PRODUCT MONOGRAPH

CERETEC™

Kit for the Preparation of Technetium-99m Exametazime Injection

Reagent for Preparation of a Radiodiagnostic Agent

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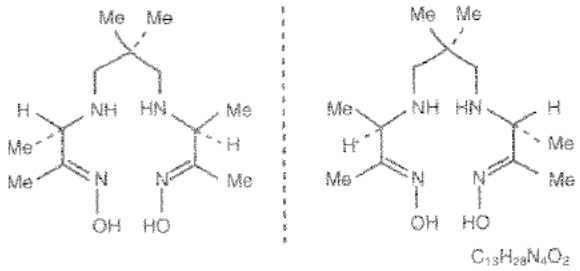
DESCRIPTION

The Ceretec kit is supplied as a five-unit package. Each unit consists of two vials: Ceretec reagent and cobalt stabilizer solution. These sterile, non-pyrogenic, non-radioactive ingredients are necessary to prepare Technetium-99m Exametazime intravenous injection with cobalt stabilizer solution, or to prepare Technetium-99m Exametazime intravenous injection without cobalt stabilizer solution.

The Ceretec kit is also supplied as a 5 vial kit, containing 5 vials of Ceretec reagent, without the cobalt stabilizer solution.

Each Ceretec multi-dose reagent vial contains a pre-dispensed, sterile, non-pyrogenic, freeze dried mixture of 0.5 mg exametazime, 7.3 µg stannous chloride dihydrate and 4.5 mg sodium chloride. Following freeze-drying, the vial is filled with inert nitrogen atmosphere to a pressure just below atmospheric and is sealed with a rubber closure. The product contains no antimicrobial preservative.

The structural formula of exametazime is:



$C_{13}H_{28}N_4O_2$

Prior to publication of the USAN, exametazime was formerly known as hexamethylpropylene amine oxime (HM-PAO). The name HM-PAO appears in many publications. Also known as (RR,SS)-4,8 diaza-3,6,6,9-tetramethyl undecane-2,10-dione bisoxime.

Each vial of sterile, non-pyrogenic cobalt stabilizer solution contains 200 µg cobalt chloride 6-hydrate stabilizer solution in 2 ml of Water for Injection. When used according to the preparation instructions (see Dosage and Administration), cobalt stabilizer solution acts as a stabilizer.

Physical Characteristics

Technetium Tc99m decays by isomeric transition with a physical half-life of 6.02 hours[(18)]. Photons that are useful for detection and imaging studies are listed in Table 1.

Table 1. Principal Radiation Emission Data

Radiation	Mean %/ Disintegration	Mean Energy (KeV)
Gamma-2	87.87	140.5

External Radiation

The specific gamma ray constant for Tc99m is 206µCkg^{-1}/37MBq-hr.(0.78R/millicurie-hr.) at l cm. The first half-value layer is 0.02 cm of Pb. A range of values for the relative attenuation of the radiation emitted by this radionuclide that results from interposition of various thicknesses of Pb is shown in Table 2**.** For example, the use of a 0.25 cm thickness of Pb will attenuate the radiation emitted by a factor of about 1,000.

Table 2. Radiation Attenuation by Lead Shielding

Shield Thickness (Pb) cm	Coefficient of Attenuation
0.02	0.5_{-1}
0.08	10^{-2}
0.16	10^{-3}
0.25	10^{-4}
0.33	

To correct for physical decay of this radionuclide, the fractions that remain at selected intervals after time of calibration are shown in Table 3.

Table 3. Physical Decay Chart: Tc99m, Half-Life 6.02 Hours

Hours	Fraction Remaining	Hours	Fraction Remaining
*0	1.000	5	0.562
1	0.891	6	0.501
2	0.794	8	0.398
3	0.708	10	0.316
4	0.631	12	0.251

*Calibration time

CLINICAL PHARMACOLOGY

Regional cerebral blood flow scintigraphy

Conventional radioisotope brain scanning uses polar radiopharmaceuticals such as Tc99m pertechnetate which do not penetrate the normal brain. They image brain pathology by crossing the damaged blood brain barrier but even using single photon emission computed tomography (SPECT) are unable to match the resolution and detailed morphology provided by X-ray computerized tomography (CT).

The development of positron emission tomography (PET) and novel radiopharmaceuticals based on the short-lived positron emitting nuclides ^{11}C, ^{18}F, ^{15}O and ^{13}N made functional imaging of the brain possible and produced spectacular results[1]. This led to the search for agents which can cross the blood brain barrier and provide information on regional cerebral blood flow using conventional gamma camera and SPECT imaging.

