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Special article

[Translated article] Guideline to radiopharmaceutical procedures for *in vitro* labeling of leukocytes

Inmaculada Romero-Zayas[a,*], María de Arcocha-Torres[b], Iván Peñuelas[c], Elena Martínez Montalbán[d], María Carmen Plancha Mansanet[e], Antonio de Jesús Fernández Sánchez[f] and Juan Antonio Pérez Iruela[g]

[a] *Unidad de Radiofarmacia, Servicio de Medicina Nuclear, Hospital Clínic de Barcelona, IDIBAPS, Barcelona, Spain*
[b] *Unidad de Radiofarmacia, Hospital Universitario Marqués de Valdecilla, Grupo de Imagen Molecular IDIVAL Santander, Spain*
[c] *Unidad de Radiofarmacia, Clínica Universidad de Navarra, Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain*
[d] *Unidad de Radiofarmacia, Hospital Universitario La Paz, Madrid, Spain*
[e] *Unidad de Radiofarmacia, Hospital Universitario Doctor Peset, Valencia, Spain*
[f] *Servicio de Farmacia, Hospital Central de la Defensa Gómez Ulla, Madrid, Spain*
[g] *Unidad de Radiofarmacia, Hospital Universitario Ramón y Cajal, Madrid, Spain*

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ABSTRACT

This guide outlines the radiopharmaceutical procedure for the *in vitro* labelling of leukocytes. Scintigraphy using leukocytes labeled with [^{99m}Tc]Tc-exametazime or [^{111}In]In-oxine is a highly valuable diagnostic tool in infectious and inflammatory diseases due to its high sensitivity and specificity. It is based on established guidelines and consensus protocols, which incorporate the recent Spanish regulations on the extemporaneous preparation of radiopharmaceuticals, this document provides detailed and precise instructions for the safe and effective preparation and quality control of *in vitro* labeled leukocytes. The clinical indications for leukocyte scintigraphy are described, along with the technical requirements for both facilities and equipment. The various stages of the radiolabelling procedure are developed and substantiated, including blood sample collection, leukocyte fraction separation and isolation, incubation and subsequent radiolabelling with either [^{99m}Tc]Tc-exametazime or [^{111}In]In-oxine, radiopharmaceutical conditioning for administration, and the necessary quality control procedures. The document concludes with a section describing drug interactions reported in the literature. Two annexes are included, providing example labeling protocols for both radiopharmaceuticals.

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Guía de procedimientos radiofarmacéuticos para el marcaje *in vitro* de leucocitos

Palabras clave:
Leucocitos marcados
Radiofarmacia
Radiofármacos
Medicina nuclear
Técnicas *in vitro*
Gammagrafía

RESUMEN

En esta guía se describe el procedimiento radiofarmacéutico de preparación de leucocitos marcados *in vitro*. La gammagrafía con leucocitos marcados con [^{99m}Tc]Tc-exametazima o [^{111}In]In-oxina es una prueba diagnóstica de gran utilidad en enfermedades infecciosas e inflamatorias debido a su elevada sensibilidad y especificidad. Basándonos en las guías y protocolos consensuados, a los que se han incorporado las directrices de la reciente regulación de la preparación extemporánea de radiofármacos en España, esta guía proporciona instrucciones detalladas y precisas para una segura y eficaz preparación y control de calidad de los leucocitos marcados *in vitro*. Se detallan las indicaciones clínicas de la gammagrafía con leucocitos marcados y se describen los requerimientos técnicos tanto de las instalaciones como del equipamiento necesario. Se desarrollan y se fundamentan las diferentes etapas que comprenden el procedimiento de radiomarcaje, desde la obtención de la muestra sanguínea, la separación y el aislamiento de la fracción leucocitaria, la incubación y consiguiente marcaje radioisotópico tanto con [^{99m}Tc]Tc-exametazima como con [^{111}In]In-oxina, el acondicionamiento del radiofármaco para su administración, así como los controles de calidad necesarios. El documento concluye con un apartado donde se describen las interacciones medicamentosas descritas en la literatura. Se incluyen 2 anexos con ejemplos de protocolos de marcaje para ambos radiofármacos.

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* Corresponding author.
E-mail address: icromero@clinic.cat (I. Romero-Zayas).

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Introduction

In vitro-labeled leukocytes are widely used to detect sites of infection and inflammation, which involve leukocyte infiltration.

In the 1970s, McAfee and Thakur developed the technique for labeling leukocytes with [^{111}In]In-oxine,[1] and in the 1980s, Peters et al. introduced [^{99m}Tc]Tc-exametazime for leukocyte labeling.[2] The superior physical properties of technetium-99m, as well as its availability, cost, and lower radiation dose, have led to the widespread use of leukocytes labeled with [^{99m}Tc] Tc-exametazime, while leukocytes labeled with [^{111}In]In-oxine are only used in very specific indications.

In vitro labeling of blood cells requires aseptic conditions to preserve cellular function and viability.

The aim of this guide was to provide information that serves as a reference for the preparation and quality control of *in vitro*-labeled leukocytes, both with [^{99m}Tc]Tc-exametazime (also known as [^{99m}Tc]Tc-HMPAO) and with [^{111}In]In-oxine. This document is an initiative of the Spanish Society of Radiopharmacy (SERFA), through the Hospital Radiopharmacy Working Group, with the aim of updating Guide No. 1 on Radiopharmaceutical Procedures published by the Spanish Agency for Medicines and Healthcare Products (AEMPS).

