Lin and Winchell 1972; Whateley and Steele 1985).

Factors causing low colloid formation (low specific activity) are primarily related to pH, inadequate reaction time, or a defect of kit formulation. ^{99m}Tc eluate used for colloid preparation should be obtained from a generator by daily elution in order to minimize ^{99}Tc carrier (Ponto et al. 1987).

Clinical Applications

Intravenous injection: Liver and spleen scintigraphy

Time of Examination

- 10–15 min after the intravenous injection

Recommended Activities for Indications

Liver and spleen scintigraphy: 75–185 MBq (2–5 mCi), injected intravenously, slowly
200 MBq maximal activity for single-photon emission computed tomography (SPECT)
0.2 mg tin colloid/kg body weight
The manufacturer's instructions should be followed.

Pediatric Dose. The amount of radioactivity for infants and children administered for liver/spleen scintigraphy is based on body weight, using the scaling factors given in Appendix 1, Table A1.2. The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

^{99m}Tc-tin colloid should not be injected together with other drugs or components.

Diffuse pulmonary accumulation of radiocolloids may result from elevated plasma levels of aluminum in patients with antacid therapy, because the ^{99m}Tc-tin colloid co-precipitates with larger aggregates of aluminum phosphate (Ponto et al. 1987). A number of clinical conditions associated with pulmonary uptake of ^{99m}Tc-labeled colloids have been reported (Hladik et al. 1987 a).

Hepatotoxic substances interfere with phagocytosis, causing transient changes, such as inhomogeneous or irregular distribution of radiocolloids in the liver/spleen or a shift of uptake from liver to spleen and/or bone marrow. Anesthetics (halothane) affect both phagocytic and catabolic reticuloendothelial system (RES) function, resulting in an increased uptake in the spleen (Hladik et al. 1987 b). The plasma expander dextran or protein deficiency may also reduce uptake of ^{99m}Tc-labeled colloids by the Kupffer cells (Hodges 1987).

Androgen therapy may stimulate the phagocytic activity of the RES, so that macrophages are released from storage sites and trapped in the lung. In the lung, these phagocytic cells extract the ^{99m}Tc-labeled colloid (Hladik et al. 1987 b).

Quality Control

Radiochemical Purity. The *European Pharmacopeia* requires thin-layer chromatography on silica gel fiberglass sheets and a migration distance 10–15 cm for the identification of impurities, using 0.9% sodium chloride solution (saline) as solvent. Free ^{99m}Tc-sodium pertechnetate is measured at an R_f of 1.0, and ^{99m}Tc-tin colloid is identified at the origin. The radiochemical purity of ^{99m}Tc-tin colloid should not be less than 95% (Council of Europe 2005)

The analysis of radiocolloids is based on the determination of free ^{99m}Tc-Na-pertechnetate, since colloidal activity remains at the start. Hydrolized ^{99m}Tc activity cannot be distinguished from the ^{99m}Tc-tin colloid.

Recommended Methods

Thin-layer chromatography using saline ore acetone as solvent

Thin-layer chromatography (*Ph. Eur.*)		
Stationary phase:	Gelman ITLC-SG (fiberglass), 2×10 cm	
Solvent:	Saline	
Developing time:	10 min	
R_f values:	^{99m}Tc-tin colloid:	0.0–0.1
	^{99m}Tc-reduced, colloidal:	0.0–0.1
	^{99m}Tc-pertechnetate:	0.9–1.0 (< 5%)

TLC using saline is described for: ^{99m}Tc-tin colloid (Amerscan Hepatate II).

TLC using acetone has been in clinical use for: ^{99m}Tc-tin colloid, ^{99m}Tc–Nanocoll and ^{99m}Tc-(Re)-sulphide colloids.

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter), and the regional radioactivities are expressed as a percentage of the total recovered counts:

$$^{99m}\text{Tc-(tin) colloid (\%)} = 100 - F$$

where F (%) = ^{99m}Tc-Na-pertechnetate (free).

Results of analysis *(12 samples)*
Results were obtained using thin-layer chromatography and saline as solvent.

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-tin colloid	99.0 ± 0.37	98.7 ± 0.35
^{99m}Tc-Na-pertechnetate	1.0 ± 0.39	1.3 ± 0.39

Bioassay of Colloidal Suspensions. To assure a particle size in the colloidal range (≤ 1 μm), the *European Pharmacopeia* recommends a physiological test in mice. At least 80% of the intravenously injected radiocolloid should localize in the liver and spleen of three mice. Lung uptake should not exceed 5%.

Pharmacokinetic Data

Intravenously injected colloids distribute within the body according to the phagocytic function of the reticuloendothelial system (RES). Size has considerable influence on the biodistribution of colloidal particles. With a particle size of 0.3–0.6 μm, 80–90% of the radioactivity is seen in the liver, with 5–10% seen in the spleen and 5–9% in the bone marrow (Adams et al. 1980; Colombetti 1974; Lin and Winchell 1972; Nelp 1975; Whateley and Steele 1985). Larger colloidal particles show increased splenic uptake, whereas smaller particles localize in the bone marrow (Schuind et al. 1984; Subramanian and McAfee 1970).

Increased splenic uptake has been seen with decreased liver function, i.e., cirrhosis (Atkins et al. 1975). Extrahepatic accumulation of ^{99m}Tc-labeled colloids is also seen in the bone marrow in the case of hyperplastic bone marrow and with certain hematological disorders (Höfer and Egert 1963; Höfer et al. 1964; Nelp and Bower 1969). Occasionally, uptake has been observed in the lung and kidneys (Klingensmith et al. 1976).

In healthy persons, the colloid is rapidly removed from the blood by phagocytosis, mainly in the liver. The colloid disappearance rate has been used to estimate liver blood flow (Vetter et al. 1954). The clearance half-time of colloidal radiogold in male and female patients without liver or circulatory disorders was measured as 2.57 and 2.64 min, respectively; patients with liver cirrhosis showed a considerable increase of clearance half-times (mean 7.37 and 7.29 min).

Inorganic colloids may remain within the macrophages indefinitely. However, ^{99m}Tc-tin colloid is eliminated from the fixed macrophages of the liver and spleen with half-times of approximately 71 and 37 h, respectively (Lin and Winchell 1972).

The metabolism of soluble bivalent tin was studied in rats after intravenous injection of $[^{113}\text{Sn}]\text{SnCl}_2 \cdot 2\text{H}_2\text{0}$ (30 μCi, no carrier added). It was apparent that tin(II)-chlo-

ride is rapidly cleared from the circulation, accumulating mainly in the liver (56.2% after 24 h) and spleen (6.9% after 24 h). The elimination from the liver followed an effective half-time of 85 days, from the spleen, of 50 days (Marciniak 1981).

Toxicity studies. Fourteen-day studies in mice and dogs (intravenous injection) produced no significant signs of toxicity at levels of 3,108 and 490 times the maximum human dose (≤0.2 mg/kg).

The acute chemical toxicity of $SnCl_2 \cdot 2H_2O$ was determined after the intravenous injection of 1–12 mg Sn^{2+}/kg body weight in rats. The animals were observed for 30 days for signs of toxicity or death. The LD_{50} value of $SnCl_2 \cdot 2H_2O$ was determined in rats as 7.83 mg Sn^{2+}/kg body weight. Lethality was observed with doses exceeding 3 mg Sn^{2+}/kg body weight; death occurred within 24 h after the intravenous injection (Marciniak 1981).

Radiation Dose

The liver, spleen, and red marrow are the most exposed organs. Calculations of the absorbed radiation dose resulting from liver and spleen scintigraphy are based on technetium-labeled colloids (International Commission on Radiological Protection 1987). The effective dose equivalent is 0.014 mSv/MBq. The effective whole-body dose in adults (70 kg) resulting from an intravenous injection of 185 MBq of ^{99m}Tc-tin colloid is 2.6 mSv.

The effective dose equivalent per ICRP 62 has been replaced by the quantity effective dose. Values per unit administered activity were published in Addendum 1 (International Commission on Radiological Protection 1991). Values calculated accordingly are slightly lower than the effective whole body doses presented here.

Storage and Stability

Storage. The lyophilized kit is stored at 2–8 °C.

Stability. ^{99m}Tc-tin colloid injection is stable for 6 h after the preparation.

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12.3.1.2 ^{99m}Tc-Rhenium Sulfide Colloid (Size Range: 0.3–1.0 μm)

I. Zolle

Chemical name	**Listed trade names**
Technetium ^{99m}Tc colloidal rhenium sulfide injection (*Ph. Eur.*) Technetium Tc 99m sulfur colloid injection (*USP*) ^{99m}Tc-(Re)-sulfide colloid ^{99m}Tc-sulfur colloid	HepatoCis (TCK-1) (1) Sulfotec Sorin (2)

Kit components(1)		**Kit components(2)**	
Potassium perrhenate	4.17 mg	Potassium perrhenate	4.17 mg
Sodium thiosulphate·5 H_2O	10 mg	Sodium thiosulphate·5 H_2O	10 mg
Gelatin	166 mg	Gelatin	100 mg
Water for injection	6 ml		

Preparation

Each kit consists of one vial with two syringes, one syringe containing 1 ml of 4.6 N HCl, the other 2 ml of 0.65 M citrate buffer (pH 12.0).

Each vial contains a sterile, pyrogen-free solution of the ingredients. After adding 5 ml of sterile ^{99m}Tc eluate under aseptic conditions to the vial, the reaction is acidified by adding 1 ml of 4.6 N HCl (syringe 1). The reaction vial is placed into a boiling water bath for 5 min. After cooling, 2 ml of 0.65 M citrate buffer solution (syringe 2) are injected into the vial. The resulting pH should be between 3.5 and 6.0 on pH paper. The manufacturer's instructions should be followed. ^{99m}Tc-(Re)-sulfide colloid is a sterile, pyrogen-free, light brown, solution (14 ml), suitable for intravenous injection.

Description of the Kit

^{99m}Tc-(Re)-sulfide colloid is formed at acidic pH in the boiling water bath. The reaction is based on the formation of colloidal sulfur and technetium heptasulfide (Tc_2S_7) at acidic pH. Rhenium is used as a carrier (Larson and Nelp 1966; Patton et al. 1966). Ten milligrams of sodium thiosulfate pentahydrate generate 2 mg of colloidal sulfur (Stern et al. 1966). The added ^{99m}Tc eluate should not be less than 5 ml, the ^{99m}Tc activity not less than 370 MBq (10 mCi).

In principle, ^{99m}Tc-sulfur colloid and ^{99m}Tc-(Re)-sulfide colloid are prepared using similar conditions (Patton et al. 1966; Larson and Nelp 1966); however, the original for-

mulation did not contain K-perrhenate carrier. Gelatin was used as a stabilizer (Stern et al. 1966; Haney et al. 1971).

^{99m}Tc-(Re)-sulfide colloid and ^{99m}Tc-sulfur colloid, respectively, show a particle size distribution mainly between 0.3 and 0.8 μm.

Factors causing low colloid formation (low specific activity) are primarily related to pH, incorrect order of mixing, low heating temperature, heating a large volume, inadequate boiling time, or a defect of kit formulation. ^{99m}Tc eluate used for colloid preparation should be obtained from a generator by daily elution in order to minimize ^{99}Tc carrier (Ponto et al. 1987). A flocculent precipitate is formed in the presence of Al^{3+} cation (1 μg/ml) (Haney et al. 1971; Ponto et al. 1987).

Clinical Applications

Intravenous injection:	Liver and spleen scintigraphy
	Bone marrow scintigraphy
Oral application:	Digestive transit scintigraphy
	Gastroduodenal motor activity

Time of Examination

Liver and spleen scintigraphy:	15–20 min after the intravenous injection
Bone marrow scintigraphy:	1 h after the intravenous injection
Digestive transit scintigraphy:	Dynamic imaging is started immediately after oral administration of test meal.

Recommended Activities for Indications

Liver and spleen scintigraphy:	75–150 MBq (2–4 mCi), injected intravenously, slowly 200 MBq maximal activity for single-photon emission computer tomography (SPECT) ≤0.2 mg sulfur colloid/kg body weight
Bone marrow scintigraphy:	150 MBq (400 MBq maximum activity)
Digestive transit scintigraphy:	37 MBq (1 mCi) in 30 ml water, followed by 300 ml water The manufacturer's instructions should be followed.

Pediatric Dose. The amount of radioactivity for infants and children administered for liver and spleen, and bone marrow scintigraphy is based on body weight, using the scaling factors given in Appendix 1, Table A1.2. The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

^{99m}Tc-(Re)-sulfide colloid should not be injected together with other drugs or components.

Diffuse pulmonary accumulation of radiocolloids may result from elevated plasma levels of aluminum in patients with antacid therapy, because the ^{99m}Tc-sulfur colloid

coprecipitates with larger aggregates of aluminum phosphate (Ponto et al. 1987). A number of clinical conditions associated with pulmonary uptake of ^{99m}Tc-labeled colloids have been reported (Hladik et al. 1987 a).

Hepatotoxic substances interfere with phagocytosis, causing transient changes such as inhomogeneous or irregular distribution of radiocolloids in the liver and spleen or a shift of uptake from liver to spleen and/or bone marrow. Anesthetics (halothane) affect both phagocytic and catabolic reticuloendothelial system (RES) function, resulting in an increased uptake in the spleen (Hladik et al. 1987 b). The plasma expander dextran or protein deficiency may also reduce uptake of ^{99m}Tc-labeled colloids by the Kupffer cells (Hodges 1987).

Androgen therapy may stimulate the phagocytic activity of the RES, so that macrophages are released from storage sites and trapped in the lung. In the lung these phagocytic cells extract the ^{99m}Tc-labeled colloid (Hladik et al. 1987 b).

Renal accumulation of ^{99m}Tc-sulfur colloid has been reported in transplant patients during rejection and during episodes of acute tubular necrosis. Kanamycin is known to cause acute tubular necrosis (Hodges 1987).

Gastric emptying has been studied using solid meals to which the labeled colloid (^{99m}Tc-(Re)-sulfide colloid or ^{99m}Tc-sulfur colloid) was added (Stacher and Bergmann 1992). After 6 h of fasting, the patient is allowed to eat the test meal. From the plot of percent activity versus time, the half-time of gastric emptying is determined. Depending on the consistency of the test meal (liquid or solid), a shorter (10–15 min) or longer (50–80 min) half-time is measured. Meal size and composition must be standardized. Delayed emptying is associated with a number of disease states.

Orally administered colloids are not absorbed from the gastrointestinal tract.

Quality Control

Radiochemical Purity. The *European Pharmacopeia* requires paper chromatography (distance 10–15 cm) for the identification of impurities using 0.9% sodium chloride solution (saline) as solvent. Free ^{99m}Tc-pertechnetate is measured at R_f 0.6 and ^{99m}Tc-(Re)-sulfide colloid is identified at the origin.

The radiochemical purity of ^{99m}Tc-(Re)-sulfide colloid and ^{99m}Tc-sulfur colloid, respectively, should not be less than 92% (Council of Europe 2005 a, b).

The analysis of radiocolloids is based on the determination of free ^{99m}Tc-Na-pertechnetate, since colloidal activity remains at the start. Hydrolized ^{99m}Tc activity cannot be distinguished from the ^{99m}Tc-(Re)-sulfide colloid.

Paper chromatography (*Ph. Eur.*)		
Stationary phase:	Whatman No. 1 paper, 2×10 cm	
Solvent:	Saline (0.9% NaCl)	
Developing time:	10 min	
R_f values:	^{99m}Tc-(Re)-sulfide colloid:	0.0–0.1 (>92%)
	^{99m}Tc reduced, hydrolized	0.0–0.1
	^{99m}Tc-pertechnetate:	0.6–0.7

Recommended Methods

Thin-layer chromatography on Gelman silica gel fiberglass sheets using acetone as solvent has been recommended for the identification of free ^{99m}Tc-pertechnetate at the solvent front ($R_f = 1.0$) and ^{99m}Tc-(Re)-sulfide colloid at the origin.

This method has been in clinical use for: ^{99m}Tc-(Re)-sulfide colloid, ^{99m}Tc-tin colloid, and ^{99m}Tc-Nanocoll.

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter), and the regional radioactivities are expressed as a percentage of the total recovered counts:

$$^{99m}\text{Tc-(Re)-sulfide colloid (\%)} = 100 - F$$

where F (%) = ^{99m}Tc-Na-pertechnetate (free).

Results of analysis *(12 samples)*

Results were obtained using thin-layer chromatography and acetone as solvent.

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-(Re)-sulfide colloid	97.3 ± 0.47	96.7 ± 0.57
^{99m}Tc-pertechnetate	2.7 ± 0.38	3.3 ± 0.97

Bioassay of Colloidal Suspensions. To assure a particle size in the colloidal range (≤1 μm), the *European Pharmacopeia* recommends a physiological test in mice. At least 80% of the intravenously injected radiocolloid should localize in the liver and spleen of three mice. Lung uptake should not exceed 5%.

Pharmacokinetic Data

Intravenously injected colloids distribute within the body according to the phagocytic function of the RES. Size has considerable influence on the biodistribution of colloidal particles. With a particle size of 0.3–0.6 μm, 80–90% of the radioactivity is seen in the liver, 4–8% in the spleen, and 3–5% in the bone marrow (Adams et al. 1980; Nelp 1975). Larger colloidal particles show increased splenic uptake, whereas smaller particles localize in the bone marrow (Nelp and Bower 1969; Schuind et al. 1984). Increased splenic uptake has been seen with decreased liver function, i.e., cirrhosis (Atkins et al. 1975). Extrahepatic accumulation of ^{99m}Tc-labeled colloids is also seen in the bone marrow in the case of hyperplastic bone marrow and with certain hematological disorders (Höfer and Egert 1963; Höfer et al. 1964). Occasionally, uptake has been observed in the lung and kidneys (Klingensmith et al. 1976).

^{99m}Tc-(Re)-sulfide colloid is rapidly extracted from the blood circulation by phagocytosis, approximately 94% in one passage. The colloid disappearance rate has been used to estimate liver blood flow (Vetter et al. 1954). The clearance half-time of colloidal radiogold in male and female patients without liver or circulatory disorders was measured as 2.57 and 2.64 min, respectively; patients with liver cirrhosis showed a considerable increase of clearance half-times (means of 7.37 and 7.29 min). For the mea-

surement of plasma clearance, a high radiochemical purity of the colloid is essential (Alavi 1982).

Inorganic colloids may remain within the macrophages indefinitely. It has been reported, however, that ^{99m}Tc-labeled sulfur colloid may be metabolized and excreted (Warbick-Cerone and Phythian 1982). However, the urinary excretion (in 48 h) measured in three patients was less than 4% of the injected radioactivity (Larson and Nelp 1966).

No signs of toxicity were observed with large doses of ^{99m}Tc-sulfur colloid (40 mg sulfur/kg) after intravenous injection in mice over a period of 4–7 days (Haney et al. 1971).

Radiation Dose

The most exposed organs are the liver, spleen, and red marrow. Calculations of the absorbed radiation dose resulting from liver and spleen scintigraphy are based on technetium-labeled colloids (International Commission on Radiological Protection 1987). The effective dose equivalent is 0.014 mSv/MBq. The effective whole-body dose in adults (70 kg) resulting from an intravenous injection of 185 MBq of ^{99m}Tc-(Re)-sulfide colloid is 2.6 mSv.

Calculations of the absorbed dose resulting from the oral application of ^{99m}Tc-(Re)-sulfide colloid are based on ICRP Publication 53 (International Commission on Radiological Protection 1987b) for nonabsorbable markers. The effective dose in adults (70 kg) resulting from 37 MBq (1 mCi) of orally administered ^{99m}Tc-(Re)-sulfide colloid is approximately 1 mSv.

The effective dose equivalent per ICRP Publication 62 has been replaced by the quantity effective dose. Values per unit administered activity were published in Addendum 1 (International Commission on Radiological Protection 1991). Values calculated accordingly are slightly lower than the effective whole-body doses presented here.

Storage and Stability

Storage. The kit is stored at room temperature.

Stability. The ^{99m}Tc-(Re)-sulfide colloid injection is stable for 6 h after preparation.

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12.3.1.3 ^{99m}Tc-Albumin Microcolloid (Size Range: 0.2–2.0 μm)

I. Zolle

Chemical name	**Listed trade names**
Human albumin microaggregates	AlbuRes Solco (1)
Technetium albumin colloid injection (*USP*)	Microlite DuPont Merck (2)
^{99m}Tc-albumin colloid	
^{99m}Tc-HSA-microcolloid	

Kit components(1)		**Kit components(2)**	
HSA-microcolloid	2.5 mg	HSA-microcolloid	1.0 mg
Stannous chloride·2 H_2O	0.4 mg	Stannous chloride·2 H_2O	0.17 mg
Monobasic sodium phosphate	0.458 mg	Human albumin	10 mg
Disodium phytate	0.25 mg	Disodium medronate	0.12 mg
Glucose anhydrous	15 mg	Sodium phosphate	10 mg
Poloxamer 238	2.5 mg	Poloxamer 188	1.1 mg

Preparation

The kits contain the sterile, lyophilized, preformed microcolloid in a multidose vial. Labeling with ^{99m}Tc-pertechnetate is carried out under aseptic conditions by adding 1–5 ml of ^{99m}Tc eluate (maximum of 3 GBq). The reaction is allowed to proceed at room temperature for 5 min. ^{99m}Tc-microaggregates form a sterile, pyrogen-free, milky-white solution suitable for intravenous injection. The pH is 4.0–7.0 on pH paper.

Description of the Kit

Preformed albumin microcolloid is easily labeled with reduced technetium at room temperature, provided the amount of stannous chloride is not less than 6 μg. No heating or pH adjustments are required. More than 90% of ^{99m}Tc-albumin microcolloids show a size distribution between 0.2 and 2.0 μm (Chia 1986; Honda et al. 1970; Taplin et al. 1964a; Yamada et al. 1969).

^{99m}Tc eluate used for labeling the preformed colloid should be obtained from a generator by daily elution in order to minimize ^{99}Tc carrier and should comply with specifications stated in the *European Pharmacopeia*. A flocculent precipitate is formed in the presence of Al^{3+} ion (>1 μg/ml) (Ponto et al. 1987).

Clinical Applications

Intravenous injection: Liver and spleen scintigraphy

Time of Examination. The time of examination should be 15–60 min after intravenous injection.

Recommended Activities for Indications

Liver and spleen scintigraphy: 40–150 MBq (1–4 mCi), injected intravenously
200 MBq (5.4 mCi) maximal activity for single-photon emission computer tomography (SPECT)
≤0.025 mg microaggregates/kg body weight
The manufacturer's instructions should be followed.

Pediatric Dose. The amount of radioactivity for infants and children administered for liver/spleen scintigraphy is based on body weight, using the scaling factors given in Appendix 1, Table A1.2. The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

The use of ^{99m}Tc-albumin microcolloid in patients with a history of hypersensitivity to human albumin is contraindicated. ^{99m}Tc-albumin microcolloid should not be injected together with other drugs or components.

Albumin aggregates in the colloidal size range are obtained by heat denaturation of a 1% solution of human serum albumin (HSA) in alkaline medium (pH 10.0) (Honda et al. 1970). The size of the albumin colloid is affected by the pH, temperature, and the mode of agitation (Taplin et al. 1964a).

Diffuse pulmonary accumulation of radiocolloids may result from elevated plasma levels of aluminum in patients with antacid therapy (Ponto et al. 1987). A number of clinical conditions associated with pulmonary uptake of ^{99m}Tc-labeled colloids have been reported (Hladik et al. 1987a).

Hepatotoxic substances interfere with phagocytosis, causing transient changes, such as inhomogeneous or irregular distribution of radiocolloids in the liver/spleen or a shift of uptake from liver to spleen and/or bone marrow. Anesthetics (halothane) affect both phagocytic and catabolic reticuloendothelial system (RES) function, resulting in an increased uptake in the spleen (Hladik et al. 1987b). The plasma expander dextran or protein deficiency may also reduce uptake of ^{99m}Tc-labeled colloids by the Kupffer cells (Hodges 1987).

Quality Control

Radiochemical Purity. ^{99m}Tc-albumin microcolloid is not described in the *European Pharmacopeia*. Thin-layer chromatography is recommended by the manufacturer, using 85% methanol as solvent. Free ^{99m}Tc-sodium pertechnetate is measured at R_f 0.7 and

^{99m}Tc-albumin microcolloid is identified at the origin. The radiochemical purity of ^{99m}Tc-albumin microcolloid should not be less than 95%.

The analysis of radiocolloids is based on the determination of free ^{99m}Tc-Na-pertechnetate, since colloidal activity remains at the start. Hydrolized ^{99m}Tc-activity cannot be distinguished from ^{99m}Tc-HSA colloid.

Recommended Methods

Thin-layer chromatography (USP 28)

Thin-layer chromatography (*USP*)		
Stationary phase:	Gelman ITLC-SG (fiberglass), 2×9.5 cm	
Solvent:	Methyl ethyl ketone (MEK)	
Developing time:	5 min	
R_f values:	^{99m}Tc-HSA microcolloid:	0.0–0.1
	^{99m}Tc reduced, hydrolized	0.0–0.1
	^{99m}Tc-pertechnetate:	0.9–1.0 (<5%)

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter), and the regional radioactivities are expressed as a percentage of the total recovered counts:

$$^{99m}\text{Tc-HSA microcolloid (\%)} = 100 - F$$

$$F\ (\%) = {}^{99m}\text{Tc-Na-pertechnetate (free)}.$$

Results of analysis *(12 samples)*

Results were obtained using the analytical method described by the manufacturer.

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-HSA microcolloid	95.9 ± 0.26	94.7 ± 0.32
^{99m}Tc-Na-pertechnetate	4.1 ± 0.10	5.3 ± 0.12

Pharmacokinetic Data

Intravenously injected colloids distribute within the body according to the phagocytic activity of the RES. Size has considerable influence on the biodistribution of colloidal particles. With a particle size of 0.3–0.6 μm, 80–90% of the radioactivity is extracted by the liver, 4–8% by the spleen, and <1% by the bone marrow (Nelp 1975). Larger colloidal particles show increased splenic uptake, whereas smaller particles (nanocolloids) localize in the bone marrow (10–15%) (Nelp and Bower 1969; Schuind et al. 1984).

In healthy persons, ^{99m}Tc-HSA microcolloid is rapidly removed from the blood by phagocytosis, approximately 94% in one passage (Shaldon et al. 1961). Fifteen minutes after the intravenous injection, only approximately 1% of the radioactivity is measured in blood (Chia 1986). The colloid disappearance rate has been used to estimate liver blood flow. Following the intravenous injection of tracer quantities (less than 0.25 mg/kg body weight), elimination half-times of 2–3 min were measured (Kitani and Taplin

1972). Using a smaller colloid (10–20 nm), a mean half-time of 2.6 min was reported (Iio et al. 1963). Values exceeding 3.0 min indicate reduced hepatic blood flow or a decreased liver function (cirrhosis), as observed in patients with various liver diseases (Iio et al. 1963; McAfee et al. 1975; Palmer et al. 1971; Taplin et al. 1964b; Wagner et al. 1963). In patients with reduced hepatic uptake, the concentration of radioactivity within the spleen is increased.

^{99m}Tc-albumin microcolloid is metabolized in the Kupffer cells by proteolytic enzymes and eliminated from the liver (Reske et al. 1981; Taplin et al. 1964a). In the case of ^{99m}Tc-albumin millimicrospheres, biological half-times of 10 min and 4.7 h were measured (Reske et al. 1981). In patients with overactive digestive function, RES ^{99m}Tc-labeled degradation products have been observed in the bile, accumulating in the gallbladder during the first 2–3 h post-intravenous injection (Kitani and Taplin 1972).

Repeated injections of high doses (5 mg/kg) of aggregated albumin (≤ 80 nm) over a 2-month period have been well tolerated in three subjects, showing no effect on the clearance rate when compared with control subjects (Iio et al. 1963). Subcutaneous injection of small doses (0.02 mg/kg) as well as a saturation dose of 4 mg/kg showed no evidence of hypersensitivity (Iio et al. 1963).

Radiation Dose

The liver, spleen, and red marrow are the most exposed organs. Calculations of the absorbed radiation dose resulting from liver and spleen scintigraphy are based on technetium-labeled colloids (International Commission on Radiological Protection 1987). The effective dose equivalent is 0.014 mSv/MBq. The effective whole-body dose in adults (70 kg) resulting from an intravenous injection of 185 MBq of ^{99m}Tc-albumin microcolloid is 2.6 mSv.

The effective dose equivalent per ICRP Publication 62 has been replaced by the quantity effective dose. Values per unit administered activity were published in Addendum 1 (International Commission on Radiological Protection 1991). Values calculated accordingly are slightly lower than the effective whole-body doses presented here.

Storage and Stability

Storage. The lyophilized kit is stored at 2–8 °C.

Stability. ^{99m}Tc-albumin microcolloid injection solution is stable for 3 h after preparation.

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12.3.1.4 ^{99m}Tc-Albumin Millimicrospheres (Size Range: 0.3–1.0 μm)

I. Zolle

Chemical name

Albumin millimicrospheres

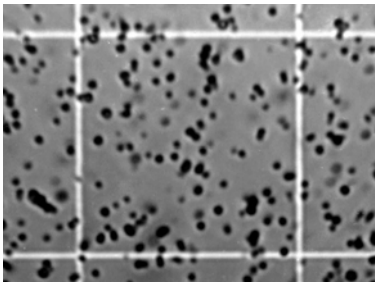
HSA millimicrospheres

milli-HAM

^{99m}Tc-HSA millimicrospheres

^{99m}Tc-millimicrospheres

^{99m}Tc-milli-HAM



Albumin millimicrospheres (< 1 μm)
(Small square 50×50 μm, 600-fold)

Kit components	
HSA millimicrospheres	2.0 mg
Stannous chloride · 2 H_2O	0.5 mg
Cysteine	0.2 mg
Pluronic F68	2.0 mg
Sodium chloride	18.0 mg

Listed trade names

Nanotec Sorin

Preparation

The kit contains sterile, lyophilized, preformed millimicrospheres in a multidose vial. Labeling with ^{99m}Tc-pertechnetate is carried out under aseptic conditions by adding 1–5 ml of ^{99m}Tc eluate (maximum of 3 GBq (80 mCi) to obtain a specific activity of 600 MBq/ml/0.4 mg). The reaction is allowed to proceed at room temperature for 15 min. ^{99m}Tc-millimicrospheres are a sterile, pyrogen-free, opalescent solution suitable for the intravenous injection. The pH is 6.0–7.5.

Description of the Kit

Preformed albumin millimicrospheres are easily labeled with reduced technetium at room temperature (Zolle et al. 1970, 1973). No heating or pH adjustments are required. Consistently high labeling yields were obtained with milli-HAM containing tin (II) salt (Villa et al. 1976), and when milli-HAM were labeled by electrolytic reduction using a tin anode and a platinum cathode (Angelberger et al. 1985; Köhn et al. 1985). More than 90% of ^{99m}Tc-albumin millimicrospheres show a size distribution between 0.3 and 0.8 μm (Köhn et al. 1985; Scheffel et al. 1972).

^{99m}Tc eluate used for labeling the preformed millimicrospheres should be obtained from a generator by daily elution in order to minimize ^{99m}Tc carrier and should comply with specifications stated in the *European Pharmacopeia*. The presence of Al^{3+} ion (>1 μg/ml) in the eluate may cause aggregation of the colloidal particles (Ponto et al. 1987).

For inhalation of ^{99m}Tc-albumin millimicrospheres, the vial (Nanotec) is transferred to an aerosol generator. Dry aerosols are obtained with ^{99m}Tc-milli-HAM suspended in ethanol (Angelberger et al. 1985; Köhn et al. 1985). These have advantages over moist aerosols, showing higher lung penetration between 20 and 30%, while in the case of aqueous nebulization, only 1–10% of the radioactivity are deposited in the lung (Santolicandro and Giuntini 1979).

Clinical Applications

Intravenous injection:	Liver and spleen scintigraphy
	Regional liver perfusion and RES function in cases of impaired liver function (cirrhosis, metal poisoning, transplantation)
	Bone marrow scintigraphy
Nebulization as aerosol:	Inhalation scintigraphy
	Assessment of pulmonary ventilation in patients with chronic obstructive lung disease, and for the differential diagnosis of acute pulmonary embolism in combination with lung perfusion scintigraphy
	Measurement of mucociliary function

Time of Examination

Liver and spleen scintigraphy:	10–60 min after the intravenous injection
Bone marrow imaging:	45–60 min after the intravenous injection
Inhalation scintigraphy:	Immediately after inhalation

Recommended Activities for Indications

Liver and spleen scintigraphy:	40–150 MBq (1–4 mCi), injected intravenously
	200 MBq (5.4 mCi) maximal activity for single-photon emission computed tomography (SPECT)
	≤0.025 mg millimicrospheres/kg body weight
Bone marrow scintigraphy:	185–370 MBq (5–10 mCi), injected intravenously
	400 MBq maximum recommended activity
Inhalation scintigraphy:	150 MBq (4 mCi) inhaled radioactivity
	The manufacturer's instructions should be followed.

Pediatric Dose. The amount of radioactivity for infants and children administered for liver/spleen and bone marrow scintigraphy is based on body weight, using the scaling factors given in Appendix 1, Table A1.2. The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

The use of ^{99m}Tc-albumin millimicrospheres in patients with a history of hypersensitivity to human albumin is contraindicated.

^{99m}Tc-albumin millimicrospheres should not be injected together with other drugs or components.

For intravenous injection (preferably not through a lying catheter), ^{99m}Tc-albumin millimicrospheres should be homogenously suspended to avoid in vivo aggregates. For this reason, the aspiration of blood into the syringe should be avoided.

Albumin millimicrospheres are obtained by heating a dispersed solution of human serum albumin (25%) in oil (Zolle et al. 1970, 1973). The size of the albumin particles depends mainly on the degree of homogenization, generally a size distribution between 0.3 and 3 µm is obtained (Zolle et al. 1970). For intravenous injection, particles larger than 1 µm have been removed by differential centrifugation (Angelberger et al. 1985; Köhn et al. 1985).

Hepatotoxic substances interfere with phagocytosis causing transient changes, such as inhomogeneous or irregular distribution of radiocolloids in the liver/spleen, or a shift of uptake from liver to spleen and/or bone marrow. Anesthetics (halothane) affect both phagocytic and catabolic RES function, resulting in an increased uptake in the spleen (Hladik et al. 1987).

Quality Control

Radiochemical purity. ^{99m}Tc-HSA-millimicrospheres are not described in the *European Pharmacopeia*. Thin-layer chromatography is recommended by the manufacturer using 85% methanol as solvent. Free ^{99m}Tc-pertechnetate is measured at an R_f of 0.6, and ^{99m}Tc-millimicrospheres are identified at the origin. The radiochemical purity of ^{99m}Tc-milli-HAM should not be less than 95%.

The analysis of radiocolloids is based on the determination of free ^{99m}Tc-Na-pertechnetate, since colloidal activity remains at the start. Hydrolized ^{99m}Tc-activity cannot be distinguished from ^{99m}Tc-milli-HAM.

