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Radioactive technetium-99m labelling of *Salmonella abortusovis* for the assessment of bacterial dissemination in sheep by in vivo imaging

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We report the development and validation of a 99mTc-labelling technique of bacteria, applied to Salmonella abortusovis. The radioactive labelling is obtained using a pre-tinning step of the cells followed by direct incubation of S. abortusovis suspension with 99mTc-pertechnetate. Several procedures with different amounts of stannous tin (SnF_2 or $SnCl_2$) were evaluated. The selected method, respectful of bacterial viability, provided a 30% labelling yield. Viability of 99mTc-labelled bacteria was assessed by flow cytometry using rhodamine 123 and was demonstrated to be unchanged, turbidimetric measurements showing only a slight increase in the growth rate for radiolabelled cells. Incubation of 99mTc-labelled S. abortusovis with pronase, saponine and urea demonstrated labelling stability and suggested an intra-cellular localization for 99mTc. A prelimi nary study was also conducted in sheep to evaluate the value of the imaging of radiolabelled S. abortusovis. Spatial and temporal patterns of their in vivo dissemination in the lymphatic system after a sub-cutaneous injection were compared with control lymphoscintigraphic agents. These imaging data supported the assumption that the radioactivity detected in vivo was proportional to the number of 99mTc-labelled bacteria.

Keywords: Salmonella abortusovis; Sheep-bacteria; Radio-labelling; Technetium

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1. Introduction

In vivo imaging of the recruitment of phagocytes and immune cells is an unique method to document the host response towards pathogens during the time course of an infection. For granulocytes and lymphocytes sub-populations, this objective can be achieved using in vitro purification, labelling of cells by suitable gamma emitters (99m technetium or 111 indium) then reinjection (Guilloteau et al., 1990; Mc Afee et al., 1984; Thakur et al., 1984). For imaging of macrophages, an in vivo targeting of the cells recruited by the lesions is required and a specific probe based on CD14 and CD11b recognition was developed for such a purpose (Perin et al., 1993; Pittet et al., 1995). To date, experimental studies on the dissemination of bacteria in the body have mostly been performed using invasive surgical techniques followed by either culture or staining of the specimens. The ability to label micro-organisms with gamma emitters suitable for external scintigraphic imaging should greatly expand capabilities in the study of the mechanisms of infection. Few authors have envisaged labelling of micro-organisms with ^{99m}Tc or ^{111}In for in vivo imaging of bacterial dissemination despite its potential value for biological research purposes (Ardehali and Mohammad, 1993; Arden et al., 1993; Aziz et al., 1993; Bernardo-Filho et al., 1991; Plotkowski et al., 1987; Thakur, 1987). This paper reports the development and the validation of a labelling technique for *Salmonella abortusovis*, using 99m technetium in conditions preserving cell viability. Attention was paid mainly to the documentation of the labelling stability and to the control of bacterial viability which is a strict requirement for a functional imaging of bacterial dissemination. In addition, preliminary data is given on the time course by which a ^{99m}Tc-labelled vaccinal strain of *S. abortusovis* disseminates in the lymphatic system of sheep after sub-cutaneous injection.

2. Materials and methods

2.1. Bacterial strain and growth conditions

The Rv6 strain of *S. abortusovis* was used for this study. This attenuated vaccinal strain (Rv6) was selected as a reverse mutant of a streptomycin dependant strain (Lantier et al., 1981; Pardon et al., 1990). Bacteria were grown on solid trypticase soya agar (TSA) at 37°C for 18h.