As reference texts, we used 2 guides published by the European Association of Nuclear Medicine (EANM) on the labeling of leukocytes with [^{99m}Tc]Tc-HMPAO[3] and [^{111}In]In-oxine,[4] the International Atomic Energy Agency (IAEA)[5] guide on radiolabeled autologous cells, and other relevant publications. The guidelines incorporate the provisions of the recent regulation on the extemporaneous preparation of radiopharmaceuticals (Ministerial order SND/939/2022).[6]

We describe the methodological aspects corresponding to each phase of the radiolabeling procedure. Additionally, appendices are included that provide practical examples of both labeling protocols.

Indications

The diagnostic objective of *in vitro* leukocyte scintigraphy is to locate structures or regions where leukocyte uptake occurs as a result of an infectious or inflammatory process. This examination is primarily used for the following indications[7]:

- Detection and location of foci of infection or inflammation in patients with fever of unknown origin.
- Assessment of the presence and extent of infectious osteomyelitis.
- Diagnosis of spondylodiscitis and paravertebral soft tissue infections.
- Diagnosis of joint and vascular prosthetic infections.
- Evaluation of inflammatory bowel disease, including assessment of extent, prognostic evaluation, monitoring of treatment response, and detection of recurrence.[8]
- Identification of soft tissue infections, such as postoperative abscesses.
- Diagnosis of endocarditis.
- Assessment of pulmonary infections.
- Evaluation of central nervous system infections.

Leukocytes labeled with [^{111}In]In-oxine are particularly useful in detecting abdominal inflammatory foci and renal infections. In these locations, leukocytes labeled with [^{99m}Tc]Tc-exametazime may have limitations due to increased intestinal and renal uptake associated with the elution of [^{99m}Tc]Tc-exametazime from the leukocytes and its subsequent hepatobiliary and urinary clearance[3,4,7] (Figs. 1 and 2).

Legal framework and applicable regulations

In Spain, the extemporaneous preparation of radiopharmaceuticals, including cellular labeling of autologous samples with radioisotopes, is governed by the general framework for medicinal products established by the Spanish Royal Legislative Decree 1/2015,[9] which approves the consolidated text of the Law on Guarantees and Rational Use of Medicines and Healthcare Products. Article 48 of this law stipulates that the preparation of autologous samples involving radionuclides does not require authorization for industrial manufacturing, provided that they are prepared in authorized radiopharmacy units, under the supervision and control of a physician specializing in radiopharmacy, for use in a center or institution legally authorized to do so.

These regulations are further detailed in Spanish Royal Decree 1345/2007, of 11 October,[10] in which Article 2.26 defines the extemporaneous preparation of radiopharmaceuticals as the preparation, at the time of use, of a ready-to-use radiopharmaceutical, based on the radioisotope labeling of a sample or the patient's own autologous samples. Exemption from authorisation and registration of these radiopharmaceutical preparations is established when they are carried out in authorized units, in accordance with the manufacturer's instructions, upon medical prescription, and provided that the rules of good extemporaneous radiopharmaceutical preparation practice are met.

These rules of good extemporaneous preparation practice are established in the ministerial order SND/939/2022, of 29th September, which sets out the essential requirements for personnel, premises and equipment, documentation, quality control, and dispensing that must be met in radiopharmacy units to ensure the quality, safety, and traceability of preparations.

Facilities and equipment

Cell labeling requires specific equipment:

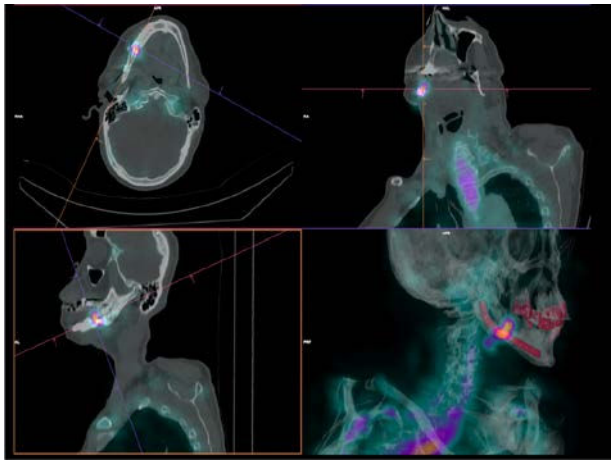

Figure 1. Leukocyte scintigraphy with [^{99m}Tc]Tc-exametazime-labeled leukocytes. Intense focal uptake of leukocytes in right mandibular osteosynthesis material, in keeping with a known active infectious process. *Source:* authors (courtesy of Hospital Clínic de Barcelona).