Recommended Methods

Thin-layer chromatography		
Stationary phase:	Gelman ITLC-SG (fiberglass), 2×9.5 cm	
Solvent:	Methanol (85%)	
Developing time:	5 min	
R_f values:	^{99m}Tc-milli-HAM:	0.0–0.1
	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-pertechnetate:	0.6–0.7 (<5%)

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter) and the regional radioactivities are expressed as a percentage of the total recovered counts:

^{99m}Tc-milli-HAM (%) = 100 – F

where F (%) = ^{99m}Tc-Na-pertechnetate (free).

Results of analysis *(12 samples)*
Results were obtained using the analytical method described by the manufacturer.

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-HSA millimicrospheres	96.7 ± 0.57	96.3 ± 0.49
^{99m}Tc-Na-pertechnetate	3.2 ± 0.59	3.7 ± 0.48

^{99m}Tc-milli-HAM are labeled with a high radiochemical yield and show high stability of the radioactive label.

Pharmacokinetic Data

Trace amounts of intravenously injected ^{99m}Tc-albumin millimicrospheres (0.02 mg/kg body weight) are rapidly removed from the blood with a half-time of 1.4–2.0 min (Reske et al. 1981; Scheffel et al. 1972). From 83 to 86% is taken up in the liver by phagocytosis; the maximum activity in the liver is reached 5–10 min after the intravenous injection (Scheffel et al. 1972; Zolle et al. 1973). The plasma disappearance rate is related to the particle size of colloids. Using aggregated albumin (10–20 nm), a mean clearance half-time of 2.6 min was reported (Iio et al. 1963). In patients with reduced hepatic uptake, the spleen shows a high concentration of radioactivity (McAfee et al. 1975).

^{99m}Tc-albumin millimicrospheres are metabolized in the Kupffer cells. The elimination of ^{99m}Tc-milli-HAM from the liver was shown as a reliable indicator of specific Kupffer-cell function (Reske et al. 1981). A biphasic elimination was observed in man, with a fast ($T_{1/2}$< 10 min) and a slowly degrading component ($T_{1/2}$ > 4 h) (Reske et al. 1981; Szabo et al. 1984). In tumor patients without detectable liver metastases or direct tumor invasion, elimination from the liver by phagocytosis was markedly delayed ($T_{1/2}$ of 8.55 h vs 4.28 h), while the clearance of ^{99m}Tc-millimicrospheres from the blood was increased ($T_{1/2}$ of 1.3 min vs 1.8 min) (Reske et al. 1981).

In the rat, ^{99m}Tc-milli-HAM have shown a monoexponential removal from the liver ($T_{1/2}$ of 8.8 h) with increasing intestinal activity 1–6 h after the intravenous injection (Villa et al. 1976). Biliary excretion of degradation products was observed with ^{99m}Tc-albumin microaggregates (Kitani and Taplin 1972; Wetterfors et al. 1960).

For the application of ^{99m}Tc-milli-HAM as an aerosol, preparations with an average diameter of approximately 0.5 μm have shown free diffusion into the lung periphery and high alveolar retention (Agnew et al. 1981; Köhn et al. 1985). Larger millimicrospheres (1–5 μm) sediment preferentially in the trachea and upper bronchial tree (Weiss et al. 1981).

^{99m}Tc-albumin millimicrospheres are well tolerated without complications. No acute reactions were seen when injecting 5–15 mg/kg body weight in 80 mice (Scheffel et al. 1972).

Hypersensitivity reactions are rare and caused mainly by agents used for suspension of ^{99m}Tc-milli-HAM. No cases of anaphylaxis have been reported after aerosol inhalation.

Radiation Dose

The liver, spleen, and red marrow are the most exposed organs. Calculations of the absorbed radiation dose resulting from liver and spleen scintigraphy are based on technetium-labeled colloids (ICRP Publication 53, International Commission on Radiological Protection 1987a). The effective dose equivalent is 0.014 mSv/MBq. The effective whole-body dose in adults (70 kg) resulting from an intravenous injection of 185 MBq (5 mCi) of ^{99m}Tc-millimicrospheres is 2.6 mSv.

Calculations of the absorbed radiation dose resulting from inhalation of ^{99m}Tc-millimicrospheres are based on technetium-labeled aerosols (ICRP Publication 53, International Commission on Radiological Protection 1987b). It is assumed that the label is released in the lung slowly, with a biological half-time of 24 h, and that the activity is excreted by the kidneys. The effective dose equivalent is 0.015 mSv/MBq. The dose to the bladder wall after inhalation of 150 MBq (4 mCi) is 1.95 mGy. The effective whole-body dose in adults (70 kg) resulting from inhalation of 150 MBq of ^{99m}Tc-millimicrospheres is 2.3 mSv.

The effective dose equivalent per ICRP 62 has been replaced by the quantity effective dose. Values per unit administered activity were published in Addendum 1 (International Commission on Radiological Protection 1991). Values calculated accordingly are slightly lower than the effective whole-body doses presented here.

Storage and Stability

Storage. The lyophilized kit is stored at 2–8 °C.

Stability. The ^{99m}Tc-milli-HAM injection is stable for 6 h after preparation.

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12.3.2 ^{99m}Tc-Labeled Nanocolloids

12.3.2.1 ^{99m}Tc-Rhenium Sulfide Nanocolloid (Size Range: 10–100 nm)

I. Zolle

Chemical name		**Listed trade names**	
Rhenium sulfide nanocolloid		NanoCis (TCK-17) (A+B)	CIS Bio
Tin(II)-sulfide nanocolloid		Lymphoscint (Solco)	GE Healthcare
^{99m}Tc-(Re)-sulfide nanocolloid			
^{99m}Tc-(Sn)-sulfide nanocolloid			
Kit components A		**Kit components B**	
Rhenium sulfide	0.48 mg	Sodium pyrophosphate · 10 H_2O	3.0 mg
Ascorbic acid	7.0 mg	Stannous chloride · 2 H_2O	0.5 mg
Gelatin	9.6 mg		
Water for injection	1.0 ml		
Hydrochloric acid (conc.)	37.4 µl		

Preparation

The NanoCis kit (TCK-17) consists of two vials, A and B. Vial A contains a sterile, pyrogen-free solution of ingredients. Vial B contains lyophilized sodium pyrophosphate and stannous chloride. Two milliliters of sterile water for injection are added to vial B to dissolve its content. Half a milliliter of solution B is transferred to vial A, and mixed well. Then, 1–2 ml of ^{99m}Tc eluate is added under aseptic conditions (not less than 370 MBq [10 mCi] to assure a specific activity of ≥ 100 MBq/ml/0.15 mg). The reaction vial is placed into a boiling water bath for 15–30 min. After cooling, the labeled colloid is ready for use. ^{99m}Tc-(Re)-sulfide nanocolloid is a sterile, pyrogen-free, brown solution, suitable for subcutaneous (interstitial) injection. The pH is between 4.0 and 7.0 on pH paper.

^{99m}Tc-Sn(II)-sulfide colloid (Lymphoscint) is prepared by adding 0.2–2 ml of sterile ^{99m}Tc eluate under aseptic conditions (not more than 1.5 GBq [40 mCi]). A volume of approximately 0.5 ml of the ^{99m}Tc eluate is recommended by the manufacturer to minimize ionic aluminum. The final volume may be adjusted with oxidant-free saline. The reaction vial is placed into a boiling water bath for 4 min. After cooling, the labeled colloid is ready for use. ^{99m}Tc-Sn(II)-sulfide nanocolloid is a sterile, pyrogen-free solution, suitable for subcutaneous (interstitial) injection. The pH is between 4.0 and 7.0 on pH paper.

Description of the Kit

^{99m}Tc-(Re)-sulfide colloid is formed by reduction with tin pyrophosphate in the boiling water bath. Rhenium sulfide is used as a carrier (Larson and Nelp 1966; Patton et al. 1966). The added ^{99m}Tc eluate should not exceed 2 ml, the ^{99m}Tc activity should not be less than 370 MBq (10 mCi). Gelatin is used as a stabilizer. The size distribution of ^{99m}Tc-(Re)-sulfide colloid is between 40 and 80 nm, comparable with the previously available ^{99m}Tc-(Sb)-sulfide colloid (5–15 nm).

^{99m}Tc-(Sn)-sulfide colloid is prepared by labeling a preformed colloid in the boiling water bath; gelatin is used for stabilization. ^{99m}Tc-tin(II)-sulfide colloid has a favorable particle size, namely $\leq$50 nm.

Factors causing low colloid formation (low specific activity) are primarily related to pH, incorrect order of mixing, low heating temperature, heating a large volume, inadequate boiling time, or a defect of kit formulation. ^{99m}Tc eluate used for colloid preparation should be obtained from a generator by daily elution in order to minimize ^{99}Tc carrier; preferably, a small volume is added in order to reduce the concentration of Al^{3+} ion (Ponto et al. 1987).

Clinical Applications

Subcutaneous (interstitial) injection:	Lymphoscintigraphy
	Visualization of lymphatic flow and regional lymph nodes in the extremities and the trunk
Subdermal or peritumoral injection:	Sentinel lymph node (SLN) scintigraphy
Oral application:	Gastroesophageal scintigraphy
	Esophageal motility disorders
	Gastroduodenal motor activity

Lymphoscintigraphy is performed for the diagnosis of peripheral edema caused by recurring erysipel or by affected lymph nodes as a result of metastatic infiltration, lymphogranuloma, lymphosarcoma, damage as a result of radiation treatment, or other causes. Other indications include secondary edema caused by blockade of the lymphatic flow, and thrombosis (Lofferer et al. 1974; Mostbeck et al. 1984). Lymphatic drainage from a primary tumor was studied in patients with breast cancer (Ege 1983). Several colloidal preparations have been evaluated (Kaplan et al. 1979, 1985; Nagai et al. 1982; Strand and Persson 1979).

SLN imaging can identify the first infected lymph node of a primary tumor before surgery. If the SLN is histologically tumor free, no other lymph node will contain metastatic disease (Alazraki et al. 1997).

Time of Examination

Lymphoscintigraphy:	Sequential scintigraphy 15 min after subcutaneous injection up to 1 h Visualization of lymph nodes between 2 and 6 h after injection
SLN imaging:	Sequential planar scintigraphy 5–10 min postinjection and 1–6 h postinjection (late images)
Gastroesophageal scintigraphy:	Sequential scintigraphy and static imaging may be performed

Recommended Activities for Indications

Lymphoscintigraphy of the extremities:	20–75 MBq (0.5–2 mCi) in a volume of 0.2–0.3 ml, subcutaneous injection into the interdigital spaces of the hands or feet. For a better resorption and to reduce the radiation burden to the injection site, the total dose (≤185 MBq [5 mCi]) may be divided between two and three injection sites. 40 MBq (1 mCi) maximum activity per injection site The volume of injection should not exceed 0.5 ml
Parasternal lymphatics:	20 MBq (0.5 mCi) in a volume of 0.3–0.5 ml, single subcostal injection at both sides
SLN:	50–80 MBq (1.3–2.2 mCi) in a volume of 0.1–0.5 ml, multiple subdermal injections (peritumoral) Guidelines for SLN detection should be followed (Cox et al. 1998)
Gastroesophageal reflux:	20 MBq (0.5 mCi) of ^{99m}Tc-(Re, Sn)-sulfide nanocolloid in a suitable liquid according to local practice 40 MBq (1 mCi) maximum recommended activity

Pediatric Dose. The amount of radioactivity for infants and children administered for lymphoscintigraphy is based on body weight, using the scaling factors given in Appendix 1 (Table A1.2). The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

^{99m}Tc-(Re, Sn)-sulfide colloid is not used for intravenous injection. A comparison with ^{99m}Tc-albumin nanocolloid showed considerable residual radioactivity in blood; approximately 11% were measured 3 h after the intravenous injection (De Schrijver et al. 1987).

The stability of colloids is affected by electrolytes, thus ^{99m}Tc-(Re, Sn)-sulfide colloid should not be injected together with other drugs or components.

Interstitial injection of a hypertonic colloidal solution may be associated with pain at the injection site. Therefore, ^{99m}Tc-(Re)-sulfide nanocolloid should be injected slowly.

^{99m}Tc-(Sn)-sulfide nanocolloid is supplied as an iso-osmolar solution, warranting a painless subcutaneous injection.

For interstitial injection ^{99m}Tc-(Re, Sn)-sulfide nanocolloid must be applied in a small volume not exceeding 0.5 ml. To ensure high specific activity, labeling should be performed with a small volume of ^{99m}Tc eluate (≤2 ml) and not less than 370 MBq (10 mCi).

The use of local anesthetic agents or hyaluronidase prior to administering the labeled preparation have been shown to negatively affect lymphatic uptake.

Iodinated contrast media used in lymphangiography may interfere with lymphatic imaging with ^{99m}Tc-(Re, Sn)-sulfide nanocolloid.

Quality Control

Radiochemical Purity. ^{99m}Tc-(Re, Sn)-sulfide nanocolloid is not described in the *European Pharmacopeia*. Paper chromatography on Whatman 1 paper is recommended by the manufacturer, using methyl ethyl ketone (MEK) as solvent. Free ^{99m}Tc-sodium pertechnetate is measured at an R_f of 1.0 and ^{99m}Tc-(Re, Sn)-sulfide nanocolloid is identified at the origin. The radiochemical purity of ^{99m}Tc-(Re, Sn)-sulfide nanocolloid should not be less than 95%.

The analysis of radiocolloids is based on the determination of free ^{99m}Tc-Na-pertechnetate, since colloidal activity remains at the start. Hydrolized ^{99m}Tc activity cannot be distinguished from the ^{99m}Tc-(Re, Sn)-sulfide nanocolloid.

Recommended Methods

Paper chromatography		
Stationary phase:	Whatman No. 1 paper, 2×9.5 cm	
Solvent:	Methyl ethyl ketone (MEK)	
Developing time:	5 min	
R_f values:	^{99m}Tc-(Re, Sn)-sulfide, nanocolloid:	0.0–0.1 (>95%)
	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-pertechnetate:	0.9–1.0

Thin-layer chromatography on Gelman silica gel fiberglass sheets using acetone as solvent has been recommended for the identification of ^{99m}Tc-(Re)-sulfide microcolloid at the origin, and free ^{99m}Tc-pertechnetate at the solvent front (R_f = 1.0). This method is also recommended for the analysis of the nanocolloids.

Quantification of labeled components

Each chromatogram is measured (TLC linear analyzer or gamma counter) and the regional radioactivities are expressed as a percentage of the total recovered counts:

$$^{99m}\text{Tc-(Re, Sn)-sulfide nanocolloid (\%)} = 100 - F$$

where F (%) = ^{99m}Tc-Na-pertechnetate (free).

Results of analysis *(12 samples)*
Results were obtained using thin-layer chromatography and acetone as solvent.

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-(Re, Sn)-sulfide nanocolloid	98.5 ± 0.67	96.3 ± 0.97
^{99m}Tc-Na-pertechnetate	1.4 ± 0.61	3.6 ± 1.07

Pharmacokinetic Data

After subcutaneous injection into the interdigital spaces of the hands or feet, ^{99m}Tc-(Re, Sn)-sulfide nanocolloid is transported with the interstitial liquid through the lymphatic capillaries into the lymph ducts, and is almost quantitatively retained by the regional lymph nodes (Mostbeck et al. 1984). Release of the colloid from the lymph nodes is slow and increasing with movement of the extremities. After 15 min of slow walking, 6–15% of the injected radioactivity has been measured in the regional lymph nodes (Mostbeck et al. 1984). Accumulation of the colloid in the liver is negligible.

Following the subcostal injection at both sides of the xyphoid (2–2.5 cm under the xyphoid) into the musculus rectus abdominalis, ^{99m}Tc-(Re, Sn)-sulfide nanocolloid is transported through the parasternal lymphatics into mediastinal and diaphragmal lymph nodes. The maximal accumulation is reached 3 h after injection (Ege 1983). Drainage from the interstitial injection site was observed between 1 and 35% in 24 h (Ege 1976).

Basic concepts for quantitative lymphoscintigraphy were derived from experimental studies in rabbits by correlating the uptake of different radiocolloids in the parasternal lymph nodes with measurements of the particle size (Strand and Persson 1979). For colloidal gold (Au-198) with a well defined particle diameter of approximately 5 nm, highest uptake in regional lymph nodes was recorded. ^{99m}Tc-(Sb)-sulfide colloid (Sb_2S_3), with colloidal particles between 5 and 15 nm, showed less uptake (approximately 60%), yet the highest uptake of all ^{99m}Tc-colloids that were evaluated. Larger particles cannot pass through the lymphatic capillary pores and are trapped in the interstitial fluid. Thus ^{99m}Tc-tin colloid, ^{99m}Tc-phytate, and ^{99m}Tc-sulfur colloid remain primarily at the injection site and show little lymphatic migration (Strand and Persson 1979).

^{99m}Tc-(Re, Sn)-sulfide nanocolloids show optimal physical characteristics (40–80 nm) for reliable visualization of anatomic lymph nodes.

Subdermal, peritumoral injection of ^{99m}Tc-(Re, Sn)-sulfide nanocolloid is used for visualization of the lymphatic drainage of a primary tumor and for identification of the SLN (Alazraki et al. 1997; Keshtgar et al. 1999; Nitz and Heidenreich 1999).

Radiation Dose

Calculations of the absorbed radiation dose after the subcutaneous administration of ^{99m}Tc-(Re)-sulfide nanocolloid into the extremities are based on the assumption that approximately 5–15% of the radioactivity are distributed over 10–20 lymph nodes (Mostbeck et al. 1984). The highest radiation dose is thus delivered at the injection site. If 37 MBq (1 mCi) are injected into each foot, the radiation absorbed dose at the injec-

tion site has been calculated as 400–700 mGy, and 22–27 mGy for each lymph node (Mostbeck et al. 1984). The weight of a lymph node was assumed to be 5 g.

Based on a model for the subcutaneous injection of ^{99m}Tc-(Sb)-sulfide colloid into the umbilical region, Bergquist et al. (1982) have calculated the radiation dose at the injection site assuming a tissue volume of 10 ml. Injection of 37 MBq (1 mCi) results in an average radiation dose of approximately 300 mGy at the injection site. The effective dose equivalent was determined as 0.005 mSv/MBq (Bergqvist et al. 1982).

Based on this information, the effective dose in adults (70 kg) resulting from the subcutaneous injection of 185 MBq (5 mCi) of ^{99m}Tc-(Re, Sn)-sulfide colloid is approximately 1 mSv.

Storage and Stability

Storage. The lyophilized kit is stored at 2–8 °C.

Stability. The ^{99m}Tc-(Re)-sulfide nanocolloid injection is stable for 4 h after preparation.

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12.3.2.2 ^{99m}Tc-Albumin Nanocolloid (Size Range: 10–80 nm)

I. Zolle

Chemical names	**Listed trade names**	
Human albumin nanocolloid	Solco Nanocoll	GE Healthcare
Albumin nanoaggregates		
Aggregated albumin (AA)		
^{99m}Tc-HSA-nanocolloid		

Kit components	
Human albumin nanocolloid	0.5 mg
Stannous chloride·2 H_2O	0.2 mg
Glucose anhydrous	
Dibasic sodium phosphate, anhydrous	
Sodium phytate, anhydrous	
Poloxamer 238	

Preparation

The kit contains the sterile, lyophilized, preformed colloid in a multidose vial. Labeling with ^{99m}Tc-pertechnetate is carried out under aseptic conditions by adding 1–5 ml of ^{99m}Tc eluate (maximum 5.5 GBq: approximately 150 mCi). The reaction is allowed to proceed at room temperature for 10 min. ^{99m}Tc-HSA nanocolloid is a sterile, pyrogen-free, opalescent solution suitable for the intravenous or subcutaneous injection. The pH is 4.0–7.0 on pH paper.

Description of the Kit

Preformed albumin nanocolloid is easily labeled with reduced technetium at room temperature. No heating or pH adjustment is required. Once formed, albumin nanocolloid is unaffected by time. More than 95% of the ^{99m}Tc-albumin nanocolloid show a size distribution between 10 and 80 nm (Chia 1986; Solco Nanocoll 1992).

^{99m}Tc eluate used for labeling the preformed colloid should be obtained from a generator by daily elution in order to minimize ^{99}Tc carrier and should preferably be in a small volume to avoid any interference of Al^{3+} ion (Ponto et al. 1987). The manufacturer recommends a minimum volume of ^{99m}Tc eluate, which is adjusted to the final volume with oxidant-free saline.

Labeling with a small volume (< 1 ml) of ^{99m}Tc-pertechnetate is also required if the colloid is used for lymphoscintigraphy.

Clinical Applications

Intravenous injection:	Bone marrow scintigraphy Scintigraphy of inflammation
Subcutaneous (interstitial) injection:	Lymphoscintigraphy Visualization of lymphatic flow and regional lymph nodes in the extremities and the trunk
Subdermal or peritumoral injection:	Sentinel lymph node (SLN) scintigraphy

Bone marrow scintigraphy with nanocolloids is based on a high extraction efficiency in bone marrow by phagocytosis (approximately 15% of the injection dose [ID]) (Hotze et al. 1984; McAfee et al. 1982; Munz 1984a; Nagai et al. 1982.). In areas where no phagocytes are present, no uptake is observed. This is the case in the fatty marrow, or where the phagocyte population has been replaced by other structures, such as metastatic growth. Enhanced phagocytic activity is causing increased colloid uptake. Defects in bone marrow distribution, nonuniform marrow distribution, or an expansion of active marrow into long bones has been diagnosed. Both, hot and cold lesions are visualized in the scintigram.

Biodegradable HSA nanocolloids offer considerable advantages over inorganic colloids. Large quantities labeled with I-131 have been used to study the phagocytic capacity of the reticuloendothelial system (RES) in healthy man and in patients with certain infections (Iio et al. 1963; Wagner et al. 1963).

Nanocolloids preferentially accumulate in inflammatory lesions associated with a number of conditions, i.e., osteomyelitis, osteitis, rheumatoid arthritis, arthrosis, joint prostheses, and wound healing, thus nanocolloid is clinically useful for detecting osteomyelitis and other bone or joint infections (De Schrijver et al. 1987; Froehlich 1985; Vorne et al. 1989).

Lymphoscintigraphy is performed for the diagnosis of peripheral edema caused by recurring erysipel or by affected lymph nodes as a result of metastatic infiltration, lymphogranuloma, lymphosarcoma, damage as a result of radiation treatment, or other causes. Other indications include secondary edema caused by blockade of the lymphatic flow, and thrombosis (Lofferer et al. 1974; Mostbeck et al. 1984). Lymphatic drainage from a primary tumor was studied in patients with breast cancer (Ege 1983). Several colloidal preparations have been evaluated for lymphoscintigraphy (Kaplan et al. 1979; Strand and Persson 1979; Kaplan et al. 1985).

SLN imaging can identify the first infected lymph node of a primary tumor before surgery. If the SLN is histologically tumor-free, no other lymph node will contain metastatic disease (Alazraki et al. 1997; Nitz and Heidenreich 1999).

Time of Examination

Bone marrow scintigraphy:	30 min after the intravenous injection
Inflammation imaging:	Static imaging 30–60 min after the intravenous injection
Lymphoscintigraphy:	Dynamic imaging immediately after the injection Static imaging 30–60 min after subcutaneous injection
SLN imaging:	Sequential planar scintigraphy 5–10 min postinjection and 1–6 h postinjection (late images)

Recommended Activities for Indications

Bone marrow scintigraphy:	185–370 MBq (5–10 mCi), injected intravenously 400 MBq maximum recommended activity 1.5–7 μg nanocolloid/kg body weight
Inflammation scintigraphy:	370–555 MBq (10-15 mCi), injected intravenously
Lymphoscintigraphy of the extremities:	20–75 MBq (0.5–2 mCi) in a volume of 0.2–0.3 ml, subcutaneous injection into the interdigital spaces of the hands or feet. For a better resorption and to reduce the radiation burden to the injection site, the total dose (≤185 MBq; 5 mCi) may be divided between two and three injection sites. 40 MBq (1 mCi) maximum activity per injection site The volume of injection should not exceed 0.5 ml
Parasternal lymphatics:	20 MBq (0.5 mCi) in a volume of 0.3–0.5 ml, single subcostal injection at both sides The manufacturer's instructions should be followed.
SLN:	50–80 MBq (1.3–2.2 mCi) in a volume of 0.1–0.5 ml, multiple subdermal injections (peritumoral) Guidelines for sentinel lymph node detection should be followed (Cox et al. 1998)

Pediatric Dose. The amount of radioactivity for infants and children administered for bone marrow and inflammation imaging or lymphoscintigraphy is based on body weight, using the scaling factors given in Appendix 1 (Table A1.2). The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

The use of ^{99m}Tc-albumin nanocolloid in patients with a history of hypersensitivity to human albumin is contraindicated.

The stability of colloids is affected by electrolytes; thus, ^{99m}Tc-albumin nanocolloid should not be injected together with other drugs or components. The use of local anes-

thetic agents or hyaluronidase prior to administering the labeled preparation has been shown to negatively affect lymphatic uptake.

Albumin aggregates in the size range ≤100 nm are obtained by heating a 1% solution of human serum albumin (HSA) in alkaline medium (pH 10.0) while shaking (Iio et al. 1963). Controlling the pH, temperature, agitation rate, and optical density can assure a high degree of uniformity in size.

Albumin nanocolloid is less affected by the concentration of Al^{3+} ion in the generator eluate than inorganic colloids. A reaction volume containing 1 μg Al^{3+} ion per milliliter had no effect; 2 μg/ml showed an increase in the particle diameter (80–100 nm) from 1–23% (Chia 1986; Haney 1971).

For interstitial injection ^{99m}Tc-albumin nanocolloid must be applied in a small volume not exceeding 0.5 ml. To ensure high specific activity, labeling should be performed with a small volume of ^{99m}Tc eluate (≤2 ml) and not less than 370 MBq (10 mCi).

Interstitial injection of a hypertonic colloidal solution may be associated with pain at the injection site. Therefore, ^{99m}Tc-albumin nanocolloid should be injected slowly.

Iodinated contrast media used in lymphangiography may interfere with lymphatic imaging with ^{99m}Tc-albumin nanocolloid.

Quality Control

Radiochemical Purity. ^{99m}Tc-albumin nanocolloid is not described in the *European Pharmacopeia*. Paper chromatography using Whatman 31 ET paper and saline as solvent is recommended by the manufacturer. Free ^{99m}Tc-sodium pertechnetate is measured at an R_f of 0.75 and ^{99m}Tc-albumin nanocolloid is identified at the origin. The radiochemical purity of ^{99m}Tc-albumin nanocolloid should not be less than 95%.

The analysis of radiocolloids is based on the determination of free ^{99m}Tc-Na-pertechnetate, since colloidal activity remains at the start. Hydrolized ^{99m}Tc activity cannot be distinguished from the ^{99m}Tc-HSA nanocolloid.

Recommended Methods

Thin layer chromatography on Gelman silica gel fiberglass sheets using acetone as solvent has been in clinical use for the analysis of ^{99m}Tc-(Re)-sulfide microcolloid and is also recommended for ^{99m}Tc-nanocolloids.

Thin-layer chromatography		
Stationary phase:	Gelman ITLC-SG (fiberglass), 2×9.5 cm	
Solvent:	Acetone (or MEK)	
Developing time:	5 min	
R_f values:	^{99m}Tc-HSA nanocolloid:	0.0–0.1 (>95%)
	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-pertechnetate:	0.9–1.0

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter), and the regional radioactivities are expressed as a percentage of the total recovered counts:

^{99m}Tc-HSA nanocolloid (%) = 100 – F

where F (%) = ^{99m}Tc-Na-pertechnetate (free).

Results of analysis *(12 samples)*
Results were obtained using thin-layer chromatography and acetone as solvent.

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-HSA nanocolloid	96.7 ± 0.15	96.3 ± 0.16
^{99m}Tc-Na-pertechnetate	3.3 ± 0.50	3.7 ± 0.40

Pharmacokinetic Data

^{99m}Tc-albumin nanocolloid is removed from the circulation by phagocytosis. More than 95% of the intravenously injected nanocolloid is accumulated in the liver, spleen, and bone marrow 15 min after administration (Chia 1986; Iio et al. 1963). Nanocolloids show high accumulation in bone marrow (10–15%) (Hotze et al. 1984; McAfee et al. 1982).

Plasma clearance of colloidal particles is related to size; nanocolloids are normally cleared with a half-time of 2.6 min (Iio et al. 1963). Ten minutes after the intravenous injection, 10% of the injected dose was measured in blood (2.5% at 60 min), showing a constant decrease of radioactivity. In comparison, ^{99m}Tc-albumin microcolloid (0.2–1.0 μm) showed approximately 1.6% of the injected dose at 10 min (Chia 1986). The maximal rate of phagocytosis of aggregated albumin (AA) in man was determined as 1.07 mg per minute per kilogram of body weight (Iio et al. 1963).

An increase of extrahepatic accumulation of nanocolloid in the bone marrow is seen with hyperplastic bone marrow and with certain hematological disorders (polycytemia, leukemia) (Höfer et al. 1964). In patients with malignant disease, bone marrow scintigraphy may offer early detection of bone marrow infiltration (Hotze et al. 1984; Munz 1984 b).

After subcutaneous injection into the interdigital spaces of the hands or feet, nanocolloid is transported with the interstitial liquid through the lymphatic capillaries into the lymph ducts and taken up almost quantitatively by the regional lymph nodes. Drainage from the interstitial injection site was observed between 1 and 35% in 24 h (Ege 1976).

Release of the colloid from the lymph nodes is slow and increasing with movement of the extremities. After 15 min of slow walking, 6–15% of the injected radioactivity has been measured in the regional lymph nodes (Mostbeck et al. 1984). Accumulation of the nanocolloid in the liver is negligible. Approximately 80% of the nanocolloid is transported by the lymphatic system (Saha 1987).

After the subcostal injection at both sides of the xyphoid (2–2.5 cm under the xyphoid) into the musculus rectus abdominalis, ^{99m}Tc-albumin nanocolloid is transported through the parasternal lymphatics into mediastinal and diaphragmal lymph nodes. The maximal accumulation is reached 3 h after injection (Ege 1983).

Subdermal, peritumoral injection of ^{99m}Tc-albumin nanocolloid is used for visualization of the lymphatic drainage of a primary tumor and for identification of the sentinel lymph node (Alazraki et al. 1997; Cox et al. 1998).

Data on the acute toxicity of intravenously or subcutaneously injected ^{99m}Tc-albumin nanocolloid have not been reported. Repeated injections of high doses of colloidal albumin aggregates (5 mg/kg) over a 2-month period have been well tolerated in three subjects, showing no effect on the clearance rate when compared with control subjects (Iio et al. 1963). Subcutaneous injection of small doses (0.02 mg/kg) as well as a saturation dose of 4 mg/kg showed no evidence of hypersensitivity.

Radiation Dose

After intravenous injection, the liver, spleen, and red bone marrow are the most exposed organs. The effective (whole body) dose equivalent is 0.014 mSv/MBq (International Commission on Radiological Protection 1987).

The effective dose in adults (70 kg) resulting from 370 MBq (10 mCi) of ^{99m}Tc-albumin nanocolloid is 5.2 mSv. The dose to liver and spleen after intravenous injection of 185 MBq (5 mCi) is 13.7 and 14.2 mGy, respectively. The dose to the bone marrow after intravenous injection of 370 MBq (10 mCi) is 5.5 mGy.

The effective dose equivalent (per ICRP 62) has been replaced by the quantity effective dose. Values per unit administered activity were published in Addendum 1 (International Commission on Radiological Protection 1991). Values calculated accordingly are slightly lower than the effective whole body dose presented here.

Calculations of the absorbed radiation dose following the subcutaneous administration of ^{99m}Tc-albumin nanocolloid into the extremities are based on the assumption that approximately 5–15% of the radioactivity is distributed over 10–20 lymph nodes (Mostbeck et al. 1984). The highest radiation dose is thus delivered at the injection site. If 37 MBq (1 mCi) are injected into each foot, the radiation absorbed dose at the injection site has been calculated as 400–700 mGy, and 22–27 mGy for each lymph node. The weight of a lymph node was assumed to be 5 g (Mostbeck et al. 1984).

Storage and Stability

Storage. The lyophilized kit is stored at 2–8 °C.

Stability. The ^{99m}Tc-albumin nanocolloid injection is stable for 6 h after preparation.

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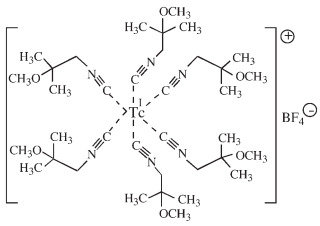
12.4 ^{99m}Tc-Labeled Myocardial Perfusion Agents

12.4.1 ^{99m}Tc-MIBI (Methoxyisobutyl Isonitrile)

F. Rakiás and I. Zolle

Chemical name	**Chemical structure**
2-Methoxy-isobutyl-isonitrile (MIBI) Tc(I)-Hexakis(2-methoxy-isobutyl-isonitrile) tetrafluroroborate Sestamibi (*Ph. Eur., USP*) Technetium Tc 99m sestamibi injection ^{99m}Tc-MIBI ^{99m}Tc-sestamibi injection	 ^{99m}Tc(I)-sestamibi complex

Kit components		**Commercial products**	
$Cu(MIBI)_4 \cdot BF_4$	1.0 mg	Cardiolite	Bristol-Myers Squibb (BMS)
Tin(II)-chloride dihydrate	0.075 mg	Miraluma	DuPont Merck/BMS
L-Cysteine hydrochloride hydrate	1.0 mg	Cardiospect	Rotop
Sodium citrate dihydrate	2.6 mg		
Mannitol	20 mg		

Preparation

The Cardiolite kit contains the lyophilized, sterile, pyrogen-free, inactive ingredients in a nitrogen atmosphere, ready for labeling with ^{99m}Tc-sodium pertechnetate (Council of Europe 2004). Labeling is performed according to the instructions given by the manufacturer (Bristol-Myers Squibb 2001).

A volume of 1–3 ml of ^{99m}Tc-pertechnetate (0.925–5.55 GBq, resp. 25–150 mCi) is added aseptically to the reaction vial. Before removing the syringe, an equal volume of headspace to normalize the pressure in the vial should be withdrawn. The shielded vial should be agitated vigorously to dissolve the lyophilized material, and then is placed in a boiling water bath, which should be shielded, for 10 min. After heating, the vial is placed into the lead shield and cooled at room temperature for approximately 15 min.

^{99m}Tc-sestamibi is a clear, colorless solution for intravenous injection, and the pH value is 5.3–5.9.

Another diagnostic kit for the preparation of ^{99m}Tc-sestamibi is Miraluma, approved for breast imaging (Bristol-Myers Squibb 2001).

Description of the Kit

The Cardiolite kit contains 2-methoxy-isobutyl-isonitrile (MIBI) as a preformed copper(I) complex, $Cu(MIBI)_4^+ \cdot BF_4^-$ [tetrakis (2-methoxy-isobutyl-isonitrile) Cu(I) tetrafluoroborate], which facilitates labeling by ligand exchange at elevated temperature.