In order to cross the blood brain barrier a substance must be uncharged, lipophilic and of low molecular weight. However, the ideal tracer must not only cross the blood brain barrier, but remain with a fixed distribution within the brain for a sufficiently long time to allow the acquisition of data for reconstruction of tomographic images. Although Xenon-133 has been widely used to measure regional cerebral blood flow it cannot be imaged without specialized instrumentation as it is rapidly washed out of the brain [2].

Though Iodine-123 labeled amines have been shown to cross the blood brain barrier and be retained[3], the ideal radiopharmaceutical product is one that complexes with a readily available radionuclide such as technetium-99m. The Tc99m complex of hexamethylpropylene amine oxime (HM-PAO) is not only taken into the brain but also shows long term retention [4,5,6]. In particular the RR,SS (d,l) diastereoisomer of HM-PAO (exametazime) provides a Tc99m complex which exhibits near-ideal characteristics [7,8,9].

When pertechnetate is added to the exametazime ligand in the presence of stannous reductant a lipophilic Tc99m complex is formed. This converts with time to a secondary complex which is less lipophilic. This secondary complex does not cross the blood brain barrier. A consequence of the conversion of lipophilic to secondary complex is that the useful life of the reconstituted agent is limited. The *in vitro* addition of cobalt stabilizer solution to the Tc99m exametazime will stabilize the complex for 5 hours. Cobalt stabilizer solution may be added to the Tc99m for cerebral imaging. Cobalt stabilizer solution must not be used in the preparation of Tc99m exametazime labeled leukocytes.

Studies in normal volunteers have shown that the Tc99m exametazime complex is rapidly cleared from the blood after intravenous injection. Uptake in the brain reaches a maximum of 3.5-7.0% of the injected dose within one minute of injection. Up to 15% of the cerebral activity washes out of the brain by 2 minutes post injection after which there is little loss of activity for the following 24 hours except by physical decay of Tc99m. The activity not associated with the brain is widely distributed throughout the body particularly in muscle and soft tissue. About 30% of the injected dose is found in the GI tract immediately after injection and about 50% of this is excreted through the gut over 48 hours. About 40% of the injected dose is excreted through the kidneys and urine over the 48 hours after injection resulting in a reduction in general muscle and soft tissue background.

In Vitro Tc99m leukocyte labeling

Leukocytes are involved in a number of the body's responses to disease including infection, inflammation and infarction. Techniques have been developed to tag leukocytes with a radiolabel using In 111, in order to subsequently assess sites of localization and consequently pathology using a gamma camera. In 111 labeled leukocytes are an established noninvasive means of diagnosing a variety of inflammatory conditions in which granulocyte migration is a prominent feature.[10-13]

The superior imaging characteristics of Tc99m have led to a search for a suitable method to label leukocytes with this nuclide. Labeling by means of complexes such as Tc99m oxine, Tc99m pyrophosphate and medronate and the incorporation of Tc99m colloids by phagocytes have been

proposed, but all suffer deficiencies either in label stability or in "activation" or damage to leukocytes during the labeling procedure, leading to an unnatural biodistribution on reinjection [14-15]

The small lipophilic nature of the Tc99m exametazime complex facilitates its uptake into leukocytes, following which the Tc99m is selectively retained in neutrophils. Provided the recommended cell separation and labeling procedures are carried out, the Tc99m labeled leukocytes do not appear to suffer significant damage or "activation", as evidenced by their *in vivo* recovery and lack of lung and liver uptake. Label elution rate is up to 10% in the first hour, declining thereafter.

Following cell separation and radiolabeling, according to the package insert instructions, a labeling efficiency of around 55% may be expected with around 78% of the label associated with the neutrophil population. Studies of elution rates indicate that Tc99m exametazime shows relative selectivity for granulocytes [16] and acts as an effective radiolabeling agent. Following reinjection of the Tc99m labeled leukocytes the functional integrity of the granulocytes appears to be well maintained as the recovery of radiolabeled granulocytes (i.e., the circulatory granulocyte associated activity as a percentage of injected granulocyte associated activity) at 40 minutes after injection gave a mean value of 37% [17] which compares favorably with pure granulocytes labeled with In 111 tropolonate.[12] The initial biodistribution is similar to that of In 111 tropolonate labeled pure granulocytes. During the first hour following injection of Tc99m labeled leukocytes, activity is seen in the lungs, liver, spleen, blood pool and bone marrow as well as in the bladder. The kidneys (parenchyma and/or renal pelvis) and gallbladder may also be visualized. This pattern of activity continues to be seen at 4 hours post-injection except that lung activity is greatly reduced and faint bowel activity may be visible. At 24 hours post-injection substantial colonic activity is seen, in addition to the normal areas visualized in earlier scans.

