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METHODOLOGY FOR RADIONUCLIDES QUANTIFICATION THROUGH "IN VITRO" BIOASSAY

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Abstract – In Brazil, the radionuclides used for therapy are: ^{131}I, ^{153}Sm, ^{90}Y and ^{177}Lu, both for routine or research protocols. The radionuclide activity excreted by patients may be quantified by bioassay analysis and constitutes a powerful tool for individual treatment planning. The Bioassay Laboratory (*LBIOVT*) of the Institute of Radiation Protection and Dosimetry (*IRD*) has equipments for gamma and beta spectroscopy. These systems are calibrated in energy and efficiency using reference sources supplied by the National Laboratory of Radiation Metrology (*LMNRI/IRD*). The *LBIOVT* has operational procedures according ISO-ABNT-17025 recommendations and participates of international and national intercomparisons. The patient samples are collected immediately after radiopharmaceutical administrations, at the hospital or at the patient residence, and are handled, stored and transported according national radiation protection regulations. The radionuclide specific activity (Bq/L) is referenced to date and time of excretion, for the estimation of the individual biological half-live. The volume of excreta may carefully manipulated in order to avoid losses and misinterpretation in the activity quantification. The process of the *LBIOVT* accreditation and its participation in intercomparisons may guarantee the confidence of the results, allowing the minimization of the uncertainties in the individual monitoring.

Key words: Radionuclides, bioassay, internal dosimetry, gamma spectroscopy.

INTRODUCTION

In the last years, it has been developed therapies with new radiopharmaceuticals and occurred great improvement of Good Manufacturing Practices (GMP) of monoclonal antibodies and peptides. In Brazil, the radionuclides used for therapy both in routine or research protocols are: ^{131}I, ^{153}Sm, ^{90}Y and ^{177}Lu. Radionuclides activities excreted by workers or patients may be quantified by "in vitro" bioassay techniques. In the last year, the distribution of internal monitored workers involving intakes of radionuclides was: nuclear fuel cycle and nuclear power plants (67%), experimental research (16%), radioisotope production (7%) and regulatory activities (7%). Less than 3% referred to medical workers or patients. Considering the laboratory resources and the improvement of new therapies in the country, IRD invested in developing human capacities for internal dosimetry in nuclear medicine.

MATERIAL AND METHODS

Equipments

For quantification of radionuclide activities in biological samples, the equipment is elected according the radionuclide emission. For gamma emitters up to 100 keV, may be used High Pure Germanium Detector (HPGe) (Canberra) 30% efficiency (Fig.1) and sodium iodine detector. These systems should be calibrated before sampling. For beta emitters, it is used liquid scintillator detector (Perkin Elmer Quantulus) with low background counting. The energy calibration consists in establishing the relation between the photo peak energy and the number of channels corresponding to the centre of the photo peak. For energy calibration of the gamma spectroscopy are selected radionuclides with its corresponding energies in the interval of interest or may be used ^{152}Eu source, which has distinct energies. These measures should be performed within sufficient time in order to have adequate statistical counting in the photo peak of interest (at least 1,000 counts) for determining with precision its position. The efficiency of detection depends on the detector as long as the geometry of the sample to be measured. The counting system should be calibrated for one or more geometries (Marinelli, bottles, etc) as appropriated. The standard geometry is the combination of the type and volume of the collecting recipient, type of sampling and the distance between the detector and the sampling. The standard solutions should be prepared with water, and it may be necessary correcting factors for samples with densities other than water. Each system is calibrated with reference sources supplied by the LMNRI/IRD. The standard sources are certified and have

rastreability to the primary standards. The procedures are established according ISO-ABNT 17025 (1).

Figure 1. High Pure Germanium (HPGe) System

Methodology

The urine samples are collected immediately after the radiopharmaceutical administration at the hospital. For each excretion, the urine is collected separately in standard plastic bottles supplied by *LBIOVT*. Depending on the physical half-live of the radionuclide, samples also may be collected at the patient residence. Each bottle should be previously identified with patient name, date and time of collection. The bottles should be maintained refrigerated until transportation to the laboratory. All samples should be handled, stored and transported according the national or international regulations, as applicable, for clinical research (4), radiation protection (3) and transport of radioactive materials (2).