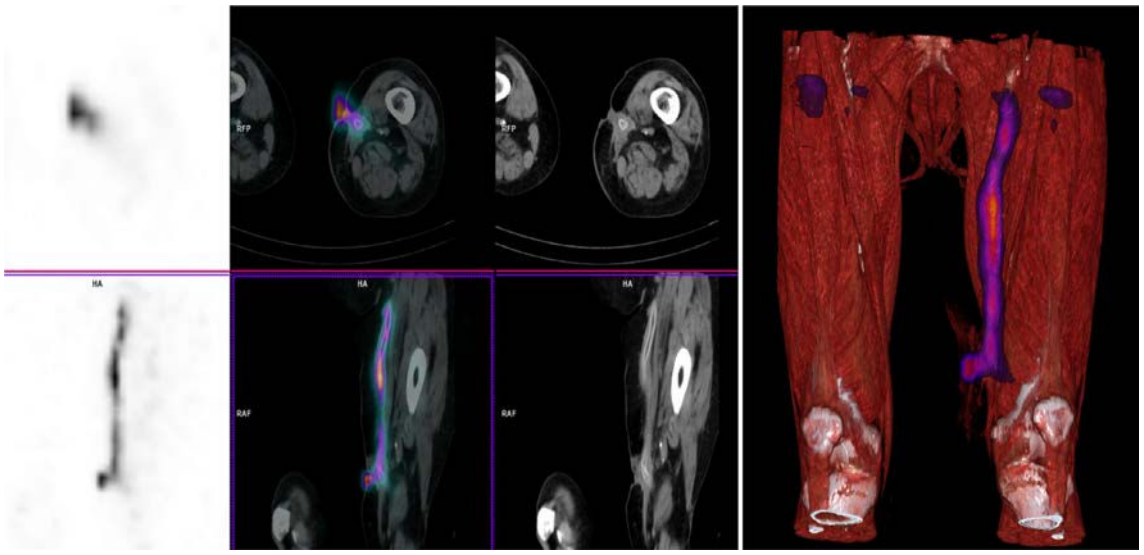

Figure 2. Scintigraphy with leukocytes labeled with [^{111}In]In-oxine. Pathological leukocyte uptake is observed due to infection of a vascular prosthesis. *Source:* authors (courtesy of Hospital Clínic de Barcelona)).

Biological safety cabinet

The biological safety cabinet must be used exclusively for cell labeling and should preferably be located in a room separate from the rest of the radiopharmacy unit.

The ministerial order specifies that this cabinet must provide grade A conditions in the working environment and must be, at minimum, class II type B1, located in a grade B environment, or grade C if administration is immediate. A Grade A isolator with a minimum Grade D environment may be used.

If the cabinet is located in a room where other radiopharmaceuticals are prepared, the compatibility of both activities must be assessed and documented through a risk analysis. In the event of incompatibility, there must be a temporal separation of the activities carried out in the same room.

Most biological safety cabinets installed in radio pharmacy units comply with the European Class II standard EN 12469, which outlines a single design that functionally corresponds to subtype A2 of the U.S. National Sanitary Foundation standard.

In cell labeling procedures, aqueous solutions are handled in closed containers; no volatile reagents are used, and no heating is performed. Therefore, Class II EN 12469 cabinets offer the same protection for the product, operator, and environment as other subclasses, with high unidirectional airflow stability and no critical dependence on the building's extraction system.

Since the partial air extraction characteristic of B1 cabinets only has advantages when handling volatile substances—and considering that there is no volatility in cell labeling with radioisotopes—as specialists in radio pharmacy, we endorse the use of Type IIA cabinets, based on a risk analysis that ensures the required radiological and microbiological protection.

Centrifuge

The centrifuge used for cell separation must ensure containment in the event of a spill or breakage, either by using closed buckets or by following in-depth cleaning protocols, which should be performed before and after each preparation.

It is important to understand the relationship between relative centrifugal force (g) and the revolutions per minute (RPM) of the centrifuge, since the RPM depends directly on the rotor radius. The conversion between g and RPM is given by the equation:

$$\mathrm{RPM} = \sqrt{\frac{g}{(r \times 1.118) \times 10^{-5}}}$$

where r is the radius of the centrifuge rotor expressed in centimeters.

Dose calibrator (activimeter)

The dose calibrator is needed to measure radioactive activity. In cell labeling, it is used to measure the radioactivity bound to the cells, radioactivity in the supernatant (unbound to the cells), and the final radioactive activity of the radiopharmaceutical.

Optical microscope

The optical microscope is needed for performing the cell viability assay.

Radiocromatography or radio-TLC (Thin-Layer Chromatography) equipment

Equipment used to perform quality control of [^{99m}Tc]Tc-exametazime via thin-layer chromatography.

Precautions

This procedure involves handling patient blood samples that may be a biological risk to both the operator and the patient.

Staff must be fully aware of this risk and specially trained in the safe handling of such samples. During the labeling process, the use of needles should be avoided whenever possible. If needles are necessary, wide-bore plastic needles must be used to minimize the risk of accidental needlestick injury to the operator.

There is a risk of blood contamination by pathogens; therefore, strict aseptic handling conditions are required.

The following measures are required to prevent cross-contamination between samples from different patients:

- No more than one labeling procedure may be performed at the same time in a single booth or isolator.
- It is essential to correctly identify every container holding a patient's blood product.
- It is essential that, during the dispensing of the final radioactive dose to be administered, appropriate measures are taken to

unambiguously identify the patient (name or medical record number), in addition to other information, such as the type of radiopharmaceutical, activity, date and time of calibration, radioactive symbol, etc.