An alternate method of heating by exposure of the reaction vial in a microwave oven has been reported (Hung et al. 1991). Using a reaction volume of 3 ml, the time for heating was reduced to 10 s, obtaining consistently high labeling yields. Separate studies using 3 ml saline have shown that heating for 8 s produced a temperature of $98.7 \pm 0.3\,^{\circ}C$.

The Cardiolite kit contains no bacteriostatic agent. Only eluates from generators eluted within 24 h after the previous elution may be used for labeling, keeping the volume small (Van Duzee and Bugaj 1981).

Clinical Applications

^{99m}Tc(I)-sestamibi is used after intravenous injection:

- Myocardial perfusion studies
  - Diagnosis of ischemic heart disease
  - Diagnosis and localization of myocardial infarction
  - Assessment of global ventricular function (first pass technique for determination of ejection fraction and/or regional wall motion)
- Breast imaging in patients with an abnormal mammogram
- Parathyroid imaging in patients with hyperfunctioning adenoma

Myocardial perfusion imaging with ^{99m}Tc-sestamibi has been evaluated by multicenter clinical studies (Wackers et al. 1989). The advantages of ^{99m}Tc-sestamibi have been demonstrated in normal subjects (Marcass et al. 1990), in patients with ischemic heart disease by comparison with thallium-201 (Maisey et al. 1990; Sporn et al. 1988; Villanueva-Meyer et al. 1990); and validated by single-photon emission computer tomography (SPECT) (Berman et al. 1993).

Time of Examinations

Rest and stress scintigraphy:	1–1.5 h after the intravenous injection
Breast imaging:	Planar imaging is begun 5–10 min postinjection
Parathyroid imaging:	10–15 min postinjection

Recommended Activities for Indications. The activity-range for intravenous administration in patients (70 kg) is:

Diagnosis of reduced regional perfusion and myocardial infarction:	250–1,000 MBq (7–27 mCi)
Assessment of global ventricular function:	600–800 MBq (16–25 mCi), injected as a bolus
Miraluma breast imaging:	555–925 MBq (15–25 mCi)
Parathyroid imaging:	185–740 MBq (5–20 mCi), injected as a bolus

The performance of myocardial scintigraphy requires a strict protocol. Usually, the first examination is performed with exercise or pharmacological stress, with the intravenous injection of ^{99m}Tc-sestamibi administered at the highest heart rate (250–370 MBq, resp. 7–10 mCi). The stress scintigram is performed 1–1.5 h after the injection.

Three hours after the initial injection, a second dose of ^{99m}Tc-sestamibi (550–750 MBq) (15–20 mCi) is administered intravenously to examine the patient at rest. The rest scintigram is performed 1–1.5 h after the injection.

Two injections (stress and rest) are required in order to differentiate a transient (reversible) from a persistent perfusion defect, which is typical for ischemic heart disease (Borges-Neto et al. 1990; Büll et al. 1996). For verification of a scar after myocardial infarction (nonreversible perfusion defect), one injection at rest (185–300 MBq, resp. 5–8 mCi) may be sufficient. However, it is usually not possible to differentiate an acute myocardial infarction from ischemic defects, which are observed in patients with angina pectoris during chest pain (Tatum et al. 1997).

Based on diagnostic reference levels for radiopharmaceuticals adopted by the European member states, the total activity administered on a single day should not exceed 1,000 MBq (27 mCi) in the case of the combined rest–exercise protocol and 600 MBq (16.2 mCi) in the case of the one-day protocol (European Commission 1999). A rather large variation in the recommendations from country to country has been reported (Hesse et al. 2005).

Pediatric Dose. The amount of radioactivity for infants and children administered for myocardial scintigraphy is based on body weight, using the scaling factors given in Appendix 1, Table A1.2. The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

Cardiac imaging. Patients should fast for at least 4 h prior to the study. It is recommended that patients take a light fatty meal or drink a glass of milk after each injection prior to SPECT imaging. This will promote rapid hepatobiliary clearance of ^{99m}Tc-sestamibi, resulting in lower liver activity in the image.

The heart-to-background ratio will increase with time; thus, the imaging time is the best compromise between heart count rate and surrounding organ uptake. Scintigraphy is performed 1–2 h after rest and stress injections. There is no evidence for significant changes in myocardial tracer concentration or redistribution.

Either planar or tomographic imaging is performed for diagnosis of ischemic heart disease and myocardial infarction. Both may be performed electrocardiogram (ECG)-gated.

For assessment of global ventricular function the same standard techniques and projections are used, as established for ^{99m}Tc first-pass ejection studies.

Adequate hydration and frequent urination are necessary to reduce the radiation exposure of the bladder wall.

Breast imaging. Another diagnostic purpose is the investigation of patients suspected of breast cancer, particularly patients with an abnormal mammogram or a palpable breast mass (Imbriaco et al. 2001; Palmedo et al. 1996 and 1998).

Miraluma breast imaging is performed 10 min after the intravenous injection of 555 MBq (15 mCi) of ^{99m}Tc-sestamibi in the arm contralateral to the site of the breast

abnormality. Then, planar images are obtained for 10 min in different positions according to the protocol. A malignant breast lesion is shown by increased uptake of the radiotracer. The detection of primary breast carcinoma may be carried out with either planar or SPECT acquisition.

Parathyroid imaging is performed for localization and identification of hyperfunctioning adenoma (Coakley 1991) by two scintigraphic procedures:

- Subtraction: Previously injected activity in thyroid gland is subtracted from the total neck image (^{99m}Tc-sestamibi + ^{123}I- or ^{99m}Tc-thyroid image) (Wei et al. 1992).
- Washout (planar or SPECT): Neck and thorax images are obtained at specified times (up to 4 h) after the injection of ^{99m}Tc-sestamibi (McBiles et al. 1995; Taillefer et al. 1992).

In order to assure high quality of the injection solution, the radiochemical purity is determined before administration of ^{99m}Tc(I)-sestamibi to patients (Hung 1991).

Quality Control

Radiochemical Purity. ^{99m}Tc-sestamibi is described in the *European Pharmacopeia* (Council of Europe 2005). Thin-layer chromatography using reverse-phase silica gel plates (octadecylsilyl-silica gel) and a mixture of acetonitrile, methanol, ammonium acetate, and tetrahydrofuran as solvent system is recommended for the separation of free ^{99m}Tc-sodium pertechnetate (R_f=0.9) and reduced, hydrolized ^{99m}Tc-activity (R_f=0–0.1). These impurities should not exceed 5% of the measured radioactivity.

The radiochemical purity is analyzed prior to administration of ^{99m}Tc-sestamibi. If the labeling yield is less than 90%, the preparation should be discarded.

Recommended Methods

The commonly used method is thin-layer chromatography on aluminum oxide-coated plastic TLC plates and absolute ethanol as solvent. Free ^{99m}Tc-sodium pertechnetate and reduced, hydrolized ^{99m}Tc-activity remain at the start. ^{99m}Tc-sestamibi is measured at an R_f of 0.9, moving with the solvent front. The radiochemical purity of ^{99m}Tc-sestamibi should not be less than 94%.

Thin-layer chromatography		
Stationary phase:	Baker-flex Alox 1B-F (precut to 2.5×7.5 cm)	
Solvent:	Ethanol (99.8%)	
Developing time:	10 min	
R_f values:	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-pertechnetate:	0.0–0.1
	^{99m}Tc-MIBI complex:	0.9–1.0 (>94%)

Procedure:

- Pour enough ethanol into the TLC tank (beaker) to have a depth of 3–4 mm of solvent.
- Cover the tank (beaker) with parafilm and allow it to equilibrate for approximately 10 min.
- Dry the plates at 100 °C for 1 h and store in a desiccator. Remove predried plate from the desiccator just prior to use.

- Apply 1 drop of ethanol (95%), using a 1-ml syringe with a 22–26 gauge needle onto the aluminum oxide TLC plate, 1.5 cm from the bottom. Do not allow the spot to dry.
- Add two drops of ^{99m}Tc-MIBI injection solution side by side on top of the ethanol spot. Return the plate to a desiccator and allow the sample to dry (typically, 15 min).
- Develop the plate in the covered TLC tank in ethanol (99.8%) for a distance of 5.0 cm from the point of application.
- Cut the TLC plate 4.0 cm from the bottom and measure the ^{99m}Tc activity of each piece in the dose calibrator.

Calculate the percent radiochemical purity as:

$$^{99m}\text{Tc-sestamibi}\ (\%) = \frac{\text{Activity of upper piece}}{\text{Activity of both pieces}} \times 100$$

The time required to complete the entire procedure is approximately 35 min.

Results of analysis *(12 samples)*
Results were obtained using TLC and ethanol as solvent.

Labeling and stability	15 min (%)	6 h (%)
^{99m}Tc-Na-pertechnetate	5.4 ± 0.3	5.6 ± 0.3
^{99m}Tc-sestamibi	94.6 ± 0.3	94.4 ± 0.3

Paper chromatography
Another method, which is faster and offers a high separation of ^{99m}Tc-MIBI from impurities was introduced by Patel et al. (1995). Whatman 3MM paper strips are used and ethyl acetate as solvent. ^{99m}Tc-sestamibi moves with an R_f of 0.55–0.75, while ^{99m}Tc-pertechnetate and reduced, hydrolized ^{99m}Tc-activity remain at the start.

Stationary phase:	Whatman 3MM paper strips (cut to 0.5×6.0 cm)
Solvent:	Ethyl acetate
Developing time:	3 min
R_f values:	^{99m}Tc reduced, hydrolized: 0.0–0.1 ^{99m}Tc-Na-pertechnetate: 0.0–0.1 ^{99m}Tc-MIBI, 0.5–0.8 (>94%)

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter), and the regional radioactivities are expressed as a percentage of the total recovered counts:

$$^{99m}\text{Tc-sestamibi}\ (\%) = 100 - \%\ (F + H)$$

where % $(F + H) = ^{99m}$Tc activity at origin.

Solvent extraction
A rapid and simple method to obtain information about the labeling yield of lipophilic ^{99m}Tc-sestamibi has found wide acceptance in nuclear medicine. Several solvents (e.g.,

ethyl acetate, chloroform, *n*-octanol) have been employed for extraction. Labeled impurities remain in the aqueous phase (saline).

Procedure:

- Add 0.1 ml of the ^{99m}Tc-sestamibi injection solution to a vial containing 3 ml of chloroform and 2.9 ml of saline.
- Close the vial, mix with a Vortex for 10 s, and let the phases separate for 1 min.
- Transfer the top layer (saline) to another vial and measure the activities in a dose calibrator. Lipophilic ^{99m}Tc-MIBI is in the chloroform fraction and the contaminants are in the saline layer.

The radiochemical purity is given by:

$$^{99m}\text{Tc-MIBI}\ (\%) = \frac{\text{Activity of chloroform fraction}}{\text{Activity of both fractions}} \times 100$$

Results of analysis. The activity in the chloroform fraction should not be less than 90% within 6 h after labeling.

Pharmacokinetic Data

Like thallous chloride [$^{201}T1^{+}$], the cationic technetium complex accumulates in the viable myocardial tissue proportional to blood flow. Studies using cultures of myocardial cells have shown that uptake is not dependent on the functional capability of the sodium/potassium pump (Maublant et al. 1988). Cationic membrane transport inhibitors did not affect 1-min ^{99m}Tc-MIBI uptake kinetics when cells were preincubated for 1 min in solutions containing saturating concentrations of quabain (100 μM), a sodium/potassium ATPase inhibitor (Piwnica-Worms et al. 1990).

After the intravenous injection, ^{99m}Tc-sestamibi is distributed in the myocardium according to blood flow and diffusion. Myocardial extraction at rest is 65% (vs 85% Tl^{+}). One-hour-postinjection myocardial uptake at rest is 1.2%; during stress, it is 1.4% (Wackers et al. 1989).

The elimination from blood is fast: 3 min after the intravenous injection, 23% of the radioactivity is measured in blood, decreasing to 9% at 5 min, and 2.5% at 10 min. The effective half-life at rest of the fast early component is 2.18 min (2.13 min during exercise) (Wackers et al. 1989). Protein binding is low (less than 1%).

Elimination of ^{99m}Tc-sestamibi from the myocardium is 27% in 3 h; the biological half-time of elimination is approximately 6 h at rest and during stress. No redistribution of ^{99m}Tc-sestamibi is observed (Wackers et al. 1989).

The major metabolic pathway for clearance of ^{99m}Tc-sestamibi is the hepatobiliary tract. Activity from the gallbladder appears in the intestine within 1 h of injection. Twenty-nine percent of the injected dose is cleared unchanged through renal elimination in 24 h, and approximately 37% of the injected dose is cleared through the feces in 48 h (at rest) (Wackers et al. 1989).

The elimination during 3 h from the liver is 76%; from the spleen, 67%; and from the lung, 49% (Wackers et al. 1989).

The mechanism of localization in various types of breast tissue (benign, inflammatory, malignant, fibrous) has not been established. Malignant breast lesions show the highest uptake of ^{99m}Tc-sestamibi (sensitivity: 79–96%, specificity: 80–94%).

Preclinical safety. Acute intravenous toxicity studies were performed in mice, rats, and dogs. The lowest dose of the reconstituted Cardiolite kit that resulted in any deaths was 7 mg/kg (expressed as $Cu(MIBI)_4 \cdot BF_4$ content) in female rats. This corresponds to 500 times the maximal human dose (MHD) of 0.014 mg/kg for adults (70 kg). Neither rats nor dogs exhibited treatment related effects at reconstituted Cardiolite kit doses of 0.42 mg/kg (30 times MHD) and 0.07 mg/kg (5 times MHD), respectively, for 28 days.

Radiation Dose

The gallbladder wall, liver, spleen, and lung are the most exposed organs. The effective dose is 0.0085 mSv/MBq at rest and 0.0075 mSv/MBq after exercise (International Commission on Radiological Protection 1991). The values are calculated assuming a 3.5-h bladder voiding period.

When two separate injections of ^{99m}Tc-sestamibi are administered, namely 250 MBq (7 mCi) (exercise) and 750 MBq (20 mCi) (at rest) the effective whole-body dose in patients (70 kg) is 8.25 mSv. The effective dose resulting from an administered radioactivity of 250 MBq (7 mCi) at rest (verification of a scar) is 2.12 mSv.

The absorbed radiation dose to the gallbladder wall resulting from an intravenous injection of 750 MBq (20 mCi) of ^{99m}Tc-sestamibi corresponds to 29.3 mGy (at rest).

Storage and Stability

Storage. Cardiolite (Miraluma) kits should be stored at 15–25 °C.

Stability. ^{99m}Tc-sestamibi injection solution is stable for 6 h.

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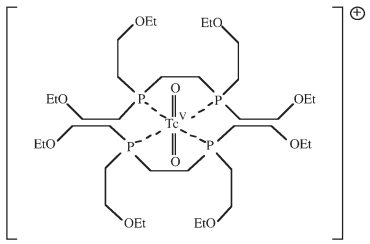
12.4.2 ^{99m}Tc-Tetrofosmin

J. Imre and I. Zolle

Chemical name	**Chemical structure**
1,2-bis[bis(2-ethoxyethyl)-phosphino]-ethane Tetrofosmin (*USP*) Technetium Tc 99m tetrofosmin injection (*USP*) Tc(V) dioxo diphosphine complex ^{99m}Tc-tetrofosmin injection	 ^{99m}Tc(V)-tetrofosmin complex

Kit components		**Commercial products**	
Tetrofosmin	0.23 mg	Myoview	GE Healthcare
Stannous chloride dihydrate	0.03 mg		
Disodium sulfosalicylate	0.32 mg		
Sodium D-gluconate	1.0 mg		
Sodium hydrogen carbonate	1.8 mg		

Preparation

The Myoview kit contains the sterile, lyophilized components in a nitrogen atmosphere. Labeling with ^{99m}Tc-sodium pertechnetate is performed by adding aseptically 4–8 ml of ^{99m}Tc activity to the vial, not exceeding 1.11 GBq/ml (30 mCi/ml). Before the syringe is removed from the vial, a volume of gas should be withdrawn from the space above the solution to normalize the pressure inside the vial. The shielded vial should be gently agitated to dissolve the lyophilized material, and allowed to react at room temperature for 15 min (Nycomed Amersham 1998).

^{99m}Tc(V)-tetrofosmin is a clear, colorless solution for intravenous injection, the pH value is 7.5–9.0.

Description of the Kit

The cationic Tc(V) dioxodiphosphine complex consists of a Tc-*trans*-oxo core having four phosphorus atoms of the bidentate diphosphine ligands arranged in a plane. A comparison with DMPE [1,2-bis(dimethylphosphino)ethane], originally introduced as the Tc(III) dichlorodiphosphine complex, may elucidate the effect of derivatization on complex structure and in vivo performance (Deutsch et al. 1981, 1989). The tetrofosmin ligand is characterized by four ether functional groups (Kelly et al. 1993).

Studies of the rate of formation of the $[^{99m}Tc(tetrofosmin)_2O_2]^+$ complex have indicated that complex formation depends on the ligand concentration and could also be enhanced by heating (Kelly et al. 1993). However, using the Myoview formulation, the Tc(V) dioxodiphosphine complex is formed rapidly at room temperature in high radiochemical purity at a ligand concentration lower than 30 μg/ml.

High chemical purity of the generator eluate is a prerequisite to obtain high labeling yields. To assure optimal reaction conditions, the eluate should be fresh (not older than 6 h), preferably obtained from a generator eluted regularly every 24 h. An interval of more than 72 h from the last elution excludes any generator eluate from being suitable for labeling Myoview.

The volume of ^{99m}Tc eluate should be at least 4 ml and not more than 8 ml. The activity concentration must not exceed 1.11 GBq/ml and should be diluted before addition, if necessary. The amount of ^{99m}Tc activity should not exceed 8.88 GBq (240 mCi) (Nycomed Amersham1998).

Clinical Applications

^{99m}Tc(V)-tetrofosmin is used for myocardial perfusion studies in patients with coronary artery disease:

- Diagnosis of ischemic heart disease
- Diagnosis of reduced regional perfusion and localization of myocardial infarction
- Detection of perfusion defects in myocardium at rest

^{99m}Tc-tetrofosmin has been evaluated as a radiotracer for myocardial perfusion imaging (Higley et al. 1993; Jain et al. 1993). Stress–rest imaging was performed using 1-day and 2-day protocols (Sridhara et al. 1994). Planar and tomographic imaging has been correlated with thallium-201 and coronary angiography (Heo et al. 1994). Pharmacological stress perfusion imaging with single-photon emission computer tomography (SPECT) was used as an alternative to dynamic exercise in patients with coronary artery disease (Cuocolo et al. 1996) and evaluated in a multicenter clinical trial (He et al. 1997). Left ventricular volumes and ejection fraction have been calculated from quantitative electrocardiographic-gated ^{99m}Tc-tetrofosmin myocardial SPECT (Yoshioka et al. 1999). ^{99m}Tc-tetrofosmin imaging at rest has been compared with rest redistribution of thallium-201 for predicting functional recovery after revascularization (Matsunari et al. 1997).

Time of Examinations. The rest and stress scintigraphy is performed 15–30 minutes after the intravenous injection (Higley et al. 1993).

Recommended Activities for Indications. The activity range for intravenous administration in patients (70 kg) is:

Diagnosis of reduced regional perfusion and myocardial infarction:	250–1,000 MBq (7–27 mCi)
First injection at peak exercise:	250–370 MBq (7–10 mCi)
Second injection at rest (4 h after the first injection):	550–750 MBq (15–20 mCi)
Single injection at rest:	185–300 MBq (5–8 mCi)

The performance of myocardial scintigraphy requires a strict protocol (He et al. 1997; Jain et al. 1993; Sridhara et al. 1994)

Usually, the first examination is performed with exercise or pharmacological stress, the intravenous injection of ^{99m}Tc-tetrofosmin is administered at the highest heart rate (250–350 MBq). The stress scintigram is performed 15 min after injection.

Four hours after the initial injection, a second intravenous injection of ^{99m}Tc-tetrofosmin (550–750 MBq) is administered to examine the patient at rest. The rest scintigram is performed 30 min after injection.

Two injections (stress and rest) are required in order to differentiate a transient (reversible) from a persistent perfusion defect, which is typical for ischemic heart disease (Jain et al. 1993; Sridhara et al. 1994). For verification of a scar after myocardial infarction (nonreversible perfusion defect), one injection at rest may be sufficient (Higley et al. 1993).

However, it is usually not possible to differentiate an acute myocardial infarction from ischemic defects, which are observed in patients with angina pectoris during chest pain (Tatum et al. 1997).

Based on diagnostic reference levels for radiopharmaceuticals adopted by the European member states, the total activity administered on a single day should not exceed 1,000 MBq in the case of the combined rest–exercise protocol and 600 MBq in the case of the 1-day protocol (European Commission 1999). A rather large variation in the recommendations from country to country has been reported (Hesse et al. 2005).

Pediatric Dose. Myoview is not recommended for use in infants and children since data are not available for these age groups.

Additional Information

Patients should fast overnight or have only a light breakfast prior to the study. Patients should drink sufficient water, and frequent bladder emptying should be encouraged in order to reduce the radiation exposure to the bladder wall.

Since no redistribution of ^{99m}Tc-tetrofosmin is observed, separate injections are required for stress and rest scintigraphy.

Planar or preferably SPECT imaging should begin no earlier than 15 min postinjection.

No significant changes in myocardial concentration or redistribution of ^{99m}Tc-tetrofosmin have been observed; therefore, images may be acquired up to at least 4 h postinjection (Higley et al. 1993; Jain et al. 1993).

Quality Control

Radiochemical Purity. ^{99m}Tc-Tetrofosmin is not described in the *European Pharmacopeia*. A rapid instant thin-layer chromatography (ITLC) procedure for determination of the radiochemical purity of ^{99m}Tc-tetrofosmin has been published (Van Hemert et al. 2001).

Thin-layer chromatography using Gelman silica gel strips and an organic solvent is described in the *United States Pharmacopeia* (United States Pharmacopeial Convention 2000) and is recommended by the manufacturer. Free ^{99m}Tc-pertechnetate moves with

the solvent front. Reduced, hydrolized ^{99m}Tc activity and any hydrophilic complexes remain at the start. ^{99m}Tc-tetrofosmin is measured at an R_f of 0.4–0.7. The radiochemical purity of ^{99m}Tc-tetrofosmin should not be less than 90%.

The radiochemical purity is analyzed prior to administration of ^{99m}Tc-tetrofosmin. If the labeling yield is less than 90%, the preparation should be discarded.

Method Recommended by the Manufacturer

Thin-layer chromatography		
Stationary phase:	Gelman ITLC-SG strips (2×20 cm)	
Solvent:	Acetone-dichloromethane, 35:65 (v/v) Developing time: 20 min	
R_f values:	^{99m}Tc-reduced, hydrolized; hydrophilic complexes:	0.0–0.1
	^{99m}Tc-tetrofosmin complex (lipophilic):	0.4–0.7 (>90%)
	^{99m}Tc-pertechnetate:	0.9–1.0

Procedure:

- Pour enough solvent into the TLC tank (beaker) to have a depth of 1 cm.
- Cover the tank (beaker) with a lid and allow it to equilibrate for approximately 10 min.
- Mark the origin on the ITLC-SG strip with a pencil line 3 cm from the bottom.
- Apply 10–20 µl of sample at the origin of the silica gel strip. Do not allow the spot to dry.
- Place the strip into the chromatography tank and cover it immediately. Ensure that the strip is not adhering to the walls of the tank.
- Develop the plate in the covered TLC tank for a distance of 15 cm from the point of application.
- Cut the TLC strip at 3 cm and at 12 cm from the origin and measure the ^{99m}Tc activity of each piece in the dose calibrator.

Calculate the percent radiochemical purity as:

$$^{99m}\text{Tc-tetrofosmin}\ (\%) = \frac{\text{Activity of center piece}}{\text{Total activity of all three pieces}} \times 100$$

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter), and the regional radioactivities are expressed as a percentage of the total recovered counts:

$$^{99m}\text{Tc-tetrofosmin}\ (\%) = 100 - \%(F + H)$$

where F (%) = free ^{99m}Tc-pertechnetate, and H (%) = hydrolized ^{99m}Tc activity and hydrophilic complexes.

Results of analysis *(12 samples)*

Labeling and stability	15 min (%)	8 h (%)
^{99m}Tc-tetrofosmin complex	97.5 ± 0.40	96.6 ± 0.29
^{99m}Tc-hydrolized and hydrophilic complexes	2.1 ± 0.13	2.5 ± 0.19
^{99m}Tc-Na-pertechnetate	0.3 ± 0.08	0.8 ± 0.31

Pharmacokinetic Data

The lipophilic ^{99m}Tc-tetrofosmin complex accumulates in viable myocardial tissue proportional to blood flow. Uptake is due to diffusion; retention is based on viable myocytes (Platts et al. 1995; Takahashi et al. 1996). After the intravenous injection, the myocardial extraction of ^{99m}Tc-tetrofosmin at rest is 65% (Tl^+ 85%). Five minutes postinjection, myocardial uptake is 1.2%; during stress, 1.3%. The myocardial concentration of ^{99m}Tc-tetrofosmin remains unchanged up to at least 4 h postinjection (Higley et al. 1993; Jain et al. 1993).

The elimination from blood is fast; 10 min after the intravenous injection less than 5% of the radioactivity are measured in blood, corresponding to 3.5% in the plasma (Higley et al. 1993).

The major metabolic pathway for clearance of ^{99m}Tc-tetrofosmin is the hepatobiliary tract. Activity in the gallbladder reaches a maximum (10% of the injected dose) within 2 h of injection, falling to below 1% at 24 h (Higley et al. 1993).

Accumulation in the liver is 4.9–10.6% of the injected radioactivity; this value falls to 1.6% within 2 h, and after 8 h elimination is complete. Following exercise uptake is reduced to half the value with enhanced sequestration in skeletal muscle (Higley et al. 1993).

Initially, the lung show 0.7–3.0% uptake; elimination of radioactivity is fast, showing only traces of activity 4 h postinjection.

Urinary excretion is 39% in 48 h. Approximately 34% of the injected dose is cleared through the feces in 48 h (Higley et al. 1993).

Preclinical safety. Acute intravenous toxicity studies were performed in rats and rabbits, using up to 1,500 times the maximum single human dose. Repeat dose toxicity was assessed in rats and rabbits at 0, 10, 100, and 1,000 times the maximum human dose daily for 14 days.

No toxicologically significant findings were made on single-dose administration of 1,500 times the maximum equivalent human dose, or on 14-day repeat dose studies at a level of 100 times the maximum human dose. No mortalities have occurred (Kelly et al. 1993).

No significant mutagenic potential was seen in any of the tests used.

Radiation Dose

The gallbladder wall, intestinal tract, kidneys, bladder wall, the salivary glands, and the thyroid, are most exposed organs. Calculations of the effective dose were published by Higley et al. (1993), presenting effective dose values of 0.0089 mSv/MBq at rest and 0.0071 mSv/MBq after exercise, comparable with the effective dose values for technetium-MIBI (International Commission on Radiological Protection 1991). The values are calculated assuming a 3.5-h bladder voiding period.

When two separate injections of ^{99m}Tc-tetrofosmin are administered, namely 250 MBq (7 mCi) (exercise) and 750 MBq (20 mCi) (at rest), the effective whole-body dose in patients (70 kg) is 8.45 mSv. The effective dose resulting from an administered radioactivity of 250 MBq (7 mCi) at rest (verification of a scar) is 2.2 mSv.

The absorbed radiation dose to the gallbladder wall resulting from an intravenous injection of 750 MBq (20 mCi) of ^{99m}Tc-tetrofosmin is corresponding to 29.3 mGy (at rest).

Storage and Stability

Storage. The Myoview kit is stored at 2–8 °C.

Stability. ^{99m}Tc-tetrofosmin injection solution is stable for 8 h. It should be kept at 2–8 °C.

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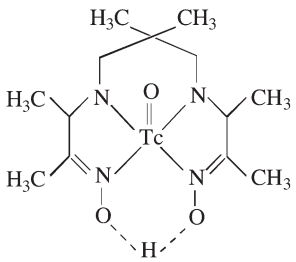
12.5 ^{99m}Tc-Labeled Brain Perfusion Agents

12.5.1 ^{99m}Tc-HMPAO (Hexamethylpropylene Amine Oxime)

F. Rakiás and I. Zolle

Chemical name	**Chemical structure**
4,8-diaza-3,6,6,9-tetramethyl-undecane-2,10-dione-bisoxime (HMPAO) D,L-Hexamethylpropylene amine oxime (D,L-HMPAO) Exametazime (*Ph. Eur., USP*) Technetium ^{99m}Tc exametazime injection (*Ph. Eur., USP*) ^{99m}Tc(V)oxo-D,L-HMPAO complex	 ^{99m}Tc(V)-D,L-HMPAO complex

Kit components		**Commercial products**	
Exametazime	0.5 mg	Ceretec	GE Healthcare
Tin(II)-chloride dihydrate	7.6 μg	Neurospect	Rotop
Sodium chloride	4.5 mg		

Preparation

The Ceretec kit contains the lyophilized, sterile ingredients in a multidose vial. Labeling with ^{99m}Tc eluate is carried out under aseptic conditions by adding 5 ml of sterile sodium ^{99m}Tc-pertechnetate to the vial (0.37–1.11 GBq). The shielded vial should be gently inverted for 10 s to ensure complete dissolution of the lyophilisate. The reaction is allowed to proceed at room temperature for 5 min.

^{99m}Tc-hexamethylpropylene amine oxime (HMPAO) is a sterile, pyrogen-free, clear, colorless solution suitable for intravenous injection. The resulting pH is between 9.0 and 9.8.

In addition, each package consisting of five labeling units contains also five vials of methylene blue injection 1% USP (10 mg methylene blue USP in 1 ml water for injection), and five vials containing 0.003 *M* monobasic sodium phosphate USP and dibasic sodium phosphate USP in 4.5 ml of 0.9% sodium chloride injection USP. Each milliliter contains 0.276 mg monobasic sodium phosphate monohydrate, 0.142 mg dibasic sodium phosphate anhydrous, and 9 mg sodium chloride in water for injection for a sufficient amount. A mixture of methylene blue in phosphate buffer is used for stabilization of the ^{99m}Tc-HMPAO complex (Amersham Healthcare 1995).

Description of the Kit

^{99m}Tc-D,L-HMPAO complex is formed rapidly with reduced technetium at room temperature. The stannous tin content per vial should not decrease to less than 6 μg. Thus, high labeling is depending on maintaining tin in the reduced state. Any oxidant in the ^{99m}Tc eluate should be avoided. Only eluates from generators eluted regularly within 24 h after the previous elution may be used for labeling (Amersham Healthcare 1995). Isotopic dilution is observed with higher concentrations of ^{99}Tc in the first eluate of new generators or after weekends, reducing the labeling efficiency (Ponto et al. 1987). The formulation contains no antimicrobial preservative.

^{99m}Tc-exametazime injection solution should be used within 60 min after labeling. In order to maintain high stability of the ^{99m}Tc-HMPAO complex for up to 6 h after preparation, the producer provides methylene blue injection USP 1% or alternatively, cobalt(II)-chloride aqueous solution (250 μg/2.5 ml, *European Pharmacopeia* [*Ph. Eur.*]) for stabilization.

Stabilization of the ^{99m}Tc-HMPAO complex for intravenous use with methylene blue. Half a milliliter of methylene blue injection 1% USP should be withdrawn into a sterile syringe and injected into the 4.5-ml vial containing the buffer solution. Two milliliters of the methylene blue/phosphate buffer mixture should be gently swirled and withdrawn into a syringe for use as stabilizer. This mixture must be used within 30 min of preparation.

Within 2 min after labeling, add 2.0 ml of the methylene blue/phosphate buffer mixture to the Ceretec vial (Amersham Healthcare 1995). The volume of stabilized Ceretec injection solution is 7.0 ml, and the resulting pH between 6.5 and 7.5.

The ^{99m}Tc-HMPAO complex stabilized with methylene blue is stable for 4 h.

Stabilization of the ^{99m}Tc-HMPAO complex with cobalt(II)-chloride. Two milliliters of cobalt stabilizer solution (250 µg cobalt(II)-chloride·6 H_2O dissolved in 2.5 ml of water for injection) should be withdrawn with a 3-ml sterile syringe.

Within 1–5 min after labeling, 2.0 ml of the stabilizer solution should be injected into the Ceretec vial, and the vial agitated for 10 sec. The volume of stabilized Ceretec injection solution is 7.0 ml, and the resulting pH between 5.0 nd 8.0.

The ^{99m}Tc-HMPAO complex stabilized with cobalt(II)-chloride is stable for 6 h.

Clinical Applications

Technetium-99m exametazime injection is indicated for brain scintigraphy for the diagnosis of perfusion abnormalities of regional cerebral blood flow (rCBF):

- Detection of focal perfusion abnormalities
- Diagnosis of acute cerebral infarction (stroke) when computer tomography (CT) is negative
- Classification of defects of ischemic stroke
- Diagnosis of cerebrovascular disease and differentiation of focal abnormalities in CBF typical in multi-infarct dementia and degenerative dementia

The kinetic parameters of ^{99m}Tc-HMPAO retention in the human brain have been studied (Lassen et al. 1988), and measurements of CBF have been compared to ^{133}Xe (Andersen et al. 1988). CBF was quantified using dynamic single-photon emission computer tomography (SPECT) (Murase et al. 1992). The role of SPECT with ^{99m}Tc-HMPAO in ischemic stroke has been evaluated (Heiss 1983; Podreka et al. 1987). Reflow hyperemia in subacute stroke has been described as "luxury perfusion" (Lassen 1966) and was later explained by hyperfixation of ^{99m}Tc-HMPAO (Lassen and Sperling 1993). Increased regional blood flow in subacute stroke has been measured with ^{99m}Tc-HMPAO (Moretti et al. 1990). The effect of acetazolamide on CBF was reported in patients with severe internal carotid artery stenosis/occlusion (Asenbaum et al. 1995), and has been studied in primates (Dormehl et al. 1997). ^{99m}Tc-HMPAO SPECT was evaluated in patients with cerebrovascular disease by comparison with ^{18}F-fluoromethane positron emission tomography (PET) (Heiss et al. 1990). A pattern of focal abnormalities in CBF was derived in patients with degenerative dementia (Holman et al. 1992).

Time of Examination

Dynamic imaging:	Right after intravenous injection up to 10 min postinjection
Planar/tomographic imaging:	15 min up to 6 h postinjection

Recommended Activities for Indications

Cerebral perfusion scintigraphy:	370–740 MBq (10–20 mCi), injected intravenously 500 MBq (13.5 mCi) maximum recommended activity (Administration of Radioactive Substances Advisory Committee 1993) The manufacturer's instructions should be followed.