2.2. Radiolabelling of bacteria

Fifteen hours before labelling, a colony was harvested from TSA medium and placed in 5 ml trypticase soya broth (TSB). This suspension (10^6 bacteria) was then submitted to three successive centrifugations (30 min, 1200 g, 4°C) in order to eliminate the culture medium. The supernatant was discarded each time and the final volume adjusted to 5 ml with 0.9% NaCl (Laboratoires Aguettant, France) to eliminate metabolites and ions likely to interfere with ^{99m}Tc-labelling. Finally, the pelleted bacteria, resuspended in 1 ml 0.9% NaCl, were mixed with 2 ml of a solution containing various amounts of

stannous ions (SnF_2 or $SnCl_2$ in the range: 0–200 μg; Aldrich, USA) for an extemporaneous reduction of ^{99m}Tc from +VII to +IV oxidation state. The ^{99m}Tc labelling was performed by adding 650 MBq ^{99m}Tc-pertechnetate (Elumatic III, CIS Biointernational, France). After stirring and 20 min incubation at 37°C, bacteria were centrifuged three times at 1200 g and 4°C. To prevent reduced Tc reoxidization, the pellet was resuspended each time in 0.9% NaCl solution containing 0.25 mg ml^{-1} ascorbic acid as reducing agent. Finally, radiolabelled bacteria were resuspended in 400 μl 0.9% NaCl.

2.3. *Determination of* ^{99m}Tc-labelling efficiency

Labelling efficiency was measured by counting the last pellet containing ^{99m}Tc-labelled bacteria with a CRC-15R activimeter (Capintec Instruments Inc., USA) and expressing the results as percentages of the radioactivity initially added.

2.4. *Assessment of bacterial viability*

This parameter was assessed immediately after ^{99m}Tc labelling. For each preparation, 30,000 cells were analyzed by flow cytometry with a FACScan (Becton Dickinson, USA) using rhodamine 123 as fluorochrome. Bacteria were washed with Tris-EDTA (10 mM Tris, 1 mM EDTA) buffer pH 8.0, then resuspended in 1 ml Tris-EDTA buffer containing 5 μg rhodamine 123 and incubated at 37°C for 30 min. Fluorescence intensity was measured at 530 nm and expressed in arbitrary units on a logarithmic scale. Bacteria killed by heating 1 h at 60°C were labelled with the same fluorochrome and used as controls.

The rate of bacterial growth was also determined by continuous turbidimetric measurements at 600 nm and 37°C using an aggregometer (Labor gmbh, Germany). The number of bacteria was evaluated from a calibration curve and measured doubling times were expressed in minutes.

2.5. *Evaluation of* ^{99m}Tc binding to bacteria

Suspensions of 10^8 bacteria labelled with 50 MBq ^{99m}Tc were first incubated in vitro for 1 h at 37°C with 1 ml of: (i) 500 μg ml^{-1} pronase dissolved in phosphate buffered saline, pH 7.3; (ii) 1% saponine; (iii) 10% saponine; and (iv) 2, 4 and 6 M urea. After centrifugation at 1200 g, radioactivity of the supernatant was measured and expressed as a percentage of the initial activity of the bacterial preparation. A control experiment was performed to evaluate the effect of incubation and centrifugation alone on the release of ^{99m}Tc.

Additional in vitro experiments were performed to document the stability of ^{99m}Tc labelling. Radiolabelled bacteria were incubated with 20% fetal bovine serum for 1 h at 37°C and then centrifuged. Radioactivity of the supernatant was counted and an aliquot was filtered (Millipore 0.22 μm) and analyzed by exclusion–diffusion radio-HPLC using a 7.5×30 mm TSK2000SWXL column (Tosoh Corporation, Japan) isocratically eluted with 0.9% NaCl at 1 ml min^{-1} flow rate. Detection of the radioactivity was performed with a Berthold LB506-C1 monitor (EG and G Instruments, Germany) equipped with a BGO-150 μl flow cell.

2.6. Scintigraphic imaging

Animal investigations were carried out according to EEC guidelines. Ten male Pré-Alpes du Sud sheep (INRA Nouzilly, France) aged 2 months were anaesthetized by intra-peritoneal administration of 15–20 mg kg^{-1} of sodium pentobarbitone (Sanofi, France). All animals were subcutaneously injected with 10^8 viable *S. abortusovis* bacteria in the hindlegs (injection volume: 0.2 ml; mean activity per site of injection: 50 MBq). Anterior images (300 s, 128^2 format) of the lower part of animals were acquired at various times post-injection using an Orbiter-75 gamma camera (Siemens, Germany) equipped with a parallel low energy-high resolution collimator. The spectral width was 15% centered around the 140 keV photopeak of ^{99m}Tc. Bladder was emptied by transcutaneous puncture when necessary in order to avoid the masking of iliac and inguinal lymph nodes by radioactivity elimination in urine.