TOXICOLOGY

Toxicity studies have been performed on intravenously administered Ceretec in male and female rats and rabbits.

Ceretec without cobalt stabilizer solution

No adverse reactions or mortalities were observed at a dose level equivalent to the single injection of 1200 times the maximum human equivalent dose (MHD). Similarly, 14-day repeat-dose studies in rats and rabbits at a cumulative dose of up to 14,000 times the maximum human equivalent dose resulted in no adverse reactions or mortalities. At termination, thorough histopathology, hematology and blood chemistry revealed no abnormalities.

Ceretec with cobalt stabilizer solution

There are no indications that the gross toxicity profile of the stabilized formulation of technetium-99m exametazime is significantly different from that of the non-stabilized formulation.

In-vitro mutagenicity studies indicate that the stabilized formulation of technetium-99m exametazime is weakly mutagenic in the Ames (bacterial mutation) test, human lymphocyte chromosome aberration assay and mouse lymphoma thymidine kinase assay. The stabilized formulation is found not to be mutagenic in two *in-vivo* assays (rat bone marrow micronucleus and rat liver micronucleus).

At quantities such as those encountered in stabilized technetium-99m exametazime preparations, cobalt ions or complexed forms of cobalt have no known adverse effects and are rapidly eliminated from the circulation by urinary excretion.

No signs of toxicity were observed in single dose toxicity studies in rats or rabbits administered 1000 or 2000 x MHD of cobalt-containing stabilized Ceretec. In repeated dose studies, no toxicologically significant effects were observed after 14 days of daily cobalt-containing stabilized Ceretec exposure to rats and rabbits up to 1000 x MHD.

INDICATIONS AND CLINICAL USES

Regional cerebral blood flow scintigraphy

Tc99m exametazime intravenous injection is used for regional cerebral blood flow scintigraphy. In stroke, reduced cerebral blood flow appears as photopenic areas on scintigrams. Tc99m exametazime scintigraphy may also be useful in investigations of transient ischemic attack, migraine and tumors of the brain.

In epilepsy, areas of both ictally increased and interictally decreased perfusion have been demonstrated. Characteristic areas of reduced perfusion have been demonstrated in Alzheimer's disease which may provide the basis for differential diagnosis of dementia.

In Vitro Tc99m Leukocyte Labeling

Tc99m Exametazime is an effective agent for *in vitro* Tc99m leukocyte radiolabeling. Tc99m labeled leukocytes are useful in the detection of sites of focal infection, especially abdominal abscess and as an adjunct in the investigation of pyrexia of unknown origin (PUO), and in the evaluation of inflammatory conditions not associated with infection such as inflammatory bowel disease (IBD).

CONTRAINDICATIONS

There are no specific contraindications.

WARNINGS

The possibility of hypersensitivity including serious signs and symptoms of anaphylaxis should always be considered. Advanced life support facilities should be readily available.

Care should be taken when handling blood specimens to be labeled using this radiopharmaceutical. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Imunodeficiency Virus (HIV) or other infectious agents are absent. All human blood samples should be considered potentially infectious. Precautions for handling are as those for handling radioactive materials.

The contents of the Ceretec kit are intended for use in the preparation of Tc99m exametazime injection and are NOT to be directly administered to the patient.

The contents of the kit are not radioactive. However, after the sodium pertechnetate Tc99m is added, adequate shielding of the final preparation must be maintained to minimize radiation exposure to occupational workers and patients.

Ideally, examinations using radiopharmaceuticals, especially those elective in nature, of women of childbearing capability should be performed during the first ten days following the onset of menses.

PRECAUTIONS

General

The Tc99m labeling reactions involved depend on maintaining the tin (stannous ion) in the reduced state. Hence, sodium pertechnetate Tc99m containing oxidants should not be employed.

Sodium Chloride Injection, USP must be used as the diluent. Do not use bacteriostatic sodium chloride as a diluent for sodium pertechnetate Tc99m injection because it will increase the oxidation products and adversely affect the biological distribution of Ceretec.

Radiopharmaceuticals should be used only by those medical practitioners who are appropriately qualified in the use of radioactive prescribed substances in or on humans.

As in the use of any other radioactive material, care should be taken to minimize radiation exposure to patients consistent with proper patient management, and to minimize radiation exposure to occupational workers.

The components of the reagent vials are sterile and nonpyrogenic. It is essential that the user follows directions carefully and adheres to strict aseptic technique.

It should also be noted that materials used in cell separation may cause hypersensitivity reactions. It is essential that cells are washed free of sedimentation agents before they are reinjected into the patient.

