
Assay of prick test inoculum volume. I. Use and reliability of a gamma camera-based method

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Introduction: In dermatology and allergy there are clinical research circumstances where very small amounts of substances introduced into the skin have to be measured “in vivo.” An example is the assay of reagents injected by prick test. As injected volumes are very small, it is necessary to use indicators that can be measured at very low concentrations. In *in vitro* studies, gamma-emitting radioisotopes have been shown suitable for use as the indicators. In *in vivo* studies, except for instruments devised for specific research requirements, the measurement of small sources is taken with a common gamma camera.

Objective: The purpose of the present study is to evaluate the experimental reliability of a gamma camera-based method to measure microvolumes labeled with radioisotopes and its suitable application in *in vivo* studies.

Methods: Using a solution of 99m Tc-pertechnetate, we prepared, with precision pipettes, some sets of scalar volumes ranging from 1 micro to 200 picoliters, which correspond to activities between some micros and some hundreds of picocuries. The volumes were measured with a gamma camera both with and without a collimator. The overall reliability of the method under different experimental conditions was evaluated for sensitivity, precision, and accuracy. Last, a blind measurement was taken as a final check on the overall reliability of the method.

Results: The volume-activity correlation appeared to be linear, with a Spearman coefficient higher than 0.99. The correlation straight lines of the measurements taken with and without a collimator proved that, in both cases, the linearity of the system did not change. The method showed a high degree of precision and accuracy. The maximum variation coefficient never exceeded 1.5% and the standard error 2%. The sampling error of the measured volumes was less than 8% in all the sets; up to 7% was due to the manual operations and to the technical characteristics of the micropipettes. The gamma camera measurement error ranged from 1% to 3%. The blind tests experimentally confirmed the overall reliability of the method.

Conclusions: The method we studied proved highly reliable and inexpensive. Measurement errors are almost exclusively due to sampling errors. The gamma camera is a device any nuclear medicine department is equipped with, and a solution of 99m Tc-pertechnetate is readily available.

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INTRODUCTION

In clinical studies it is often necessary to measure precisely very small amounts (volumes) of substances or active principles introduced into the organism. In

most cases these substances can be recorded by sampling biologic liquids. In other cases, especially in clinical research, this is not possible. Examples would be in dermatology, when studying the adsorption of liquids or active principles into intact or damaged skin, or in allergy in the assays of reagents injected into the skin by prick tests. The size of the inoculum is a critical point for the reproducibility of the prick test. Directly and precisely measuring the inoculum volume by prick

testing could be very useful in studies concerning the standardization and grading of the different techniques of prick-puncture tests. This, however, is an objective which has yet to be achieved. To the best of our knowledge, no assay methods suitable for this purpose have been described or validated in literature. As such volumes are usually extremely small, they can be estimated by indicators labeled at very low concentrations. Gamma-emitting radioisotopes have proved suitable in *in vitro* studies for this purpose. The isotopes are precisely measured with well counters even at very low activity levels, approximately 10 E-6 microcuries.^{1,2} Excluding high-sensitivity Whole Body Counter or high-resolution probes devised ad hoc, radioactivity is routinely recorded with a gamma camera in *in vivo* studies. In fact, nuclear medicine departments routinely connect gamma cameras to data processing systems to examine organ function quantitatively.³ The aim of the present work is to check whether the gamma camera is suitable for measurements of microvolumes—that is volumes smaller than a microliter—labeled with radioisotopes. To determine its potential use in *in vivo* assays, we designed a laboratory study to validate the method scientifically, and to check its overall degree of reliability. Consequently, as commonly required for the statistical quality control of laboratory-based measurement methods, it was examined and described in terms of sensitivity (estimate of the smallest quantity of reagent that can be measured), precision (consistency of the results obtained with repeated measurements of the same sample), and accuracy (consistency of the real value with the measured value).

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METHODS

The gamma camera is an imaging system. It consists of a scintigraphic camera that records the radioactivity from gamma-emitting radionuclides simultaneously from the entire operative field. It can convert the gamma radiations that strike the crystal detector into as many electric pulses. The electron-beam is transferred to a point on an oscilloscope: the point corresponding to the one where the scintillation of the crystal has taken place. The image of the radioactive source is the result of the sum of these punctiform traces. The image is processed through the following steps:

- (1) definition of the background radioactivity resulting from the scintillations produced by radiations not coming from the source under examination, which must be subtracted from the activity of the latter;
- (2) delimitation of the region of interest and automatic edge detection of the source; and
- (3) recording of the counts (gamma emissions) from the source under examination, and drawing of the relative activity curve in a determined unit of time.