Procedure

Blood sample collection

To achieve optimal labeling efficiency and an adequate number of radiolabeled leukocytes for high image quality, the blood sample drawn from the patient must contain at least 2×10^8 leukocytes.[3,4] This can be achieved with a minimum of 30 mL in patients whose leukocyte concentration is 5000 leukocytes/mm^3 or higher.[11]

It is recommended to know the patient's white blood cell count in order to draw a larger volume of blood in cases of neutropenia ($<2 \times 10^3$ neutrophils/mm^3).[3,4,11]

The patient does not need to be fasting at the time of blood draw; however, potential interactions with the labeling process should be considered if cholesterol and glucose levels are high.[5] Fasting is required for abdominal studies (such as inflammatory bowel disease).[12]

The anticoagulant of choice is ACD-A (Ph. Eur 01/2020:0209, consisting of 0.73 g of anhydrous citric acid, 2.2 g of sodium citrate dihydrate, and 2.45 g of glucose monohydrate in 100 mL of water for injection). ACD-A prevents leukocyte aggregation and adhesion to plastic surfaces, resulting in increased leukocyte recovery. In addition, the citrate it contains inhibits leukocyte degranulation and oxidative stress. The recommended ratio is 1 mL of ACD-A per 6–7.5 mL of blood.[5,11]

Alternatively, preservative-free heparin can be used at a concentration of 10 IU/mL of blood. Heparin increases erythrocyte sedimentation rate.[5,11]

In the pediatric population, the volume of blood to be drawn should be adjusted according to weight, clinical status, and total blood volume, following minimal-risk guidelines that recommend not exceeding 2.5% of total blood volume in a single draw.[13] In practice, leukocyte labeling procedures with [^{99m}Tc]Tc-exametazime typically require a minimum volume of 10–15 mL, depending on the patient's cell count.[14]

The needles used for collection must be at least 20 G to minimize leukocyte damage.[3,4]

Blood is drawn into one or more syringes containing the appropriate proportion of anticoagulant. The draw should be performed slowly and gently, avoiding bubble formation.[3,4]

Once obtained, the sample is gently mixed by inverting the syringe several times to ensure proper homogenization with the anticoagulant.[3,4]

Obtaining cell-free plasma

An additional sample of anticoagulated blood should be drawn to obtain cell-free plasma (CFP), using the same ratio of 1 mL of ACD-A per 6–7.5 mL of blood (e.g., 15 mL of blood and 2 mL of ACD-A). CFP is used as a medium during the labeling process and in the final resuspension of the leukocytes.[3,4,11]

It is obtained by centrifuging for 10 min at 2000*g*, followed by separation of the supernatant CFP from the cell pellet.

Alternatively, phosphate-buffered saline (PBS) at pH 7.4 or physiological saline may be used.[3,4]

Isolation of leukocytes

Obtaining cell-rich plasma (CRP)

The most common technique for separating plasma from erythrocytes in a blood sample involves the use of macromolecules that accelerate cell sedimentation. These compounds act as plasma expanders, and the most commonly used agent is high-molecular-weight hydroxyethyl starch (HES) (e.g., 200/0.6 or 450/0.7).[3–5]

Formulations with lower molecular weights are not effective as sedimentation agents. The most widely used solution is 6% HES. It is a starch-derived colloid composed of polymerized glucose units modified with hydroxyethyl ether groups. For HES 450/0.7, the average molecular weight is approximately 450,000 Da, with a degree of substitution of 0.7, which indicates the presence of 7 hydroxyethyl groups for every 10 glucose units.

The recommended ratio is one part HES for every 3–9 parts of blood (e.g., 8 mL of HES for 30 mL of blood).[3–5,11]

HES accelerates the erythrocyte sedimentation process by affecting the charge of the erythrocyte outer membrane—which is carried by sialic acid groups—thereby promoting erythrocyte aggregation and improving leukocyte recovery. It has not been associated with allergic reactions and has the additional advantage of preventing leukocyte aggregation.

Sedimentation time varies depending on patient characteristics, typically ranging from 30 to 60 min (Fig. 3).

In Spain, 6% HES is not commercially available at the time of writing; therefore, other sedimentation agents are used, such as succinylated gelatine (modified fluid gelatine or GFM), a colloid approved as a plasma expander. The negative charges of the succinyl groups decrease the zeta potential of erythrocytes, which promotes their aggregation and accelerates sedimentation.

Roca et al.[15] evaluated 4% succinylated gelatine (Gelafundina®, Braun Medical)[16] and found no significant differences in leukocyte and platelet recovery, labeling efficiency, cell viability, or erythrocyte-to-leukocyte ratio, concluding that it is a suitable alternative to HES.

In the Leucokit® medical device, 10% HES has been replaced by 4% succinylated gelatine, with an average molecular weight of 26,500 Da, and marketed as Gelafundina® or Gelafusine®, depending on the country. Auletta et al.[17] also observed no differences in leukocyte recovery, labeling efficiency, chemotactic properties, or diagnostic accuracy, supporting its use as a valid alternative to HES.

Gelaspan® is largely available in hospital settings;[18] it contains the same 4% succinylated gelatine but with a balanced electrolyte profile, which gives it more physiological properties and makes it better suited to human plasma.