Carcinogenesis, Mutagenesis and Impairment of Fertility

Since adequate reproduction studies have not been performed in animals to determine whether this drug affects fertility in males and females, has teratogenic potential, or has other adverse effects on the fetus, this radiopharmaceutical preparation should not be administered to pregnant or nursing women unless it is considered that the benefits to be gained outweigh the potential hazards.

Nursing

Where an assessment of the risk/benefits ratio suggests use of this product in lactating mothers, nursing should be stopped.

Pediatric Use

Adequate studies do not exist to support the use in children. As in pregnancy and lactating mothers, the benefits to risk ratio should be assessed before consideration is given to the use of this product in this age group.

The safety and effectiveness of Technetium 99m exametazime with the cobalt stabilizer solution has not been established in the pediatric population and therefore is not recommended for administration to children.

Use in Pregnancy

In women of childbearing age the possibility of pregnancy should always be taken into account. It would be prudent to treat as pregnant any woman of reproductive age presenting for a nuclear medicine examination at a time when a menstrual period is overdue or missed, unless there is information that precludes pregnancy. If the menstrual cycle is irregular, a pregnancy test may be indicated before proceeding.

ADVERSE REACTIONS

Safety profile of Ceretec with cobalt stabilizer solution characterizes by mild to moderate hypersensitivity reactions evidenced by the development of rash, pruritus or erythema.

Reports of hypersensitivity reactions, possibly anaphylactic in nature, following administration of Tc99m labeled leukocytes prepared using Tc99m exametazime have been received.

Immune system disorders
Hypersensitivity including rash, erythema, urticaria, angioedema, pruritus.

Re-injected CERETEC labelled leukocytes only:
Hypersensitivity, anaphylactoid reaction or anaphylactoid shock

Nervous system disorders
Headache, dizziness, paraesthesia

Vascular disorders
Flushing

Gastrointestinal disorders
Nausea, Vomiting

General disorders and administration site conditions
Asthenic conditions (e.g. malaise, fatigue)

In case of side effects following administration of radiopharmaceuticals, users should ensure the availability of appropriate medical treatment at the time of administration of any radiopharmaceutical to the patient. Users are requested to report to GE Healthcare Canada Inc. any instances of suspected adverse drug reactions or side effects associated with the use of this product.

DOSAGE AND ADMINISTRATION

Regional cerebral blood flow scintigraphy

Shielding should be used at all times when handling both vial and syringes. Please refer to Cautionary Notes for injection procedure.

For adults (70kg), the usual administered activity is 555-1110 MBq (15 to 30 mCi) [19-20] by intravenous injection.
Brain imaging may commence from 2 minutes after injection.

Although gross abnormalities of regional cerebral blood flow may be visualized by planar imaging it is strongly recommended that SPECT imaging be carried out to maximize the value of the study.

In Vivo localization of Tc99m labeled leukocytes

For adults (70kg), the usual administered activity is 185-370 MBq (5 to 10 mCi) [21-22] of Tc99m labeled leukocytes by intravenous injection. Administer the Tc99m labeled leukocyte suspension using a 19G needle as soon as possible after labeling. Dynamic imaging may be performed for the first 60 minutes after injection to assess lung clearance and to visualize immediate cell migration.

Static imaging at 0.5-1.5 hours**,** 2-4 hours and if necessary, at 18-24 hours post injection should be performed to detect focal accumulation of activity. Care should be taken to distinguish between leukocyte localization and normal biodistribution.

INSTRUCTIONS FOR PREPARATION

Procedure for the Preparation of Tc99m Exametazime Injection with Cobalt Stabilizer Solution for Intravenous Injection

(Use aseptic technique and wear waterproof gloves throughout entire procedure)

NOTE: DO NOT USE THIS PROCEDURE FOR LEUKOCYTE LABELING. SEE PROCEDURE FOR THE PREPARATION OF Tc99m EXAMETAZIME INJECTION WITHOUT COBALT STABILIZER SOLUTION

1) Place the exametazime vial in a shielding container and sanitize the closure with an isopropyl alcohol swab.

2) Using a 10 ml syringe, inject into the shielded vial 5 ml of sterile eluate from a technetium-99m generator (see notes 1 - 6). Before withdrawing the syringe from the vial withdraw 5 ml of gas from the space above the solution to normalise the pressure in the vial. Shake the shielded vial for 10 seconds to ensure complete dissolution of the powder.