Control lymphoscintigraphies were performed in six additional animals using: (i) ^{99m}Tc-labelled antimony sulphide colloids (Mallinckrodt Diagnostica, Netherlands) (n = 3) and ^{99m}Tc-labelled albumin nanocolloids (Solco Nuclear, Switzerland) ($n = 3$), prepared and administered as previously described (Pittet et al., 1995); (ii) free ^{99m}Tc-pertechnetate (CIS Biointernational, France) in one out of the six sheep according to the scintigraphic protocol described above. These imaging controls were then compared with the patterns obtained with ^{99m}Tc-labelled bacteria.

3. Results

3.1. Bacteria labelling with ^{99m}Tc

The absence of ^{99m}Tc labelling in the control test performed on bacteria directly incubated with pertechnetate demonstrated that the use of a reducing agent such as stannous ion was necessary (Fig. 1). Among the protocols tested for radiolabelling of *S. abortusovis* bacteria with ^{99m}Tc, the best efficiency was observed when 200 μg SnF_2

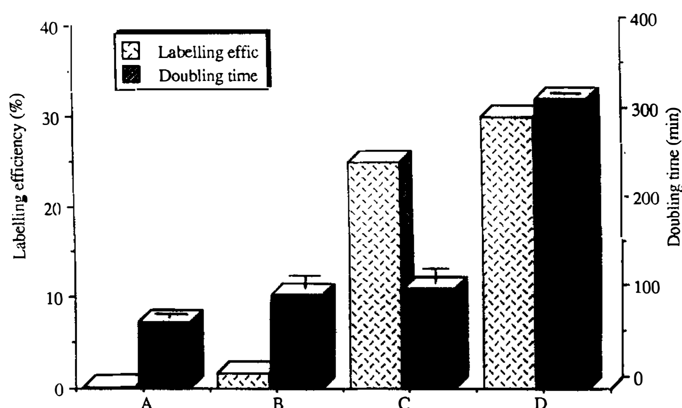

Fig. 1. Influence of ^{99m}Tc-labelling protocol upon radiolabelling efficiency and doubling time of *Salmonella abortusovis*. *A, B, C* and *D* correspond to ^{99m}Tc-labelling experiments differing only in the reduction mode of the pertechnetate, e.g., by using no reducing agent, 80 μg $SnCl_2$, 80 μg SnF_2, or 200 μg SnF_2 respectively. Data are mean values ± s.d. ($n = 3$).

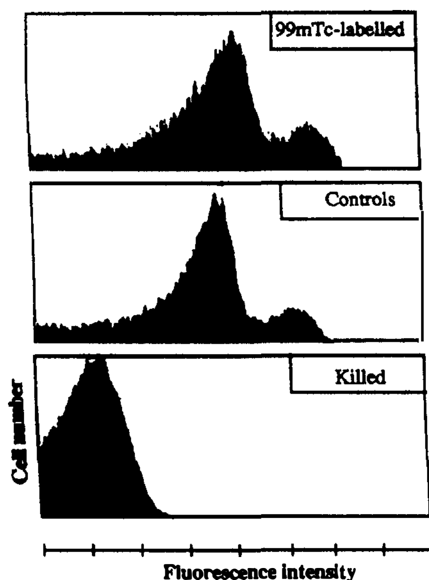

Fig. 2. A composite of fluorescence intensity histograms (expressed in arbitrary units) obtained for ^{99m}Tc-labelled (upper), control (middle) and killed bacteria (lower). These results demonstrate that the selected ^{99m}Tc-labelling technique (80 μg SnF_2) was not detrimental to the viability of the *S. abortusovis* bacteria.

were used (Fig. 1). Unfortunately, a highly significant increase in doubling time (320 min) was observed when such an amount of stannous tin was used since doubling time of viable non-radiolabelled bacteria was 71 ± 3.8 min ($p < 0.01$). In order to be close to the normal growth rate of bacteria, 80 μg SnF_2 was therefore considered as the best compromise since it provided a 25% labelling efficiency without significant modification of the bacterial growth rate (Fig. 1).