The preparation of urine samples for measurements includes homogenisation and determination of its volume by comparison with its mass, which is obtained by using a precision balance. For testing the dead time of counting, each sample is counted for a short time. If the dead time is higher than 5%, it should be calculated an aliquot of the sample and the volume of the bottle completed with 1 L of HNO_3 1M. The bottle is sealed in a plastic bag, positioned on the HPGe detector and measured for one hour. All spectrums are recorded (Fig.2) as well as the complete identification of the patient and time of sampling.

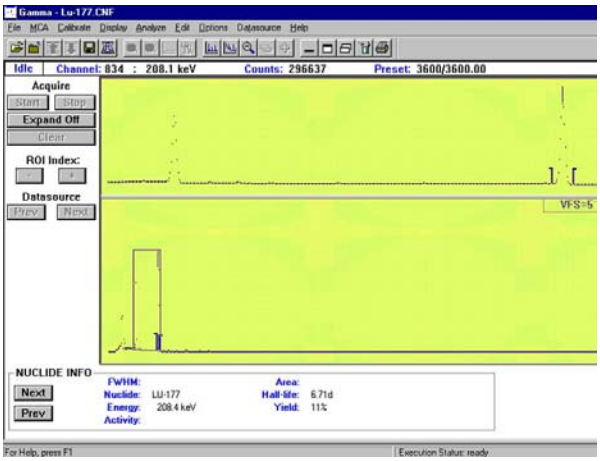

Figure 2. ^{177}Lu gamma spectroscopy with HPGe detector

Considering that the radionuclide activity is referenced to the time of sampling, the initial registered activity should be reliable and the dose meters should be calibrated using a standard source supplied by the LMNRI/IRD (5). Since the results are registered in specific activity (Bq/L), it is mandatory to guarantee that no losses in the process have occurred.

Table 1. Number of "In vitro" studies (workers and patients) per radionuclides

Radionuclide	Workers (n)	Patients (n)
^{131}I	7	8
^{177}Lu	-	11
Total	7	19

RESULTS AND DISCUSSION

After 1998, the *LBIOVT* has participated of 11 international intercomparisons of the "Association for the Promotion of Quality Controls in Radiotoxicological Bioassay" (PROCORAD) for beta and gamma spectroscopy and, after 2000, it has participated of 27 intercomparisons of the Program of National Intercomparisons (PNI) for gamma spectroscopy. For beta spectroscopy, the evaluated radionuclides were: ^{3}H and ^{14}C. For gamma spectroscopy, the evaluated radionuclides were: ^{65}Zn; ^{40}K; ^{133}Ba; ^{134}Cs; ^{137}Cs; ^{54}Mn; ^{152}Eu; ^{125}I; ^{129}I; ^{139}Ce; ^{22}Na; ^{57}Co and ^{60}Co.

It was shown that "In vitro" bioassay is a sensitive method to quantify intakes of ^{131}I in 8 patients (7) and 7 nuclear medicine workers (6) (Table 1). For eleven patients treated with ^{177}Lu, although beta radiation represents the major contribution to the tumour absorbed doses, it was demonstrated strong correlation between patients activities excreted and their individual clinical achievements, according to the physician personal communication (Correa J., 2008).

The process of the *LBIOVT* accreditation and participation in intercomparisons may ensure the confidence of the results, allowing the minimization of the uncertainties in the individual monitoring. At the moment, the *LBIOVT* is planning research with therapy beta emitters using the liquid scintillator detector.

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Other articles in this theme issue include references (8-15).