Alternatively, erythrocyte separation can also be performed by low-speed centrifugation. Various centrifugation times have been described, for example, $5g \times 15$ min.[19] This shortens the procedure time, but as a trade-off, the CRP contains more red blood cells and fewer leukocytes.

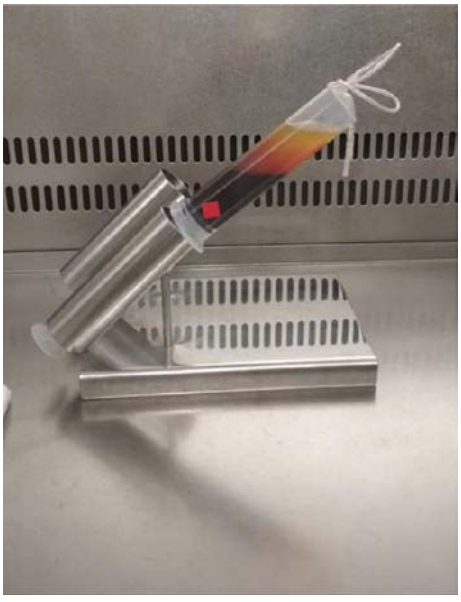

Figure 3. Obtaining cell-rich plasma (supernatant or the clearer upper fraction) using macromolecules. *Source:* authors.

Obtaining the leukocyte pellet and resuspension

The CRP is transferred to a conical-bottom tube and centrifuged at 150*g* for 5 min. This relatively low speed keeps the platelets in the supernatant, while the leukocytes and red blood cells settle.

Centrifuging at higher speeds should be avoided, as it increases platelet contamination of the leukocyte pellet.[3,4,11]

Platelet-rich plasma (PRP) is carefully removed and can be used for the post-labeling wash step.

The leukocyte pellet is resuspended in 0.5–1 mL of CFP or, alternatively, in PBS or saline salution.[3,4,11] Resuspension is performed by gentle aspiration with a Pasteur pipette, avoiding the formation of foam.

Using saline, rather than plasma, to resuspend may increase labeling efficiency, although it could decrease cell viability.

If the leukocyte pellet is to be labeled with [^{111}In]In-oxine, it should be noted that [^{111}In]In + 3 has a higher affinity for plasma transferrin than for oxine; therefore, the labeling process should be performed in the absence of plasma.[4,11]

In this case, an additional step of washing the pellet with normal saline is recommended (for example, resuspend with 3 mL, centrifuge again at 150*g* for 5 min, and separate the supernatant from the pellet). Finally, resuspend the cell pellet in a plasma-free medium, such as saline solution or Hank's balanced salt solution, a mixture of salts that preserves the structural and physiological integrity of cells *in vitro*.[4,11]

Radioisotope labeling of cells

The mechanism for labeling relies on the passive diffusion of lipophilic and neutral chelates containing the radioactive isotope.

[^{111}In]In-oxine is a 3:1 complex of oxine (8-hydroxyquinoline) and indium-111, resulting in a neutral, highly lipophilic compound that crosses the cell membrane (Fig. 4). Once inside the cell, the complex dissociates and the indium binds to intracellular proteins (Fig. 6).[4,11]

[^{99m}Tc]Tc-exametazime or [^{99m}Tc]Tc-d,l-HMPAO (hexamethylpropyleneaminoxime) (Fig. 5) is also a neutral complex that, in its lipophilic form, diffuses into leukocytes. It has been suggested that it converts to its hydrophilic form via intracellular glutathione and the higher pH of the cytoplasm, thereby becoming trapped inside the cell (Fig. 4).[5,20]

Due to its instability in aqueous solution, [^{99m}Tc]Tc-exametazime must be prepared immediately before use. Stabilizing agents, such as cobalt chloride, should not be used.[3,5] Prior to use, the radiopharmaceutical must undergo quality control in accordance with the manufacturer's summary of product characteristics (SmPC) [21,22] or another validated procedure.

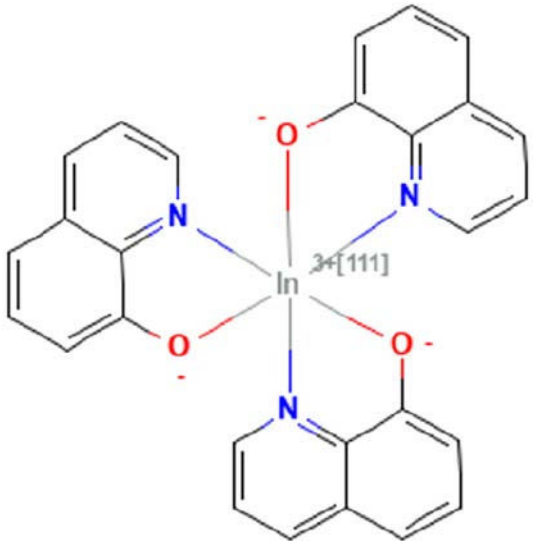

Figure 4. Chemical structure of [^{111}In]In-oxine. It is a complex consisting of 3 oxine (8-hydroxyquinoline) molecules and one ^{111}In atom. *Source:* PubChem Compound Database, 119117. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Indium-In-111-Oxyquinoline.