3.2. Viability of ^{99m}Tc-labelled bacteria

Comparison of fluorescence histogram profiles of control bacteria, ^{99m}Tc-labelled bacteria (using 80 μg SnF2) and heat-killed bacteria obtained by flow cytometry with rhodamine 123 clearly showed that labelling of *S. abortusovis* with ^{99m}Tc did not disturb their membrane potential (Fig. 2). Culture of bacteria labelled in the same conditions confirmed the viability of the cells with a doubling time very close to that of control bacteria tested in the absence of ^{99m}Tc or reducing agents (Fig. 1).

3.3. ^{99m}Tc binding to bacteria

When compared to the spontaneous release of ^{99m}Tc from control bacteria, only the non-specific protease pronase and denaturing agents such as 10% saponine and 6M urea were effective in releasing some ^{99m}Tc radioactivity. This effect was moderate since 6 M urea, a highly denaturing agent, resulted in only a small increase in released

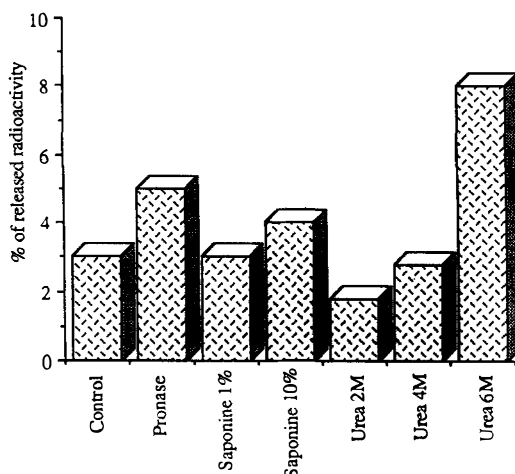

Fig. 3. Evaluation of the percentages of release of ^{99m}Tc-radioactivity from labelled bacteria after 1 h incubation at 37°C with enzymatic (pronase), detergent (saponine) and denaturing (urea) agents.

radioactivity as compared to controls (Fig. 3). At 37°C in the presence of serum, the release of radioactivity reached only 8–12% after 1 h and HPLC analysis showed that 50% of the radioactivity released by bacteria was associated with albumin, the other half being equally distributed between free pertechnetate and hydrolyzed reduced technetium.

3.4. Scintigraphic imaging

Dissemination of bacteria was studied in ten sheep with static images recorded every 20 min during the first 3 h after injection and then every 30 min for the following 3 h. Radioactivity was always detected in the popliteal lymph node as early as 10 min post-injection. *S. abortusovis* was found to pass across the first lymph node, i.e., the popliteal one in 50% of animals. In five animals, bacteria were drained from the injection sites but remained fully trapped in the popliteal lymph node. A control lymphoscintigraphy was performed in the same conditions after s-c injection of free pertechnetate to document the potential contribution of radioactivity released from bacteria. Rapid clearance from the injection site resulting from passage in the blood was observed; however, no accumulation of radioactivity was seen in any lymph nodes, thus suggesting a minimal contribution of free technetium in the constitution of the images obtained after injection of labelled bacteria (Fig. 4). Second, lymphoscintigraphic colloidal agents (antimony sulphide and albumin nanocolloids) were used to document the potential role of radioactive colloids likely to be generated during the labelling steps due to the use of stannous tin. These two radiopharmaceuticals demonstrated much lower diffusibility in the lymphatic system of the sheep, with massive trapping in the popliteal lymph node (30% of the injected dose at 2h for Sb_2S_3 colloids; 20% of I.D. for albumin nanocolloids) with less than 2% of the radioactivity found in the downstream lymph nodes. These colloidal agents were unable to image both the popliteal and the