Pediatric Dose. The amount of radioactivity for infants and children administered for brain scintigraphy is based on body weight, using the scaling factors given in Appendix 1 (Table A1.4). The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

The ^{99m}Tc eluate used for labeling should be obtained from a ^{99m}Tc-generator, which is eluted daily at a 24-h interval. When using the stabilizing protocol, generator eluates older than 30 min should not be used. Highest radiochemical purity is obtained with ^{99m}Tc-pertechnetate right after elution.

The lipophilic ^{99m}Tc-HMPAO complex is transformed by hydrolysis (in vitro) to the "secondary" hydrophilic ^{99m}Tc-HMPAO complex, which cannot cross the blood–brain barrier (BBB) when injected. Since a high extraction fraction is essential for quantitation of CBF, high radiochemical purity of lipophilic ^{99m}Tc-HMPAO is required. The average value of lipophilic ^{99m}Tc-HMPAO has been reported as 83% (Lassen et al. 1988) and 80.8% (Murase et al. 1992), respectively. Care has been taken to minimize the time between the quality control measurements and administration of the ^{99m}Tc-HMPAO complex (Murase et al. 1992).

Stabilization of the ^{99m}Tc-HMPAO complex with methylene blue/phosphate buffer or with cobalt(II)-chloride will extend in vitro stability up to 6 h after labeling.

The patient should be asked to drink water frequently in order to stimulate excretion of radioactivity.

The ^{99m}Tc-exametazime complex may be used for radiolabeling of leukocytes. In this case, stabilization with methylene blue/phosphate buffer should not be used. A generator eluate more than 2-h-old should not be used for complex formation.

Quality Control

Radiochemical Purity. ^{99m}Tc-exametazime is included in the *Ph. Eur.* (Council of Europe 2005). Thin-layer chromatography on silica gel fiberglass plates using two solvent systems is described to determine the radiochemical purity of the injection solution. Free ^{99m}Tc-Na-pertechnetate (B) is determined in saline, the lipophilic ^{99m}Tc-HMPAO complex (D) is quantified indirectly by a subtraction method (Tab. 1). Previously, three solvent systems had been used, as recommended by the manufacturer.

The labeled product must be tested before application in patients. The radiochemical purity of lipophilic ^{99m}Tc-exametazime should not be less than 80% (*Ph. Eur.*).

Thin-layer chromatography. Aliquots are spotted and analyzed within 2 minutes after labelling, before stabilisation. The entire procedure of analysis takes approx. 15 minutes.

- System I: Gelman ITLC silica gel fiberglass plates and 2-butanone (methyl ethyl ketone [MEK]) as solvent: Reduced, hydrolized ^{99m}Tc-technetium (C) and the secondary ^{99m}Tc-HMPAO complex (A) remain at the start (A+C); the lipophilic ^{99m}Tc-HMPAO complex (D) and unbound ^{99m}Tc-Na-pertechnetate (B) are measured at the solvent front (B+D).

- System II: Gelman ITLC silica gel fiberglass plates and saline as solvent: Reduced, hydrolized ^{99m}Tc-technetium (C), the lipophilic and the secondary ^{99m}Tc-HMPAO complexes (A+D) remain at the start (A+C+D); unbound ^{99m}Tc-Na-pertechnetate (B) moves with the solvent front.

Table 1. Thin-layer chromatography on silica gel fiberglass plates using two solvent systems

System I (MEK)	Sum of reduced, hydrolized ^{99m}Tc activity and the secondary ^{99m}Tc-HMPAO complex at the start:	**(A + C)**
	Sum of lipophilic ^{99m}Tc-HMPAO complex and free ^{99m}Tc-pertechnetate at the solvent front:	**(B + D)**
System II (Saline)	Free ^{99m}Tc-pertechnetate at the solvent front:	**(B)**
	^{99m}Tc activity at origin corresponding to:	**(A + C + D)**
	A, B, and C represent labeled impurities, D is the lipophilic ^{99m}Tc-HMPAO complex	
	D (%) = 100 – % (A + C + B) (*Ph. Eur.*)	
	D (%) = % (A + C+D) – %(A+C) (*USP*)	

Impurities (A+C) measured in solvent I (MEK) and free ^{99m}Tc-pertechnetate (B), quantified in system II at the solvent front are subtracted from the total recovered activity, which is assumed as 100%. Free ^{99m}Tc-pertechnetate (B) should not exceed 10% of the total radioactivity, the lipophilic ^{99m}Tc-HMPAO complex should not be less than 80% (*Ph. Eur.*).

The difference between activity measured at the start (A+C+D) in solvent II (saline) minus activity measured at the start (A+C) in solvent I (MEK) corresponds to the percentage of the lipophilic ^{99m}Tc-HMPAO complex. Radioactivity measured at the solvent front (B) in system II indicates the amount of free ^{99m}Tc-pertechnetate (*USP*).

The percentage of the lipophilic ^{99m}Tc-HMPAO complex is calculated according to the specifications described in the pharmacopeias (Tab. 1).

Methods recommended by the manufacturer*:*

Thin-layer chromatography on Gelman silica gel sheets and paper chromatography on Whatman 1 strips is recommended by the manufacturer, using three solvent systems for the analysis of the lipophilic ^{99m}Tc-HMPAO complex (D), the secondary hydrophilic complex (A), unbound ^{99m}Tc-Na-pertechnetate (B), and reduced, hydrolized ^{99m}Tc activity (C) (Tab. 2). Since reduced, hydrolized ^{99m}Tc activity is determined separately, each impurity is quantified. The radiochemical purity of lipophilic ^{99m}Tc-exametazime should not be less than 80% (USP 28).

System I and II are identical with the methods recommended in the *Ph. Eur.*

System III is based on paper chromatography using Whatman 1 paper strips and acetonitrile-water (1:1) as solvent. Reduced, hydrolized ^{99m}Tc-technetium (C) remains at the start; both ^{99m}Tc-HMPAO complexes (A+D) and unbound ^{99m}Tc-Na-pertechnetate (B) are measured at the solvent front (A+B+D).

Table 2. Thin-layer chromatography and paper chromatography using three solvent systems (recommended for Ceretec)

System I	Sum of reduced, hydrolized ^{99m}Tc activity and the secondary ^{99m}Tc-HMPAO complex at the origin	**(A + C)**
System II	Free ^{99m}Tc-pertechnetate at the solvent front	**(B)**
System III	Reduced, hydrolized ^{99m}Tc-activity at the origin	**(C)**
	D (%) = 100 – % (A + B + C)	

The added impurities % (A + B + C) are subtracted from the total recovered activity (100%) according to the recommended methods (Tab. 2).

Lipophilic ^{99m}Tc-HMPAO complex (% D) = 100–%(A + B + C)

A = Secondary ^{99m}Tc-HMPAO complex
B = ^{99m}Tc-pertechnetate
C = Reduced, hydrolized ^{99m}Tc activity
D = Lipophilic ^{99m}Tc-HMPAO complex

Results of analysis *(12 samples)*
Results were obtained using the analytical methods originally described for Ceretec (Amersham Healthcare 1995) outlined in Tab. 2.

Labeling and stability	15 min (%)	1 h (%)
^{99m}Tc-HMPAO lipophilic complex **(D)**	90.1±1.06	85.6±1.23
^{99m}Tc-HMPAO secondary complex **(A)**	4.5±0.90	6.5±1.79
^{99m}Tc-Na-pertechnetate **(B)**	2.9±0.51	3.9±0.62
^{99m}Tc-reduced, hydrolized **(C)**	2.4±0.47	4.0±1.42

Solvent extraction
A more rapid method to obtain information about the labelling yield (percentage of lipophilic ^{99m}Tc-exametazime) has found wide acceptance in nuclear medicine (Ballinger et al. 1988). Several solvents (ethyl acetate, chloroform, n-octanol) have been employed for the extraction of the lipophilic ^{99m}Tc-HMPAO complex. Labelled impurities remain in the aqueous phase.

Procedure: A sample of 0.1 ml of labeled Ceretec is added to a mixture of 3 ml of chloroform and 2.9 ml of saline. Close vial and mix well using a vortex mixer for 30 seconds. Let phases separate for 1 min, then transfer the top layer (saline) to another vial and measure both vials in a dose calibrator.

The radiochemical purity of the lipophilic ^{99m}Tc-HMPAO complex is expressed by:

$$^{99m}\text{Tc-HMPAO}\ (\%) = \frac{\text{Activity of chloroform fraction}}{\text{Sum of activities in both fractions}} \times 100$$

A comparison of results obtained with both methods (ITLC and solvent extraction) indicates excellent correlation between both separation methods:

ITLC separation ($n=12$)	$87.0 \pm 2.0\%$
Solvent extraction ($n=12$)	$85.5 \pm 3.7\%$

Pharmacokinetic Data

Neutral, lipophilic molecules with log *P*-values between 0.9 and 3.5 may cross the BBB by diffusion or active process, depending on the structural configuration (Holm et al. 1985; Troutner et al. 1984).

The lipophilic ^{99m}Tc-D,L-HMPAO complex (log $P=1.2$) can cross the BBB and is extracted with high efficiency ($E=0.8$) from blood at normal flow levels (Holmes et al. 1985; Lassen and Andersen 1988; Lassen et al. 1987). Cerebral extraction corresponds to approximately 5% of the injected radioactivity (Leonard et al. 1986; Neirinckx et al. 1987). The mesoform is not accumulated in the brain due to the stereospecificity of uptake (Sharp et al. 1986).

The lipophilic ^{99m}Tc-D,L-HMPAO complex is decomposed rapidly in vivo, both in the blood and in the brain. Due to this instability, the secondary ^{99m}Tc-D,L-HMPAO complex, a charged complex, is formed (Neirinckx et al. 1988). The secondary complex cannot pass the BBB and is trapped inside the brain and in blood cells, i.e., the ionized molecule is trapped.

After intravenous injection of a bolus of ^{99m}Tc-D,L-HMPAO complex, 50% of the radioactivity are eliminated from the circulation within 2–3 min; thus, when the lipophilic complex has been eliminated from the blood, no further exchange is observed (Lassen and Andersen 1988).

After approximately 5 min, when the lipophilic radiotracer has disappeared both in the blood and in brain, the distribution of radioactivity in the brain is a true image of the initial blood flow (Lassen et al. 1987). The radioactivity pattern remains constant for 24 h. Elimination from the brain is very slow, approximately 1% per hour (Ell et al. 1987). After 24 h, >70% of the tracer is still in the brain.

The kidneys excrete 41% of the injected radioactivity over the first 48 h. Between 8.5 and 13.0% pass through the liver, thereby showing the main bile ducts and the gall bladder. Lung uptake averages 9%, and about 2% of the injected radiotracer localize in the myocardium (Ell et al. 1987).

The lipophilic ^{99m}Tc-D,L-HMPAO complex accumulates in blood cells (80% in red cells, less in white cells and platelets); the blood shows approximately 12% radioactivity 1 h postinjection (Ell et al. 1987).

A relatively high concentration of activity was observed in lacrimal glands of volunteers (Meyer et al. 1990).

Radiation Dose

The excretory organs such as the kidneys and urinary bladder, along with the gallbladder wall, the upper large intestinal wall, the lower large intestinal wall, the small intestine, and the liver are the most exposed organs (ICRP 62). The effective (whole body)

dose is 0.0093 mSv/MBq (International Commission on Radiological Protection 1991). The effective dose in adults (70 kg) resulting from 500 MBq (13.5 mCi) of intravenously injected ^{99m}Tc-HMPAO complex is 4.65 mSv.

Storage and Stability

Storage. The lyophilized kit should be stored at 15–25 °C.

Stability. The stabilized ^{99m}Tc-HMPAO complex may be used 4–6 h after preparation.

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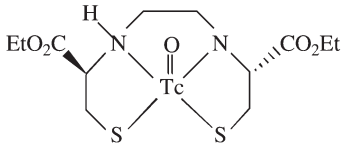
12.5.2 ^{99m}Tc-ECD (Ethyl Cysteinate Dimer)

J. Imre and I. Zolle

Chemical name	**Chemical structure**
(*N*,*N*′-1,2-ethylenediyl-bis-L-cysteine) diethyl ester dihydrochloride (ECD) Ethyl-Cysteinate-Dimer (L,L-ECD) Bicisate (*USP*) Technetium Tc 99m bicisate injection (*USP*) Tc(V)oxo-*N*,*N*′-ethylene di-cysteinate diethyl ester ^{99m}Tc(V)oxo-L,L-ECD complex	 Tc(V)O-L,L-ECD

Kit components		**Listed trade names**	
Vial A:		Neurolite	Bristol-Myers Squibb
Bicisate dihydrochloride	0.9 mg		
Stannous chloride, dihydrate	0.072 mg		
Edetate disodium, dihydrate	0.36 mg		
Mannitol	24 mg		
Vial B:			
Sodium phosphate dibasic Heptahydrate	4.1 mg		
Sodium phosphate monobasic monohydrate	0.46 mg		
Water for injection	ad 1 ml		

Preparation

The Neurolite kit consists of two nonradioactive vials, vial A and vial B.

Vial A contains the lyophilized active ingredients in a nitrogen atmosphere. A volume of 3 ml of saline is added to vial A, and the vial inverted to dissolve the kit content. Within 30 s, 1 ml from vial A should be withdrawn and injected into vial B.

Vial B contains 1 ml phosphate buffer, pH 7.6 ± 0.4. It is placed into a lead shield. A volume of 2 ml of ^{99m}Tc-pertechnetate (925 MBq–3.7 GBq; 25–100 mCi) is added aseptically to vial B. Labeling is performed by adding aseptically 1 ml from vial A to vial B, and allowed to react for 30 min at room temperature (DuPont Merck Pharmaceutical 1995).

^{99m}Tc-ethyl cysteinate dimer (ECD) is a clear, colorless, sterile, solution suitable for intravenous injection. The pH value is between 6.6 and 7.8.

Description of the Kit

The kit formulation (vial A) contains bicisate dihydrochloride exclusively as the L,L-enantiomer (DuPont Merck Pharmaceutical 1995). After reconstitution with 3 ml saline, the pH of vial A is 2.7 ± 0.25. One third of bicisate (0.3 mg) is used for labeling. The rest is discarded. Storage of residual portions of Neurolite in a freezer and subsequent labeling within 4 weeks has been suggested (Verbeke et al. 1997).

Initially, reduced ^{99m}Tc activity and ethylenediaminetetraacetic acid (EDTA) form an intermediary complex, which is transformed slowly by ligand exchange to the ^{99m}Tc(V)-ECD complex, showing high in vitro stability. Originally, glucoheptonate had been used instead of EDTA (Cheesman et al. 1988).

The diaminodithiol (DADT)-derived ligand ECD forms a stable complex with pentavalent oxo-technetium (Cheesman et al. 1988). ^{99m}Tc-ECD is a neutral, lipophilic complex with the chemical formula [TcO-L,L-ECD]^0.

Clinical Applications

Technetium-99m bicisate injection is indicated for brain scintigraphy to delineate focal perfusion abnormalities:

- Diagnosis of acute cerebral infarction (stroke) when computer tomography (CT) is negative
- Detection of inflammatory conditions in the brain
- Detection of an abnormal focus in patients with head trauma after accidents
- Differentiation of focal abnormalities in cerebral blood flow typical in multi-infarct dementia and degenerative dementia

^{99m}Tc-bicisate uptake follows linearity, with blood flow values up to 20 ml/100 g/min; however, ECD underestimates higher flow rates (Tsuchida et al. 1992). A considerable disadvantage is 3–4% washout per hour, which is difficult to correct for. Another deficit of ECD is an overestimation of infarct size in patients with subactute stroke (Lassen and Sperling 1994). The role of single-photon emission computer tomography (SPECT) with ^{99m}Tc-bicisate in ischemic stroke has been evaluated (Brass et al. 1994; Moretti et al. 1990).

The kinetic parameters of ^{99m}Tc-ECD in the human brain have been compared with regional cerebral blood flow measurements with ^{133}Xe SPECT (Devous et al. 1993) and with positron emission tomography (PET) (Tsuchida et al. 1992). The response of ^{99m}Tc-ECD, ^{99m}Tc-examethylpropylene amine oxime HMPAO and ^{123}I-IMP (*N*-isopropyl-*p*-[^{123}I]-iodoamphetamine) to changes in cerebral blood flow was studied in primates (Dormehl et al. 1997).

Time of Examination. Images of the brain are obtained from 10 min up to 6 h after injection. Optimal images occur 30–60 min after injection.

Recommended Activities for Indications

Cerebral perfusion scintigraphy: 370–740 MBq (10–20 mCi), injected intravenously
500 MBq (13.5 mCi) maximum recommended activity (Administration of Radioactive Substances Advisory Committee 1993)
The manufacturer's instructions should be followed.

Pediatric Dose. The amount of radioactivity for infants and children administered for brain scintigraphy is based on body weight, using the scaling factors given in Appendix 1 (Table A1.2). The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

Patients should drink sufficient water before and after the study, and frequent bladder emptying should be encouraged in order to reduce the radiation exposure to the bladder wall.

Technetium ^{99m}Tc-bicisate is eliminated primarily by renal excretion. It should be used with caution in patients with renal or hepatic impairment.

The effect of acetazolamide on cerebral blood flow is underestimated with ^{99m}Tc-ECD (Dormehl et al. 1997).

Quality Control

Radiochemical Purity. ^{99m}Tc-bicisate is not described in the *European Pharmacopeia* (United States Pharmacopeial Convention 2005). Thin-layer chromatography (TLC) using Baker-flex silica gel strips and an organic solvent is recommended by the manufacturer. Free ^{99m}Tc-pertechnetate and reduced, hydrolized ^{99m}Tc activity remain at the start, ^{99m}Tc-bicisate is measured at an R_f of 0.9–1.0. The radiochemical purity of ^{99m}Tc-bicisate should not be less than 90%.

The radiochemical purity should be determined prior to administration of ^{99m}Tc-ECD complex. If the labeling yield is less than 90%, the preparation should be discarded.

Methods Recommended by the Manufacturer

Thin-layer chromatography		
Stationary phase:	Baker-flex SG 1B-F (precut to 2.5×7.5 cm)	
Solvent:	Ethyl acetate (high-performance liquid chromatography [HPLC] grade)	
Developing time:	15 min	
R_f values:	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-pertechnetate:	0.0–0.1
	^{99m}Tc-ECD complex:	0.9–1.0 (>90%)

Procedure:

- Pour enough ethyl acetate into the TLC tank (beaker) to have a depth of 3–4 mm of solvent.
- Cover the tank (beaker) with parafilm and allow it to equilibrate for approximately 10 min.
- Dry the plates at 100 °C for 1 h and store in a desiccator. Remove predried plate from the desiccator just prior to use.
- With a pencil, draw a faint line across the TLC plate at heights of 2, 4.5, and 7 cm from the bottom of the TLC plate.
- Apply 5 µl of ^{99m}Tc-ECD injection solution at the center of the 2-cm mark. The diameter of the spot should not be greater than 10 mm.
- Allow the spot to dry for 5–10 min, no longer.
- Develop the plate in the covered TLC tank in fresh ethyl acetate to the 7.0-cm line (about 15 min). Remove the plate and dry in a ventilated hood.
- Cut the TLC plate at the 4.5-cm mark with scissors.
- Count the activity on each plate using a dose calibrator or a gamma counter.

The time required to complete the entire procedure is approximately 30 min.

The portion 4.5–7.0 cm contains the ^{99m}Tc-ECD complex and the bottom portion contains all labeled impurities.

Calculate the percent radiochemical purity as:

$$^{99m}\text{Tc-bicisate}\ (\%) = \frac{\text{Activity of upper piece}}{\text{Activity of both pieces}} \times 100$$

Results of analysis *(12 samples)*

Results were obtained by thin-layer chromatography described by the manufacturer.

Labeling and stability	15 min (%)	6 h (%)
^{99m}Tc-ECD complex	95.1 ± 0.41	93.2 ± 0.34
^{99m}Tc-Na-pertechnetate and ^{99m}Tc-reduced, hydrolized	4.8 ± 0.24	6.0 ± 0.17

Pharmacokinetic Data

After intravenous injection of a bolus of activity, ^{99m}Tc-L,L-ECD is distributed in the normal brain proportional to regional blood flow (Friberg et al. 1994). The lipophilic complex crosses the blood–brain barrier (BBB); cerebral uptake at 5 min postinjection is approximately 6.5% of the injected radioactivity (Holman et al. 1989; Leveille et al. 1989; Vallabhajosula et al. 1989). Within 4 h after injection, activity in the brain decreased to 3.7 ± 0.3% injection dose (ID).

The highest concentration of radioactivity in blood was measured at 1 min after intravenous injection, representing 19% of the injected dose (Vallabhajosula et al. 1989). The elimination from blood is rapid ($T_{1/2} = 0.8$ min), falling to 10% of the injected radioactivity at 2 min. One hour after the intravenous injection, less than 5% of the

radioactivity is present, mainly as the nonlipophilic complex. ^{99m}Tc-bicisate is rapidly metabolized in blood; the hydrophilic acid metabolites are excreted through the kidneys, resulting in high brain to soft tissue concentration ratios early after injection and increasing over several hours (Holman et al. 1989).

Brain retention of ^{99m}Tc-L,L-ECD is caused by intracellular stereospecific hydrolysis of the ester, producing the monoethyl ester (Walovitch et al. 1988). The D,D-isomer showed back-diffusion ($T_{1/2}$ 30 min). Once the metabolite is formed in the brain, it cannot cross the BBB in either direction (Walovitch et al. 1989). Effect of hypoxia on esterase function might interfere with reflow hyperemia in subacute stroke (Dormehl et al. 1997). Elimination from brain is expressed by two exponential functions, corresponding to half-times of 1.3 h (40%) and 42.3 h (60%, 3.8% ID), respectively (Vallabhajosula et al. 1989).

^{99m}Tc-L,L-ECD is rapidly metabolized in the liver to the monoacid, and also to the diacid, ^{99m}Tc-L,L-EC. At 5 min postinjection, only a third of the blood activity (2.4% ID) was identified as the neutral, parent complex. The remaining activity was a mixture of polar metabolites. The amount of ^{99m}Tc-bicisate is decreasing rapidly, 10 min after injection there remains little parent complex in the blood for extraction by the brain. Metabolites were identified in urine by HPLC as 19.5% monoacid and 37.8% diacid (^{99m}Tc-EC) and 26.3% monoacid and 48.9% diacid at 2 hr and at 6 hr after administration of ^{99m}Tc-L,L-ECD, respectively (Walovitch et al. 1991).

Excretion of ^{99m}Tc-L,L-ECD from the body is primarily by the kidneys, approximately 50% during the first 2 h, a total of 74% in 24 h. In feces 11.2 ± 6.2% were measured in 48 h. Total body retention of ^{99m}Tc activity was less then 30% at 4 hr (Vallabhajosula et al. 1989).

Radiation Dose

The excretory organs such as the kidneys and urinary bladder, along with the gallbladder wall, the upper large intestinal wall, the lower large intestinal wall, the small intestine, and the liver are the most exposed organs (International Commission on Radiological Protection 1991). The effective (whole body) dose for HMPAO is 0.0093 mSv/MBq (International Commission on Radiological Protection 1991). Based on this value, the effective dose in adults (70 kg) resulting from 500 MBq of intravenously injected ^{99m}Tc-ECD complex is 4.65 mSv.

Storage and Stability

Storage. The lyophilized kit should be stored at 15–25 °C. Vial A should be protected from light. ^{99m}Tc-ECD injection is kept at room temperature with adequate shielding.

Stability. The ^{99m}Tc-ECD injection is stable for 8 h after preparation.

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12.6 ^{99m}Tc-Labeled Leukocytes

I. Zolle and Gy. Jánoki

Chemical name	**Kit components, Ceretec**	
D,L-Hexamethylpropylene amine oxime	Exametazime	0.5 mg
D,L-HM-PAO	Tin(II)-chloride dihydrate	7.6 μg
Exametazime (*Ph. Eur., USP*)	Sodium chloride	4.5 mg
^{99m}Tc-D,L-HMPAO complex		

Preparation

The Ceretec kit contains the lyophilized, sterile ingredients in a multidose vial, sealed under nitrogen atmosphere. Labeling with ^{99m}Tc eluate is carried out under aseptic conditions by adding 5 ml of sterile sodium ^{99m}Tc-pertechnetate to the vial (0.37–1.11 GBq). The shielded vial should be inverted gently for 10 sec to ensure complete dissolution of the lyophilisate. The reaction is allowed to proceed at room temperature for 5 min. The resulting pH is between 9.0 and 9.8.

^{99m}Tc-hexamethylpropylene amine oxime (HMPAO) injection solution (0.1 mg/ml) is suitable for labeling leukocytes (GE Healthcare 2005).

Description of the Kit

^{99m}Tc-D,L-HMPAO complex is formed rapidly with reduced technetium at room temperature. A high labeling yield depends on maintaining tin in the reduced state. Any oxidant in the ^{99m}Tc eluate should be avoided. Only eluates from generators eluted regularly within 24 h after the previous elution may be used for labeling (GE Healthcare 2005). Isotopic dilution is observed with higher concentrations of ^{99}Tc in the first eluate of new generators or after weekends, reducing the labeling efficiency (Ponto et al. 1987).

The mean separation efficiency expressed as a percentage of the number of leukocytes present in the patients' blood is 40%. Only one fifth of the ^{99m}Tc-HMPAO injection solution (0.1 mg) is used for labeling. A maximum labeling efficiency of 80% during a 20-min incubation period was reported using 50 μg of D,L-HMPAO ligand in 1 ml of saline and 5 μg of tin(II)-salt, corresponding to an activity of 370–740 MBq (10–20 mCi) of ^{99m}Tc-D,L-HMPAO (Mortelmans et al. 1989).

Procedure for labeling autologous leukocytes. For cell labeling, aseptic techniques have to be used throughout (Danpure et al. 1988; Segall et al. 1994). The original labeling procedure described by the manufacturer has been adapted to using smaller amounts of blood.

1. Draw 2 ml of acid-citrate-dextrose (ACD) solution and 3 ml of a sedimentation agent into each of four 20-ml plastic nonheparinized syringes.

2. Withdraw 15 ml of patient's blood into each syringe (a total of 60 ml), and mix gently by inversion.
3. Allow tubes to stand for 30–40 min at room temperature for erythrocytes to sediment.
4. When red cells have sedimented to approximately half the original volume of the blood, carefully draw up the leukocyte-rich, platelet-rich plasma (LRPRP) into a sterile tube and centrifuge at 150×g for 10 min.
5. While centrifuging the tubes, reconstitute one vial of HMPAO with 1.5 ml of ^{99m}Tc eluate containing 700–750 MBq (19–20 mCi) of ^{99m}Tc-pertechnetate (400–500 MBq/ml, resp. 11–13 mCi/ml)). Gently invert the shielded vial for 10 sec to dissolve the lyophilisate. The generator eluate should not be more than 2 h old and the generator must have been eluted within the past 24 h.
6. Remove the supernatant PRP platelet-rich plasma (PRP) from the pellet of "mixed" leukocytes, leaving the pellet almost dry. Save 10–15 ml of PRP for step 8. Agitate the tube gently to loosen the cells, and then pool all the cells into one tube.
7. Add exactly 1 ml of ^{99m}Tc-HMPAO to the tube with pooled leukocytes (the radioactivity of ^{99m}Tc-HMPAO is 400–500 MBq (11–13 mCi)). Mix gently and incubate the cells for 10 min at room temperature.
8. While incubating the leukocytes, centrifuge the PRP (obtained in step 6) for 5 min at 2000×*g* to produce cell-free plasma (CFP).
9. After incubation, carefully add 3–5 ml of cell-free plasma (obtained in step 8) to the labeled cell suspension and mix.
10. Centrifuge at 150×*g* for 10 min.
11. Add 3–5 ml of CFP containing ACD to the pellet of leukocytes; gently swirl for mixing.
12. Measure the radioactivity in the cells and in the supernatant (from step 10) and calculate the labeling efficiency (defined as the radioactivity in the cells expressed as a percentage of the sum of activities measured in the cells and in the supernatant).
13. The labeled leukocytes are ready to be reinjected. This should be performed without delay.

Clinical Applications. Lipophilic ^{99m}Tc-exametazime has been shown to label leukocytes without affecting cell viability (Mortelmans et al. 1989; Peters et al. 1986; Roddie et al. 1988). HMPAO-labeled leukocytes have been used to locate site(s) of focal infection (e.g., abdominal abscess, abdominal sepsis) (Kelbaek et al. 1985); it is also indicated in conditions of fever of unknown origin, and in conditions not associated with infection such as inflammatory bowel disease (Arndt et al. 1993; Lantto et al. 1991). Labeled leukocytes have offered superior information when compared with bone scanning for the detection of osteomyelitis in children (Lantto et al. 1992). In a retrospective study in 116 patients with infection suspected to involve orthopedic implants, osteomyelitis, and septic arthritis, HMPAO-labeled leukocytes have been an effective tool in the diagnosis of chronic osteomyelitis and joint infection involving implants (sensitivity: >97%, specificity: >89%) (Devillers et al. 1995).

Time of Examination. Planar imaging is performed at 1, 2 or 24 h after injection of labeled leukocytes.

Recommended Activities for Indications. Abdominal scintigraphy: 185–370 MBq (5–10 mCi) by intravenous injection

Additional Information

Use only ^{99m}Tc eluate for labeling which has been obtained less than 2 h before use.

Do not use methylene blue for stabilization, if ^{99m}Tc-HMPAO injection is used for leukocyte labeling (GE Healthcare 2005).

^{99m}Tc-labeled leukocytes should be used within 1 h after preparation.

A prolonged and delayed lung transit time and/or an abnormally high liver uptake are indications that some damage of the labeled cells occurred (Bowring 1986).

Quality Control

Radiochemical Purity. ^{99m}Tc-exametazime is included in the *European Pharmacopeia* (Council of Europe 2002). Thin-layer chromatography on silica gel fiberglass plates, using two solvent systems is described to determine the radiochemical purity of the injection solution. In analogy, ^{99m}Tc-HMPAO-labeled leukocytes are analyzed under similar conditions. However, since cells do not migrate, free ^{99m}Tc-Na-pertechnetate and the unbound ^{99m}Tc-HMPAO complex are the major impurities to be quantified.

The labeled product is analyzed before application to the patient. The radiochemical purity of ^{99m}Tc-labeled leukocytes is generally 95%.

Thin-layer chromatography

Aliquots are taken after incubation (step 7 above) to determine the labeling yield and also of the resuspended labeled leukocytes (step 11 above).

Leukocyte labeling efficiency must be at least 52 ± 4.1%. Higher labeling efficiencies have been reported (Mortelmans et al. 1989).

- System I: Gelman instant thin-layer chromatography (ITLC) silica gel fiberglass plates and 2-butanone (methyl ethyl ketone [MEK]) as solvent: Reduced, hydrolized ^{99m}Tc-technetium (C), the secondary ^{99m}Tc-HMPAO complex (A) and the ^{99m}Tc-HMPAO-labeled leukocytes (E) remain at the start (A + C + E); unbound ^{99m}Tc-HMPAO complex (D) and free ^{99m}Tc-Na-pertechnetate (B) are measured at the solvent front (B + D).
- System II: Gelman ITLC silica gel fiberglass plates and saline as solvent: Reduced, hydrolized ^{99m}Tc-technetium (C), the secondary ^{99m}Tc-HMPAO complexes (A), lipophilic ^{99m}Tc-HMPAO complex (D), and ^{99m}Tc-HMPAO-labeled leukocytes (E) remain at the start (A + C + D + E); unbound ^{99m}Tc-Na-pertechnetate (B) moves with the solvent front.

The difference between activity measured at the start (A + C + D + E) in solvent II (saline) minus activity measured at the start (A + C + E) in solvent I (MEK) corresponds to the percentage of unbound lipophilic ^{99m}Tc-HMPAO complex (D). Radioactivity measured at the solvent front in system II indicates the amount of free ^{99m}Tc-pertechnetate (B). Thus, the major radiochemical impurities (B and D) are used as an approximation of the purity of ^{99m}Tc-HMPAO-labeled leukocytes (E).

D (%) = %(A + C + D + E) – % (A + C + E)

B + D (%) = Unbound ^{99m}Tc-activity

^{99m}Tc-labeled leukocytes (%) = 100–%(B+D)

A = Secondary ^{99m}Tc-HMPAO complex
B = ^{99m}Tc-pertechnetate (free)
C = Reduced, hydrolized ^{99m}Tc activity
D = Lipophilic ^{99m}Tc-HMPAO complex
E = ^{99m}Tc-HMPAO-labeled leukocytes

Pharmacokinetic Data

Following reinjection of ^{99m}Tc-labeled leukocytes, normal distribution shows an initial transitory uptake in the lungs, and later, in the spleen, liver, and bone marrow. ^{99m}Tc-labeled leukocytes represent approximately 37% of the circulating pool 40 min after injection. The kidneys and the gall bladder may also be visualized. During the first hours (1–6 h) nonspecific bowel activity is seen, 24 h after injection activity in the colon predominates. ^{99m}Tc activity is slowly released from the cells, with an observed elution rate of 20% during the first 24 h (Mortelmans et al. 1989; Segall et al. 1994), excreted partly by the kidneys and partly by the liver into the gall bladder.

Damage to cells or clumping as a result of the labeling procedure will produce an abnormal distribution of ^{99m}Tc-labeled leukocytes (see Chap. 8).

Radiation Dose

Intravenously injected leukocytes are distributed in liver, spleen, bone marrow, and other tissues; 40% are assumed to circulate in the blood with a half-time of 7 h, after which they are taken up in the same organs and tissues and in the same proportions as for the early uptake. The total uptake in the liver is 20%, in the spleen 25%, in red bone marrow 30%, and 25% in other tissues. Whole-body elimination is assumed to be 50% in 70 days. The model is based on granulocytes, which form the majority of cells in a preparation of mixed leukocytes (International Commission on Radiological Protection 1987).

The effective (whole body) dose value is 0.011 mSv/MBq (International Commission on Radiological Protection 1991). The effective dose in adults (70 kg) resulting from an intravenous injection of 185 MBq of ^{99m}Tc-labeled leukocytes is 2.04 mSv. Good abscess images were reported with 100 MBq of ^{99m}Tc-labeled granulocytes, resulting in an effective dose to the patient of 1.2 mSv (Skretting et al. 1988).

Storage and Stability

Storage. Kits should be stored at room temperature, and not stored above 25 °C.

Stability. ^{99m}Tc-labeled leukocytes should be reinjected without delay after preparation. They should not be refrigerated or frozen.