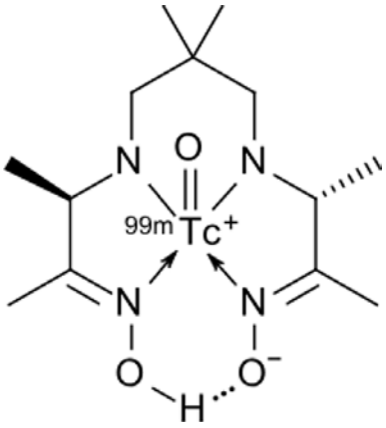

Figure 5. Chemical structure of [^{99m}Tc]exametazime. *Author:* X, Wikipedia, CC BY-SA 4.0, https://commons.wikimedia.org/wiki/File:Tc-99m_exametazime_skeletal.svg.

When labeling the leukocyte pellet with [^{99m}Tc]Tc-exametazime, add a volume of 0.5–1 mL with an activity of less than 740 MBq (20 mCi). Small volumes improve labeling efficiency.[23]

It is recommended to carry out the labeling in a medium composed of 50% plasma.[11] If the leukocyte pellet has been resuspended in 0.5 mL of CFP, add 0.5 mL of [99mTc]Tc-exametazime, mixing gently.

Incubate for 10 min at room temperature (or 5 min if the resuspension was performed in saline).

Incubation at 37 °C for 15–20 min may increase the labeling efficiency, but reference guidelines do not recommend its use.[3–5]

During incubation, it is advisable to gently shake the suspension periodically to prevent cell sedimentation.[3,4]

To remove radiopharmaceutical activity not bound to the cells after incubation, add at least 3 mL of CFP (preferably up to 10 mL) and centrifuge at 150*g* for 5 min.[3,4]

Alternatively, PRP obtained during the separation of the leukocyte pellet may be used after being centrifuged for 10 min at 2000*g*.[3] The use of PRP without centrifugation is standard practice, and no adverse effects on cell labeling have been observed.

To label leukocytes with [^{111}In]In-oxine, approximately 20 MBq (540 μCi) is added to the leukocyte pellet.[4]

The commercially available preparation of [^{111}In]In-oxine[24] consists of 2 vials, one containing 37 MBq (1 mCi) of the precursor and the other containing Tris buffer. The buffer must be added at 0.4 mL per mL of

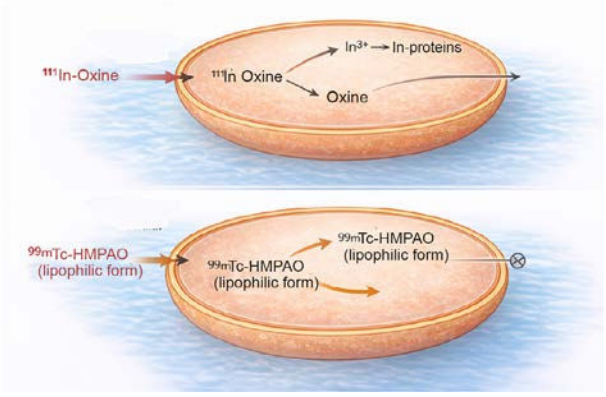

Figure 6. Schematic of the passive diffusion mechanism for leukocyte labeling. *Source:* Prepared by the authors.

precursor, immediately before addition to the leukocyte pellet, to prevent adsorption of the complex to the vial or syringe.

The required incubating time is 10–15 min at room temperature.

For the washing step, add at least 3 mL of PBS or saline (preferably up to 10 mL) and centrifuge at 150*g* for 5 min.

After centrifugation, the supernatant—which contains the unbound activity —is removed and set aside to calculate the labeling efficiency.

Preparation of leukocytes for administration

Once centrifugation is complete, the supernatant containing the unbound radiopharmaceutical is separated, and the leukocyte pellet is resuspended in 3–5 mL of CFP.[3–5,11] Aspirate using plastic cannulas or needles with a minimum gauge of 19 G.

Administration

Labeled leukocytes must be administered as soon as possible. The stability of the preparation is limited to a maximum of 1 h after labeling.[3,4] The viability and functionality of the leukocytes progressively decline afterward, which may reduce migration and inflammatory response capacity, affecting the sensitivity and specificity of the study. Up to 10% elution of the radioisotope from the leukocytes occurs within the first hour, most pronounced in leukocytes labeled with [^{99m}Tc]Tc-exametazime.[3,4]

It should be administered slowly, preferably using needles of at least 22G to minimize stress-induced cellular damage.[3,4]

The activity of leukocytes labeled with [^{99m}Tc]Tc-exametazime ranges from 185 to 370 MBq (5–10 mCi).[3] In the pediatric population, the activity is calculated according to EANM's recommendations by multiplying the baseline activity (35 MBq) by a body weight-dependent factor.[25]

For leukocytes labeled with [^{111}In]In-oxine, typical activity is 10–18.5 MBq (0.27–0.50 mCi).[4] Due to the greater dosimetric impact, especially at the splenic level,[26] its use is restricted in the pediatric population in favor of alternatives with lower dosimetry, such as leukocytes labeled with [^{99m}Tc]Tc-exametazime.

Quality control

Radiopharmaceutical quality control

The radiochemical purity of [^{99m}Tc]Tc-exametazime is determined by thin-layer chromatography (radio-TLC) according to the SmPC[21,22] or a validated procedure. Quality control must be confirmed before adding it to the leukocyte suspension.