3) Between 1 and 5 minutes after reconstitution, inject 2 ml of cobalt stabilizer solution into the shielded vial using a 3 ml syringe. Before withdrawing the syringe from the vial, withdraw 2 ml of gas from the space above the solution to normalise the pressure in the vial. Shake the shielded vial for 10 seconds to ensure complete mixing.

4) Assay the total radioactivity and calculate the volume to be injected.

5) Complete the label provided and attach to the vial.

6) Use the stabilized product within 5 hours after preparation.
Individual patient doses may be stored aseptically in a capped syringe if required (see note 7).

7) Discard any unused material.

Note:

1) For the highest radiochemical purity reconstitute with freshly eluted technetium-99m generator eluate.

2) The technetium-99m generator eluate must be used within 4 hours of elution from a generator that has already been eluted within the previous 24 hours.

3) 0.37 - 1.11 GBq technetium 99m may be added to the vial.

4) Before reconstitution the generator eluate may be adjusted to the correct radioactive concentration (0.37 – 1.11 GBq in 5 ml) by dilution with sodium chloride for injection.

5) (^{99m}Tc) pertechnetate complying with the specifications prescribed by the USP and BP/Ph.Eur. Monographs on Sodium Pertechnetate (^{99m}Tc) Injection should be used.

6) The cobalt stabilized technetium-99m exametazime is a pale straw-coloured solution and the pH is in the range 5.0 - 8.0.

7) When stabilized Ceretec preparations are transferred to individual patient syringes, a small volume of the headspace gas must be withdrawn from the vial into the syringe after solution transfer to ensure that no solution remains in contact with the syringe needle prior to administration to the patient.

8) The shelf life of the reconstituted Ceretec component without the addition of the cobalt stabilizer solution is only 30 minutes.

Radiochemical Purity Measurement

Three potential radiochemical impurities may be present in prepared Technetium (^{99m}Tc) Exametazime Injection. These are a secondary ^{99m}Tc-exametazime complex, free (^{99m}Tc)-pertechnetate and reduced-hydrolysed-technetium-99m. A combination of two chromatographic systems is necessary for the determination of the radiochemical purity of the injection.

Test samples are applied by needle approximately 2.5 cm from the bottom of two Glass Microfiber Chromatography Paper impregnated with Silicic Acid (GMCP-SA) strips (2 cm (±2 mm) x 20 cm).

The strips are then immediately placed in prepared ascending chromatography development tanks, one containing butan-2-one and the other 0.9 % sodium chloride (1cm depth fresh solvent).

After a 14 cm elution the strips are removed, solvent fronts marked, the strips dried and the distribution of activity determined using suitable equipment.

Interpretation of chromatograms

System 1 (GMCP-SA:butan-2-one (methyl ethyl ketone))
Secondary ^{99m}Tc-exametazime complex and reduced-hydrolysed-technetium-99m remain at the origin.
Lipophilic ^{99m}Tc-exametazime complex and (^{99m}Tc)-pertechnetate migrate at R_f 0.8-1.0.

System 2 (GMCP-SA:0.9% sodium chloride)
Lipophilic ^{99m}Tc-exametazime complex, secondary ^{99m}Tc-exametazime complex and reduced-hydrolysed-technetium-99m remain at the origin.
(^{99m}Tc)-pertechnetate migrates at R_f 0.8-1.0.

1) Calculate the percentage of activity due to both secondary ^{99m}Tc exametazime complex and reduced-hydrolysed-technetium-99m from System 1 (A %). Calculate the percentage of activity due to (^{99m}Tc)-pertechnetate from System 2 (B %).

2) The radiochemical purity (as percentage lipophilic technetium-99m exametazime complex) is given by:

   100 - (A %+B %) where:

   A % represents the level of secondary technetium-99m exametazime complex plus reduced-hydrolysed technetium-99m.
   B % represents the level of (^{99m}Tc)-pertechnetate.

A radiochemical purity of at least 80 % may be expected provided the test samples have been taken and analysed within 5 hours of the preparation of the stabilized product.

Procedure for Preparation of Tc99m Exametazime Injection without Cobalt Stabilizer Solution for Intravenous Injection or *In Vitro* Leukocyte Labeling

(Use aseptic technique and wear waterproof gloves throughout the entire procedure).

1) Place the vial in a suitable shielding container and sanitize the rubber septum with an isopropyl alcohol swab.

2) Using a l0mL syringe, inject into the shielded vial 5mL of sterile additive-free eluate from a Tc99m generator (see cautionary notes 1-5). Before withdrawing the syringe from the vial withdraw 5 mL of gas from the space above the solution to normalize the pressure in the vial. Gently swirl the shielded vial for 10 seconds to ensure complete dissolution of the powder.

