# $^{111}$In oxine leukocytes

## 1. Indications

$^{111}$In oxine (8-hydroxyquinoline or oxyquinoline) labelled leukocytes or labelled white blood cells are prepared using a registered radiopharmaceutical precursor Indium ($^{111}$In) Oxinate. $^{111}$In oxine leukocytes can be used to detect and localise sites of infection and to detect and determine the extent of inflammatory conditions not associated with infection. A complete list of indications is given in chapter 'Leucocyte scintigraphy'.
When comparing $^{111}$In oxine leukocytes with $^{99m}$Tc exametazime leukocytes: scintigraphy with $^{99m}$Tc exametazime leukocytes is preferred for most indications, because the advantage of earlier and shorter imaging times and lower radiation dose. $^{111}$In oxine leukocytes scintigraphy is preferred in patients with inflammatory bowel disease and kidney infections because, unlike $^{99m}$Tc exametazime leukocytes, there is normally no excretion into gastrointestinal or urinary tracts. Also in more chronic processes, where late imaging is needed, $^{111}$In oxine leukocytes can be used.

## 2. Preparation

The preparation of $^{111}$In oxine leukocytes is described in the the SmPC and guidelines. The preparation can be summarized in the following steps:

a. Fill a syringe with anticoagulant (ACD-A) and sedimentation agent (HES).
b. Collect blood using a needle with an inner diameter of 19 G. Needles with a smaller inner diameter will damage the cells. Make sure that the blood is well mixed with the anticoagulant.
c. Bring either the blood-mixture over in a tube or turn the syringe with the plunger facing downwards.
d. Allow the red cells to sediment for 45-60 min.
e. Separate the leukocyte rich and platelet rich plasma (LRPRP) upper layer from the layer of red cells.
f. Centrifuge the LRPRP at 150 g for 5 min.
g. Separate all of the platelet rich plasma (PRP) upper layer from the layer of the leukocytes.
h. Re-suspend the leukocytes in a 0,9% aqueous solution of sodium chloride (saline) or alternatively isotonic phosphate-buffered saline (pH 7,4).
i. Centrifuge the PRP at 1500 g for 10 min.
j. Separate the cell free plasma upper layer from the platelets and keep for later use.
k. Add a buffer solution to the $^{111}$In oxine solution as required by the manufacturer.
l. Add the $^{111}$In oxine solution to the suspended leukocytes.
m. Incubate for 15 min at room temperature.
n. Centrifuge for 5 min at 150 g.
o. Remove the supernatant and keep for determination of labelling efficiency.
p. Re-suspend the radiolabelled cells in cell free plasma.
q. Draw the needed activity of $^{111}$In oxine leukocytes for the patient dose.

*Special considerations:*

- Working with blood can introduce risks to both the operator and the patient. The department labelling the cells should comply with all regulations. Adequate facilities, equipment, procedures and training for operators should be present. Additional the risks for blood contamination should be recognized and precautionary measures should be implemented to minimise those risks.
- Sedimentation may be affected by a variety of factors such as number of cells and certain diseases like sickle cell anaemia.
- The amount of plasma in the labelling medium will affect the labelling efficiency. If the cells are labelled in a medium containing plasma, $^{111}$In oxine will bind to plasma proteins resulting in a labelling efficiency as low as 5%. It is therefore required to label the cells in a plasma free medium such as saline or isotonic phosphate-buffered saline.
- In some countries Hydroxyethyl-starch (HES) solutions might not be available. The reason for this is the endorsement of the EMA to allow the use of HES only in restricted patient populations. In the case HES is no longer available, alternatives like succinylated gelatin and methylcellulose could be considered.

**3. Quality control**

- Before the blood cell labelling is started and throughout the procedure a check on the absence of blood clots needs to be performed.
- The labelling efficiency of the $^{111}$In oxine labelled leukocytes should be determined after labelling. The labelling efficiency is defined as the total radioactivity measured in the cells as a percentage of the total radioactivity measured in both the cells and the supernatant. The method is described in the SmPC. A labelling efficiency above 50% might be expected. Although labelling efficiency of the radiolabeled leukocytes have been reported from approximately 37% to 90% or more. The labelling efficiency depends on several aspects such as: presence of disease in patient, concentration and number of cells, cell damage, plasma concentration in the labelling medium, pH, concentration of ligand and radionuclide, temperature, operator inter-variability and drugs (see 'interactions').

Several other quality control tests have been described. Some of these tests are time-consuming. The following tests are recommended to be performed periodically or for validation purposes:

- Tryptan blue exclusion test, clumping and cell counting
- Cell subset recovery test
- Measurement of cell efflux of $^{99m}$Tc
- In vivo in lung uptake
- In vivo liver-to-spleen ratio

The tests are described in the EANM guidelines

**4. Interactions, contraindications & adverse reactions**

*Interactions*

- Antibiotics like penicillins could cause pseudomembranous colitis. This might give a higher activity in the colon. A possible effect in images should be taken into account.