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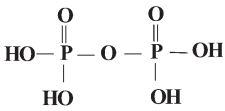
12.7 ^{99m}Tc-Labeled Bone Imaging Agents

12.7.1 ^{99m}Tc-Pyrophosphate (PYP)

S. Kladnik and I. Zolle

Chemical name	**Chemical structure**
Sodium pyrophosphate·10 H_2O (PYP) Tin(II) diphosphate Technetium ^{99m}Tc tin pyrophosphate injection (*Ph. Eur.*) Technetium Tc 99m (pyrophosphate injection (*USP*) ^{99m}Tc-(Sn)-pyrophosphate injection ^{99m}Tc-PYP	 Diphosphoric acid

Kit components		**Commercial products**	
TechneScan PYP		TechneScan PYP	Mallinckrodt/Tyco
PYP	11.9 mg	Pyroscint	Bristol-Meyers Squibb
Tin(II)-chloride-dihydrate	3.4 mg	AngioCis (TCK-7)	CIS Bio
AngioCis		HematoCis (TCK-11)	CIS Bio
PYP	100 mg		
Tin(II)-chloride dihydrate	1.6 mg		
HematoCis			
PYP	0.67 mg		
Tin(II)-chloride dihydrate	0.01 mg		

Preparation

Each vial contains the freeze-dried, sterile components under nitrogen atmosphere in a multidose vial. Depending on the intended use, the vial is reconstituted with sterile saline or with sterile sodium ^{99m}Tc-pertechnetate injection solution. All transfers and vial stopper entries must be done using aseptic techniques. Following reconstitution, the vial is agitated to dissolve the lyophilized material.

Labeling with ^{99m}Tc-pertechnetate is performed by adding 1–5 ml of sterile sodium ^{99m}Tc-pertechnetate solution to obtain a suitable radioactivity concentration for intravenous application (Cis International 1985 a).

^{99m}Tc-(Sn)-pyrophosphate (^{99m}Tc-PYP) injection solution is clear and free of particulate matter, the pH value is 5.–7.0.

Pretreatment of red blood cells (RBC) with PYP for in vivo labeling with ^{99m}Tc-Na-pertechnetate. Stannous pyrophosphate cold kits (except HematoCis) are used for in vivo labeling of erythrocytes with ^{99m}Tc-pertechnetate. In this case, the vial is reconstituted with sterile, nonpyrogenic saline or water, containing no preservatives. A volume of 3 ml resp. 10 ml is recommended by the manufacturer. The vial is agitated to dissolve the lyophilized material. After two resp. 5 min at room temperature, the preparation is ready for injection. A volume corresponding to 3.0–4.0 mg of stannous pyrophosphate is injected intravenously; 30 min later, ^{99m}Tc-Na-pertechnetate (555–740 MBq) (15–20 mCi) is injected, also intravenously, for in vivo labeling of pretreated erythrocytes. The amount of PYP should not exceed 0.5 mg/kg body weight (Mallinckrodt Medical 1993). Factors to be considered for in vivo labeling have been presented (Zimmer et al. 1979).

In vitro labeling of RBC with ^{99m}Tc-Na-pertechnetate. HematoCis (TCK-11) consists of two vials. Vial A contains a lyophilized, sterile formulation of 0.67 mg sodium pyrophosphate decahydrate and 0.01 mg stannous chloride dihydrate under nitrogen atmosphere. Vial B contains 10 ml of sterile saline under nitrogen atmosphere. Three milliliters of saline from vial B should be injected into vial A (this reducing solution must be used within 1 h). It should be stirred with a vortex for complete dissolution of freeze-dried material (Cis International 1985b).

One and a half milliliters of the reducing solution A should be withdrawn and added to 2 ml of blood. After 5 min's incubation at room temperature and gentle agitation, the preparation is centrifuged and the plasma is removed. From vial B, 1–2 ml of saline should be added, the vial centrifuged, and then the supernatant withdrawn.

For labeling, 0.8–1.4 ml of ^{99m}Tc-Na-pertechnetate (74–740 MBq) (2–20 mCi) is to be added to the RBC and incubated at room temperature for 5 min. The vial contents should be mixed well and then centrifuged to remove the supernatant. Measurements of both vials are then taken, and the labeling yield calculated. Generally, the labeling yield is approximately 97%.

Labeled RBC may be resuspended with 0.5–1 ml of saline from vial B or with patient's plasma. The preparation is ready for intravenous injection of ^{99m}Tc-RBC.

After heat alteration at 49.5 °C for 15 min, in vitro labeled RBC may be used for spleen scintigraphy. For this procedure, ^{99m}Tc-RBC should not be resuspended in plasma.

Advantages of in vitro labeling:

- High specific activity of ^{99m}Tc-RBC
- Controlled unbound ^{99m}Tc activity
- Bolus injection
- Heat alteration for spleen scintigraphy

For different kit applications, the manufacturer's instructions should be followed.

Description of the Kit

Kits containing tin-pyrophosphate as a sterile, nonpyrogenic formulation are either reconstituted with sterile saline or with sterile sodium ^{99m}Tc-pertechnetate solution. ^{99m}Tc-PYP has been used for imaging myocardial infarction; the cold kits serve as stannous agent for in vivo labeling of RBC. No bacteriostatic preservative is present in kits.

Stannous pyrophosphate and ^{99m}Tc-(Sn)-pyrophosphate are sterile, pyrogen-free, clear, colorless solutions suitable for intravenous injection.

Two stable complexes of technetium pyrophosphate have been identified at carrier levels by polarography, namely Tc(III) and Tc(IV) (Russell and Cash 1979). At pH below 6.0, ^{99m}Tc-(Sn)-pyrophosphate is described as a stable complex with Tc(IV).

The active ingredient is sodium pyrophosphate ($Na_4P_2O_7 \cdot 10\ H_2O$). For reduction of ^{99m}Tc-pertechnetate to lower oxidation states, tin(II) chloride or tin(II) fluoride is used. An optimal ratio of reducing agent/pyrophosphate must be maintained to prevent ^{99m}Tc-Sn-colloid formation (Srivastava et al. 1977). Technical problems with kit production have been reported (Kowalsky and Dalton 1981).

Clinical Applications

^{99m}Tc(tin)-pyrophosphate has been introduced for skeletal imaging (Subramanian et al. 1972) and was evaluated in patients with bone disease (Cohen et al. 1972; Fletcher et al. 1973; Rampon et al. 1974). The diagnostic value of bone scintigraphy has been evaluated retrospectively (Kuntz et al. 1975) and compared with compounds showing higher in vivo stability, namely diphosphonate derivatives (Henne et al. 1975; Rudd et al. 1977). The advantages of ^{99m}Tc-PYP for imaging damaged myocardial tissue have been demonstrated (Bonte et al. 1974; Buja et al. 1977; Cowley et al. 1977; Davis et al. 1976; Kelly et al. 1979; Willerson et al. 1975, 1977; Zaret et al. 1976). Application of the cold kit PYP as an agent for labeling RBC with technetium has gained general acceptance (Hegge et al. 1978; Pavel et al. 1977; Thrall et al. 1978).

^{99m}Tc-RBC for radionuclide angiography

- Regional imaging of blood pools (deep vein visualization)
- Electrocardiogram (ECG)-triggered cardiac radionuclide ventriculography (ejection fraction, wall motion)
- Detection of gastro intestinal hemorrhage, blood loss
- Determination of RBC mass or blood volume
- Spleen scintigraphy (heat-treated labeled RBC)

Time of Examination

Acute myocardial necrosis:	90–120 min after intravenous injection of ^{99m}Tc(tin)-pyrophosphate Patients are imaged 24 h up to 6 days following the acute episode.
Blood pool scintigraphy:	10 min after intravenous injection of sodium ^{99m}Tc-pertechnetate
Spleen scintigraphy:	60 min after intravenous injection of ^{99m}Tc-RBC (denatured)

Recommended Activities for Indications

Myocardial infarct imaging: 555–740 MBq (15–20 mCi) ^{99m}Tc(tin)-pyrophosphate: 0.5 mg/kg body weight (BW)
Blood pool imaging: 555–740 MBq (15–20 mCi) ^{99m}Tc-pertechnetate
Spleen scintigraphy: 37–75 MBq (1–2 mCi) ^{99m}Tc-RBC

Pediatric Dose. The amount of radioactivity for infants and children is based on BW, using the scaling factors given in Appendix 1, Table A1.2. The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

Patients should drink sufficient water and frequent bladder emptying should be encouraged, to reduce the radiation exposure to the bladder wall.

Pyrophosphate in aqueous solutions slightly hydrolyzes to monophosphates, causing the formation of free pertechnetate and hydrolized colloidal technetium.

Alteration of body distribution of ^{99m}Tc-pyrophosphate has been observed under various conditions (Crawford and Gumerman 1978; Hladik et al. 1982, 1987).

Early studies in experimental models suggested that hydroxyapatite deposition is not detectable until 12–24 h after infarction (Bonte et al. 1974).

Patients with cardiotoxic lesions resulting from an excess treatment with Adriamycin (doxorubicin) showed a diffuse accumulation of ^{99m}Tc-PYP in the heart, which otherwise is known only as localized uptake in focal lesions of myocardial infarction. Similar images have been observed in patients after defibrillation and reanimation treatment (Chacko et al. 1977).

Excess aluminum (in patients taking drugs for peptic ulcer, containing aluminum hydroxide) could interfere with the biodistribution of ^{99m}Tc-PYP; almost all the radioactivity was found in the liver and spleen, possibly due to formation of a colloidal ^{99m}Tc-species (Hladik et al. 1987).

A reduction in the labeling yield of RBC has been reported for heparin, tin-overload, aluminum, prazocin, methyldopa, hydralazine, digoxin, quinidine, β-adrenergic blockers, (e.g., propanolol), calcium channel blockers (e.g., verapamil), nitrates (e.g., nitroglycerin), doxorubicin, iodinated contrast agents, and Teflon tubing (catheter) (Hladik et al. 1987).

It is recommended to perform in vivo labeling of RBC prior to the administration of iodinated contrast media (Tatum et al. 1983).

Diminished cardiac activity and increased renal activity has been observed when a heparinized catheter was used for in vivo red cell labeling with pyrophosphate. This was interpreted as formation of a ^{99m}Tc-heparin complex that localizes avidly in the kidneys (Hegge et al. 1978). Injection of Sn-pyrophosphate and ^{99m}Tc-pertechnetate through a heparin lock should be avoided.

Legal Aspects. Quality requirements of ^{99m}Tc-PYP are stated in the official monographs of the *United States Pharmacopeia* (*USP*) and the *European Pharmacopeia* (*Ph. Eur.*):

Technetium ^{99m}Tc-tin pyrophosphate injection (Ph. Eur.) (Council of Europe 2005)
Technetium Tc-99m pyrophosphate injection (USP) (United States Pharmacopeial Convention 2005)

Quality Control

Radiochemical Purity. The *Ph. Eur.* requires thin-layer chromatography (TLC) on instant (I)TLC-silica gel (SG) fiberglass sheets for the identification of free ^{99m}Tc-Na-pertechnetate, using methyl ethyl ketone (MEK) as solvent. Unbound ^{99m}Tc-pertechnetate is measured at an R_f of 1.0. ^{99m}Tc-PYP and reduced, hydrolized ^{99m}Tc activity is identified at the origin.

Reduced, hydrolized ^{99m}Tc activity is determined separately using sodium acetate (13.6%) as solvent. Hydrolized ^{99m}Tc-oxide is measured at the origin, and ^{99m}Tc-PYP and ^{99m}Tc-pertechnetate move with the solvent front.

The sum of the measured radioactivity due to impurities obtained in two-solvent systems should not exceed 10% of the total radioactivity, according to the limits stated in the *Ph. Eur.* Analysis of various Tc-99m-labeled bone scanning agents has been reported (Krogsgaard 1976).

The radiochemical purity of ^{99m}Tc-(Sn)-pyrophosphate should not be less than 90%.

Conditions for thin-layer chromatography are shown below (Table 1 and 2) as are typical results of ^{99m}Tc-PYP analyses at different times after preparation.

Table 1. Thin-layer chromatography on silica gel plates using two solvent systems

	System 1	System II
Stationary phase	ITLC-SG (fiberglass)	ITLC-SG (fiberglass)
Solvent:	MEK	Sodium acetate 13.6%
Developing time:	10 min	10 min

Table 2. Relative migration of labeled components and quantification of impurities

System I	^{99m}Tc-reduced, hydrolized and ^{99m}Tc-PYP at the start Free ^{99m}Tc-pertechnetate at the solvent front	(A)
System II	Reduced, hydrolized ^{99m}Tc activity at the start ^{99m}Tc-pertechnetate and ^{99m}Tc-PYP at the solvent front	(B)
	^{99m}Tc-PYP (%) = 100–%(A+B)	

Results of analysis *(12 samples)*
Results were obtained using the analytical methods described in the *Ph. Eur.*

Labeling and stability	15 min (%)	6 h (%)
^{99m}Tc-PYP complex	96.8	93.7
^{99m}Tc-Na-pertechnetate	0.8	1.4
^{99m}Tc reduced, hydrolized	2.3	4.9

Pharmacokinetic Data

After intravenous injection in patients, ^{99m}Tc-(Sn)-pyrophosphate accumulates in regions of active osteogenesis, and also in injured myocardium, mainly in necrotic tissue (Buja et al. 1977). Uptake in infarcted myocardium in experimental model (dog) was reported as 4.4% injected dose (ID)/g, compared with 0.31% in normal myocardial tissue (Bevan et al. (1980).

Due to in vivo instability, ^{99m}Tc-PYP is no longer used for bone scintigraphy.

Uptake of ^{99m}Tc-PYP in acute myocardial infarction was detected with higher sensitivity than were the known ^{99m}Tc-diphosphonate complexes (Kelly et al. 1979). Using a rat model, the percentage of injected activity per gram of infarct was approximately 2.4 times higher than with ^{99m}Tc-MDP (Davis et al. 1976). Visualization of the infarct can be effected from 24 h–7 days after the onset of symptoms and with maximum sensitivity (96%) between 48 and 72 h (Kelly et al. 1979).

Pyrophosphate is subject to enzymatic hydrolysis by pyrophosphatase, which has been demonstrated in bone, the kidneys, and other tissues (Kornberg 1962; Russell et al. 1970). Several ^{99m}Tc-phosphate species are formed, showing a different biological distribution and causing poor image quality of the bone scintigram (Eckelman and Volkert 1982).

Two hours after the intravenous injection of ^{99m}Tc-PYP, 10–30% of the injected radioactivity is taken up by bone structures; approximately 10% remain in the vascular space, declining to within 2–3% 24 h postinjection. (Krishnamurthy et al. 1975; Subramanian et al. 1975). The average urinary excretion is 60% of the administered dose in 24 h.

The slow clearance of ^{99m}Tc-PYP is due to high binding to plasma proteins, 42% at 2 h postinjection (Saha and Boyd 1978).

Stannous pyrophosphate has an affinity for RBC. It has been reported that stannous ion binds to the β-chain of hemoglobin. When ^{99m}Tc-Na-pertechnetate is injected 20–30 min after the intravenous injection of the reducing solution, high labeling of the preloaded erythrocytes is obtained. Normally, ^{99m}Tc-Na-pertechnetate will diffuse freely in and out of the RBC; however, in the presence of stannous ion, it is reduced intracellularly and reacts with hemoglobin. Intravenous injection of 10–20 μg/kg BW of stannous pyrophosphate results in efficient labeling of the blood pool. At 10 min postinjection, 77 ± 15% of the injected radioactivity remains in the circulation, and at 100 min postinjection, 71 ± 14%. The radioactivity is decreasing slowly, approximately 6% in 2 h (Mallinckrodt Medical 1993).

The effect of tin is long lasting. Preloaded RBC show labeling with ^{99m}Tc-Na-pertechnetate for up to 8 days after the injection of stannous pyrophosphate (Ancri et al. 1977). With doses of 0.02 mg of Sn(II)/kg BW, no negative effects were observed (Mallinckrodt Medical 1993). In vivo RBC labeling should not be repeated within 3 months.

Radiation Dose

^{99m}Tc-PYP is used for myocardial scintigraphy after intravenous injection. ^{99m}Tc-labeled erythrocytes are used for blood pool imaging, heat-denatured RBC for imaging the spleen. The most exposed organs are bone, bone marrow, bladder, kidneys, heart, and spleen. The radiation absorbed dose values previously obtained using the medical internal radiation dose (MIRD) scheme (Weber et al. 1989) correlate well with the effective (whole body) dose equivalent given in the ICRP 53 (International Commission on Radiological Protection (1987 a). A voiding period of 2 h was assumed.

The effective (whole body) dose equivalent for ^{99m}Tc(Sn)-pyrophosphate is 0.008 mSv/MBq (ICRP 53, International Commission on Radiological Protection 1987 a). The effective dose in adults (70 kg) resulting from 740 MBq (20 mCi) of intravenously injected ^{99m}Tc(Sn)-pyrophosphate for cardiac imaging is approximately 5.9 mSv.

The effective (whole body) dose equivalent for ^{99m}Tc-labeled erythrocytes is 0.0085 mSv/MBq (International Commission on Radiological Protection 1987 b). The effective dose in adults (70 kg) resulting from 740 MBq (20 mCi) of intravenously injected ^{99m}Tc-pertechnetate for angioscintigraphy corresponds to approximately 6.3 mSv. The absorbed radiation dose to the heart resulting from ^{99m}Tc-labeled RBC is 17.0 mGy and to the kidneys, 7.4 mGy. Approximately 15% of the activity is excreted in the urine during the first day (Porter et al. 1983). Activity is assumed to be distributed in the blood, being removed with a half-time of 60 h by renal excretion.

The effective dose value for the intravenous injection of denatured RBC is 0.019 mSv/MBq (International Commission on Radiological Protection 1991). The effective dose in adults (70 kg) resulting from 75 MBq (2 mCi) of ^{99m}Tc-RBC (denatured) for spleen scintigraphy is approximately 1.4 mSv. The dose to the spleen after intravenous injection of 75 MBq (2 mCi) of denatured ^{99m}Tc-RBC is 42 mGy.

Storage and Stability

Storage. The lyophilized kit is to be stored in the refrigerator at 2–8 °C.

Stability. ^{99m}Tc-pyrophosphate injection solution is stable for 4 h at room temperature.

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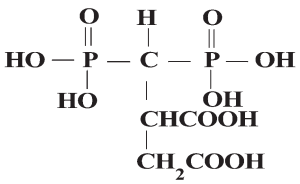
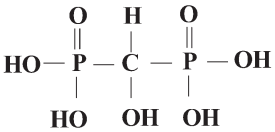
12.7.2 ^{99m}Tc-Diphosphonates

I. Zolle and S. Kladnik

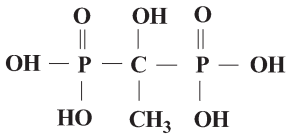
^{99m}Tc-DPD (Dicarboxypropane diphosphonate)

Chemical name	**Chemical structure**
3,3-Diphosphono-1,2-propane-dicarboxy acid, tetrasodium salt (DPD) 1,2-dicarboxypropane diphosphonate	 Dicarboxypropane diphosphonic acid
Kit components	**Commercial products**
DPD 13.0 mg Tin(II)-chloride dihydrate 0.23 mg *N*-(4-aminobenzoyl)-L-glutamic acid, monosodium salt 1.0 mg	Teceos CIS bio international

^{99m}Tc-HDP (Hydroxymethylene diphosphonate)

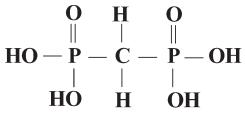
Chemical name	**Chemical structure**
Hydroxymethylene diphosphonic acid, disodium salt (HMDP, HDP) Hydroxymethylene diphosphonate Oxidronate (*USP*) Technetium Tc 99m oxidronate injection (*USP*)	 Hydroxymethylene diphosphonic acid
Kit components	**Commercial products**
Oxidronate 3.0 mg Tin(II)-chloride dihydrate 0.24–0.45 mg Gentisic acid resp. 0.84 mg Ascorbic acid 0.75 mg Sodium chloride 10 mg	TechneScan HDP Mallinckrodt Medical OsteoCis (TCK-21) CIS bio international

^{99m}Tc-HEDSPA (Hydroxyethylidene diphosphonate)

Chemical name	**Chemical structure**
1-Hydroxyethylidene-1,1-diphosphonic acid, disodium salt (HEDSPA) Ethylidene-1-hydroxy-1,1-disodium phosphonate (EHDP) Hydroxyethylidene diphosphonate Etidronate (*USP*) Technetium Tc-99m etidronate injection (*USP*)	 Hydroxethylidene diphosphonic acid
Kit components	**Commercial products**
Etidronate disodium salt 0.75 mg Stannous tartrate 0.18 mg Hydrochloric acid q.s. pH = 4	HEDSPA Union Carbide* Osteoscan Proctor and Gamble No longer commercially available

* HEDSPA Unit dose kit: Etidronate disodium tin kit (80 μg stannous ion)

^{99m}Tc-MDP (Methylene diphosphonate)

Chemical name	**Chemical structure**
Methylene diphosphonic acid, disodium salt (MDP) Methylene diphosphonate Medronate (*Ph. Eur.; USP*) Technetium Tc 99m medronate injection (*Ph. Eur.; USP*)	 Methylene diphosphonic acid
Kit components	**Commercial products**
MedroCis: Methylene diphosphonate 10.0 mg Tin(II)-chloride dihydrate 1.0 mg Ascorbic acid 1.8 mg	MedroCis (TCK-14) CIS bio international Lenoscint Bristol-Myers Squibb Amerscan MDP GE Healthcare Amerscan Stannous Agent GE Healthcare

Preparation

Kits for the preparation of diphosphonate complexes contain the lyophilized ingredients in a multidose vial. Labeling with ^{99m}Tc-pertechnetate is performed by adding 2–10 ml of sterile ^{99m}Tc eluate by aseptic techniques. When calculating the amount of radioactivity to be added, the labeling efficiency, number of patients, time of injection and radioactive decay must be taken into account. Activities corresponding to 6.6–18.5 GBq

(180–500 mCi) are recommended by the manufacturer. The vial is agitated to dissolve the lyophilized material. After 5–20 min at room temperature, the preparation is ready for injection.

^{99m}Tc-diphosphonate injection solutions are clear and free of particulate matter; the pH value is 3.5–7.5 (3,3-diphosphono-1,2-propane-dicarboxy acid [DPD] 6.5–7.5).

A special formulation of medronate (Amerscan Stannous Agent) is used for in vivo loading of red blood cells with stannous ion, preparatory to labeling with sodium ^{99m}Tc-pertechnetate.

Amerscan Stannous Agent contains a freeze-dried, sterile formulation of 6.8 mg sodium medronate, corresponding to 5.4 mg medronic acid as disodium salt, and 4.0 mg stannous fluoride. Upon reconstitution with 6 ml saline, the recommended amount for adults (70 kg) by intravenous injection is 2 ml, corresponding to 1.8 mg medronic acid and 1.3 mg stannous fluoride.

Description of the Kit

Each vial contains the freeze-dried, sterile components under nitrogen atmosphere in a multidose vial. ^{99m}Tc-Na-pertechnetate used for labeling must be free from any oxidizing agent, using a solution of 0.9% sodium chloride (saline) as diluent. No bacteriostatic agents are present in kits. In order to prevent oxidative reactions, kits are purged with nitrogen gas, the amount of tin reducing agent might be increased, and/or antioxidants might be added (Saha 1998).

The active ingredients are diphosphonic acids – dicarboxypropane diphosphonic acid (DPD), hydroxymethylene diphosphonic acid (HDP or HMDP), hydroxyethylidene diphosphonic acid (EHDP), methylene diphosphonic acid (MDP) – or their sodium salts. As stabilizers are used *N*-(4-aminobenzoyl)-L-glutaminic acid, gentisic acid (2,5-dihydroxybenzoic acid), ascorbic acid, or carbamide. For reduction of ^{99m}Tc-pertechnetate to lower oxidation states, tin(II) chloride and tin(II) fluoride are employed.

Commercial kit formulations contain various quantities of active ingredients; some formulations use gentisic acid as a stabilizer of stannous ion (Tofe and Francis 1976; Tofe et al. 1980). Reduced, hydrolized ^{99m}Tc activity has been a common impurity. Colloid formation is also caused by aluminum in the ^{99m}Tc eluate (Ponto et al. 1987). An optimal ratio of reducing agent/diphosphonate must be maintained to prevent ^{99m}Tc-Sn-colloid formation (Srivastava et al. 1977). Optimal labeling conditions require carrier-free ^{99m}Tc eluate obtained from a generator that is eluted daily (Van Duzee and Bugaj 1981).

Generally, diphopsphonate kits are used for several patient doses, keeping the injected mass of the ^{99m}Tc-diphosphonate complex below 1.0 mg (Castronovo and Callahan 1972). Thus, also in cases, when a single dose is prepared, the standard labeling conditions are applied.

Pretreatment of red blood cells (RBC) with MDP for in vivo labeling with ^{99m}Tc-Na-pertechnetate. Stannous medronate freeze-dried formulation is reconstituted with sterile, nonpyrogenic saline. A volume of 6 ml is added (product information for Amerscan Stannous Agent). The vial is agitated to dissolve the lyophilized material. After 5 min at room temperature, the preparation is ready for injection (2 ml for one patient). The injection solution should be clear and free of particulate matter with a pH value between

5.5 and 7.5. Thirty minutes later, ^{99m}Tc-Na-pertechnetate (555–740 MBq) (15–20 mCi) is injected, also intravenously, for in vivo labeling of pretreated erythrocytes.

Factors to be considered for in vivo labeling have been presented (Zimmer et al. 1979).

For different kit applications, the manufacturer's instructions should be followed.

Historical background. HEDSPA was the first Tc-99m-diphosphonate complex introduced to clinical practice by Castronovo and Callahan (1972). The use of EHDP was proposed by several groups (Pendergrass et al. 1973; Tofe and Francis 1972; Subramanian et al. 1972; Yano et al. 1973). Large numbers of patients were studied and compared with polyphosphate and pyrophosphate (Ackerhalt et al. 1974; Dunson et al. 1973; Genant et al. 1974; Hughes et al. 1975; Krishnamurthy et al. 1974); however, not all reports were favorable.

MDP was introduced as a superior agent (Subramanian et al. 1975) and served as a reference for later developments (Russel and Cash 1979).

HMDP, or HDP, showed favorable characteristics in early studies (Bevan et al. 1980; Domstad et al. 1980; Francis et al. 1980; Fogelman et al. 1981); after critical evaluation it was concluded that there was no significant difference between HMDP and MDP.

DPD was developed at Höchst, Germany (Schwarz and Kloss 1981; Schwarz et al. 1991), and compared favorably with MDP (Buell et al. 1982; Godart et al. 1986; Hale et al. 1981; Pauwels et al. 1983; Schroth et al. 1984). DPD is another efficient phosphonate complex that has been widely used as Teceos. The merits of available diphosphonate bone scanning agents have been discussed by Fogelman 1982.

Clinical Applications

Skeletal scintigraphy with ^{99m}Tc-diphosphonate complexes.

- Diagnosis of bone disease, e.g., primary bone tumors, metastatic bone tumors
- Metabolic bone disease, osteomyelitis
- Localization of fractures (stress and hairline fractures, fractures of the small bones of the hands and feet)
- Evaluation of painful arthroplasties of the knee and hip
- Evaluation of bone pain in patients with negative x-ray

Radionuclide angiography with ^{99m}Tc-RBC

- Regional imaging of blood pools (deep vein visualization)
- Electrocardiogram (ECG)-triggered cardiac radionuclide ventriculography (ejection fraction, wall motion)

An overview on the clinical application of radionuclide imaging in skeletal disease provides methodological detail (McAfee 1987).

Amerscan Stannous Agent facilitates in vivo labeling of RBC; therefore, ^{99m}Tc-medronate is also used for blood pool scintigraphy (Gray et al. 1979).

Time of Examination

Bone imaging: 2 h after intravenous injection

Blood pool scintigraphy: 10 min after intravenous injection of sodium ^{99m}Tc-pertechnetate

Recommended Activities for Indications

Bone scintigraphy: 370–740 MBq (10–20 mCi)
Blood pool imaging: 555–740 MBq (15–20 mCi) (^{99m}Tc-pertechnetate)

Pediatric Dose. The amount of radioactivity for infants and children administered for bone scintigraphy is based on body weight, using the scaling factors given in Appendix 1, Table A1.2. The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

Patients should be encouraged to drink sufficient water and to empty the bladder before scintigraphy is started. Frequent bladder emptying is recommended to reduce the radiation exposure to the bladder wall.

When kits containing large amounts of stannous ion were used for bone imaging, ^{99m}Tc-labeling of RBC for up to 2 weeks after administration of ^{99m}Tc-PYP was observed (Ancri et al. 1977).

Alteration of body distribution of ^{99m}Tc-phosphonates has been observed under various conditions (Hladik et al. 1982, 1987).

Presence of aluminum in the generator eluate and treatment with aluminum-containing drugs may result in colloid formation, with liver and spleen uptake and trapping of particles in lung capillaries (Zimmer and Pavel 1978). Colloid formation is also observed at elevated pH above 8.5 (Chaudhuri 1976).

The influence of iron and iron-containing drugs on the retention of diphosphonates has been observed frequently (Hladik 1987). A localized muscular accumulation of iron-dextran at the injection site was reported (Byun et al. 1976).

After intravenous infusion of iron containing drugs or in cases of chronic iron overload in chronic diseases, a change in the bone-to-kidney ratio of diphosphonate complexes has been reported, namely, a decrease in bone uptake and an increase in accumulation in renal parenchyma (McRae et al. 1976). Dissociation of the ^{99m}Tc-diphosphonate complex and a conversion into the renaltropic gluconate was proved in the presence of ionic iron(II) and calcium. In vivo alteration of the complex was concluded from an increase in renal uptake even after infusion of dextrose.

Long-term treatment with glucocorticosteroids results in a decrease in bone uptake. This phenomenon may not be explained as interference between a pharmaceutical and a radiopharmaceutical but by the drug-induced osteoporosis (Conklin et al. 1983).

Another case report described the diffuse accumulation of ^{99m}Tc-MDP in the liver of a patient with methotrexate-induced hepatotoxicity. The authors postulated that inhibition of protein synthesis by methotrexate caused a disruption of the cell membranes, resulting in the inflow of calcium ions. The high affinity of MDP for calcium leads to diffuse uptake of tracer in the liver (Hladik et al. 1982).

Since the stability of ^{99m}Tc-diphosphonate complexes may be affected, ^{99m}Tc-diphosphonates should not be mixed with other drugs or components nor injected simultaneously.

Legal Aspects. Quality requirements of ^{99m}Tc-diphosphonates are stated in the official monographs of the *European Pharmacopeia* (*Ph. Eur.*) and the *United States Pharmacopeia* (*USP*):

^{99m}Tc-MDP Technetium Tc-99m medronate injection in *USP* 28 (United States Pharmacopeial Convention 2005)
Technetium ^{99m}Tc medronate injection in *Eur. Ph.* 5.0 (Council of Europe 2005)

^{99m}Tc-HDP Technetium Tc-99m oxidronate injection in *USP* 28

^{99m}Tc-EHDP Technetium Tc-99m etidronate injection in *USP* 28

Quality Control

Radiochemical Purity. ^{99m}Tc-medronate is included in the pharmacopoeias and may serve as an example for the analysis of other ^{99m}Tc-diphosphonates. The *Ph. Eur.* requires thin-layer chromatography (TLC) on instant (I)TLC-silica gel (SG) fiberglass sheets, using two separate solvent systems.

- System I: Separation of ^{99m}Tc-pertechnetate in organic solvent methyl ethyl ketone (MEK): ^{99m}Tc-diphosphonates and hydrolized ^{99m}Tc activity remain at the start; free ^{99m}Tc-pertechnetate is measured at the solvent front.
- System II: Separation of reduced, hydrolized ^{99m}Tc activity using sodium acetate (13.6%): ^{99m}Tc-diphosphonates and free ^{99m}Tc-pertechnetate move with the solvent front; colloidal activity is measured at the start.

Impurities. Free ^{99m}Tc-pertechnetate and colloidal activity are expressed as a percentage of the sum of the radioactivity measured in systems A and B, not exceeding 5%. ^{99m}Tc-pertechnetate does not exceed 2%.

The radiochemical purity of ^{99m}Tc-medronate is not less than 95% (*Ph. Eur.*).

Thin-layer chromatography

Analysis of various Tc-99m-labelled bone scanning agents has been reported (Krogsgaard 1976).

Conditions for thin-layer chromatography are shown below (Table 1 and 2), as are results of ^{99m}Tc-MDP analyses at different times after preparation. Three different solvents were evaluated in each TLC system.

Table 1. Thin-layer chromatography using silica gel and three solvents in each system

	System I	System II
Stationary phase:	ITLC-SG (fiberglass)	ITLC-SG (fiberglass)
Solvent: (a)	MEK	Sodium acetate 13.6%
(b)	Methanol–acetone, 1:1	Saline
(c)	Acetone	Saline
Developing time:	10 min	10 min

Table 2. Relative migration of labeled components and quantification of impurities

System I	^{99m}Tc-reduced, hydrolized and ^{99m}Tc-MDP at the start Free ^{99m}Tc-pertechnetate at the solvent front	(A)
System II	Reduced, hydrolized ^{99m}Tc activity at the start ^{99m}Tc-pertechnetate and ^{99m}Tc-MDP at the solvent front	(B)
	^{99m}Tc-MDP (%) = 100–%(A+B)	

Results of analysis *(12 samples)*
Shown are results of ^{99m}Tc-MDP analyses obtained with three different solvents, as described in Table 1. The consistent values of free ^{99m}Tc-pertechnetate and hydrolized ^{99m}Tc activity measured at different times after preparation (*a*), favor the use of MEK and sodium acetate (13.6%), as stated in the Ph. Eur. However, saline may be used instead.

Labeling and stability	15 min (%)			6 h (%)		
Solvent no.	a	b	c	a	b	c
^{99m}Tc-MDP complex	98.0	96.7	96.4	97.4	95.2	95.0
^{99m}Tc-Na-pertechnetate	1.4	2.8	3.2	1.9	4.3	4.5
^{99m}Tc-reduced, hydrolized	0.6	0.4	0.4	0.6	0.5	0.5

Biological Test (*Ph. Eur.*). The biodistribution of ^{99m}Tc-MDP should be determined in three rats, according to the procedure in the *Ph. Eur.* to demonstrate that not less than 2.5% of the radioactivity is found in the femur and not more than 1.0% is found in the liver. Calculate the radioactivity per unit mass in the femurs (A_1), muscle (A_2) and blood (A_3). The ratio of radioactivity per unit mass in the femur and muscle (A_1/A_2) is not less than 100, and in the femur and blood (A_1/A_3) is not less than 40.

Pharmacokinetic Data

Following intravenous injection, 45–50% of ^{99m}Tc-diphosphonates (MDP, HDP, DPD) accumulate in the skeleton, while most of the rest is excreted in the urine. Maximum bone accumulation occurs 1 h after injection and remains constant for 72 h. The determining factors for bone uptake of ^{99m}Tc-phosphonate complexes are an increased blood flow to the skeleton and reactive bone formation, causing avid extraction by the bone mineral matrix (Jones et al. 1976; Sahni et al. 1993). Symmetric areas of increased activity concentration are seen in the metaphyseal zones in the growing skeleton. Ankles, knees, elbows, wrists, shoulder joints, pelvic bones, and vertebrae show increased uptake in the normal anterior bone scintigram (Saha 1987).