3) Assay the total activity and calculate the volume to be injected. The patient dose should be measured in a suitable radioactivity calibration system immediately prior to administration.

4) Complete the label provided and attach to the vial shield. The technetium Tc99m exametazime injection is ready for quality control.

5) Maintain adequate shielding of the radioactive preparation.

6) Use within a maximum of **30 minutes** after reconstitution. Discard any unused material in accordance with Canadian radioactive waste regulations.

7) Visually inspect the reconstituted material and do not use if there is evidence of foreign matter.

8) The injection may be prepared for use in cerebral scintigraphy or in the preparation of Tc99m labeled leukocytes.

9) The pH of the prepared injection is 9.0-9.8

10) The patient dose should be measured in a suitable radioactivity calibration system immediately prior to administration

Cautionary Notes

1) 0.37 - 2.0 GBq (10 - 54 mCi) technetium Tc99m sodium pertechnetate may be added to the vial. Before reconstitution the technetium Tc99m generator eluate may be adjusted to the correct radioactive concentration to a volume of 5 mL by dilution with preservative-free, non-bacteriostatic saline for injection.

2) For radiolabeling of non-stabilized exametazime, generator eluate more than 2 hours old should not be used.

3) Use only eluate from a technetium Tc99m generator which was previously eluted within 24 hours. For the highest radiochemical purity, reconstitute with freshly eluted Tc99m generator eluate.

4) 5 mL of eluate is necessary due to the limited solubility of exametazime.

5) Oxidant-free Tc99m eluate must be used due to the minimal amount of stannous ion present in the product.

6) Radiochemical purity testing must be performed prior to patient administration. A radiochemical purity greater than 80% is necessary for product acceptance.

Procedure for Separation of Leukocytes and Subsequent *In Vitro* Labeling with Tc99m Exametazime <u>without</u> Cobalt Stabilizer Solution

(Use aseptic technique throughout).

i) Draw 9mL of acid-citrate-dextrose(a) into each of two 60 mL plastic non-heparinized syringes.

ii) Withdraw 51 mL of patient's blood into each syringe using a 19G Butterfly needle infusion set. Close the syringes with sterile hubs.

iii) Dispense 2mL of sedimentation agent(b) into each of 5 Universal containers or tubes.

iv) Without attaching a needle to the syringes dispense 20mL of blood into each of the 5 Universal tubes containing sedimentation agent. Dispense the remaining 20 mL of blood into a tube without sedimentation agent.

TIP *To avoid bubbles and frothing run the blood **gently** down the sides of the tubes.*

v) Mix the blood and sedimentation agent with one gentle inversion. Remove the cap of the Universal tube and burst the bubble formed at the top using a sterile needle. Replace the cap and allow the tubes to stand for 30-60 minutes for erythrocyte sedimentation to take place.

TIP *The period of time for erythrocyte sedimentation depends on the patient's condition. As a guideline it should be stopped when the blood has sedimented to give approximately half the volume* as *sedimented red cells.*

vi) Meanwhile centrifuge the tube containing 20mL of blood and no sedimentation agent at 2000 G for 10 minutes. This will yield supernatant cell-free plasma (CFP) containing ACD which is retained, at room temperature, for use as a cell labeling and reinjection medium.

vii) When sufficient red cell sedimentation has taken place (see v), carefully transfer 15mL aliquots of the cloudy straw-colored supernatant into clean Universal tubes. Take care to avoid withdrawing any sedimented erythrocytes. The supernatant is leukocyte-rich, platelet-rich plasma (LRPRP).

TIP *Do not use needles on sampling syringes to avoid unnecessary cell damage.*

viii) Centrifuge the LRPRP at 150 G for 5 minutes to give supernatant, platelet-rich

plasma (PRP) and a pellet of "mixed" leukocytes.

ix) Remove as much of the PRP as possible into clean Universal tubes and further centrifuge at 2000 G for 10 minutes to give more supernatant, cell-free plasma (CFP) containing sedimentation agent. This will be used to wash the cells after labeling.

x) Meanwhile, loosen the pellets of "mixed" leukocytes by **very gently** tapping and swirling the Universal tubes. Using a syringe, without an attached needle, pool all the cells into one tube, then, using the same syringe, add 1 mL of cell-free plasma containing ACD (from vi) and **gently** swirl to resuspend.

xi) Reconstitute a vial of Ceretec® with 5mL of Tc99m generator eluate containing approximately 185 – 370 MBq/ 5 – 10 mCi of Tc99m sodium pertechnetate (using the procedure described above).

xii) Immediately following reconstitution add 4mL of the resulting Tc99m exametazime solution to the "mixed" leukocytes in CFP (from x).

xiii) **Gently** swirl to mix and incubate for 10 minutes at room temperature.

xiv) On completion of incubation **carefully** add 10mL of CFP containing sedimentation agent (from ix) to the cells, in order to stop labeling. Gently invert the cells to mix.

xv) Centrifuge at 150 G for 5 minutes.

xvi) Remove and retain all of the supernatant.