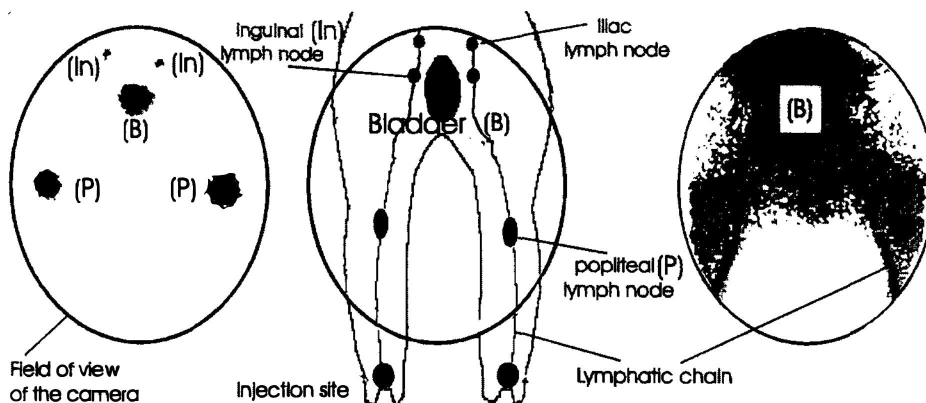

Fig. 4. Scintigrams obtained 20 min after sub-cutaneous injection of ^{99m}Tc-labelled SAO (left) or after subcutaneous administration of $^{99m}TcO_4^-$ (right). In the middle, as an help for interpretation, is represented a schematic illustration of lymphoscintigraphy showing the lymphatic system likely to be imaged in sheep (B: urinary elimination of ^{99m}Tc in bladder; P: popliteal lymph node; In: inguinal lymph node).

inguinal lymph nodes within the first 20 min following the injection. In contrast, with ^{99m}Tc-labelled bacteria, as soon as 10 min after injection, radioactivity was detected in both lymph nodes and even in the third node (iliac) of the lymphatic chain draining the site of injection for one sheep.

4. Discussion

To date, only seven studies have been reported about radiolabelling of bacteria with gamma emitters. Technetium 99m has been used to label *Pseudomonas aeruginosa* (Plotkowski et al., 1987), *Klebsiella pneumoniae* (Bernardo-Filho et al., 1991) and *Escherichia coli* (Arden et al., 1993). Labelling with ^{111}In has been previously undertaken with *Escherichia coli* (Thakur, 1987), for some strains of *Streptoccocus* (Aziz et al., 1993) and for *Staphyloccocus aureus* (Ardehali and Mohammad, 1993) whereas ^{75}Se-selenomethionine was used by Kishore et al. (1980) to label bacteria in order to study phagocytosis. Due to its physical characteristics ($E = 140$ keV, $T_{1/2} = 6.03$ h), ^{99m}Tc, available as $^{99m}TcO_4^-$, is the most suitable radiolabelling agent for in vivo imaging because it gives minimal radiation dose and is inexpensive and readily available. The absence of direct ^{99m}Tc labelling in the first test performed on bacteria directly incubated with pertechnetate demonstrated that the use of a reducing agent was necessary (Fig. 1). $SnCl_2$ is the usual reducing agent for $^{99m}TcO_4^-$ (Dewanjee, 1990; Lin et al., 1971). However, a low labelling efficiency (1.7%) was obtained when 80 μg $SnCl_2$ were used (Fig. 1). In order to optimize the usable chemical form of tin for the reduction of pertechnetate (Srivastava et al., 1977), SnF_2, exhibiting better aqueous solubility and ionization at neutral pH, was therefore considered with 80 μg demonstrating the best compromise between labelling efficiency (25.0%) and bacterial viability (Fig. 1).