The image is recorded as it is seen on the persistence screen of the gamma camera. The image is stored, together with the mathematical parameters defining it, in the computer interfaced with the gamma camera for subsequent processing. The space resolution of the gamma camera is optimized by a collimator, which consists of a lead plate with a great number of holes. The holes permit only perpendicularly incident radiations to strike the crystal detector. This, however, means a reduced counting sensitivity, and for this reason the collimator is not generally used to detect punctiform, low-radioactivity sources. When the collimator is not used, we can place punctiform sources in contact with the crystal detector so as to avoid significant losses of the space resolution capacity of the gamma camera. The gamma emissions that strike the crystal can be precisely

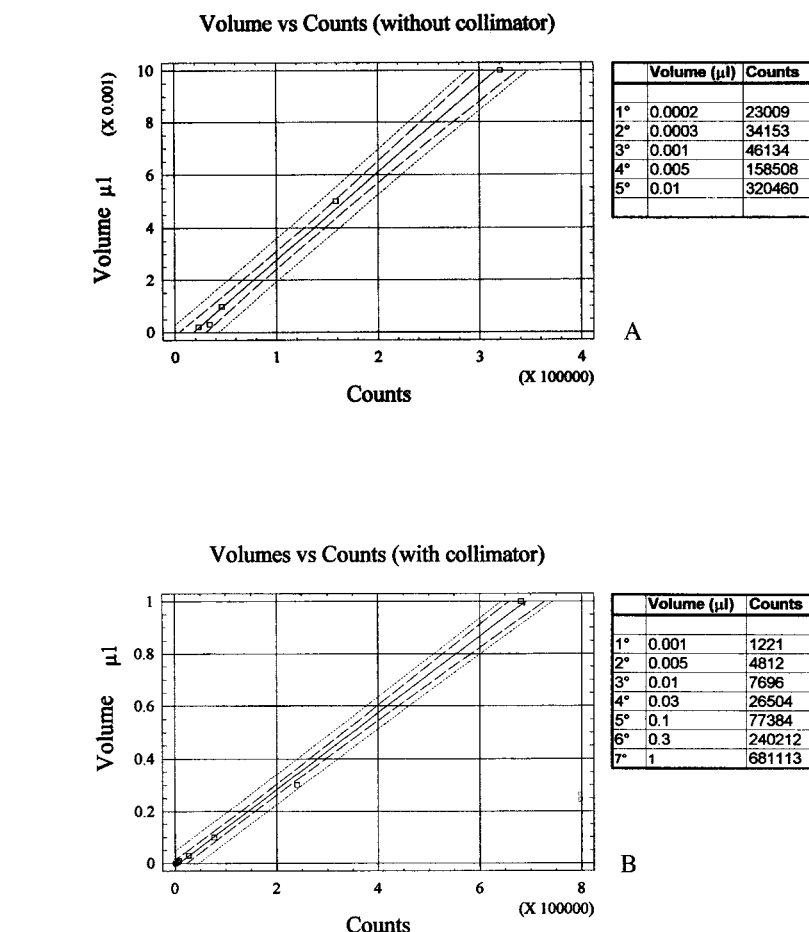


Figure 1. Volume versus count linear regression of punctiform sources recorded by the gamma camera (A) without a collimator (interpolable values between 200 picoliters and 0.01 microliter) and recorded (B) by a high-sensitivity collimator (interpolable values between 0.01 and 1 microliter). For (A), $r^2 = 0.998$ and $Y = 3.3 \times 10^{-8} \times X$. For (B), $r^2 = 0.998$ and $Y = 1.46 \times 10^{-6} \times X$.

counted down to very low activity values. As the activity of a source is directly proportional to the concentration of the existing radionuclide, these substances can be used as indicators for quantitative analyses. The assay of an unknown volume of a solution containing a radionuclide can be easily performed if we know both the activity of the volume to be measured and the activity of a known reference volume of the same solution. Following this precept, we used a solution of 99mTc-pertechnetate, which is a radioisotope usually available in any department of nuclear medicine. This solution has physiochemical properties that make it ideal for use in *in vivo* studies:

6-hour half-life and 140 keV gamma energy. With precision micropipettes (Hirschmann Laborgerate, Germany) we prepared some sets of scalar factor-10 volumes, ranging from 1 micro to 100 picoliters. The volumes corresponded to activities between microcuries and hundreds of picocuries. Such volumes were counted with a gamma camera (SP4 Elscint, Haifa, Israel) and volume-activity correlation straight lines were calculated to check the linearity of the system. The low-activity volumes were counted without a collimator, while the high-activity ones, which would quickly bring the counting system to saturation point, were measured by fitting a high-sensitivity collimator

(99m Tc 1105 cps/ μ Ci) to the recording head of the gamma camera. The intermediate-activity volumes (tens to nanocuries) were counted with and without a collimator, enabling us to detect relationships between the two different correlation straight lines.

The measurements of the single activities of the various sets of volumes were taken by computerized technique for the automatic search of contours, with background subtraction. To evaluate the precision of the method we counted the same sources (respectively 0.1, 0.01, and 0.001 microliters) five times each. The 1-microliter volume was considered a real value by which to estimate the degree of accuracy of the system, as it is the least affected by manual sampling error due to repeated fractionings. The micropipette imprecision for volumes of 1 microliter was calculated by the producer as equal to ± 0.02 microliters.

The sampling error was estimated by measuring five different sources of the same volume. Four scalar volumes [1, 0.1, 0.01, and 1000 picoliters, (instead of a single one)] were taken into consideration to correct for manual errors by the operator. The sampling error could be regarded as equal to the estimated error after subtracting the ones due to imprecision and lack of accuracy and the theoretical error of measurement resulting from the characteristics of the gamma camera. We then considered the influence of the form variability of the sources on the precision and accuracy of the counts. When the signal intensity is very low, as in the case of the smallest volumes we prepared, it can be difficult to define the region of interest because of the low ratio of the source to the background. To evaluate this possible influence, we calculated volume-activity curves by letting the volumes deposit onto blotting paper instead of in the common microcuvettes. This simulates more of a spread and a less homogeneous source. The overall adequacy of the method for the description of the observed data was also evaluated by the "Lack-of-FIT Test." Finally, the overall reliability of the system and its

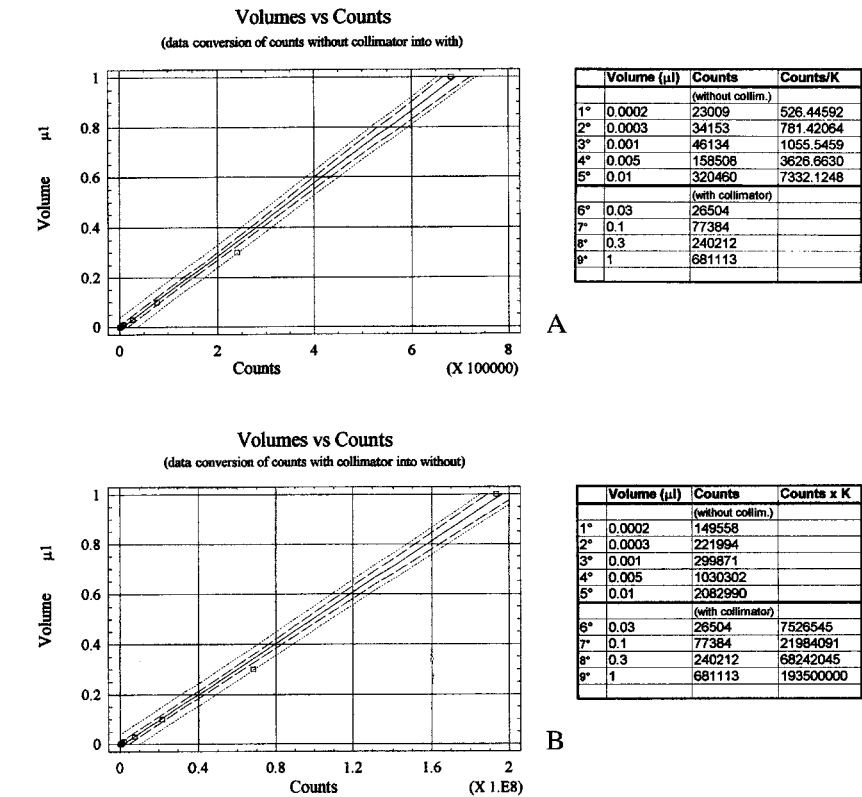


Figure 2. Volume versus count linear regression of punctiform sources obtained after converting the data recorded without (A) and with (B) a collimator. Cnt with a collimator = Cnt without a collimator/K (K = constant coefficient depending on the type of collimator). For A, $r^2 = 0.99$ and $Y = 1.46 \text{ E-6} \times X$. For B, $r^2 = 0.99$ and $Y = 5.13 \text{ E-9} \times X$.