TIP *It is critical that all the supernatant which contains unbound Tc99m exametazime is removed at this stage. This can be best achieved using a syringe with a wide Bore (19G) needle.*

xvii) Gently resuspend the Tc99m labeled mixed leukocyte preparation in 5-10mL of CFP containing ACD from (vi). Gently swirl to mix.

xviii) Measure the radioactivity in the cells and in the supernatant from (xvi). Calculate the labeling efficiency (LE) which is defined as the activity in the cells as a percentage of the sum of the activity in the cells and the activity in the supernatant.

TIP *Labeling efficiency depends on the patient's leukocyte count and will vary according to the volume of the initial blood sample. Using the volumes in (ii), a LE of about* 55% *might be expected.*

xix) Without attaching a needle, carefully draw up the labeled cells into a plastic, non-heparinized syringe and close it with a sterile hub. Measure the radioactivity.

xx) Labeled cells are now ready for reinjection. This should be performed without delay.

xxi) The patient dose should be measured in a suitable radioactivity calibration system immediately prior to administration.

NOTE:

(a) Acid-citrate-dextrose (ACD) - commercial preparations are available.

(b) 6% hydroxyethyl starch is recommended. Alternatively, a sterile preparation of 2% methyl cellulose in 0.9% saline may be used.

Radiochemical Purity Measurement

Three potential radiochemical impurities may be present in the prepared injection of the lipophilic complex Tc99m exametazime. These are secondary Tc99m exametazime complex, free pertechnetate and reduced hydrolyzed Tc99m. A combination of 3 chromatographic systems is necessary for the complete definition of the radiochemical composition of the injection.

Test samples are applied by needle approximately 2.5 cm from the bottom of two GMCP SA strips (2 cm (± 2 mm) x 20cm) and one Whatman No. 1 strip (2.5cm x 30cm) and then immediately placed in prepared ascending chromatography development tanks containing fresh solvent (1cm depth). The two GMCP-SA strips are run in butanone and 0.9% aqueous sodium chloride respectively and the Whatman No. 1 in 50% aqueous acetonitrile. After a 14 cm elution the strips are removed, solvent fronts marked, dried and the distribution of activity determined using suitable equipment.

Interpretation of Chromatograms

System 1 (GMCP-SA: butan-2-one(MEK))
Secondary Tc99m exametazime and reduced hydrolyzed-Tc99m remain at the origin.
Lipophilic Tc99m exametazime complex and Tc99m pertechnetate migrate at Rf 0.8-1.0

System 2 (GMCP-SA: 0.9% sodium chloride)
Lipophilic Tc99m exametazime complex, secondary Tc99m exametazime complex and reduced-hydrolyzed-Tc99m remain at the origin. Tc99m pertechnetate migrates at Rf 0.8-1.0

System 3
(Whatman No. 1: 50% aqueous acetonitrile)
Reduced-hydrolyzed-Tc99m remains at the origin.
Lipophilic Tc99m exametazime complex, secondary Tc99m exametazime complex and Tc99m pertechnetate migrate at Rf 0.8-1.0

i) Calculate the percentage of activity due to both secondary Tc99m exametazime complex and reduced-hydrolyzed-Tc99m from System 1 (A% + C%). Calculate the percentage of activity due to Tc99m pertechnetate from System 2 (B%). Calculate the percentage of activity due to the reduced-hydrolyzed-Tc99m from System 3 (C%).

ii) The radiochemical purity (as percentage lipophilic Tc99m exametazime complex) is given by: 100-(A% + B% +C%) where:
A% represents the level of secondary Tc99m exametazime complex.
B% represents the level of Tc99m pertechnetate.
C% represents the level of reduced-hydrolyzed-Tc99m.

RADIATION DOSIMETRY

(1) Brain Scintigraphy

Based on human data, the absorbed radiation doses to an average human adult (70 kg) from an intravenous injection of this product are estimated in Table 4.