- Inhalation anesthetics Halothane and Isoflurane are known to cause liver dysfunction. This could give rise to an irregular distribution of the radiopharmaceutical with a higher uptake around the diaphragm. When possible the scintigraphy with $^{111}$In oxine leukocytes should be planned a week after use of these inhalation anesthetics.
- The following drugs could alter chemotaxis of the leukocytes and thereby interfere with the leukocyte labelling. This might cause less uptake of $^{111}$In oxine in the leukocytes and a disturbed imaging.

- Azathioprine
- Cephalosporins
- Cyclosporine
- Cyclophosphamide
- Heparin
- Iron preparations
- Low molecular weight heparins (LMWHs)
- Mesalazine (Mesalamine, 5-ASA)
- Methotrexate
- Nifedipine
- Prednisolone
- Ranitidine
- Sulfasalazine
- Total parenteral nutrition (TPN)

For those drugs, with the exception of nifedipine and ranitidine, a possible effect in images should be taken into account. Nifedipine should be discontinued 12 h before the study (32 h for sustained release/retard preparations). Ranitidine should be discontinued 10 h for the study.

*Adverse reactions*
Hypersensitivity (including fever, rash, urticaria, pruritus, anaphylactoid reaction)

**5. Biodistribution & pharmacokinetics**
After injection of $^{111}$In labelled leukocytes, about 60% will distribute to liver, spleen and bone marrow. Part of the normal biodistibution is a temporarily lung accumulation immediately after injection, with almost no activity left in the lungs after around 4h. The radiolabeled cells have an exponential clearance from the blood with a halflive between 5-10 h, with a final distribution of around 20% in the liver, 25% in the spleen, 30% in the bone marrow and 25% in other organs. The renal and bowel elimination of $^{111}$In oxine leukocytes is 1% of the injected dose in the first 24 h.

**6. Stability**
It has been reported that the loss of label for $^{111}$In oxine leukocytes range from 3% after 1 h to 24% at 24 h. It is essential that cells are viable when returned to the patient. Labelled cells may be damaged from the collection and labelling procedures. Re-suspension of cells in cell free plasma optimises their viability. The SmPC does not mention a specific maximum shelf live. In practice, longer periods of time between blood being taken from a patient and the cells being re-injected have provided no evidence of problems. However it is recommended to re-inject as soon as possible, preferably within 1-2 h after labelling.

**7. Literature**

- Indium (In-111) Oxinate [SmPC]. Petten: Mallinckrodt Medical; 2010.
- Roca M, De Vries EF, Jamar F, Israel O, Signore A. Guidelines for the labelling of leucocytes with 111In-

oxine. Eur J Nucl Med Mol Imaging 2010:37:835-41.

- Palestro CJ, Brown ML, Forstrom LA, et al. Society of Nuclear Medicine Procedure Guideline for 111In-Leukocyte Scintigraphy for Suspected Infection/Inflammation. The Society of Nuclear Medicine, Version 3.
- Peters AM. Imaging Inflammation: current role of labelled autologous leukocytes. J Nucl Med. 1992;33:65-7.
- Guidelines for the safe prepraration of radiolabelled blood cells. UK Radiopharmacy Group. 2009.
- Ellis BL, Sampson CB. Radiolabelling of blood cells-Theory and practice. In: Sampson CB, ed.Textbook of radiopharmacy theory and practice. Amsterdam: Gordon and Breach Science Publishers; 1999: 83-104.
- Hydroxyethyl-starch solutions (HES) should no longer be used in patients with sepsis or burn injuries or in critically ill patients – CMDh endorses PRAC recommendations. EMA. 25 October 2013.
- McAfee JG, Thakur ML. Survey of radioactive agents for in vitro labeling of phagocytic leukocytes. I:Soluble agents. J Nucl Med. 1976;17:480-7.
- Goodwin DA, Doherty PW, McDougall IR. Clinical use of indium-111 labeled white cells: an analysis of 312 cases. In Thakur ML, Gottschalk A, eds. Indium-111 Labeled Neutophils, Platelets, and Lymphocytes. New York: Trivirum; 1981:131.
- Coleman RE, Welsch DM, Baker W, et al. Clinical experience using Indium-111 labeled leukocytes. In Thakur ML, Gottschalk A, eds. Indium-111 Labeled Neutophils, Platelets, and Lymphocytes. New York: Trivirum; 1981:131.
- Schreuder N. Radiofarmaca Medicatiebewaking. Zwolle: Probook Media; 2013:37-9.
- E. Lazzeri, A. Signore, P.A. Erba, et al. Radionuclide Imaging of Infection and Inflammation: A Pictorial Case-Based Atlas. Milan: Springer; 2013:11.
- Smith BT, Weatherman KD. Diagnostic Imaging for pharmacists. Washington D.C.: APhA; 2012:89-90.
- Ten Berge, R.J.M., Natarajan, A.T., Hardeman, M.R. , et al. Labeling with indium-111 has detrimental effects on human lymphocytes: concise communication. J Nucl Med. 1983;24:615-20.