To obtain reliable and relevant data on the in vivo time course distribution of bacteria in a given host, the radiolabelling must be stable and must also preserve the viability and biological properties of the cells. To be representative of the in vivo release of ^{99m}Tc from bacteria, the radiolabelled bacteria were incubated in vitro in the presence of serum. The released radioactivity (only 8–12% after 1 h) was ascribed to ^{99m}Tc-albumin, free pertechnetate and hydrolyzed reduced technetium after HPLC analysis. When stability of the ^{99m}Tc binding was tested against non-specific protease, denaturing and detergent agents, only the pronase and denaturing agents such as 10% saponine and 6 M urea were effective to release only 5–8% of the ^{99m}Tc radioactivity (Fig. 3). Although these results support the hypothesis of an intra-cellular localization of ^{99m}Tc and may be in keeping with previous studies (Bernardo-Filho et al., 1991), the lack of an appropriate agent able to cleave lipopolysaccharides does not allow strict exclusion of membrane ^{99m}Tc fixation. As demonstrated in the case of ^{99m}Tc-labelling of *Pseudomonas aeruginosa* (Plotkowski et al., 1987) or *Klebsiella pneumoniae* (Bernardo-Filho et al., 1991), it seems likely that tin is taken up by the bacteria and that reduction of pertechnetate occurs inside the cells.

Viability of the radiolabelled bacteria is a strict requirement for performing scintigraphic imaging study. Due to the lack of a recognized standard for flow cytometry assessment of the viability, we therefore initially considered a combination of several fluorochromes (Chemunex®, ethidium bromide, propidium bromide and rhodamine 123) directed against different bacterial functions (Diaper et al., 1995; Kaprelyants and Kell, 1992; Mc Feters et al., 1995; Shapiro, 1990). Associating flow cytometry with the determination of bacterial growth rate, we have validated that the rhodamine 123 assay alone was sufficient to monitor the effect of ^{99m}Tc labelling on the viability of the bacteria. Comparison of rhodamine 123 fluorescence histogram profiles of control bacteria, ^{99m}Tc-labelled (using 80 μg SnF_2) and heat-killed bacteria clearly showed that labelling of *S. abortusovis* with ^{99m}Tc appeared to be well tolerated (Fig. 2). Culture of bacteria labelled in the same conditions confirmed the viability of the cells with a doubling time very close to that of control bacteria tested in the absence of ^{99m}Tc or reducing agents (Fig. 1).

As a test to evaluate the potential of gamma radiolabelling of bacteria, we have documented the early dissemination of the Rv6 strain of *S. abortusovis* in the lymphatic system of sheep. Three lymphoscintigraphy controls were performed to assess the specificity of the imaging ascribed to radiolabelled bacteria. These experiments suggested a minimal contribution of both free technetium and ^{99m}Tc-tin colloidal species in the constitution of the images obtained after injection of labelled bacteria (Fig. 4). These imaging controls supported therefore the assumption that the radioactivity detected in vivo was proportional to the number of ^{99m}Tc-labelled bacteria.

5. Conclusion

This study demonstrates that *S. abortusovis* can be labelled with ^{99m}Tc and used for in vivo investigations. Due to its non-invasive nature, this technique could be useful in studies of the host defense against infection in animal models as a complementary

technique to usual microbiological and immunological investigations. Another advantage of scintigraphic studies with radiolabelled bacteria is to provide data on kinetics in the same animal, thus eliminating one cause of variability. This strategy of radiolabelling for gamma imaging is the support of an investigation currently in progress about the influence of the pathogenicity of two different strains of *S. abortusovis* upon their migration across the successive nodes of the lymphatic system.

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References

Ardehali, R., Mohammad, S.F., 1993. ^{111}Indium labeling of microorganisms to facilitate the investigation of bacterial adhesion. J. Biomed. Mat. Res. 27, 269–275.

Arden, W.A., Yacko, M.A., Jay, M., Beihn, R.M., Derbin, M., Gross, D.R., Schwartz, R.W., 1993. Scintigraphic evaluation of bacterial translocation during hemorrhagic shock. J. Surg. Res. 54, 102–106.

Aziz, S.M., Sandefer, E.P., Pauly, T.H., Gillespie, M.N., 1993. Comparative disposition kinetics of ^{111}In labeled group B Streptoccocus and neutrophils during onset of sepsis-induced pulmonary hypertension. Biol. Neonate. 63, 86–95.