REFERENCES

1. Associação Brasileira de Normas Técnicas. General requirements for establishing competency of essays and

calibration laboratories. Norma ABNT NBR/ISO/IEC 17025. ABNT, Rio de Janeiro, 2005 (In Portuguese).
2. Comissão Nacional de Energia Nuclear. Transport of Radioactive Materials. Norma CNEN-NN-5.01. CNEN, Rio de Janeiro, 1988 (in Portuguese).
3. International Atomic Energy Agency. International safety basic standards for protection against ionising radiation and for the safety of radiation sources. *Safety Series 115*, IAEA, Vienna, 1996.
4. International Commission on Radiological Protection. Radiological Protection in Biomedical Research. ICRP **62**, Pergamon Press, 1992.
5. Iwahara A., Oliveira AE., Tauhata L., Silva C.J., Silva C.P.G., Braguirolli AM.S., Lopes R.T. Performance of dose calibrators in Brazilian hospitals for activity measurements. *Applied Radiation and Isotopes*, 2002, **56**: 361-367.
6. Lucena E. A., Rebelo A.M., Araújo F., Sousa W. O., Dantas A.L.A., Dantas B.M. and R. Corbo. Evaluation of internal exposure of nuclear medicine staff through In Vivo and In Vitro bioassay techniques. *Rad Prot Dos Advance Access* Aug 6, (2007) pp. 1-4.
7. Nascimento A.C.H., Lipsztein J.L., Lucena E., Dantas B.M., Melo D. R., Oliveira E.R., Meguerian B. Biokinetic and cytogenetic studies in thyroid cancer patients after ^{131}I treatment. In: *Radiation and thyroid cancer*, Thomas G., Karaoglou A. and Williams E.D. (eds.) World Scientific, 1999.
8. Correia, M. B. L., Magnata, S. S. L. P., Silva, I. M. S., Catanho, M. T. J. A. and Lima, F. F. Biokinetics and dosimetric studies about 99MTC(V)-DMSA distribution. *Cell. Mol. Biol*., 2010, **56** (2): 1-5.
9. Couto, R. M., De Barboza, M. F., De Souza, A. A., Muramoto, E., Mengatti, J. and De Araújo, E. B. *In vivo* comparative study of hydroxyapatite labeled with different radioisotopes: evaluation of the scintigraphic images. *Cell. Mol. Biol*., 2010, **56** (2): 6-11.
10. De Araújo, E. B., Pujatti, P. B. and Mengatti, J. Radiolabeling of substance p with lutetium-177 and biodistribution study in rat pancreatic tumor xenografted *nude* mice. *Cell. Mol. Biol*., 2010, **56** (2): 12-17.
11. Pujatti, P. B., Santos, J. S., Massicano, A. V. F., Mengatti, J. and De Araújo, E. B. Development of a new bombesin analog radiolabeled with lutetium-177: *in vivo* evaluation of the biological properties in *balb-c* mice. *Cell. Mol. Biol*., 2010, **56** (2): 18-24.
12. Yano, V. F. and Lima, F. F. Radiation exposure from diagnostic nuclear medicine in alagoas (Brazil) in 2002-2005. *Cell. Mol. Biol*., 2010, **56** (2): 25-30.
13. Melo, I.B., Ueda, L.T., Araujo, E.B., Muramoto, E., Barboz, M.F., Mengatti, J., Buchpiguel, C.A. and Silva, C.P.G. Tecnetium-99m as alternative to produce somatostatin-labeled derivatives: comparative biodistribution evaluation with 111in-dtpa-octreotide. *Cell. Mol. Biol*., 2010, **56** (2): 31-36.
14. Silva, I C. O. A., Lucena, E. A., Souza, W. O., Dantas, A. L. A. and Dantas, B. M. Estimation of internal exposure to 99mo in nuclear medicine patients. *Cell. Mol. Biol*., 2010, **56** (2): 37-40.
15. Velasques De Oliveira, S. M., Carlos, M. T., Carneiro, M. P., Da Silva, J. W. E., Kasai, E. P., Oliveira, A. R. N. and Boasquevisque, E. M. Protocol for 18F-FDG quantification in PET-CT whole-body exams. *Cell. Mol. Biol*., 2010, **56** (2): 44-46.