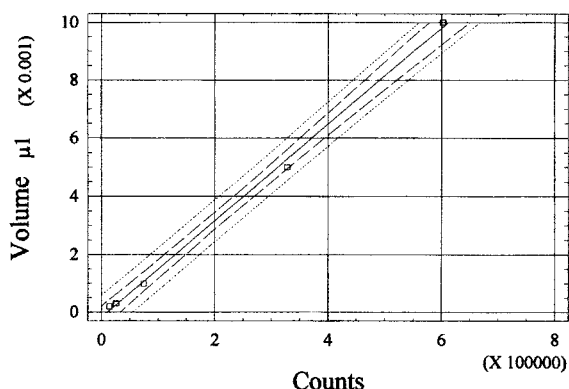
appropriateness for our future research studies were checked under different experimental conditions and by measurements in a blind test. An operator calculated the volume-activity correlation straight line and prepared a set of some scalar volumes of the same solution. Another operator measured, on the basis of the correlation straight line already calculated, the volumes that his colleague had prepared and that he did not know. All the statistical analyses were performed by computer program Statgraphic Plus 3.0 (Manugistics Inc, Rockville, Maryland, USA).

RESULTS

The analysis of the volume-activity linear regression in the range 1 micro to 200 picoliters shows the excellent linearity of the system, whether the source activity is recorded with or without a collimator. In both condi-

tions the Spearman coefficient is higher than 0.99 (Fig 1). The counts of the volumes used to calculate the single correlation straight lines were obtained after previously subtracting the background. The interpolable values ranged between 0.0002 and 0.01 microliters when recorded without a collimator, and between 0.01 and 1 microliter if obtained with the collimator. The regression lines, calculated both with and without a collimator, show that there is a simple mathematical relationship between them (Fig 2). This means that, after recording the activity of any sample of the same solution with a collimator, it is possible to calculate its corresponding volume as if it had been obtained without a collimator and vice versa. This simple, mathematical operation permits any source to be read with both recording methods, and only entails a small reduction of the

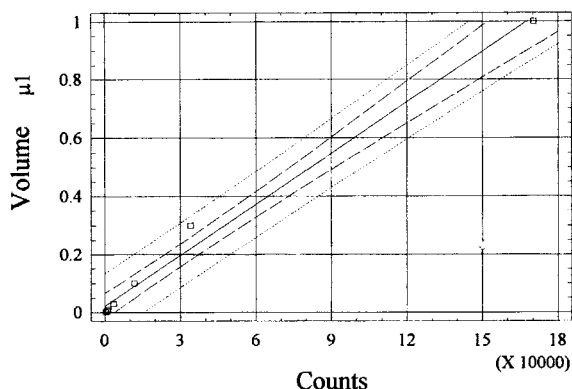
Volumes vs Counts (no point source without collimator)



	Volume (μl)	Counts
1°	0.0002	14486
2°	0.0003	20371
3°	0.001	74996
4°	0.005	317808
5°	0.01	650000

A

Volumes vs Counts (no point source with collimator)



	Volume (μl)	Counts
1°	0.001	1117
2°	0.005	4885
3°	0.01	7790
4°	0.03	22658
5°	0.1	76002
6°	0.3	233371
7°	1	1106950

B

Figure 3. Volume versus count linear regression of non-punctiform sources recorded (A) without a collimator (200 picoliters to 0.01 microliter) and (B) with a collimator (0.01 to 1 microliter). For A, $r^2 = 0.99$ and $Y = 1.66 \text{ E-}8 \times X$. For B, $r^2 = 0.99$ and $Y = 5.8 \text{ E-}6 \times X$.

range of interpolable values (0.005 to 1 microliter). The presence of non-punctiform sources does not affect the linearity of the system (Fig 3). In experiments, we discovered a different volume-count relationship that slightly

influenced the slope of the regression straight line. The calculation of the confidence intervals at 95%, however, shows that these slight differences are statistically significant (punctiform sources slope = $1.55\text{E-}8$, 95% CI =