Bernardo-Filho, M., Pereira, J.A., Boasquevisque, E.M., Hasson-Voloch, A., 1991. Technetium-99m distribution into *Klebsiella* pneumoniae. J. Nucl. Biol. Med. 35, 162–166.

Dewanjee, M.K., 1990. The chemistry of ^{99m}Tc-labeled radiopharmaceuticals. Sem. Nucl. Med. 20, 5–27.

Diaper, J.P., Tither, K., Edwards, C., 1995. Rapid assessment of bacteria viability by flow cytometry. Appl. Microbiol. Biotechnol. 38, 268–272.

Guilloteau, L., Pepin, M., Pardon, P., Le Pape, A., 1990. Recruitment of 99m technetium or 111 indium labelled polymorphonuclear leukocytes in experimentally induced pyogranulomas in lambs. J. Leuk. Biol. 48, 343–352.

Kaprelyants, A.S., Kell, D.B., 1992. Rapid assessment of bacterial viability and vitality by rhodamine 123 and flow cytometry. J. Appl. Bacteriol. 72, 410–422.

Kishore, R., Baltch, A.L., Hammer, M., Sutphen, N.T., Smith, R.P., 1980. Bacterial labeling with the gamma emitting radionuclide ^{75}Se and its use in phagocytosis. Radiochem. Radioanal. Lett. 43, 55–62.

Lantier, F., Pardon, P., Marly, J., 1981. Vaccinal properties of *Salmonella abortusovis* mutants for streptomycin: screening with a murine model. Infect. Immun. 34, 492–497.

Lin, M.S., Winchell, H.S., Shipley, B.A., 1971. Use of Fe(II) or Sn(II) alone for technetium labeling of albumin. J. Nucl. Med. 12, 204–211.

Mc Afee. J.G., Subramanian, G., Gagne, G., 1984. Technique of leukocyte harvesting and labeling: problems and perspectives. Sem. Nucl. Med. 14, 83–106.

Mc Feters, G.A., Yu, F.P., Pyle, B.H., Stewart, P.S.J., 1995. Physiological assessment of bacteria using fluorochromes. J. Microbiol. Meth. 21, 1–13.

Pardon, P., Sanchis, R., Marly, J., Lantier, F., Guilloteau, L., Buzoni-Gatel, D., Oswald, I., Pepin, M., Kaeffer, B., Berthon, P., Popoff, M., 1990. Experimental ovine Salmonellosis (*Salmonella abortusovis*): pathogenesis and vaccination. Res. Microbiol. 141, 945–953.

Perin, F., Pittet, J.C., Hoffschir, D., Normier, G., Binz, H., Le Pape, A., 1993. Scintigraphic potential of J001X acylated poly-galactoside for the imaging of inflammatory lesions in pigs. Nucl. Med. Biol. 20, 963–971.

Pittet, J.C., Pepin, M., Normier, G., Binz, H., Le Pape, A., 1995. Lymphoscintigraphy via the targeting of macrophages with ^{99m}Tc-J001X poly-galactoside in a model of pyogranulomas developed in sheep lymph nodes. Nucl. Med. Biol. 22, 355–365.

Plotkowski, M.C., Beck, G., Bernardo-Filho, M., Oliveira, E.F., Hinnrasky, J., Puchelle, E., 1987. Evaluation of the 99mtechnetium labelling effect on *Pseudomonas aeruginosa* surface properties. Ann. Inst. Pasteur/Microbiol. 138, 415–426.

Shapiro, M., 1990. Cell membrane potential analysis. Meth. Cell. Biol. 33, 25–35.

Srivastava, S.C., Meinken, G., Smith, T.D., Richards, P., 1977. Problems associated with stannous ^{99m}Tc-radiopharmaceuticals. Int. J. Appl. Radiat. Isot. 28, 83–95.

Thakur, M.L., Seifert, C.L., Madsen, M.T., Mc Kenney, S.M., Desai, A.G., Park, C.H., 1984. Neutrophil labeling: Problems and pitfalls. Sem. Nucl. Med. 14, 107–117.

Thakur, M.L., 1987. Live bacteria labeled with ^{111}In. Eur. J. Nucl. Med. 13, 266.