The disappearance of ^{99m}Tc-phosphonate complexes from blood is affected by skeletal fixation and urinary excretion. Blood clearance proceeds with three half-times of elimination, a rapid phase ($T_{1/2} = 3.5$ min), an intermediate phase ($T_{1/2} = 27$ min), and a slow phase ($T_{1/2} = 144$ min). Transfer to the bone matrix corresponds to the intermediate phase. The total radioactivity in blood at 5 min, 2, 4, and 24 h after the intravenous injection was measured as 40, 10, 5.8, and 2.3%, respectively (Subramanian et al. 1975 a).

Besides an increase in the osteogenic activity, bone uptake of ^{99m}Tc-diphosphonates is closely related to chemical structure, as demonstrated by the skeletal uptake of

EHDP, MDP, and HDP, differing by the substituents at the methylene bridge. Whole-body retention (24 h) in volunteers showed values of 18.4 (EHDP), 30.3 (MDP), and 36.6% (HDP), indicating a greater uptake of oxidronate (HDP) (Fogelman et al. 1981). These results agree with the conformational requirements derived from structure–activity relationship studies performed with mono- and disubstituted diphosphonates (Wang et al 1980).

For normal renal function, elimination by glomerular filtration of the nonfixed complex is approximately 32% in 1 h, 47% in 2 h, and 60% in 6 h. The percentage of cumulative activity excreted in the urine during 24 h is 79.2% (EHDP) and 76.5% (MDP), indicating 75–80% 24-h urinary excretion of bone scanning agents (Subramanian et al. 1975 b). The relatively lower urinary excretion of HDP in dogs, namely, approximately 59%, is primarily resulting from high skeletal uptake, indicated by a femur-to-muscle ratio of 35 (Bevan et al. 1980).

Protein binding of MDP has been reported as 22% at 2 h; in the case of EHDP, approximately 30% 3 h after injection (Saha and Boyd 1979; Subramanian et al. 1975a).

^{99m}Tc-DPD shows lower binding to plasma protein and is excreted to a smaller extent in the urine (Schwarz et al. 1991). Whole-body retention at 24 h was 40.6% for DPD and 27.0% in the case of MDP (Buell et al. 1982). The bone-to-soft tissue ratio, which is important for skeletal scintigraphy, showed consistently higher values for ^{99m}Tc-DPD, namely, an increase of 11.4% in normal volunteers and 7.3% in patients 2 h postinjection (Buell et al. 1982).

The detection of bone metastases is based on increased regional uptake of ^{99m}Tc-diphosphonates; in a comparative study the diagnostic efficacy was determined for ^{99m}Tc-MDP, ^{99m}Tc-HDP, and ^{99m}Tc-DPD, demonstrating equal detection rates for the three agents (Pauwels et al. 1983).

Diphosphonates form stable Tc(IV) complexes. Contrary to inorganic phosphorus compounds, which are degraded in vivo, the organically bound diphosphonate complexes show high in vivo stability (Davis and Jones 1976).

Stannous medronate has an affinity for RBC. It has been shown that stannous ion binds to the β-chain of hemoglobin. When ^{99m}Tc-Na-pertechnetate is injected 20–30 min after the intravenous injection of the reducing solution, high labeling of the preloaded erythrocytes is obtained (Callahan et al. 1982). Approximately 15% of the injected activity is excreted in the urine during the first day (Porter et al. 1983).

Radiation Dose

^{99m}Tc-diphosphonate complexes are injected intravenously for bone scintigraphy. ^{99m}Tc-labeled erythrocytes are used for blood pool imaging. The most exposed organs are bone, bone marrow, bladder, kidneys, and the heart. The radiation absorbed dose values previously obtained using the medical internal radiation dose (MIRD) scheme (Weber et al. 1989) correlate well with the effective (whole body) dose equivalent given in the ICRP 53 (International Commission on Radiological Protection 1987 a). A voiding period of 3 h was assumed.

The effective (whole body) dose value for ^{99m}Tc-diphosphonates is 0.0057 mSv/MBq (International Commission on Radiological Protection 1998). The effective dose in adults (70 kg) resulting from 740 MBq (20 mCi) of intravenously injected ^{99m}Tc-diphosphonate complexes for bone scintigraphy is approximately 4.2 mSv.

The effective (whole body) dose value for ^{99m}Tc-labeled erythrocytes is 0.0066 mSv/MBq (International Commission on Radiological Protection 1991). The effective dose in adults (70 kg) resulting from 740 MBq (20 mCi) of intravenously injected ^{99m}Tc-pertechnetate for angioscintigraphy corresponds to approximately 4.9 mSv. The absorbed radiation dose to the heart resulting from ^{99m}Tc-labeled RBC is 17.0 mGy, and to the kidneys, 7.4 mGy. Approximately 15% of the activity is excreted in the urine during the first day (Porter et al. 1983). Activity is assumed to be distributed in the blood, being removed with a half-time of 60 h, by renal excretion (International Commission on Radiological Protection 1987b).

Storage and Stability

Storage. The lyophilized kits are to be stored in the refrigerator at 2–8 °C.

Stability. ^{99m}Tc–diphosphonate injection solution is stable for 6 h at room temperature.

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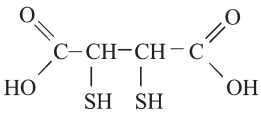
12.8 ^{99m}Tc-Labeled Renal Imaging Agents

12.8.1 ^{99m}Tc-DMSA (Dimercaptosuccinic Acid)

J. Környei and I. Zolle

Chemical name	**Chemical structure**
Dimercaptosuccinic acid (DMSA) Succimer (*Ph. Eur.; USP*) Technetium Tc 99m succimer injection (*Ph. Eur.; USP*) ^{99m}Tc(III)-dimercaptosuccinate (DMS) ^{99m}Tc(V)-DMSA	 Dimercaptosuccinic acid

Kit components		**Listed trade names**	
Amerscan DMSA:		Amerscan DMSA	GE Healthcare
Dimercaptosuccinic acid	1 mg	RenoCis (TCK-12)	CIS Bio
Tin(II)-chloride dihydrate	0.42 mg	TechneScan DMSA	Mallinckrodt/Tyco
Inositol	50.0 mg	Nephroscint	Bristol-Myers Squibb
Ascorbic acid	0.7 mg		
Sodium chloride	2.9 mg	DMSA	Rotop

Preparation

The kit contains the lyophilized ingredients in a multidose vial. Labeling is performed by adding 1–6 ml of ^{99m}Tc eluate under aseptic conditions. Sterile ^{99m}Tc-sodium pertechnetate (370–925 MBq, maximum 3.7 GBq) (10–25 mCi) is injected into the vial, and then mixed well to dissolve the lyophilisate. The reaction is allowed to proceed at room temperature for 15 min.

^{99m}Tc-dimercaptosuccinic acid (DMSA) complex is a sterile, pyrogen-free, clear colorless solution suitable for intravenous injection. The pH should be between 2.3 and 3.5 (*Ph. Eur.*).

Description of the Kit

The kit contains an isomeric mixture of DMSA as the mesoisomer (>90%) and the D,L-isomers (<10%). Upon addition of sterile sodium ^{99m}Tc-pertechnetate injection to the sterile, nonpyrogenic formulation, the renal ^{99m}Tc(III)-DMSA complex is formed.

The lyophilized formulation is under nitrogen atmosphere to avoid oxidative processes; the introduction of air into the vial must be avoided. A breather needle should not be used. It is recommended to use a separate kit preparation for each patient. If

multiple doses are obtained from the same vial, care should be taken to avoid introducing air into the reaction vial, and the doses should be drawn as close together as possible and administered immediately (Taylor et al. 1980).

The antioxidant inositol is used for stabilization of the ^{99m}Tc(III)-DMSA complex.

^{99m}Tc eluate used for labeling should be obtained from a generator that is eluted daily (Van Duzee and Bugaj 1981).

Initially, two complexes are formed under acidic conditions (pH 2.5), which yield the ^{99m}Tc(III)-DMSA complex at the end of the reaction time (15 min). An excess amount of stannous ion as well as a high molar ratio of Sn(II) to Sn(IV) have been postulated to yield Tc(III)-dimercaptosuccinate (DMS) (Ikeda et al. 1976).

At least four ^{99m}Tc-DMSA complexes have been identified. It has been shown that pH, stannous ion concentration, and the concentration and purity of ^{99m}Tc-pertechnetate added for on-site preparation affect the formation of the different complexes (Ikeda et al. 1977 a, b).

The formation of pentavalent ^{99m}Tc-DMSA complex occurs at elevated pH; therefore, 0.2 ml of a sterile solution of 3.5% sodium bicarbonate is added to the lyophilized DMSA kit before adding ^{99m}Tc-sodium pertechnetate (Ramamoorthy et al. 1987). The optimal pH for ^{99m}Tc(V)-DMSA formation is pH 7.5–8.0; the resulting concentration of stannous ion produced a complex with high stability (6 h). It was observed that addition of ^{99m}Tc-pertechnetate should follow immediately after mixing the powder with the alkaline sodium bicarbonate solution (Johannsen et al. 1979; Ramamoorthy et al. 1987; Westera et al. 1985).

The negatively charged Tc(V)-oxo complex with DMSA consists of a TcO core having four sulfur atoms of the bidentate DMSA ligands arranged in a plane. The chemical formula is $[TcO\ (DMSA)_2]^{-1}$ (Saha 1997).

Clinical Applications

Renal scintigraphy:	Static imaging (planar or tomographic) of the functional renal cortex Morphological studies of the renal cortex indicating space-occupying lesions and areas of reduced function kidney localization, delineating size and shape of the kidneys as well as defects
Scintigraphy of medullary carcinoma of the thyroid (MCT):	Detection of metastatic lesions

For static imaging of the kidneys, the radiotracer is retained in the renal parenchyma by tubular fixation. The scintigram shows solely functional parenchyma. Necrotic tissue and inflammatory processes are not imaged; a tumor, cyst or abscess appears as cold area.

Pentavalent ^{99m}Tc(V)-DMSA has been used in the diagnosis of MCT (Clarke et al. 1988; Ohta et al. 1984).

Time of Examination

Renal scintigraphy:	1–3 h after intravenous injection 6 h postinjection in the case of severely impaired kidney function
Scintigraphy of MCT:	2–4 h after intravenous injection

Recommended Activities for Indications

Renal scintigraphy: 37–120 MBq (1–3.2 mCi) of ^{99m}Tc(III)-DMSA complex (0.3–1 mg DMSA)

Scintigraphy of MCT: 370 MBq (10 mCi) of ^{99m}Tc(V)-DMSA complex

The manufacturer's instructions should be followed.

Pediatric Dose. The amount of radioactivity for infants and children is based on body weight (static imaging). Recommendations by the Pediatric Task Group of the European Association of Nuclear Medicine (EANM), based on body weight should be followed (see Appendix 1, Table A1.2).

Additional Information

Because of the slow transfer of activity from blood to kidney, imaging should be delayed for 3 h after injection.

Reduced cortical accumulation is observed in the presence of ammonium chloride, sodium bicarbonate, and mannitol based on experimental data (Yee et al. 1981).

Medication with angiotensin-converting enzyme (ACE)-inhibitors (captopril) may cause reduced uptake in the affected kidney (Hovinga et al. 1989; Kopecky et al. 1990).

A marked increase in hepatic activity may result from poor labeling conditions (Ikeda et al. 1976; Taylor et al. 1980).

The patient should be adequately hydrated before ^{99m}Tc-DMSA scintigraphy.

Quality Control

Radiochemical Purity. ^{99m}Tc-DMSA complex is described in the *Ph. Eur.* (Council of Europe 2005). Thin-layer chromatography (TLC) on silica gel (SG) fiberglass sheets in two different solvents is recommended for the detection of free ^{99m}Tc-pertechnetate and reduced, hydrolized ^{99m}Tc activity. Free ^{99m}Tc-pertechnetate is measured in methyl ethyl ketone (MEK) at the solvent front, reduced, hydrolized ^{99m}Tc activity is identified in saline at the start. The radiochemical purity of ^{99m}Tc-DMSA should not be less than 95%. The amount of free ^{99m}Tc-pertechnetate should not exceed 2% of the measured radioactivity (*Ph. Eur.*).

Thin-layer chromatography (*Ph. Eur.*)		
Stationary phase:	Gelman ITLC-SG (fiberglass), 2×9.5 cm	
Solvent:	Methyl ethyl ketone (MEK)	
Developing time:	10 min	
R_f values:	^{99m}Tc-DMSA complex:	0.0–0.1 (>95%)
	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-pertechnetate:	0.9–1.0 (<2%)

Reduced, hydrolized ^{99m}Tc activity is identified in saline at the origin. ^{99m}Tc-pertechnetate should not exceed 2% of the measured radioactivity. The sum of measured impurities (F+H) in both solvents must not exceed 5% of the total radioactivity.

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter), and the regional radioactivities are expressed as a percentage of the total recovered counts:

$$^{99m}\text{Tc-DMSA}\ (\%) = 100 - (F + H)$$

where F (%) = free ^{99m}Tc-pertechnetate, and H (%) = hydrolized ^{99m}Tc activity.

Results of analysis *(12 samples)*
Results were obtained by thin-layer chromatography in two solvent systems, at different times after labeling.

Labeling and stability	15 min (%)	6 h (%)
^{99m}Tc-DMSA complex	98.3 ± 0.13	97.8 ± 0.28
^{99m}Tc-Na-pertechnetate	0.8±0.22	0.9±0.21
^{99m}Tc-reduced, hydrolized	1.0±0.04	1.0±0.12

Bioassay of ^{99m}Tc-DMSA Complex. To assure high renal uptake, the *Ph. Eur.* recommends a physiological test in rats. The required organ uptake values at 1 h after intravenous injection (at least two of three rats) are more than 40% of the applied radioactivity is measured in the kidneys, less than 10% in the liver, < 2% in the stomach, and < 5% in the lung.

Pharmacokinetic Data

After intravenous injection, the ^{99m}Tc(III)-DMSA complex is taken up in the renal parenchyma (24% at 1 h), showing high cortical affinity (Lin et al. 1974). Uptake is related to renal cortical perfusion; the plasma clearance half-time in patients with normal kidney function is 56 min (Enlander et al. 1974).

In patients, both kidneys are visualized 1 h after intravenous injection, with significant radioactivity in the bladder and negligible amounts in the liver, stomach, thyroid gland, or other organs. Most of the circulating ^{99m}Tc-DMSA is loosely bound to plasma proteins (Arnold et al. 1975). At 1 h after injection, approximately 25% of the injected radioactivity is measured in the proximal tubules, 30% in plasma, and 10% in the urine (Arnold et al. 1975; Bingham and Maisey 1978; Handmaker et al. 1975; Lin et al. 1974). Renal extraction is 4–5% per renal passage; the maximum accumulation in the renal cortex (48.3 ± 3.0%) is reached 3 h after injection, 20% remain in the plasma, less than 10% in liver and muscle, and a urinary excretion of 11.4%. ^{99m}Tc-DMSA is exclusively excreted in the urine as the unchanged molecule; in the presence of renal tubular acidosis, urinary excretion is increased (de Lange et al. 1989). Urinary excretion in 24 h amounts to approximately 30% (Arnold et al. 1975).

Renal accumulation is unaffected by probenecid and *para*-aminohippuric acid (PAH) (Lee and Blaufox 1985). Uptake is decreased by ACE inhibitors in the presence of renal artery stenosis (Hovinga et al. 1989; Kopecky et al. 1990).

In renal failure, ^{99m}Tc-DMSA activity accumulates in the liver, the gallbladder, and the gut.

Pentavalent ^{99m}Tc(V)-DMSA differs structurally and has been evaluated for imaging medullary carcinoma of the thyroid (Ohta et al. 1984; Ramamoorthy et al. 1987). The sensitivity of lesion detection is 95%; no false positive uptake of ^{99m}Tc(V)-DMSA was seen in nine patients with a histologic diagnosis of medullary carcinoma. Accumulation of ^{99m}Tc(V)-DMSA was seen in both bone and soft tissue metastases. In comparison, ^{99m}Tc-MDP detected all known metastases in bone, but none in soft tissue (Clarke et al. 1988).

Radiation Dose

The most exposed organs are the kidneys, bladder wall, adrenals, liver, and spleen. The effective (whole body) dose equivalent is 0.016 mSv/MBq. The effective dose in adults (70 kg) resulting from an intravenous injection of 70 MBq (1.9 mCi) of ^{99m}Tc(III)-DMSA complex for renal scintigraphy is 1.12 mSv. The dose to the kidneys (renal cortex) after intravenous injection of 70 MBq (1.9 mCi) of ^{99m}Tc(III)-DMSA complex is 11.9 mGy.

The effective dose in adults (70 kg) resulting from an intravenous injection of 370 MBq (10 mCi) of ^{99m}Tc(V)-DMSA complex for scintigraphy of medullary carcinoma is 3.0 mSv (Clarke et al. 1988).

Storage and Stability

Storage. The lyophilized kit is stored at 2–8 °C in the refrigerator, protected from light.

Stability. The ^{99m}Tc(III)-DMSA injection solution is stable for 4 h at room temperature.

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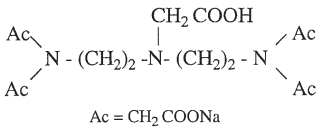
12.8.2 ^{99m}Tc-DPTA (Diethylenetriaminepentaacetate)

J. Környei and I. Zolle

Chemical name	**Chemical structure**
Diethylenetriaminepentaacetate (DTPA) as Calcium trisodium salt Pentetate (*Ph. Eur.; USP*) Technetium Tc 99m pentetate injection (*Ph. Eur.; USP*) ^{99m}Tc-DTPA complex	 Ac = CH_2COONa Pentetate
Kit components	**Commercial products**
Amerscan Pentetate II: Calcium trisodium diethylene-triaminepentaacetate 20.6 mg Tin(II)-chloride dihydrate 0.25 mg Sodium *para*-amino-benzoate 2 mg	Amerscan Pentetate II — GE Healthcare TechneScan DTPA — Mallinckrodt/Tyco TechneScan DTPA/Aerosol PentaCis (TCK-6) — CIS Bio DTPAScint — Bristol-Myers Squibb DTPA — Rotop

Preparation

The kit contains the lyophilized ingredients in a multidose vial. Labeling is performed by adding 2–10 ml of ^{99m}Tc eluate containing up to 11.1 GBq (300 mCi) under aseptic conditions. After sterile sodium ^{99m}Tc-pertechnetate has been added to the vial, the powder is dissolved by inverting the vial. The reaction is allowed to proceed at room temperature for 10–30 min. Kit composition varies between manufacturers; therefore, instructions should be followed.

^{99m}Tc-diethylenetriaminepentaacetate (DTPA) complex is a sterile, pyrogen-free, clear, colorless solution suitable for intravenous injection. The pH should be between 4.0 and 7.5 (*European Pharmacopeia* [*Ph. Eur.*]).

Description of the Kit

The ^{99m}Tc-DTPA complex for intravenous injection is used for renal studies (Atkins et al. 1971; Klopper et al. 1972). DTPA forms a negatively charged complex with reduced ^{99m}Tc-technetium in neutral or weakly acidic solutions (Russel et al. 1980). The exact oxidation state is not known, although several valency states have been suggested (III–V). Quality control is essential when ^{99m}Tc-DTPA is used for the measurement of glomerular filtration rate (GFR), since commercial kits contain reduced, hydrolized ^{99m}Tc

activity as an impurity (Carlsen et al. 1988). Some formulations use gentisic acid as a stabilizer (Tofe et al. 1980).

The ^{99m}Tc-DTPA complex may also be used as an aerosol; however, the kit composition suitable for nebulization differs from the renal agent. TechneScan DTPA/Aerosol contains 1.25 mg of DTPA and 0.12 mg tin(II)-chloride as dihydrate. Gentisic acid (0.25 mg) is used for stabilization. Labeling is performed by injecting less than 0.1 ml of ^{99m}Tc eluate, corresponding to at least 550 MBq (15 mCi) of radioactivity into the TechneScan DTPA/Aerosol vial, and then water for injection is added to obtain 1 ml of solution. After 15 min at room temperature, 0.5 ml of ethanol (98%) is added to the labeled product. The labeling yield is >95%. The kit TechneScan DTPA/Aerosol must not be used for intravenous injection.

^{99m}Tc-DTPA/Aerosol is a clear or slightly opalescent, aqueous solution, and the pH is 4.0–5.0.

Fractionated elution of the generator is required to obtain a high activity concentration of ^{99m}Tc eluate for labeling. ^{99m}Tc eluate used for labeling should be obtained from a generator that is eluted daily (Van Duzee and Bugaj 1981). Not more than 0.5 ml (1,110 MBq; 30 mCi) of the labeled product should be used for nebulization (to deliver 150 MBq (4 mCi) to the lung).

For inhalation of the ^{99m}Tc-DTPA complex, the vial is transferred to an aerosol generator. Ultrasonic nebulization of aqueous ^{99m}Tc-DTPA complex produces particles with an aerodynamic diameter of 0.5 μm (Wagner 1995).

Clinical Applications

- Renal studies providing both anatomical and functional information
- Determination of the GFR
- Cerebral scintigraphy based on leaks in the blood–brain barrier (BBB)
- Localization of inflammatory bowel disease
- Inhalation scintigraphy to measure regional lung ventilation

Measurement of separate kidney function is particularly indicated for the management of unilateral and bilateral uropathy, staging of disease, assessment of unilateral compensatory hypertrophy, and in cases where x-ray examinations (intravenous pyelography, etc.) are contraindicated (Hilson et al. 1976; Nielsen et al. 1977; Russel 1985; Wassner 1981).

^{99m}Tc-DTPA has been used for conventional cerebral scintigraphic studies for the detection of vascular and neoplastic brain lesions (Hauser et al. 1970).

^{99m}Tc-DTPA accumulates in inflammatory lesions of the gastrointestinal tract and has been used for the detection of bleeding sites. An increase in tracer activity is seen almost immediately after bolus injection in the affected segments of bowel and persists for hours (Kadir and Strauss 1979).

Inhalation scintigraphy using ^{99m}Tc-DTPA aerosol is performed for the assessment of pulmonary ventilation in patients with chronic obstructive lung disease (Coates and O'Brodovich 1986; Santolicandro and Giuntini 1979), and for the differential diagnosis of acute pulmonary embolism in combination with lung perfusion scintigraphy (Taplin and Chopra 1978; Wagner 1995). When inhaled, ^{99m}Tc-DTPA aerosol particles serve as an indicator of regional ventilation. Moist aerosols show a lung penetration between 1

and 10% of the nebulized activity. Labeled aerosols are also used to measure mucociliary clearance (Agnew 1991; Wanner 1977).

Time of Examination

Renal dynamic studies:	Collection of frames should start immediately after the intravenous injection. The total time for one examination is 30–60 min.
Brain scintigraphy:	1–3 h after intravenous injection
Abdominal images:	Images are obtained at 5, 10, 15, and 25 min; in some patients, delayed images are obtained at intervals from 45 min–4 h after the ^{99m}Tc-DTPA bolus
Inhalation scintigraphy:	This should start immediately after inhalation of the aerosol

Recommended Activities for Indications

Renal studies:	111–185 MBq (3–5 mCi) (7–20 mg DTPA)
Brain scintigraphy:	300–500 MBq (8.1–13.5 mCi) (7–20 mg DTPA)
Abdominal scintigraphy:	185–370 MBq (5–10 mCi)
Inhalation scintigraphy:	150 MBq (4 mCi) inhaled radioactivity (nebulized activity 1,110 MBq resp., 30 mCi). Breathing time 3–5 min, with an oxygen supply of 7–10 l/min

The manufacturer's instructions should be followed.

Pediatric Dose. The amount of radioactivity for infants and children is based on either body weight (static imaging) or body surface area (dynamic examinations). Recommendations by the Pediatric Task Group of the European Association of Nuclear Medicine (EANM) based on body weight should be followed (see Appendix 1, Table A2.2).

Additional Information

Free ^{99m}Tc-pertechnetate adds to increased background activity. Reduced, hydrolized ^{99m}Tc activity is bound to plasma proteins in circulating blood, interfering with the measurement of plasma clearance (Rehling 1988). Therefore, quality control of ^{99m}Tc-DTPA chelate should be performed before administration to patients. When ^{99m}Tc-DTPA is used to measure GFR, it is necessary to remove the protein-bound impurity by passing the plasma through an ultrafilter before the measurement of radioactivity (Rowell et al. 1986).

^{99m}Tc-DTPA is specific for the diagnosis of renal artery stenosis (Gruenewald and Collins 1983). Angiotensin-converting enzymes (ACE) inhibitors such as captopril reduce filtration pressure and thus cause a fall in GFR, as manifested by decreased renal uptake of ^{99m}Tc-DTPA. In patients who do not have renovascular hypertension, the GFR of each kidney is unchanged.

Furosemide (Lasix) will enhance the detection of a dilated collecting system by rapid washout of the radiotracer from the pelvis and ureter, while in the case of urinary tract obstruction, no change of pelvic retention and in the shape of the time–activity curve is seen (O'Reilly 1992).

The patient should be adequately hydrated before ^{99m}Tc-DTPA scintigraphy.

^{99m}Tc-DTPA has been used for studies of gastroesophageal reflux and gastric emptying. In this case, scintigraphy is performed after oral administration of ^{99m}Tc-DTPA (10–20 MBq, 0.3–0.6 mCi) in a suitable liquid (300 ml), according to local practice. Sequential scintigraphy and static imaging may be performed. The patient's stomach is imaged at 5 and 10 min after drinking the liquid, and then every 5–15 min until the stomach activity has reached half the original value. The gastric emptying half-time is determined from a plot of percent activity versus time. Normal values are 10–15 min; delayed emptying is observed with gastric ulcers, pyloric stenosis, vagotomy, and in the case of malignancy (Chadhuri 1974).

Quality Control

Radiochemical Purity. ^{99m}Tc-DTPA complex is described in the *Ph. Eur.* (Council of Europe 1997). Thin-layer chromatography (TLC) on silica gel (SG) fiberglass sheets in two different solvent systems is recommended for the separation of free ^{99m}Tc-pertechnetate and reduced, hydrolized ^{99m}Tc activity.

- System I: Free ^{99m}Tc-pertechnetate is measured in methyl ethyl ketone (MEK) at the solvent front.
- System II: Reduced, hydrolized ^{99m}Tc activity is separated in saline at the start. The radiochemical purity of ^{99m}Tc-DTPA chelate should not be less than 95% (*Ph. Eur.*).

Thin-layer chromatography		
Sytem I (MEK)	Free ^{99m}Tc-pertechnetate at the solvent front	**(F)**
System II (Saline)	Reduced, hydrolized ^{99m}Tc activity at the origin	**(H)**
	^{99m}Tc-DTPA complex (%) = 100–%(F+H)	

The *Ph. Eur.* states that the sum of measured impurities in both solvents must not exceed 5% of the total radioactivity. Reduced, hydrolized ^{99m}Tc activity should be analyzed before ^{99m}Tc-DTPA is used for GFR measurements:

$$^{99m}\text{Tc-DTPA complex } (\%) = 100 - (F + H)$$

F (%) = ^{99m}Tc-Na-pertechnetate (free)
H (%) = hydrolized ^{99m}Tc-activity.

Results of analysis *(12 samples)*
Results were obtained by thin-layer chromatography in two solvent systems, at different times after labeling.

Labeling and stability	15 min (%)	6 h (%)
^{99m}Tc-DTPA complex	98.5 ± 0.27	97.2 ± 0.32
^{99m}Tc-reduced, hydrolized	0.6 ± 0.12	0.8 ± 0.25
^{99m}Tc-Na-pertechnetate	0.8 ± 0.06	1.0 ± 0.17

Pharmacokinetic Data

After intravenous injection, ^{99m}Tc-DTPA penetrates capillary walls to enter the extravascular space within 4 min (McAfee et al. 1979). Because of its hydrophilicity and negative charge, ^{99m}Tc-DTPA is excluded from cells and is confined to the extracellular space. ^{99m}Tc-DTPA is removed from the circulation exclusively by the kidneys; renal clearance is unaffected by urine flow and by the administration of probenecid (Klopper et al. 1972). However, only the filtered fraction (20%) of the total renal plasma flow is excreted by glomerular filtration (Barbour et al. 1976).

The plasma disappearance of ^{99m}Tc-DTPA can be described by a double exponential function (McAfee et al. 1979). One component (0.99) is excreted with a biological half-time of 100 min, the rest (0.01) with a half-time of 7 days. The fraction excreted by the kidneys is 1.0, and the renal transit time is 5 min. For abnormal cases, the retention half-time of the major component is increased (1,000 min) and so is the transit time (20 min).

^{99m}Tc-DTPA cannot pass through the intact BBB, but in areas of the brain where structural defects permit diffusion, uptake has been used as an indicator of vascular and neoplastic brain lesions (Hauser et al. 1970).

Inhalation of ^{99m}Tc-DTPA as an aerosol shows free diffusion of the particles (diameter of 0.5 μm) to the lung periphery and alveolar retention, larger droplets (>1 μm) sediment preferentially in the trachea and upper bronchial tree (Coates and O'Brodovich 1986). In the lung, the label is released with a biological half-time of approximately 1 h. ^{99m}Tc-DTPA reaching the blood is excreted by the kidneys.

Radiation Dose

The most exposed organs are the kidneys and bladder wall. The effective (whole body) dose equivalent is 0.0063 mSv/MBq (International Commission on Radiological Protection 1987a). The effective dose in adults (70 kg) resulting from intravenous injection of 185 MBq (5 mCi) of ^{99m}Tc-DTPA complex is 1.2 mSv.

Calculations of the absorbed radiation dose resulting from inhalation of ^{99m}Tc-DTPA are based on technetium-labeled aerosols; it is assumed that the label is released fast, and that ^{99m}Tc-DTPA reaching the blood is excreted by the kidneys. The effective (whole body) dose equivalent is 0.007 mSv/MBq (International Commission on Radiological Protection 1987b). The effective dose in adults (70 kg) resulting from inhalation of 150 MBq (4 mCi) of ^{99m}Tc-DTPA is 1.05 mSv. The dose to the bladder wall after inhalation of 150 MBq (4 mCi) of ^{99m}Tc-DTPA is 7.0 mGy.

Storage and Stability

Storage. The lyophilized kit is stored at 2–8 °C in the refrigerator. ^{99m}Tc-DTPA injection solution is kept at room temperature with adequate shielding.

Stability. The ^{99m}Tc-DTPA injection is stable within 6–8 h after preparation.

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12.8.3 ^{99m}Tc-EC (Ethylene Dicysteine)

J. Környei

Chemical name

N,N-Ethylene-L,L-dicysteine (L,L-EC)

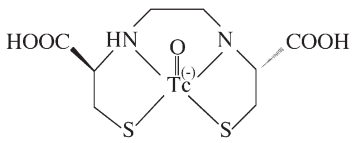
Tc(V)oxo-*N,N*-ethylene dicysteine

Technetium-99m ethylenedicysteine

^{99m}Tc(V)-L,L-ethylenedicysteine (EC)

^{99m}Tc-L,L-EC complex

Chemical structure



Tc(V)oxo-L,L-EC

Kit components

Vial A:

N,N,-Ethylene-L,L-dicysteine	2.0 mg
Ascorbic acid	0.5 mg
Sodium-ethylenediamine tetraacetic acid (EDTA)	0.35 mg
Disodium hydrogen phosphate	13.3 mg
Mannitol	30 mg

Vial C:

Stannous chloride dihydrate	0.2 mg
Potassium dihydrogen phosphate	18.2 mg
Ascorbic acid	12 mg

Kit components

Vial B:

Stannous chloride dihydrate	0.2 mg
Tartaric acid	48 mg
Ascorbic acid	12 mg

Commercial products

EC in vivo kit Institute of Isotopes (Hu)

(Kit is registered in Hungary)

Preparation

One kit consists of three vials, A, B, and C, containing the lyophilized ingredients. Labeling is performed under aseptic conditions as follows.

Procedure:

- Inject into vial A sufficient ^{99m}Tc activity (0.8–1.6 GBq; resp., 22-43 mCi) in a volume of 2.0 ml.
- Dissolve the content of vial B in 2.0 ml saline. After dissolution, withdraw 0.5 ml and inject into vial A. The resulting pH should not be below 12.0.

- Let react for 15 min at room temperature.
- Dissolve the content of vial C in 1.0 ml saline and transfer this solution to vial A.

^{99m}Tc-EC complex is a sterile, pyrogen-free, clear, colorless solution suitable for intravenous injection. The pH value is 5.5–8.0. ^{99m}Tc-EC injection solution should be used within 3 h after labeling.

Description of the Kit

Vial A contains 2 mg of lyophilized ethylene-L,L-dicysteine (L,L-EC) and several reagents to facilitate labeling with ^{99m}Tc-pertechnetate at alkaline pH. The eluate should be fresh, preferably obtained from a ^{99}Mo/^{99m}Tc generator eluted regularly, every 24 h. If necessary, the appropriate radioactivity concentration is adjusted with 0.9% NaCl solution for injection. Labeling is initiated by the addition of 0.5 ml of reducing agent containing 50 μg of stannous chloride from vial B. After 15 min incubation at room temperature, 1 ml of the buffer solution containing ascorbic acid as a stabilizer is added from vial C. Vial A contains 3.5 ml of ^{99m}Tc-EC injection solution suitable for not less than 3 patient doses.

The original kit composition reported by Verbruggen et al. (1992) consisted of the lyophilized residue of a solution of 0.5 mg of L,L-EC and 0.1 mg of stannous chloride dihydrate in 1 ml of phosphate buffer (0.05 M, pH 12.0). Labeling was performed by adding 1.850 MBq ^{99m}Tc-pertechnetate in a volume of 5 ml to the reaction vial. After incubation for at least 1 min at room temperature, the pH was adjusted with 0.2 ml of 0.5 *M* phosphate buffer (pH 5). The final pH of ^{99m}Tc-EC injection solution was 7.0–8.0; the complex was stable for 8 h. The advantage of this formulation is high specific activity labeling, keeping the amount of injected ligand below 15 μg (Stoffel et al. 1994).

At alkaline pH, an N_2S_2 complex is formed with the EC-ligand and pentavalent technetium, having a TcO core and two carboxylate functions. After neutralization, ^{99m}Tc-EC is a negatively charged complex with the chemical formula $[TcO\ EC]^{-1}$.

Clinical Applications

- Dynamic studies providing information on renal function (camera renography)
- Determination of the tubular extraction rate (TER)
- Examination of renal function in patients with transplanted kidneys

Since the introduction of ^{99m}Tc-L,L-EC as a tubular radiotracer (Verbruggen et al. 1992), clinical verification of the pharmacokinetic parameters has been obtained in normal controls (Van Nerom et al. 1993) and in patients (Gupta et al. 1995; Kabasakal et al. 1995), using standard procedures and also by comparison with the reference compounds *ortho*-iodohippurate (OIH) labeled with iodine-125 and with ^{99m}Tc-mercaptoacetyltriglycine (MAG_3) (Kabasakal et al. 1995; Özker et al. 1994; Stoffel et al. 1996). ^{99m}Tc-EC complex showed high similarity with OIH with respect to plasma clearance and elimination half-times (Özker et al. 1994; Van Nerom et al. 1993). ^{99m}Tc-EC was

also evaluated for the diagnosis of renal artery stenosis in patients with suspected renovascular hypertension (Kibar et al. 1997).