For [^{111}In]In-oxine, verify reconstitution with buffer in the proportion indicated by the manufacturer.[24]

Quality control of the labeled leukocyte suspension

Routine for each preparation:

Visual inspection

Perform a visual inspection of the sample throughout the labeling process, especially before loading the final product, to detect clots or aggregates.

If there are aggregates that do not dissolve (e.g., upon gentle resuspension with a Pasteur pipette), do not administer.[3,4]

Labeling efficiency

This is defined as the percentage of activity bound to leukocytes relative to total activity. The procedure consists of measuring the activity of both the leukocyte pellet and the supernatant (cell-unbound activity) using the dose calibrator.

$$\textbf{\textit{Efficiency}}\ (\%) = \frac{\textit{Activity of leukocyte button}}{\textit{Activity in supernatant} + \textit{activity of leukocyte button}} \times \mathbf{100}$$

The expected range for leukocytes labeled with [^{99m}Tc]Tc-exametazime is an efficiency between 40% and 80%[3] (between 50% and 80% for labeling with [^{111}In]In-oxine).[4] If it is less than 40%[3] (50% with [^{111}In]In-oxine),[4] perform the viability test with trypan blue and consider repeating the preparation based on the findings and the clinical indication.

Periodic checks/validation (procedure, technical staff, or changes)

Cell viability (Trypan Blue Exclusion Test): Viable cells with intact membranes exclude the dye, whereas non-viable cells take up the stain and appear blue under a light microscope. This test is performed by mixing 25 μL of a 0.4% trypan blue solution with 25 μL of the labeled leukocyte suspension. A drop of this mixture is then loaded onto a hemocytometer and examined. A stained (non-viable) cell count exceeding 4% contraindicates administration to the patient.[3,4]

Cell function: Chemotaxis or other specific functional assays are conducted in accordance with institutional protocols to confirm that the labeling process does not significantly impair leukocyte function.

Cell recovery: This parameter is defined as the fraction of viable leukocytes recovered after isotopic labeling relative to the initial leukocyte count in the blood sample. Recovery is assessed by quantifying cell subtypes in the final sample and after each centrifugation step. Residual contamination by erythrocytes and platelets is also monitored; an erythrocyte-to-leukocyte ratio of less than 3:1 and a platelet-to-leukocyte ratio of less than 1:1 are considered acceptable.[3,4]

Aseptic process simulation test (media fill): Routine media fill testing is performed to verify and validate microbiological control throughout the entire preparation procedure.[27]

Verification of labeling quality through biodistribution analysis[3,4]

In the lungs, early transient uptake is considered normal. Persistence after 30 min suggests cellular disruption during the labeling procedure.

Focal pulmonary uptake suggests cellular aggregates.

Splenic activity must be greater than hepatic activity. Liver uptake equal to or greater than splenic uptake suggests cellular damage.

Closed leukocyte labeling system

A closed, sterile, and disposable system is available; Leucokit® (CellTech) is a medical device that integrates the materials and reagents necessary for leukocyte labeling. Recently, the HES-based sedimentation agent has been replaced by succinylated gelatine (Gelofusine®, B. Braun), while maintaining the functionality of the procedure.[17]

The theoretical advantage of using this medical device is to simplify the infrastructure and logistics of the cell labeling process. However, from a regulatory standpoint, current legislation does not provide for any exceptions that would allow conditions other than those required for preparation using autologous samples.

Drug interactions

The leukocyte labeling process may be affected by the administration of certain drugs,[28] with several interactions reported in the literature.

- **Decreased granulocyte adhesion:** Induced by lidocaine, alcohol, and acetylsalicylic acid.

- **Altered chemotaxis:** Caused by corticosteroids, leading to reduced uptake of the radiopharmaceutical in abscesses or other inflammatory foci.
- **Altered neutrophil distribution:** Driven by nonsteroidal anti-inflammatory drugs (NSAIDs), aminoglycosides, and penicillin, which can modify biodistribution and impair the visualization of foci.
- **Induced leukopenia:** Triggered by drugs such as cyclosporine, ranitidine, azathioprine, methotrexate, or cyclophosphamide, which can lower labeling efficiency by reducing the number of available leukocytes.

Although interactions with antibiotics should be considered when interpreting labeled leukocyte scintigraphy, it is not recommended to systematically exclude patients on antibiotic therapy. Published evidence is inconsistent regarding the impact on the accuracy of labeled leukocyte scintigraphy; therefore, the decision to perform or postpone the study should be evaluated on a case-by-case basis by the prescribing nuclear medicine physician.[7]

Methodological aspects requiring clarification

Usage of non-plasma media—such as the radiopharmaceutical solution itself—to resuspend the leukocyte pellet must be evaluated and validated based on cell viability and labeling efficiency, in accordance with available evidence.

The SmPC for Ceretec (GE Healthcare),[21] as well as the SmPC for Exametazima-Radiopharmacy,[22] state that the reagent mixture should be labeled with [^{99m}Tc]TcO_4Na in a volume of 5 SmPC mL, and 4 mL of freshly prepared [^{99m}Tc]Tc-HMPAO should be added to the leukocyte pellet. However, the EANM guidelines[3] support the use of reduced volumes (0.5–1 mL), which increase labeling efficiency, likely due to the effect of cell concentration.[23]

For this reason, although the Spanish ministerial order SND/939/2022 states that "the preparation of radiopharmaceuticals from autologous samples shall be carried out as specified in the SmPC of the radiopharmaceutical used for labeling", this guide —based on scientific evidence and international recommendations (EANM)— considers that an adjustment to the volume specified in the SmPC may be justified, provided it is documented and approved by the specialist responsible for radio pharmacy.