Table 4. Estimated Absorbed Radiation Dose* for Cerebral Scintigraphy

Target Organ	Absorbed dose per unit activity	
	µGy/MBq	**rads/mCi**
Adrenals	5.3	0.020
Bone surfaces	5.1	0.019
Brain	6.8	0.025
Breast	2.0	0.007
Gallbladder wall	18.0	0.067
Gastrointestinal tract		
Stomach wall	6.4	0.024
Small Intestine wall	12.0	0.044
Colon wall	17.0	0.063
(Upper Large Intestine wall	18.0	0.067)
(Lower Large Intestine wall	15.0	0.056)
Heart wall	3.7	0.014
Kidneys	34.0	0.126
Liver	8.6	0.032
Lungs	11.0	0.041
Muscles	2.8	0.010
Esophagus	2.6	0.010
Ovaries	6.6	0.024
Pancreas	5.1	0.019
Red marrow	3.4	0.013
Skin	1.6	0.006
Spleen	4.3	0.016
Testes	2.4	0.009
Thymus	2.6	0.010
Thyroid	26.0	0.096
Urinary bladder wall	23.0	0.085
Uterus	6.6	0.024
Remaining organs	3.2	0.012
Effective dose	**9.3 µSv/MBq**	**344.1 µSv/mCi**

*International Commission on Radiological Protection, Radiation Dose to Patients from Radiopharmaceuticals: A Compendium of Current Information Related to Frequently Used Substances, Ann ICRP 2015)., ICRP Publication 128, Ann ICRP 2015).

The effective dose resulting from the administration of a (maximal recommended) activity of 1110 MBq for an adult weighing 70 kg is about 10.3 mSv. For an administered activity of 740 MBq the typical radiation dose to the target organ (brain) is 5.0 mGy and the typical radiation dose/doses to the critical organ (kidneys) is 25.2 mGy.

The biodistribution and hence the radiation dosimetry of technetium 99m exametazime is not significantly altered by *in vitro* cobalt stabilization.

2) *In vivo* localization of Tc99m labeled leukocytes.

Table 5. Estimated Absorbed Radiation Dose* for i*n vivo* localization of Tc99m labeled leukocytes

Target Organ	Absorbed dose per unit activity	
	µGy/MBq	**rad/mCi**
Adrenals	12.0	0.044
Bone surfaces	16.0	0.059
Brain	2.3	0.009
Breast	2.4	0.009
Gallbladder wall	8.4	0.031
Gastrointestinal tract		
Stomach wall	8.1	0.030
Small intestine wall	4.6	0.017
Colon wall	4.3	0.016
(Upper large intestine wall	4.7	0.017)
(Lower large intestine wall	3.7	0.014)
Heart wall	9.4	0.035
Kidneys	12.0	0.044
Liver	20.0	0.074
Lungs	7.8	0.029
Muscles	3.3	0.012
Esophagus	3.5	0.013
Ovaries	3.9	0.014
Pancreas	13.0	0.048
Red marrow	23.0	0.085
Skin	1.8	0.007
Spleen	150.0	0.555
Testes	1.6	0.006
Thymus	3.5	0.013
Thyroid	2.9	0.011
Urinary bladder wall	2.6	0.010
Uterus	3.4	0.013
Remaining organs	3.4	0.013
Effective dose	**11.0 µSv/MBq**	**407 µSv/mCi**

*International Commission on Radiological Protection, Radiation Dose to Patients from Radiopharmaceuticals: A Compendium of Current Information Related to Frequently Used Substances, Ann ICRP 2015)., ICRP Publication 128, Ann ICRP 2015).

The effective dose resulting from the administration of a (maximal recommended) activity of 370 MBq for an adult weighing 70 kg is about 4.1mSv.

HOW SUPPLIED

Each Ceretec Kit is supplied as a five-unit package containing:

5 multiple dose Ceretec vials containing a freeze-dried sterile, pyrogen-free mixture of exametazime, stannous chloride dihydrate and sodium chloride sealed under an inert nitrogen atmosphere.

5 vials of cobalt stabilizer solution.

5 labels for the reconstituted injection.

1 package insert.

Ceretec without cobalt chloride solution is supplied separately and is available as follows:

5 multiple dose Ceretec vials containing a freeze-dried sterile, pyrogen-free mixture of exametazime, stannous chloride dihydrate and sodium chloride sealed under an inert nitrogen atmosphere.

5 labels for the reconstituted injection.

1 package insert

STORAGE

Store the kit at any temperature in the range of 15-25 ^{o}C, 59-77^{o}F.
Store the reconstituted injection at 20-25 ^{o}C, 68-77 ^{o}F using appropriate radiation shielding.

EXPIRY

Kit before reconstitution: 52 weeks from the day of manufacture.

Use Tc99m exametazime injection with cobalt stabilizer solution within 5 hours after reconstitution. Use Tc99m exametazime injection without cobalt stabilizer solution within 30 minutes after reconstitution. Protect from freezing.

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