Time of Examination

Dynamic renal studies: Collection of frames should start immediately after the intravenous injection of ^{99m}Tc-EC. The total time for one examination is approximately 20 min.

Recommended Activities for Indications

Dynamic renal studies: 90–120 MBq (2.4–3.2 mCi), injected intravenously
0.3–0.7 mg of ^{99m}Tc-EC complex
The manufacturer's instructions should be followed.

Pediatric Dose. The amount of radioactivity for infants and children is based on either body weight (static imaging) or body surface area (dynamic examinations). Recommendations by the Pediatric Task Group of the European Association of Nuclear Medicine (EANM) based on body weight should be followed (see Appendix 1, Table A1.2).

Additional Information

The patient should be adequately hydrated before ^{99m}Tc-EC renography.

Certain medication may affect renal clearance even in patients with normal kidney function.

Slower washout is observed with angiotensin-converting enzymes (ACE) inhibitors.

Tubular secretion is affected by the administered amount of ^{99m}Tc-EC. The single dose should not exceed 0.7 mg of EC (use kit for three patients), since secretion may be delayed; in cases of impaired renal function, a saturation effect may be observed. Higher doses (entire kit content) should be avoided since the diagnostic value of the renal examination may be biased.

^{99m}Tc-EC complex does not contribute to hepatobiliary activity; thus, ^{99m}Tc-EC is especially suited for the examination of renal function in patients with kidney transplants.

Quality Control

Radiochemical Purity. The radiochemical purity of the ^{99m}Tc-EC complex is measured by thin-layer chromatography (TLC), using one solvent system to separate ^{99m}Tc-pertechnetate (SF) and reduced ^{99m}Tc activity (start) from the ^{99m}Tc-EC complex (a more rapid analysis using two solvent systems has been described by Stoffel et al. 1994). The radiochemical purity of the ^{99m}Tc-EC complex should not be less than 95%.

Method Recommended by the Manufacturer

Thin-layer chromatography	
Stationary phase:	Gelman ITLC SG-60 plates 1.5×20 cm
Solvent:	96% Ethanol
Developing time:	2.5 h (A more rapid analysis using 2 solvent systems has been described by Stoffel et al. [1994].)
R_f values:	^{99m}Tc reduced, hydrolized: 0.0–0.1 ^{99m}Tc-EC complex: 0.45–0.55 (>95%) ^{99m}Tc-Na-pertechnetate: 0.9–1.0

The limit of radiochemical impurities at expiry (3 h) is <10%.

Results of analysis *(12 samples)*

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-EC complex	98.7 ± 0.65	99.4 ± 0.2
^{99m}Tc-Na-pertechnetate	< 0.3	< 0.2
^{99m}Tc-reduced, hydrolized	< 1.0	< 0.4

Pharmacokinetic Data

The elimination of the ^{99m}Tc-L,L-EC complex from the blood is rapid. At 2–3 min post-injection, no heart and liver activities (due to perfusion) are observed. This is in agreement with the protein binding data of ^{99m}Tc-EC (31%) (Van Nerom et al. 1993), which is considerably less than of ^{123}I-OIH (74.1 ± 3.9%) and approximately 30% of ^{99m}Tc-MAG_3 (91.3 ± 1.9%) (Bubeck et al. 1990).

^{99m}Tc-EC is excreted by tubular secretion in the kidneys; the TER is 69–75% of the reference *para*-amino-hippurate (PAH). The maximum of radioactivity in the kidneys is observed at 4.4 ± 0.3 min after intravenous injection. The washout of ^{99m}Tc-EC is rapid but slightly slower than of OIH, indicated by the biological half-times of ^{99m}Tc-EC 6.3–7.6 min and OIH 4.7–6.3 min (Özker et al. 1994).

A comparison of ^{99m}Tc-L,L-EC with ^{125}I-OIH in normal volunteers and in a wide range of renal function values has produced data of the mean plasma clearance with a close correlation between ^{99m}Tc-L,L-EC and ^{125}I-OIH ($r = 0.99$, $p < 0.001$). The mean clearance of ^{99m}Tc-L,L-EC amounted to 70.6 ± 6.2% of the ^{125}I-OIH clearance with a range of 34.7–103.8% (Stoffel et al. 1994). The total volume of distribution of both tracers was not significantly different, corresponding to approximately 20% of the body weight.

When ^{99m}Tc-EC was compared with ^{99m}Tc-MAG_3, it showed a higher plasma clearance and considerably lower activity in the liver; there was no significant activity in bowel and gallbladder even in patients with impaired kidney function (Verbruggen et al. 1992).

Radiation Dose

Kidneys, bladder wall, and adrenals are most exposed organs. Calculations of the effective dose are based on dose equivalents for technetium-MAG_3 (International Commission on Radiological Protection 1991). Depending on the functional state of the kidneys the effective dose (mSv/MBq) is given as:

Normal function: 0.0073
Abnormal function: 0.0063
Acute unilateral: 0.01

Accordingly, the effective (whole body) dose resulting from 111 MBq of intravenously injected ^{99m}Tc-EC complex is 0.81 mSv (normal function), 0.70 mSv (abnormal function), and 1.11 mSv (acute unilateral obstruction).

Storage and Stability

Storage. The lyophilized kit is stored at 2–8 °C in the refrigerator. ^{99m}Tc-EC injection solution is kept at room temperature with adequate shielding.

Stability. The ^{99m}Tc-EC injection solution is stable for 3 or 8 h after preparation, depending on the kit formulation.

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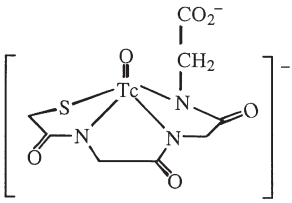
12.8.4 ^{99m}Tc-MAG$_3$ (Mercaptoacetyltriglycine)

F. Rakiás and I. Zolle

Chemical name	**Chemical structure**
Benzoylmercapto-acetyltriglycine: Betiatide (*Ph. Eur.; USP*) Tc(V)oxo-*N,N,N*-mercaptoacetyltriglycine: Mertiatide (*Ph. Eur.; USP*) Technetium Tc 99m mertiatide injection (*Ph. Eur.; USP*) ^{99m}Tc(V)O-mercaptoacetyltriglycine complex ^{99m}Tc-MAG$_3$ complex	 ^{99m}Tc(V)-MAG$_3$ complex
Kit components	**Commercial products**
Betiatide 1.0 mg Stannous chloride dehydrate 0.04 mg Disodium tartrate 16.9 mg	TechneScan MAG$_3$ Mallinckrodt/Tyco

Preparation

The kit contains the lyophilized, sterile, pyrogen-free, inactive ingredients in a nitrogen atmosphere, ready for labeling with ^{99m}Tc-sodium pertecnetate (*European Pharmacopeia* [*Ph. Eur.*]). Labeling is performed according to the instructions given by the manufacturer (Mallinckrodt 1992):

- Method 1: Use 3 ml of fresh eluate; the activity should not exceed 1.11 GBq (30 mCi). The calculated amount of ^{99m}Tc activity is diluted to a volume of 10 ml with saline. Add this volume to a TechneScan mercaptoacetyltriglycine (MAG$_3$) vial, using a thin hypodermic needle (20 gauge or higher). Place the kit in upright position into a boiling water bath for 10 min. After heating, cool the vial in cold water. ^{99m}Tc-MAG$_3$ injection solution (10 ml) is stable for 4 h.

- Method 2: To obtain higher activity concentrations, use 1 ml of fresh eluate for labeling and dilute to 4 ml with saline. The activity should not exceed 925 MBq (25 mCi). Follow the procedure as described above. This preparation (4 ml) is stable for 1 h.

^{99m}Tc-MAG_3 is a clear or slightly opalescent, colorless, sterile solution for intravenous injection. The resulting pH should be 5.0–7.5. ^{99m}Tc-MAG_3 injection solution may be used for one or multiple administrations.

Description of the Kit

^{99m}Tc-mertiatide is obtained upon heating in a boiling water bath with a labeling yield of approximately 96%. One molecule of *S*-benzoyl-protected betiatide reacts with reduced ^{99m}Tc in the +5 oxidation state to form a negatively charged ^{99m}Tc-MAG_3 complex. Initially, at pH 5.5, a labile ^{99m}Tc-tartrate complex is formed, which is readily exchanged by the benzoylmercaptoacetyl triglycine ligand during heating at 95 °C (ligand exchange). Heating in a boiling water bath is required, because at room temperature formation of the ^{99m}Tc-MAG_3 complex is slow (52% in 2 h) (Fritzberg et al. 1986). The heating process also increases the rate of hydrolysis of the protecting benzoyl group and the conversion of a bis ligand complex, which may also be formed, to single-ligand ^{99m}Tc-MAG_3.

The core ligand system N_3S and an additional carboxylate group are responsible for high tubular specificity and a high excretion rate. In addition, the triamide monomercaptide ligand does not form stereoisomers that required high-performance liquid chromatography (HPLC) purification (Fritzberg et al. 1986). ^{99m}Tc-MAG_3 is a tetradentate chelate of oxotechnetium as described for the $TcON_2S_2$ *N*,*N*-bis(mercaptoacetyl) ethylenediamine (DADS) ligands (Davison et al. 1981).

The effect of stereoisomers and of byproducts on kit preparation has been investigated extensively (Brandau et al. 1988a, b; Bubeck et al. 1986; Coveney and Robbins 1987; Fritzberg et al. 1982).

Labeling should be performed with eluates with the highest possible radioactive concentration. In order to obtain a high yield and to avoid labeled impurities, the ^{99m}Tc eluate used for labeling must be obtained from a ^{99m}Tc generator that is eluted daily at a 24-h interval (Van Duzee and Bugaj 1981). Moreover, only eluates from a ^{99m}Tc generator that has been in use for no longer than 5 days are suitable. Dilutions should be performed with saline.

Clinical Applications

Intravenous injection: Renal imaging/renography

- To obtain anatomical and functional information
- To demonstrate adequate renal perfusion
- To evaluate renal tubular function
- To determine the tubular extraction rate (TER)
- As a control after surgical intervention (kidney transplants)
- To evaluate renal artery stenosis and obstructive uropathy
- To diagnose urinary obstruction in infants

The similarity of ^{99m}Tc-MAG_3 with ^{131}I-OIH (*o*-iodohippurate) has been demonstrated in experimental animals (Fritzberg et al. 1986) and in volunteers (Taylor et al. 1986).

Since glomerular filtration accounts for less than 2% of the total clearance, ^{99m}Tc-MAG_3 has been recommended for determination of the TER (Bubeck et al. 1987, 1990).

^{99m}Tc-MAG_3 is used for the evaluation of nephrological and urological disorders, in particular for the study of renal perfusion, relative kidney function, and characterization of urinary flow. Renovascular hypertension is diagnosed after pharmacological intervention (captopril test) (Kletter 1988).

Time of Examination

Renal dynamic studies: Collection of frames should start immediately after the intravenous injection of. The total time for one examination is approximately 20 min.

Recommended Activities for Indications

Renal clearance (plasma clearance):	10–20 MBq (0.27–0.54 mCi)
Renography:	60–80 MBq (1.6–2.2 mCi)
Functional scintigraphy:	150–200 MBq (4–5 mCi), 0.3–1 mg of ^{99m}Tc-MAG_3 100 MBq (2.7 mCi) maximum recommended activity (Administration of Radioactive Substances Advisory Committee [ARSAC])

Depending on the parameters to be studied and the method to be used, 37–185 MBq (1–5 mCi) are used in adults (70 kg). Studies of renal blood flow or transport through the ureters generally require a larger dose than do studies of intrarenal transport; whereas renography requires smaller activities than dynamic sequential scintigraphy.

Pediatric Dose. The amount of radioactivity for infants and children is based on either body weight (static imaging) or body surface area (dynamic examinations). Recommendations by the Pediatric Task Group of the European Association of Nuclear Medicine (EANM) based on body weight should be followed (see Appendix 1, Table A1.2).

Additional Information

The patient should be adequately hydrated before injecting ^{99m}Tc-MAG_3. Insufficient hydration of the patient will affect the renal excretion rate. In order to reduce the radiation exposure to the bladder wall, the patient should be asked to drink water during the following hours.

Small amounts of ^{99m}Tc-labeled impurities may be formed during the labeling process, which accumulate in the liver and contribute to hepatobiliary activity. Dynamic renal studies may be affected in the late phase (after 30 min) due to an overlap of activity in the region of interest.

ACE inhibitors such as captopril may facilitate the differential diagnosis of renovascular hypertension; diuretics such as furosemide (Lasix) cause rapid washout of the radiotracer or demonstrate urinary tract obstruction (Kletter 1988).

Quality Control

Radiochemical Purity. The *Ph. Eur.* (Council of Europe 2005) requires paper chromatography using acetonitrile/water (60:40, v/v) to determine reduced, hydrolized technetium at the start, which should not exceed 2% of the measured radioactivity. For identification of ^{99m}Tc-mertiatide and ^{99m}Tc-Na-pertechnetate, high performance liquid chromatography (HPLC) is recommended. The radiochemical purity of the ^{99m}Tc-MAG_3 complex should not be less than 94%.

HPLC method
Gradient elution for the simultaneous determination of free ^{99m}Tc-pertechnetate and ^{99m}Tc-mertiatide.

- The column is a Merck octadecylsilyl-silica (5 µm), 25 cm×4 mm
- Flow rate is 1.0 ml/min
- Mobile phase A is a 19:1 mixture of phosphate solution (1000 parts 0.01 M NaH_2PO_4 and 114 parts 0.01 M Na_2HPO_4, adjusted to pH 6.0) and ethanol.
- Mobile phase B is a 1:9 mixture of water and methanol.
- Inject 20 µl of the test solution into the chromatograph and record the chromatograms by gradient elution. Use a gradient elution program.
- Retention times: ^{99m}Tc-pertechnetate, 1.8–2.2 min; ^{99m}Tc-mertiatide, 10–14 min

*Requirements (*Ph. Eur.*).* The sum of activities eluted before the major peak activity (hydrophilic impurities including ^{99m}Tc-pertechnetate) should not exceed 3% of the sum of all peaks, and the sum of activities eluted after the major peak activity (lipophilic impurities) should not be more than 4% of the sum of all peaks. The radiochemical purity of the ^{99m}Tc-MAG_3 complex should not be less than 94%.

Results of HPLC analysis of the ^{99m}Tc-MAG_3 complex, performed at different times after preparation:

Eluted peak activity	TOC (%)	4 h (%)
Mertiatide	≥96.0	≥95.0
Total front fractions	≤3.0	≤3.0
Methanol fraction	≤4.0	≤4.0

Methods Recommended by the Manufacturer. Thin-layer chromatography (TLC) on silica gel reversed phase plates (migration distance 10–15 cm) and acidified methanol-saline is used as solvent for the analysis of three components, namely the ^{99m}Tc-MAG_3 complex (R_f=0.4–0.5); reduced, hydrolized technetium at the start; and unbound ^{99m}Tc-Na-pertechnetate at the solvent front. The radiochemical purity of the ^{99m}Tc-MAG_3 complex should not be less than 90%.

Thin-layer chromatography		
Stationary phase:	Merck RP-18 F_{254} plates, 1.5×20 cm	
Solvent:	Methanol/saline/acetic acid (45:54:1, v/v/v)	
Developing time:	1.5 h	
R_f values:	^{99m}Tc-reduced, hydrolized:	0.0–0.1
	^{99m}Tc-mertiatide:	0.4–0.5 (>90%)
	^{99m}Tc-Na-pertechnetate:	0.9–1.0

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer, or gamma counter) and the regional radioactivities are expressed as a percentage of the total recovered counts.

Results of analysis *(12 samples):*

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-MAG$_3$ complex	98.3 ± 0.30	96.2 ± 0.34
^{99m}Tc-Na-pertechnetate	1.0 ± 0.45	1.0 ± 0.39
^{99m}Tc reduced, hydrolized	0.6 ± 0.27	2.7 ± 0.54

Column chromatography. This method is based on the extraction by Sep-Pak C18 cartridges (Waters, light) and elution with 50% ethanol/saline.
Procedure:

1. Rinse the cartridge first with 5 ml ethanol, and then with 5 ml 0.001 N hydrochloric acid.
2. Mix 5 ml ethanol and 5 ml saline.
3. Apply 0.1–0.3 ml of the ^{99m}Tc-MAG$_3$ injection solution onto the column.
4. Rinse the column with 5 ml 0.001 N HCl into vial A.
5. Elute cartridge with 5 ml of ethanol/saline into vial B.

Vials A and B and the eluted cartridge are measured for ^{99m}Tc activity in a dose calibrator.

Use the sum of eluted radioactivities as 100%:

$$^{99m}\text{Tc-MAG}_3 \text{ complex } (\%) = \frac{\text{B} \times 100}{\text{Sum of A} + \text{B} + \text{SEP-PAK}}$$

The radiochemical purity of ^{99m}Tc-mertiatide should not be less than 90%.

Pharmacokinetic Data

After intravenous injection, ^{99m}Tc-mertiatide is rapidly distributed in the extracellular fluid and excreted entirely by the renal system. The maximum renal accumulation of radioactivity is observed at 3–4 min (207 ± 80 s) after intravenous injection (Taylor et al. 1986).

The elimination from plasma is described by two half-times, namely, 3.2 and 16.9 min. The mean parenchymal transit time is approximately 4 min (100–270 s) (Jafri et al. 1988).

At 3 h postinjection the activity in blood is less than 1% of the injected dose. Urinary excretion of ^{99m}Tc-MAG$_3$ is 70% in 30 min (normal renal function), and 99.9 ± 4.3% at three h postinjection. ^{99m}Tc-MAG$_3$ is not metabolized and is excreted unchanged (Taylor et al. 1986).

The mechanism of excretion is predominantly based on renal tubular secretion. The TER of ^{99m}Tc-MAG$_3$ is 0.55 of the reference *para*-amino-hippurate ([PAH] 1.0); in comparison, *ortho*-iodohippurate (OIH) shows a value of 0.83 (Bubeck et al. 1987). Using this coefficient (0.55), ^{99m}Tc-MAG$_3$ may substitute for OIH as an indicator of effective renal plasma flow (ERPF) (Bubeck et al. 1990).

The renal clearance is dependent on the functional state of the kidneys and the urogenital system; however, there is a good correlation between the clearance of ^{99m}Tc-MAG_3 and ^{131}I-OIH in patients with seriously impaired renal function or transplant kidneys as well as in patients with normal tubular function (Bubeck et al. 1988c). Simultaneously obtained clearance data showed striking differences between plasma clearance and plasma concentration. The plasma clearance of ^{99m}Tc-MAG_3 was about one half that of ^{131}I-OIH, but its plasma concentration was much higher in concordance with the high protein binding; the net effect being a similar urinary excretion and qualitatively similar renogram curves (Russel et al. 1988). Using a two-compartment model, Taylor et al. (1986) reported a ^{99m}Tc-MAG_3/OIH plasma-clearance ratio of 0.69 during the first 30 min postinjection, and Bubeck et al. (1990) at steady state a ratio of 0.67.

^{99m}Tc-mertiatide shows high binding to plasma proteins (91.3 ± 1.9%). Glomerular filtration accounts for less than 2% of the total clearance (Bubeck et al. 1987, 1990).

Radiation Dose

The kidneys, bladder wall, and adrenals are most exposed organs. MAG_3 is rapidly distributed in the extracellular fluid and excreted entirely by the renal system. The renal transit time is approximately 4 min, as is for OIH (Bubeck et al. 1987).

Calculations of the effective dose are based on dose equivalents for technetium-MAG_3 (International Commission on Radiological Protection 1991). Depending on the functional state of the kidneys the effective dose (mSv/MBq) is given as:

- Normal function: 0.0073
- Abnormal function: 0.0063
- Acute unilateral blockage: 0.01

Accordingly, the effective (whole body) dose resulting from 100 MBq (2.7 mCi) of intravenously injected ^{99m}Tc-MAG_3 complex is 0.73 mSv (normal function), 0.63 mSv (abnormal function), and 1.0 mSv (acute unilateral obstruction).

The doses resulting from 100 MBq (2.7 mCi) to exposed organs (mGy) are:

- Bladder 11.0, kidneys 0.34, uterus 1.2 (normal function)
- Bladder 8.3, kidneys 1.4, uterus 1.0 (abnormal function)
- Adrenals 1.1, bladder 5.6, kidneys 20.0 (acute, unilateral obstruction)

When renal function is bilaterally impaired, it is assumed that the clearance rate of MAG_3 is one tenth of that for the normal case, the renal transit time is increased to 20 min, and a fraction of 0.04 is taken up in the liver.

In the case of acute unilateral renal blockage, it is assumed that a fraction of 0.5 of administered MAG_3 is taken up by one kidney and slowly released to the blood with a half-time of 5 days, and subsequently excreted by the other kidney, which is assumed to function normally (International Commission on Radiological Protection 1991).

Unilateral renal blockage causes a shift in the excretion pattern, with the highest radiation absorbed dose delivered to the kidneys, namely, 0.20 mGy/MBq.

Consequently, the effective (whole body) dose value is 0.01 mSv/MBq, and the effective dose in adults (70 kg) resulting from intravenous injection of 150 MBq (4 mCi) of ^{99m}Tc-MAG_3 complex is 1.5 mSv.

Storage and Stability

Storage. The TecneScan MAG_3 kit is stored at 2–8 °C, protected from light until use. ^{99m}Tc-MAG_3 injection solution is kept at room temperature with adequate shielding.

Stability. ^{99m}Tc-MAG_3 injection is stable for 1 or 4 h after labeling, depending on the volume of preparation (4 or 10 ml). If the injection solution is used for multiple administrations, the vial should be kept in the refrigerator.

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12.9 ^{99m}Tc-Labeled Hepatobiliary Agents

12.9.1 ^{99m}Tc-IDA (Iminodiacetic Acid) Derivatives

I. Zolle and A. G. Bratouss

Accepted Chemical Names

N-(2,6-diethylphenylcarbamoylmethyl)-iminodiacetic acid (Etifenin, Ph. Eur.)

N-(2,6-dimethylphenylcarbamoylmethyl)-iminodiacetic acid (Lidofenin, USP)

N-(2,4,6-trimehtyl-3-bromophenylcarbamoylmethyl)-iminodiacetic acid (Mebrofenin)

N-(2,6-diisopropylphenylcarbamoylmethyl)-iminiodiacetic acid (Disofenin)

Kit Components*

Etifenin	20.0 mg
Mebrofenin	40.0 mg
Disofenin	20.0 mg
Tin(II)-chloride-dihydrate	0.2–0.6 mg

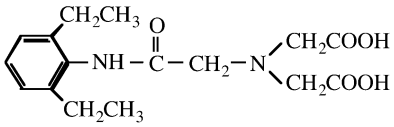
Listed Trade Names

Etifenin – EHIDA (Rotop)
as disodium salt – Hepatobida

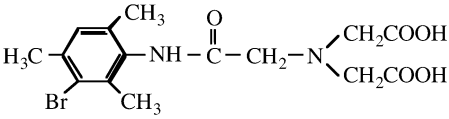
Mebrofenin – TCK-22 (CIS Bio)
CholeCIS
Choletec (Bracco)
Bridatec (Sorin)

Disofenin – DISIDA
Hepatolite

Chemical Structures



Etifenin



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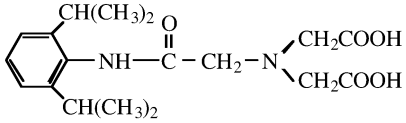

Disofenin

** The composition of the kit components may vary with new manufacturers*

Preparation

The kit contains the lyophilized, sterile ingredients in a multidose vial. Labeling with ^{99m}Tc eluate is carried out under aseptic conditions by adding 1–5 ml of sterile ^{99m}Tc-sodium pertechnetate to the vial (0.3–1.5 GBq resp., 8.1–40.5 mCi). The reaction is allowed to proceed at room temperature for 15 min. ^{99m}Tc-iminodiacetic acid (IDA) preparations

are sterile, pyrogen-free, clear, colorless solutions suitable for intravenous injection. The resulting pH should be 4.0–6.0 (TCK-22), 4.2–5.7 (Choletec), and 6.5–7.5 (Bridatec).

Description of the Kit. Etifenin is officinal in the *European Pharmacopeia* (*Ph. Eur.*) Diethyl-IDA or EHIDA is a registered radiopharmaceutical (kit) in Europe. Three IDA derivatives (lidofenin, disofenin, and mebrofenin) have been included in the *USP* since the first hepatobiliary agent dimethyl-IDA, with the generic name lidofenin, was introduced (Loberg et al. 1976).

^{99m}Tc-IDA complexes are formed easily with reduced technetium at room temperature. The size of the substituents attached to the phenyl ring does affect the labeling yield, radiochemical purity, and stability (Loberg et al. 1976; Ryan et al. 1977). Labeling is also affected by pH and the ligand concentration, showing higher labeling at low pH (Nunn and Schramm 1981). The amount of tin has no affect on the labeling yield; however, high labeling is depending on maintaining tin in the reduced state. Isotopic dilution is observed with higher concentrations of ^{99}Tc in the first eluate of new generators, reducing the labeling efficiency (Ponto et al. 1987). Increasing the reaction time to 30 min will ensure maximum labeling.

Clinical Applications

Intravenous injection: Hepatobiliary scintigraphy

- To evaluate hepatocyte function
- To demonstrate patency or obstruction of cystic duct
- To rule out acute cholecystitis
- To demonstrate common bile duct obstruction
- As a control after surgical intervention
- To verify hepatic bile duct atresia in infants

Time of Examination. Hepatobiliary scintigraphy is started 5 min after the intravenous injection. Thereafter, scintigrams are taken at 10-min intervals up to 60 min. If the gallbladder is not visualized within 60 min, delayed images up to 4 h after administration of the radiotracer are obtained.

Recommended Activities for Indications

Hepatobiliary scintigraphy: 75–185 MBq, injected intravenously
150 MBq maximum recommended activity
1–2.6 MBq/kg body weight (normal bilirubin level)
< 0.5 mg etifenin/kg body weight

Pediatric Dose. The amount of radioactivity for infants and children administered for hepatobiliary scintigraphy is based on body weight, using the scaling factors given in Appendix 1, Table A1.2. The minimum value of the scaling factor is 0.1, since activities below 10% of the value of the adult radioactivity have resulted in poor image quality.

Additional Information

The patient should not eat 2–6 h prior to the hepatobiliary scintigraphy, because hepatocyte clearance of the radiotracer and parenchymal transit time is affected by the ingestion of food. The gallbladder cannot be visualized in 65% of cases within the first 60 min of injection of the ^{99m}Tc-IDA complex, even if the cystic duct is patent (Fink-Bennett 1995). Gallbladder contractility can be provoked with a fatty meal or intravenous cholecystokinin.

Persistent nonvisualization of the gallbladder during a 4-h period is suggestive of acute cholecystitis; whereas delayed visualization of the gallbladder after 1.5 h up to 4 h is more typical of chronic cholecystitis (Weissmann et al. 1979). To enhance gallbladder visualization, cholecystokinin or sincalide have been used to induce contraction and subsequent filling of the gallbladder. The usual intravenous dose of sincalide is 0.02 μg/kg, administered either 30 min before imaging is started or after the nonvisualization of the gallbladder within 1 h. The limitations of this medication in cholescintigraphic studies have been described (Freeman et al. 1981).

Narcotic (opioid) analgesics (morphine, meperidine) and phenobarbital cause a marked increase in biliary tract pressure. The increase of intraluminal common bile duct pressure by morphine (50–60%) has been used to differentiate between acute and chronic cholecystitis. Morphine-augmented cholescintigraphy has been considered superior to conventional hepatobiliary scintigraphy in assessing cystic duct patency because diagnostic results are obtained faster. The increase in common bile duct pressure is sufficient to overcome the resistance to bile flow caused by a sludge-filled gallbladder, so that the radiotracer may penetrate the cystic duct into the gallbladder. A dose of 0.04 mg/kg morphine sulfate diluted to 10 ml with saline is administered intravenously over 3 min, when the gallbladder has not been visualized within 60 min after the administration of the ^{99m}Tc-IDA complex. Serial images are then obtained at 5-min intervals for 30 min. In the case of persistent nonvisualization of the gallbladder after morphine administration, acute cholecystitis is diagnosed (Fink-Bennett 1995). If, however, the gallbladder is visualized 5–30 min after morphine administration, chronic cholecystitis is present. Morphine should not be given to patients with a history of drug abuse, an allergy to morphine, or with pancreatitis.

Phenobarbital enhances the biliary conjugation and excretion of bilirubin. It promotes the excretion of organic anions such as ^{99m}Tc-IDA complexes that are not conjugated by the liver. The hepatic extraction of ^{99m}Tc-IDA complexes and canalicular bile flow are increased; thus, phenobarbital is used in neonates for hepatobiliary imaging primarily to increase the diagnostic accuracy of differentiating between neonatal hepatitis and biliary atresia. The administered dose to increase the rate of excretion of a ^{99m}Tc-IDA complex is approximately 5 mg/kg per day for at least 5 days prior to the imaging procedure (Majd et al. 1981).

Nicotinic acid in high doses may affect the cholescintigraphy because of poor extraction and elimination of the radiotracer (toxic effect on hepatocytes). Total parenteral nutrition may cause delayed or no visualization of the gallbladder, even in patients with no gallbladder disease caused by bile stasis and the formation of thick viscous jelly-like bile. Nonvisualization of the gallbladder may also be caused by hepatic artery infusion during chemotherapy.

Quality Control

Radiochemical Purity. The *Ph. Eur.* (Council of Europe 2005) requires thin-layer chromatography (TLC) on silica gel (SG) fiberglass sheets (migration distance of 10–15 cm) for the analysis of the ^{99m}Tc-EHIDA complex, using 0.9% sodium chloride solution (saline) as solvent. Reduced, hydrolized technetium remains at the start; the ^{99m}Tc-etifenin complex is identified at an R_f of 0.4–0.5; unbound ^{99m}Tc-Na-pertechnetate is measured at the solvent front. The radiochemical purity of the ^{99m}Tc-EHIDA complex should not be less than 95% (*Ph. Eur.*).

Recommended Methods

Thin-layer chromatography. TLC with two different solvent systems is based on the separate identification of labeled impurities (Nunn et al. 1983).

- System I: In saline (20%) unbound ^{99m}Tc-Na-pertechnetate is identified at the solvent front ($R_f = 1$). The sum of activities of reduced, hydrolized technetium and the ^{99m}Tc-IDA complex is measured at the start ($R_f = 0$).
- System II: Using Gelman ITLC-SG and acetonitrile/water as solvent, reduced, hydrolized activity is identified separately at the start ($R_f = 0$).

System I		
Stationary phase:	Gelman ITLC-SA, 1×9.5 cm	
Solvent:	Saturated NaCl solution	
Developing time:	5 min	
R_f values:	^{99m}Tc-IDA complex:	0.0–01
	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-Na-pertechnetate:	0.9–1.0
System II		
Stationary phase:	Gelman ITLC-SG, 1×9.5 cm	
Solvent:	Acetonitrile–water (3:1)	
Developing time:	5 min	
R_f values:	**^{99m}Tc reduced, hydrolized:**	0.0–0.1
	^{99m}Tc-IDA complex:	0.9–1.0
	^{99m}Tc-Na-pertechnetate:	0.9–1.0

Quantification of labeled components. Each chromatogram is measured (TLC linear analyzer or gamma counter), and the regional radioactivities are expressed as a percentage of the total recovered counts. The percentage of free ^{99m}Tc Na-pertechnetate (F) and reduced, hydrolized activity (H) are determined and the pure ^{99m}Tc-IDA complex quantified according to the equation:

$$^{99m}\text{Tc-IDA complex } (\%) = 100 - \%(F + H)$$

Results of analysis *(12 samples)*
Results were obtained using the analytical method described by Nunn et al. (1983).

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-IDA complex	96.5 ± 1.68	95.2 ± 0.71
^{99m}Tc-Na-pertechnetate	1.6 ± 1.51	2.9 ± 1.02
^{99m}Tc-reduced, hydrolized	1.9 ± 0.61	1.9 ± 0.59

Pharmacokinetic Data

Hepatobiliary excretion of ^{99m}Tc-IDA complexes is governed by molecular size (optimal molecular weight: 700–900 Da) and structural configuration. Two molecules of ligand are coordinated to one technetium atom in the +3 oxidation state. ^{99m}Tc-IDA complexes have a negative charge, high polarity, and show high binding to plasma protein (Loberg and Fields 1978).

Substituents attached to the phenyl ring affect the degree of hepatic uptake and the rate of excretion, as well as urinary elimination of the ^{99m}Tc-IDA complex (Nunn et al. 1983). Approximately 82% of the injected dose of ^{99m}Tc-EHIDA, 88% of ^{99m}Tc-disofenin, and 98% of ^{99m}Tc-mebrofenin are extracted by the hepatocytes and secreted into bile with a hepatic excretion half-time of 37.3, 19.0, and 17.0 min, respectively (Krishnamurthy and Krishnamurthy 1989; Krishnamurthy and Turner 1990). Clinical comparison of diethyl-IDA (etifenin) and diisopropyl-IDA (disofenin) was also reported (Klingensmith et al. 1981). ^{99m}Tc-DISIDA and ^{99m}Tc-mebrofenin show least hepatic retention and are best suited to delineate hepatic biliary anatomy (Krishnamurthy and Krishnamurthy 1989).

^{99m}Tc-IDA complexes are excreted into bile as the original bis-coordinated complex; they are not reabsorbed by epithelia of the biliary ducts nor metabolized, and released into the duodenum unchanged (Krishnamurthy and Krishnamurthy 1989; Loberg et al. 1976). Excretion across the bile canalicular membrane is a carrier-mediated process (Nielson and Rasmussen 1975).

Following intravenous injection, the ^{99m}Tc-IDA complex is bound to plasma protein (mainly albumin) and carried to the liver (Nicholson et al. 1980). Accumulation in the liver involves the same carrier-mediated, non–sodium-dependent organic anion transport processes as for the uptake of bilirubin. In the space of Disse, the albumin–^{99m}Tc-IDA conjugate is dissociated to facilitate active transport of the ^{99m}Tc-IDA complex into hepatocytes (Krishnamurthy and Krishnamurthy 1989). In patients with normal hepatobiliary function, maximal liver uptake is measured at 12 min (^{99m}Tc-mebrofenin, 10.9 ± 1.9 min; ^{99m}Tc-disofenin, 11.5 ± 3.1 min) (Fritzberg 1986). The radioactivity is half this value within approximately 20 min. The gallbladder is well visualized 20 min postinjection. Intestinal activity appears on the average at 15–30 min. The common bile duct may be visualized after 14 min. The upper limit of "normal" for visualization of these structures is 1 h (Weissmann et al. 1979).