Contribution to the scientific literature

This manuscript describes an updated technical protocol that includes the Spanish regulatory framework.

It reduces variability, improves safety, and provides a basis for technical decisions.

CRediT authorship contribution statement

Inmaculada Romero-Zayas: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **María de Arcocha-Torres:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Data curation, Conceptualization. **Iván Peñuelas:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Data curation, Conceptualization. **Elena Martínez Montalbán:** Writing – original draft, Visualization, Validation, Supervision, Data curation, Conceptualization. **María Carmen Plancha Mansanet:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Data curation, Conceptualization. **Antonio de Jesús Fernández Sánchez:** Visualization, Validation, Supervision. **Juan Antonio Pérez Iruela:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Data curation, Conceptualization.

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Appendix 1: Example of leukocyte labelling with [^{99m}Tc]Tc-exametazime (or [^{99m}Tc]Tc-HMPAO)

1. Draw 45 mL of blood using a 60 mL syringe with 6 mL of ACD-A anticoagulant added prior. Mix gently. Transfer 10 mL of extracted blood to a 10 mL conical-bottom tube (tube B)
2. Using a 10 mL syringe, draw up 8 mL of succinylated gelatine (Gelaspan®) and aseptically transfer it into the 60 mL syringe without creating bubbles. Mix gently. Leave the syringe in an upright position or tilted at a 45° angle for 30–60 min at room temperature to allow sedimentation. Replace the needle with a butterfly needle (19 G).
3. Centrifuge the blood from the 10 mL tube (tube B) for 10 min at 2000*g* to obtain the CFP. Set it aside for incubation and final resuspension of the leukocyte pellet.
4. Without changing the vertical position of the 60 mL syringe, transfer the supernatant containing the CRP to a conical-bottom tube, pressing the plunger gently and taking care to avoid blood contamination.
5. Centrifuge for 5 min at 150*g*.
6. Using a sterile Pasteur pipette, transfer the supernatant containing leukocyte-poor plasma to another conical-bottom tube, taking care not to disturb the leukocyte concentrate. Centrifuge for 10 min at 2000*g*. Using a 10 mL syringe, withdraw 8 mL of the supernatant. Cap the syringe and set it aside for the washing process. Place the tube containing the leukocyte pellet in a lead shield.
7. Prepare the [^{99m}Tc]Tc-exametazime and determine radiochemical purity.
8. Add 0.5 mL of CFP (from step 3) and add 0.5 mL of [^{99m}Tc]Tc-exametazime (<20 mCi; 740 MBq). Add it drop by drop to the leukocyte concentrate. Gently resuspend with a Pasteur pipette.
9. Incubate for 10 min at room temperature.
10. Once incubation is complete, add the 8 mL obtained in step 6 after centrifuging the leukocyte-poor plasma. Gently resuspend, then centrifuge for 5 min at 150*g*.
11. Using a Pasteur pipette, transfer the supernatant to a conical-bottom tube. Set it aside to calculate the labelling efficiency.
12. Resuspend the leukocyte pellet in 3–4 mL of CFP.
13. Draw up the sample into a syringe using a sterile cannula.
14. Measure the supernatant and the syringe in the dose calibrator to calculate the labelling efficiency.

Appendix 2: Example of leukocyte labelling with [^{111}In]In-oxine

Follow the steps in Appendix 1 through to step 6:

7. Resuspend the leukocyte pellet in 5 mL of normal saline or Hanks' solution.
8. Repeat centrifugation for 5 min at 150*g*.
9. Completely remove supernatant.
10. Gently resuspend the leukocyte pellet in 2 mL of normal saline or Hank's solution, taking care not to create bubbles.
11. Prepare the [^{111}In]In-oxine immediately before use by adding the appropriate amount of buffer (0.4/1 *v*/v).
12. Add 20 MBq (540 μCi) of [^{111}In]In-oxine drop by drop.
13. Incubate for 10–15 min at room temperature.
14. Add 3 mL of normal saline solution to the pellet.
15. Centrifuge for 5 min at 150*g*.

The following steps correspond to steps 11, 12, 13, and 14 in Appendix 1.

Ethical responsibilities

The authors declare that no experiments were conducted on humans or animals in the preparation of this manuscript. This work does not include identifiable patient data or clinically sensitive information; therefore, it was not necessary to obtain informed consent. The manuscript was prepared in accordance with ethical principles and standards of good scientific practice.

All authors accept the responsibilities defined by the International Committee of Medical Journal Editors (ICMJE).

Conflict of interest

None declared.

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Declaration on the use of generative artificial intelligence

During the preparation of this manuscript, the authors used generative artificial intelligence tools exclusively for the purpose of refining the conceptual schematic illustration in Fig. 6. Generative artificial intelligence was not used for the drafting, analysis, or interpretation of data, nor for the development of the scientific content of the manuscript, for which the authors bear full responsibility.

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