Table 1. Evaluation of the Precision Degree of Assays. Results of Five Repeat Counts of the Same Sample

	Volumes (microliters)	Counts (5 times each vol) (means $\pm$ SD)	Error (%)
1	0.1	23513 $\pm$ 249 (with collimator)	1.05
2	0.01	7622 $\pm$ 109 (with collimator)	1.43
3	0.001	73485 $\pm$ 582 (without collimator)	0.79

Table 2. Evaluation of the Accuracy of the Method*

	Samples	Real Counts	Comp. Volumes
1	1 microliter	8148	1.018565184
2	1 microliter	8458	1.018565184
3	1 microliter	7571	0.946435568
4	1 microliter	7718	0.964811744

* Assays of some 1-microliter volumes, regarded as the "real volume," on the basis of the regression straight lines built with the corresponding solutions.

$1.50\text{E-}8 \leftrightarrow 1.60\text{E-}8$). The repeat counts of the same sample proved to be highly precise, in fact the variation coefficient was lower than 1.5% (Table 1). Further, the method appears to be very accurate, as the measured value and the real value are basically equivalent (standard error equal to 1.85%) (Table 2). The sampling error is influenced by the operator's manual skill and by other factors, such as scattering of the solution and adhesion to the walls of the cuvettes, and the dragging of the liquid during the pipetting operations. The whole error was, however, limited to a maximum 8% (Table 3). The manual error accounted, in variable percentage terms, for 5% to 7%. The measurement error due to the gamma camera ranged from 1% to 3%, being affected by the degree of difference between the source and the background. This was proportionally higher where the smallest volumes were used at lower activity. The variance analysis by "Lack-of-FIT Test" confirmed the adequacy of the method for the description of observed data (Table 4). The final check was performed by measuring, in a blind test, different volumes in the range taken into con-

Table 3. Results of the Counts of Five Different Sources of the Same Volume, for a Series of Four Scalar Volumes

	Sources (5 samples $\times$ vol)	Counts (mean $\pm$ SD)	Error (%)
1	1 microl.	5090 $\pm$ 294	5.7
2	0.1 microl.	3322 $\pm$ 210	6.3
3	0.01 microl.	1236 $\pm$ 063	5.0
4	0.001 microl.	0370 $\pm$ 028	7.7

Table 4. Results of Analysis of Variance with Lack-of-Fit Test, Applied to the Volume Versus Count Linear Regression of Punctiform Sources Recorded by the Gamma Camera with Collimator

Source	Sum of Squares	Df	Mean Square	F Ratio	P Value
Model	1.52996E12	1	1.52996E12	6332.89	0.0000
Residual	6.28132E9	26	2.41589E8		
Lack-of-Fit	1.52904E9	5	3.05808E8	1.35	0.2821
Pure Error	4.75228E9	21	2.26299E8		
Total (Corr)	1.53624E12	27			

sideration. This definitely provided the experimental proof of the precision and reliability of the system. The measurement in all the counts was within the limits of the above-mentioned sampling error.

CONCLUSIONS

The measurement of radiolabeled microvolumes with a gamma camera proves to be highly reliable. The counts are so precise and accurate as to show even the slightest sampling errors. This reliability does not change when sources are diffuse or not very homogeneous, provided that their activity can be well defined compared with the background. In our measurement system this was possible up to concentration levels of some nanoCi/100 picoliters. When dealing with these volumes, it is essential to operate in environments with very low background activity. The gamma camera-based assay method we developed can

easily be applied to clinical allergy in vivo studies. For instance, it can be used to calculate the inoculum volume with different prick-puncture test techniques or for similar measurements. This study demonstrates that, after previously calculating a volume-activity correlation straight line with known-volume samples, it is possible to measure with a gamma camera unknown volumes of a radioactive solution, even of the order of some hundreds of picoliters. Owing to its high sensitivity, the system is also highly reliable and safe in terms of the patient's radioprotection both at the inoculation site (very low activity source) and as an effective equivalent dose to the whole body, even in the light of new Italian legislation.^{4,5} Finally, the gamma camera is a device which can be found in any department of nuclear medicine, and ^{99m}Tc-pertechnetate, used as a radio-tracer, is readily available. In conclusion, the method is certainly quick,

simple, reliable, and inexpensive. Its use in the calculation of the inoculum volume and in the study of the other variability factors affecting the reproducibility of the prick test will certainly provide useful information not only to research and clinical studies but also to allergy practice.

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