The blood clearance curve shows two major half-times of elimination, a fast component with $T_{1/2} = 2.5$ min (23–40%) and a slower component with $T_{1/2} = 17$ min (7–16%); a small percentage is excreted with $T_{1/2} \geq 16$ h (1.4–2.6%). The total radioactivity in blood at 1 and 24 h after the intravenous injection is 3%, and less than 1% of the administered dose, respectively (Brown et al. 1982).

Hepatic extraction efficiency decreases with increasing serum bilirubin levels. With impaired hepatocyte function, high plasma concentrations (> 8 mg/100 ml) may inhibit uptake of ^{99m}Tc-IDA complexes. As hepatobiliary excretion decreases, renal accumulation of certain ^{99m}Tc-IDA complexes is observed (Fink-Bennett 1995).

Normally, the renal elimination of IDA complexes is low: cumulative urinary excretion of ^{99m}Tc-mebrofenin is 1.2% in 3 h, and 7.1% in the case of ^{99m}Tc-DISIDA. With a serum bilirubin level of 15 mg/100 ml, urinary excretion increases to approximately 5 and 23%, respectively (Fritzberg 1986). ^{99m}Tc-etifenin, on the other hand, shows a cumulative urinary excretion of 17% in 5 h, with normal bilirubin levels (Nielson and Rasmussen 1975).

Among the many ligands that have been evaluated, ^{99m}Tc-mebrofenin has shown the best in vivo characteristics, namely high hepatocyte extraction (98%) and a fast clearance ($T_{1/2}$ = 17 min), a rapid hepatobiliary-to-bowel transit time, and low urinary excretion. ^{99m}Tc-mebrofenin provides excellent visualization of the common bile duct and the gallbladder at serum bilirubin levels as high as 30 mg/100 ml (Fink-Bennett 1995; Krishnamurthy and Turner 1990).

Healthy persons fasting overnight prior to the examination showed 56% of the radiotracer in the gallbladder; 44% were excreted directly into the small intestine (Brown et al. 1982). The fraction of gallbladder emptying following a whole meal has been measured as 87%.

Nonvisualization of the gallbladder with visualization of the common bile duct and the duodenum in fasting patients within 2 h is diagnostic of acute cholecystitis (Weissmann et al. 1979). It has been suggested to repeat scintigraphy with the ^{99m}Tc-IDA complex 30 min after stimulation of gallbladder contractility with intravenous cholecystokinin. Persistent nonvisualization of the gallbladder confirms cystic duct obstruction, caused by acute cholecystitis. If the gallbladder visualizes after administration of cholecystokinin, chronic cholecystitis is diagnosed (Weissmann et al. 1979). Acute cholecystitis can be virtually excluded if the gallbladder, the common bile duct, and the duodenum are visualized in fasting patients within 1 h after injection of the ^{99m}Tc-IDA complex (Weissmann et al. 1979).

An obstruction or occlusion of the common bile duct affects the clearance of the ^{99m}Tc-IDA complex into the duodenum, resulting in an increased transit time and an abnormal scintigram. The absence of biliary tract visualization in the presence of normal hepatic extraction indicates an acute common bile duct obstruction from a stone in the common bile duct (Fink-Bennett 1995).

Few reports on adverse reactions after intravenous injection of IDA-derivatives are available. The LD_{50} value of etifenin, determined in mice and in rats, is 280 and 270 mg/kg body weight, respectively. The LD_{50} value of mebrofenin is given with 285 mg/kg body weight (in mice) and 250 mg/kg body weight (in rats).

Radiation Dose

The most exposed organs are the gallbladder wall, the upper large intestinal wall, the lower large intestinal wall, the small intestine, and the liver (Brown et al. 1982). The gallbladder receives the highest absorbed dose, 0.18–0.21 mGy/MBq. The relative amount of the delivered dose is strongly dependent on the type of stimulation used to induce gallbladder emptying. In fasting subjects (no gallbladder stimulation), an in-

crease in the radiation absorbed dose to the gallbladder by approximately 170% versus whole-meal gallbladder stimulation is observed (Brown et al. 1982).

Severe hepatocellular disease (high bilirubin levels) will cause a shift in the excretion pattern, with the highest radiation absorbed dose delivered to the urinary bladder (Brown et al. 1982). The effective (whole body) dose equivalent is 0.024 mSv/MBq (International Commission on Radiological Protection 1987). The effective dose in adults (70 kg) resulting from 185 MBq (5 mCi) of intravenously injected ^{99m}Tc-IDA complex is 4.4 mSv.

Storage and Stability

Storage. The lyophilized kit is stored at 2–8 °C.

Stability. The ^{99m}Tc-IDA complex is stable for 6 h after preparation.

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12.10 ^{99m}Tc-Labeled Peptides

12.10.1 ^{99m}Tc-Depreotide

I. Zolle

Chemical name	**Chemical structure**
Depreotide-trifluoroacetate ^{99m}Tc(V)O-depreotide Technetium Tc 99m depreotide injection (*USP*)	Depreotide = synthetic cyclic decapeptide: cyclo-(*N*-Me)Phe-Tyr-(D-Trp)-Lys-Val-Hcy(CH_2CO-(β-Dap)-Lys-Cys-Lys–NH_2) Hcy = L-homocysteine β-Dap = L-1,2-diaminopropionic acid
Kit components	**Commercial products**
Depreotide 47.0 μg resp. 50.0 μg Tin(II)-chloride dihydrate 50.0 μg Sodium D-glucoheptonate (gluceptate) dihydrate 5 mg Edetate disodium dihydrate 0.1 mg HCl and/or NaOH	NeoSpect GE Healthcare NeoTect Berlex Laboratories

Preparation

The commercial kits contain the sterile, lyophilized components including preformed synthetic peptide in a nitrogen atmosphere, as a single dose. Labeling with ^{99m}Tc-pertechnetate injection is carried out under aseptic conditions by adding a volume of exactly 1 ml of eluate to the vial at ambient temperature. The ^{99m}Tc activity should not exceed 1.8 GBq (50 mCi). Before removing the syringe, an equal volume of headspace should be withdrawn to normalize the pressure in the vial. The shielded vial is agitated carefully for 10 s to dissolve the lyophilized material, and then the vial is placed into a lead-shielded boiling water bath for 10 min. After the labeling procedure, the vial is placed into the lead shield and cooled at room temperature for approximately 15 min.

The vial must not be cooled under running water (product monographs, Amersham Healthcare 2000; Berlex Laboratories 2001).

^{99m}Tc-depreotide is a clear, aqueous solution suitable for intravenous injection, used as a single dose. The pH of the injection solution is 6.0–8.0 (United States Pharmacopeial Convention 2005).

Description of the Kit

Kits NeoSpect and NeoTect have almost identical composition, labeling of the cyclic decapeptide with ^{99m}Tc is performed by ligand exchange of intermediary ^{99m}Tc-glucoheptonate, using stannous ion for reduction and heating for 10 min in a boiling water bath. The injection solution should be inspected visually for particulate matter and discoloration before administration. Oxidative processes interfere with the labeling reaction.

Only eluates from generators eluted regularly within 24 h after the previous elution may be used for labeling.

The amount of ^{99m}Tc activity (up to 1.8 GBq resp., 50 mCi) used for labeling is based on calculations recommending activities of 555–740 MBq (15–20 mCi) for injection at a certain time after labeling, and on using a total volume of 1 ml of ^{99m}Tc-depreotide (47 resp., 50 μg) for one patient.

Kits NeoSpect and NeoTect contain no antimicrobial agents. Saline used for dilutions must be prepared without addition of any bacteriostatic agent (product monographs, Amersham Healthcare 2000; Berlex Laboratories 2001).

Depreotide (P829 Diatide Inc.) is a synthetic peptide comprising two separate domains. The pharmacophore is part of a cyclic hexapeptide, to which a linear tetrapeptide is appended via the thiol group of the homocysteine residue, Hcy(CH_2CO). The linear sequence Ala-Lys-Cys-Lys-NH_2 constitutes the structural requirements for complex formation with technetium. Cyclic configuration avoids reductive cleavage during labeling with Tc-99m, which has been observed with small peptides containing an accessible disulfide bridge (Vallabhajosula et al. 1996).

^{99m}Tc-depreotide contains a triamide-thiol chelate (N_3S), which coordinates oxotechnetium, having a Tc(V)O core.

Clinical Applications

^{99m}Tc-depreotide is valuable for scintigraphic imaging of solitary pulmonary nodules in combination with computer tomography (CT) or chest x-ray, in patients suspected of malignancy.

Lung scintigraphy: Somatostatin receptor imaging of non–small cell lung cancer.

Somatostatin is a neuroregulatory peptide secreted by the hypothalamus. Human somatostatin receptors (ssts) are expressed in the brain, anterior pituitary gland, pancreas, thyroid gland, and in the mucosa of the gastrointestinal tract (Hofland and Lamberts 1997; Patel 1999). Tumors arising from sst-positive cells in these organs contain a high density of ssts (Hofland et al. 2003; Lamberts et al. 1991; Reubi 1996).

The somatostatin analog depreotide consists of ten amino acids containing the key ssts-binding amino acid residues – Tyr-(D-Trp)-Lys-Val – protected in a cyclic configuration, increasing in vivo stability. Depreotide binds with high affinity to sst-positive tumors and their metastases (Vallabhajosula et al. 1996).

The labeled analogue, ^{99m}T-depreotide (P829), has been first used in patients (Virgolini et al. 1998) and evaluated for the detection of solitary pulmonary nodules in a multicenter trial (Blum et al. 1999, 2000). Experience gained with this ssts-binding radioligand in the diagnosis of non–small cell lung cancer has been reported (Menda and Kahn 2002). Comparative studies using ^{99m}T-depreotide and ^{18}F-FDG positron emission tomography (PET) in patients suspected of non–small cell lung cancer demonstrated high sensitivity of both functional imaging methods for detecting lung cancer in primary lesions and in hilar and mediastinal lymph nodes (Kahn et al. 2004). Another study investigated the involvement of regional lymph nodes in 56 patients with lung cancer and in 30 patients with benign lung lesions, using ^{99m}T-depreotide single-photon emission computer tomography (SPECT) and CT to enhance staging accuracy. Scintigraphic results displayed high sensitivity (93.7–99%), demonstrating that a negative result with ^{99m}T-depreotide can exclude regional lymph node metastases with a high degree of probability (Danielsson et al. 2005). Response to endocrine therapy in advanced breast cancer patients could be predicted using sequential ^{99m}Tc-depreotide scintigraphy, thus selecting nonresponders as early as 3 weeks after initiation of treatment (Van den Bossche et al. 2006).

Time of Examination. The optimal time for SPECT imaging is 2–4 h after the intravenous injection.

Recommended Activities for Indications

Lung scintigraphy: 555–740 MBq (15–20 mCi), injected intravenously
≤0.7 μg/kg body weight

Pediatric Dose. The application of ^{99m}Tc-depreotide to patients less than 18 years is not recommended. No data are available for this age group.

Additional Information

The diagnostic application of ^{99m}Tc-depreotide is restricted to a single intravenous injection.

Contraindications are a known hypersensitivity against depreotide, or against another kit component.

^{99m}Tc-depreotide should not be mixed with other drugs or components and should be injected separately.

Attention should be given to patients with reduced kidney function because of decreased renal excretion causing an increased radiation exposure. Attention should also be given to patients with reduced liver function.

Patients should drink sufficient water and should be encouraged to frequent bladder emptying during the first hours after injection.

Since depreotide binds to somatostatin receptors, caution should be paid to patients with insulinoma or diabetes mellitus.

Quality Control

Radiochemical Purity. ^{99m}Tc-depreotide is not described in the *European Pharmacopeia*. Thin-layer chromatography (TLC) is recommended by the manufacturer, using instant (I)TLC-silica gel (SG) strips as a stationary phase and analysis in two solvent systems. Using methanol/1 M ammonium acetate 1:1 (MAM) as mobile phase, the insoluble ^{99m}Tc components are measured at the start ($R_f = 0–0.4$). With saturated NaCl solution as mobile phase, free ^{99m}Tc-sodium pertechnetate, ^{99m}Tc-glucoheptonate, and ^{99m}Tc-edetate move with the solvent front and are measured at $R_f = 0.75–1.0$.

The radiochemical purity of ^{99m}Tc-depreotide should not be less than 90% (United States Pharmacopeial Convention 2005).

The radiochemical purity should be analyzed prior to administration of ^{99m}Tc-depreotide.

Methods Recommended by the Manufacturer

Thin-layer chromatography

Stationary phase: Gelman ITLC-SG strips (precut to 2.0×10 cm)
Solvent I: MAM
Solvent II: Saturated sodium chloride solution (SSCS)

Procedure

Aliquots are spotted and analyzed without drying.

- System I: Gelman ITLC-SG fiberglass plates and MAM as solvent: Reduced, hydrolized ^{99m}Tc-technetium is measured at the start (A); ^{99m}Tc-depreotide and free ^{99m}Tc-Na-pertechnetate as well as the ^{99m}Tc complexes (gluceptate and edetate) move with the solvent front.
- System II: Gelman ITLC-SG fiberglass plates and saturated sodium chloride as solvent: ^{99m}Tc-depreotide and colloidal ^{99m}Tc activity remain at the start; ^{99m}Tc-Na-pertechnetate and the ^{99m}Tc-complexes (gluceptate and edetate) are measured at the solvent front (B).

Table 1. Thin-layer chromatography on silica gel plates using two solvent systems

System I (MAM)	Reduced, hydrolized ^{99m}Tc activity at the start:	**(A) < 3%**
System II (SSCS)	^{99m}Tc-depreotide and colloidal ^{99m}Tc activity at origin Free ^{99m}Tc-pertechnetate and the ^{99m}Tc-complexes (gluceptate and edetate) at the solvent front:	**(B)**
	A and B represent labeled impurities: **< 10%**	
	^{99m}Tc-depreotide (%) = 100–%(A+B)	

Preparation of saturated sodium chloride solution (SSCS)

Five grams of sodium chloride are dissolved in 10 ml distilled water in the TLC tank. Over a period of 10–15 min, the tank is swayed repeatedly. Undissolved sodium chloride should settle at the bottom. If all sodium chloride dissolves, more sodium chloride should be added.

Preparation of MAM

- 1 M ammonium acetate: 3.9 g ammonium acetate is dissolved in 50 ml distilled water, the solution is stable for one month.
- Methanol/1 *M* ammonium acetate (1:1): one part methanol is mixed with one part of the 1 M ammonium acetate solution. This solution should be prepared every day.

Method of analysis (TLC)

1. Fill the TLC tanks (beakers) each with 0.5 ml of MAM or SSCS.
2. Close the tanks (beakers) and allow them to equilibrate.
3. Apply 1 drop of ^{99m}Tc-depreotide (5–10 µl), using a 1-ml syringe with a 21-gauge needle onto each of the Gelman ITLC-SG strips, 1.0 cm from the bottom. Do not allow the spot to dry.
4. Develop each plate in the covered TLC tank, using MAM and SSCS, respectively, until the upper edge of the plates is reached.
5. Allow the plates to dry.
6. Cut the ITLC-SG MAM plate at $R_f = 0.4$ (A).
7. Cut the ITLC-SG SSCS plate at $R_f = 0.75$ (B).
8. Measure the radioactivity of each portion in a counter and record separately.
9. Calculate the percentage of A and B as a fraction of the sum of recovered counts.
10. Calculate the radiochemical purity of ^{99m}Tc-depreotide (Table 1).

Pharmacokinetic Data

After intravenous injection of ^{99m}Tc-depreotide, the elimination from blood is described by three effective half-times, namely $T_{1/2} \leq 5$ min, 45 min, and 22 h. Approximately 12% of injected ^{99m}Tc-depreotide is bound to plasma proteins (5-min plasma sample). Four hours after injection, 71–84% of activity in blood was bound to depreotide, in the urine less, namely, 61–64% (NeoTect product monograph, Berlex Laboratories 2001).

High-affinity binding to somatostatin receptors was demonstrated in vivo using Lewis rats bearing CA20948 rat pancreatic tumor implants, and in vitro using human tumor cell membranes. Tumor uptake in rats was 4.9% injected dose (ID)/g at 90 min after injection, compared with 2.9% in the case of ^{111}In-(diethylene triamine pentaacetate [DTPA])octreotide (Vallabhajosula et al. 1996).

In patients, specific uptake in single pulmonary nodules is seen 1.5–2 h postinjection, also in regional lymph nodes overexpressing somatostatin receptors. Lymphoreticular response affects initially hilar, mediastinal, supraclavicular and axillary nodes, extending to distant nodes and the spleen (Danielsson et al. 2005).

Increased uptake of ^{99m}Tc-depreotide is seen in the spine, sternum and rib ends, and somewhat lower in the hilar and mediastinal regions. Nonspecific mediastinal uptake of ^{99m}Tc-depreotide has been reported (Menda et al. 2001).

^{99m}Tc-depreotide is not metabolized and is excreted unchanged (>90%). The major route of excretion is the renal system. Four hours after injection, 12% of injected radioactivity is measured in the urine (NeoTect product monograph, Berlex Laboratories 2001). The average renal clearance amounts to approximately 0.3 ml/min/kg. The average overall clearance is 2–4 ml/min/kg. External whole-body scintigraphy localized the highest activity in the abdomen (Kahn et al. 2004). Gastrointestinal excretion is ≤5%.

A fraction of the injected activity is retained in the kidneys, indicating proximal tubular reabsorption, as has been observed with ^{111}In-(DTPA)octreotide (Vallabhajosula et al. 1996).

Radiation Dose

^{99m}Tc-depreotide is excreted by the kidneys. The most exposed organs are the kidneys and urinary bladder, as well as the spleen, the liver, and the thyroid gland.

The effective (whole body) dose equivalent for ^{99m}Tc-depreotide was calculated as 0.016 mSv/MBq (International Commission on Radiological Protection 1990). The effective dose in adults (70 kg) resulting from 740 MBq (20 mCi) of intravenously injected ^{99m}Tc-depreotide is 11.84 mSv. The values are calculated assuming a 4.8-h bladder voiding period.

The absorbed radiation dose to the lung resulting from an intravenous injection of 555 MBq (15 mCi) of ^{99m}Tc-depreotide for lung scintigraphy is 7.8 mGy.

Storage and Stability

Storage. Kits should be stored at –10 °C or below. Keep the ^{99m}Tc-depreotide injection solution at 15–25 °C.

Stability. ^{99m}Tc-depreotide injection solution is stable for 5 h.

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12.11 ^{99m}Tc-Labeled Monoclonal Antibodies

12.11.1 ^{99m}Tc-Arcitumomab

F. Rakiás

Chemical name

Arcitumomab IMMU-4 Fab′ anti-CEA monoclonal antibody fragments

Technetium Tc 99m arcitumomab injection (*USP*)

^{99m}Tc-arcitumomab injection

^{99m}Tc-CEA-Scan injection

Kit components		**Commercial products**	
Arcitumomab	1.25 mg	CEA-Scan	Immunomedics Europe
Stannous chloride	0.29 mg		
Potassium sodium tartrate tetrahydrate			
Sodium acetate trihydrate			
Sodium chloride			
Sucrose			

Preparation

The carcinoembryonic antigen (CEA)-Scan kit contains the lyophilized, sterile components in argon atmosphere, ready for aseptic labeling with ^{99m}Tc-sodium pertechnetate. Labeling is carried out by adding 1.0 ml of ^{99m}Tc activity to the vial, not exceeding 1.11 GBq/ml (30 mCi/ml). The vial is allowed to react at room temperature for at least 5 min, and then 1 ml saline is added to the labeled product and mixed well.

^{99m}Tc-CEA-Scan injection is a clear, colorless solution suitable for intravenous injection, used as a single dose (740–1100 MBq; resp., 20–30 mCi). The total volume is 2 ml. The pH of the injection solution is 5.0–7.0. The injection solution should be inspected visually for particulate matter and discoloration. If either is present, the product should be discarded (Immunomedics Europe 2000).

Description of the Kit

Each 3-ml vial contains 1.25 mg arcitumomab (IMMU-4 Fab′ anti-CEA monoclonal antibody fragments, consisting mainly of Fab′, but also containing $F(ab')_2$ at ≤5% of total protein, with heavy- and light-chain fragments), buffered at pH 5.0–7.0. Before labeling, the ^{99m}Tc activity should be adjusted to a final concentration of 1.11 GBq/ml (30 mCi/ml), using a 2-ml vial.

Isotopic dilution is observed with higher concentrations of ^{99}Tc in the first eluate; therefore, only eluates from generators eluted regularly within 24 h after the previous elution may be used for labeling (Ponto et al. 1987). The CEA-Scan kit contains no antimicrobial agents (Immunomedics Europe 2000).

Clinical applications

^{99m}Tc-CEA-Scan injection solution is indicated for the detection of malignant lesions in patients with a history of colorectal carcinoma and with evidence of recurrence and/or metastases. ^{99m}Tc-CEA-Scan is employed as an adjunct to standard noninvasive imaging techniques, such as ultrasonography or computer tomography (CT), in the following situations:

- Patients with evidence of recurrence and/or metastatic carcinoma of the colon or rectum, who are undergoing an evaluation for the extent of disease, such as prior to surgical resection and/or other therapy
- Patients with suspected recurrence and/or metastatic carcinoma of the colon or rectum in association with rising levels of CEA

The specificity of monoclonal antibodies for the detection of heterogenous carcinoembryonic antigen posed a considerable uncertainty (Primus 1983). The metabolism and kinetics of labeled antibodies affected the detection of liver metastases of colorectal cancer (Behr et al. 1995). The utility of external immunoscintigraphy with the IMMU-4 technetium-99m Fab′ antibody fragment was evaluated in patients undergoing surgery for carcinoma of the colon and rectum in a phase III clinical trial (Moffat et al. 1996). Both radioimmunoscintigraphy and computed tomography were used for predicting

the resectability of recurrent colorectal cancer (Hughes et al. 1997). The safety and efficacy of repeated administrations of arcitumomab was demonstrated in patients with colorectal cancer (Wegener et al. 2000). Of considerable importance is the diagnosis of recurrence of colorectal carcinoma (Willkomm et al. 2000) and the detection of resectable rectal cancer recurrence by CEA immunoscintigraphy (Lechner et al. 2000).

Time of Examination. A whole-body planar scan at 2–5 h postinjection can be used to localize sites of colorectal cancer.

Immunoscintigraphy, using planar and single-photon emission computer tomography (SPECT) techniques, should be performed preferably 1–5 h after injection.

Recommended Activities for Indications

Planar and SPECT imaging: 750–1100 MBq (20–30 mCi), injected intravenously (30 s)
1 mg of Fab′ fragment

Since the recommended adult dose of Fab fragment injected as a single dose is limited with 1 mg, 1.6 ml of the injection solution may be used for one patient.

Pediatric Dose. Recommendations by the Pediatric Task Group of the European Association of Nuclear Medicine (EANM) based on body weight should be followed (see Appendix 1, Table A1.2).

Additional Information

Patients should be encouraged to drink sufficient water and to empty the bladder before scintigraphy is started. Frequent bladder emptying is recommended to reduce the radiation exposure to the bladder wall.

Patients with known allergies or hypersensitivity to mouse proteins, human anti-mouse antibodies (HAMA) titers should be determined before administration of ^{99m}Tc-CEA-Scan.

Doses of arcitumomab up to 10 mg have not shown any serious adverse reaction.

^{99m}Tc-CEAScan should be used only once in each patient (Immunomedics Europe 2000).

Any remaining portion of the injection solution should be discarded.

Quality Control

Radiochemical Purity

^{99m}Tc-CEA-Scan is not described in the Eur. Pharmacopoeia. Thin-layer chromatography is recommended by the manufacturer using instant (I)TLC-silica gel (SG) fiberglass sheets for the identification of free ^{99m}Tc-Na-pertechnetate, using acetone as solvent. Unbound ^{99m}Tc-pertechnetate moves with the solvent front ($R_f = 1.0$). The insoluble ^{99m}Tc components are measured at the start.

The radiochemical purity of ^{99m}Tc-arcitumomab should not be less than 90% (*USP*).

The analysis should be performed prior to administration of ^{99m}Tc-CEA-Scan in the patient.

Thin-layer chromatography		
Stationary phase:	Gelman ITLC-SG fiberglass, 1×9.5 cm	
Solvent:	Acetone	
Developing time:	5 min	
R_f values:	^{99m}Tc-arcitumomab	0.0–0.1
	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-pertechnetate:	0.9–1.0 (<10%)

Procedure

- A 10 µl sample of the labeled antibody is diluted with 1.5 ml saline, and immediately spotted for thin-layer chromatography.
- When the solvent front is within 1 cm of the top, the strip is removed, dried, and analyzed in a radiochromatogram scanner.
- Otherwise, the strip is cut into half and placed into two test tubes for measurement with a gamma scintillation counter or a dose calibrator.

Calculate the percent impurity as follows:

$$^{99m}\text{Tc-pertechnetate}\ (\%) = \frac{\text{Activity in upper piece}}{\text{Activity in both pieces}} \times 100$$

Results of analysis *(12 samples)*
Results were obtained by thin-layer chromatography in acetone, at different times after labeling.

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-Arcitumomab	95.3 ± 0.32	92.7 ± 0.44
^{99m}Tc-Na-pertechnetate	4.7 ± 0.48	7.3 ± 0.21

Pharmacokinetic Data

Pharmacokinetic studies were performed after the intravenous administration of ^{99m}Tc-CEA-Scan. The elimination from the blood is indicated by 63% of baseline activity at 1 h after infusion, 23% after 5 h, and 7% after 24 h. The distribution half-time is approximately 1 h; the elimination from blood follows a half-time of approximately 13 h. Twenty-eight percent of the administered activity is excreted in the urine during the first 24 h after infusion (Immunomedics Europe 2000).

CEA is expressed in a variety of carcinomas, particularly of the gastrointestinal tract (e.g., Crohn's disease, inflammatory bowel disease, post–radiation therapy to the bowel) and can be detected in the serum. IMMU-4 is specific for the classical 200 000-Da CEA that is found predominantly on the cell membrane. ^{99m}Tc-CEA-Scan complexes the circulating CEA and binds to CEA on the cell surface. Imaging efficacy and safety have been evaluated in four clinical trials to evaluate the presence, location, and extent of colorectal cancer, primarily in the liver and extrahepatic abdominal and pelvic regions.

Radiation Dose

^{99m}Tc-CEA-Scan is excreted by the kidneys. The most exposed organs are the kidneys and urinary bladder, along with the spleen and liver.

The effective (whole body) dose equivalent for ^{99m}Tc-CEA-Scan was calculated as 0.0131 mSv/MBq (International Commission on Radiological Protection 1990). The effective dose in adults (70 kg) resulting from 750 MBq (20.3 mCi) of intravenously injected ^{99m}Tc-CEA-Scan is approximately 9.8 mSv. The values were calculated assuming a 2-h bladder voiding period.

The absorbed radiation dose to the kidneys resulting from an intravenous injection of 750 MBq (20.3 mCi) of ^{99m}Tc-CEA-Scan for colon scintigraphy is calculated as 75 mGy, the absorbed radiation dose to the spleen as 11.9 mGy (based on data obtained by the standard medical internal radiation dose [MIRD] method) (Immunomedics Europe 2000).

Storage and Stability

Storage. Lyophilized kits should be stored at 2–8 °C, and not frozen. The ^{99m}Tc-CEA-Scan injection solution should be kept at 15–25 °C, and not refrigerated or frozen.

Stability. ^{99m}Tc-CEA-Scan injection solution is stable for 4 h.

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12.11.2 ^{99m}Tc-Sulesomab

F. Rakiás

Chemical name	
Sulesomab, IMMU-MN3 Fab′-SH antigranulocyte monoclonal antibody fragments ^{99m}Tc-Sulesomab injection	
Kit components	**Commercial products**
Sulesomab 0.31 mg	LeukoScan Immunomedics Europe
Stannous chloride, dihydrate 0.22 mg	
Sodium chloride	
Hydrochloric acid	
Sodium potassium tartrate, tetrahydrate	
Sodium acetate, trihydrate	
Sucrose	

Preparation

The kit contains the lyophilized, sterile, pyrogen-free, inactive components in an argon or nitrogen atmosphere, ready for labeling with ^{99m}Tc-sodium pertechnetate. Labeling is carried out by first adding 0.5 ml of isotonic saline and swirling the content for 30 s; immediately after dissolution, 1 ml ^{99m}Tc-pertechnetate is added to the shielded vial, corresponding to an activity of at least 1,100 MBq (30 mCi).

^{99m}Tc-LeukoScan is a clear, colorless solution suitable for intravenous injection, used as a single dose. The total volume is 1.5 ml. The pH of the injection solution is 5.0–7.0. The injection solution should be inspected visually for particulate matter and discoloration. If either is present, the product should be discarded (Immunomedics Europe 1997).

Description of the Kit

Each 3-ml vial contains a lyophilized powder of sulesomab (IMMU-MN3 Fab′-SH antigranulocyte monoclonal antibody fragment, consisting mainly of Fab′, but also containing $F(ab')_2$ at 5% of total protein, with heavy- and light-chain fragments) buffered to pH 5.0–7.0. The powder is dissolved by agitation during 30 s; the labeling reaction is completed after 10 min at room temperature.

Isotopic dilution is observed with higher concentrations of ^{99}Tc in the first eluate; therefore, only eluates from generators eluted regularly within 24 h after the previous elution may be used for labeling (Ponto et al. 1987). The LeukoScan kit contains no antimicrobial agents (Immunomedics Europe 1997).

Clinical Applications

^{99m}Tc-LeukoScan has been evaluated as a marker of infection/inflammation in patients with suspected osteomyelitis, joint infection involving implants, inflammatory bowel disease, and diabetic patients with foot ulcers. These heterogeneous patients have been diagnosed with high sensitivity (>93%), comparable to bone scans with diphosphonates, but with considerably higher specificity (>91%), offering a reliable imaging method based on the specificity of sulesomab, a murine anti–granulocyte monoclonal antibody Fab′ fragment. In clinical trials with more than 350 patients, no induction of human anti-mouse antibody (HAMA) to antibody fragments has been observed.

When a bone scan is positive and imaging with ^{99m}Tc-LeukoScan is negative, infection is unlikely. Single-photon emission computed tomography (SPECT) imaging may aid in differentiating osteomyelitis from soft tissue infections (Becker et al. 1994). The advantages of rapid imaging in different conditions of infection have been investigated (Barron et al. 1999; Becker et al. 1996; Gratz et al. 2000, 2003; Hakki et al. 1997; Harwood et al. 1999; Kampen et al. 1999).

Time of Examination. Planar scintigraphy should be performed anytime between 1 and 8 h after injection of ^{99m}Tc-LeukoScan.

Recommended Activities for Indications

Planar and SPECT imaging: 750–1,100 MBq (20–30 mCi), injected intravenously
0.25 mg of Fab′ antigranulocyte fragment

Since the recommended adult dose of Fab′ antigranulocyte fragment injected as a single dose is limited with 0.25 mg, 1.2 ml of the injection solution may be used for one patient.

Pediatric Dose. The amount of radioactivity for infants and children is based on either body weight (static imaging) or body surface area (dynamic examinations). Recommendations by the Pediatric Task Group of the European Association of Nuclear Medicine (EANM) based on body weight should be followed (see Appendix 1, Table A1.2).

Additional Information

Patients should drink sufficient water and be encouraged to empty the bladder before scintigraphy is started to reduce the radiation exposure to the bladder wall.

HAMA titers should be determined before repeated administration of ^{99m}Tc-LeukoScan.

Any remaining portion of the injection solution should be discarded.

Quality Control

Radiochemical Purity

Thin-layer chromatography

The manufacturer recommends TLC on instant (I)TLC-silica gel (SG) fiberglass sheets for the identification of free ^{99m}Tc-Na-pertechnetate, using acetone as solvent. Unbound ^{99m}Tc-pertechnetate moves with the solvent front ($R_f = 1.0$).

The radiochemical purity of ^{99m}Tc-LeukoScan should not be less than 90% (Immunomedics Europe 1997). The radiochemical purity should be analyzed prior to administration of ^{99m}Tc-LeukoScan.

Thin-layer chromatography		
Stationary phase:	Gelman ITLC-SG (fiberglass), 1×9.5 cm	
Solvent:	Acetone	
Developing time:	5 min	
R_f values:	^{99m}Tc-sulesomab	0.0–0.1
	^{99m}Tc reduced, hydrolized:	0.0–0.1
	^{99m}Tc-pertechnetate:	0.9–1.0 (<10%)

Procedure

- A 10-µl sample of the labeled antibody is spotted undiluted and immediataly developed in acetone by thin-layer chromatography.
- When the solvent front is within 1 cm of the top, the strip is removed, dried, and analyzed in a radiochromatogram scanner.
- Otherwise, the strip is cut into half and placed into two test tubes for measurement with a gamma scintillation counter or a dose calibrator.

$$^{99m}\text{Tc-pertechnetate}\ (\%) = \frac{\text{Activity in upper piece}}{\text{Activity in both pieces}} \times 100$$

Results of analysis *(12 samples)*

Results were obtained by thin-layer chromatography in acetone, at different times after labelling.

Labeling and stability	15 min (%)	3 h (%)
^{99m}Tc-Sulesomab complex	96.8 ± 0.30	94.7 ± 0.38
^{99m}Tc-Na-pertechnetate	3.12 ± 0.15	5.3 ± 0.34

Pharmacokinetic Data

Pharmacokinetic studies were performed after the intravenous administration of ^{99m}Tc-LeukoScan. The elimination from the blood is indicated by 34% of baseline activity at 1 h after infusion, 17% at 4 h, and 7% after 24 h. The distribution half-time was approximately 1.5 h; the route of excretion is essentially renal, with 41% of the radiolabel excreted in urine over the first 24 h after injection (Immunomedics Europe 1997).

Radiation Dose

^{99m}Tc-sulesomab is excreted by the kidneys. The most exposed organs are the kidneys and urinary bladder, and the spleen and liver.

The effective (whole body) dose equivalent for ^{99m}Tc-sulesomab was calculated as 0.0103 mSv/MBq (International Commission on Radiological Protection 1990). The effective dose in adults (70 kg) resulting from 750 MBq (20.3 mCi) of intravenously injected ^{99m}Tc-LeukoScan is 7.7 mSv. The values were calculated assuming a 2-h bladder voiding period.

The absorbed radiation dose to the kidneys resulting from an intravenous injection of 750 MBq (20 mCi) of ^{99m}Tc-LeukoScan for diagnostic immunoscintigraphy is calculated as 33.7 mGy, the absorbed radiation dose to the spleen as 11.8 mGy (based on data obtained by the standard medical internal radiation dose [MIRD] method) (Immunomedics Europe 1997).

Storage and Stability

Storage. Lyophilized kits should be stored at 2–8 °C and not frozen. ^{99m}Tc-LeukoScint injection solution is kept at 15–25 °C and not refrigerated or frozen.

Stability. ^{99m}Tc-LeukoScan injection solution is stable for 4 h.

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