ASSAYS OF RADIOACTIVE MATERIALS FOR USE IN PATIENTS—A FIVE YEAR STUDY

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Since the opening of the Clinical Center in 1953, the staff of the Radiation Safety Office of the National Institutes of Health (NIH) has had the responsibility for the assay and isotopic-purity verification of all radioactive materials intended for use in patients. During this span, more than 12,000 assays have been performed on materials labeled with one of 36 radionuclides. This paper describes the NIH assay methods and summarizes the experience of the NIH with commercial radiopharmaceutical and radiochemical suppliers as well as with the national laboratories during the 5-year period from January 1, 1962, through December 31, 1966.

ASSAY METHODS

Several different counting systems have been used for the assay work. Currently these include a quartz-fiber electroscope, two multichannel analyzers, each connected to a 3 × 3-in. NaI(Tl) crystal, a multichannel analyzer connected to a 3 × 3-in. NaI(Tl) well crystal and a liquid scintillation counter. Although new equipment was purchased during the 5-year study period, the basic methods of calibration remain the same and are described below.

Electroscope system. A quartz-fiber electroscope (Model 2) from the Fred C. Henson Company of Pasadena, Calif., is used routinely to assay high-activity shipments (> 3 mc ^{131}I) and therapy doses of gamma-emitting radionuclides (Fig. 1). Sources can be placed at variable but reproducible distances from the electroscope. One of a series of radium needles calibrated by the National Bureau of Standards (NBS) is placed at a fixed distance. The time necessary for the fiber to move a given distance on the scale is determined. Several such measurements are made, and the average time is calculated. The procedure is repeated using the source to be assayed. From this information as well as the gamma-ray constants for radium and the nuclide to be assayed (*1*), one can determine the activity of the source.

In practice, we use 75 cm as the minimum source-to-electroscope distance. The minimum useful gamma intensity has been found to be 1.17 mR/hr.

Multichannel-analyzer systems. Smaller quantities of gamma-emitting radionuclides are routinely assayed using one of three multichannel-analyzer systems. A Harshaw 3 × 3-in. NaI(Tl) well crystal connected to a 200-channel Radiation Instrument Development Laboratory (RIDL) Model 34-8 analyzer is used to assay low levels (< 0.1 μc) of activity. For intermediate levels (0.1 μc ^{125}I–15 mc ^{51}Cr), a Harshaw 3 × 3-in. NaI(Tl) integral line crystal is used (Fig. 2). This is housed in a graded lead shield similar to that described by R. L. Heath (*2*) and is connected to a 512-channel Nuclear Data Model 130 or Model 180 analyzer.

The basic method of calibrating all three systems is the same. Normal counting geometry is 1 ml total volume in a 3.5-ml glass vial. Whenever possible, solution standards from the NBS are used. When these are unavailable, solution standards are obtained from more than one commercial supplier for intercomparison.

Each instrument is calibrated at 10 kev/channel for radionuclides emitting photons in the 0.25–1.4-Mev energy range. We have found that the following radionuclides make a convenient set of standards: ^{203}Hg, ^{51}Cr, ^{85}Sr, ^{137}Cs, ^{54}Mn, ^{65}Zn, ^{59}Fe, ^{22}Na and ^{60}Co. The total counting rate for 10 channels symmetrically located about the highest energy photopeak of each standard is determined and corrected for contribution from background. Log–log paper is used for the construction of a calibration curve relating sensitivity in terms of corrected counts/min/μc (100% occurrence of the photon) to photon energy in Mev (Fig. 3). This procedure is repeated at various distances from the external crystals and

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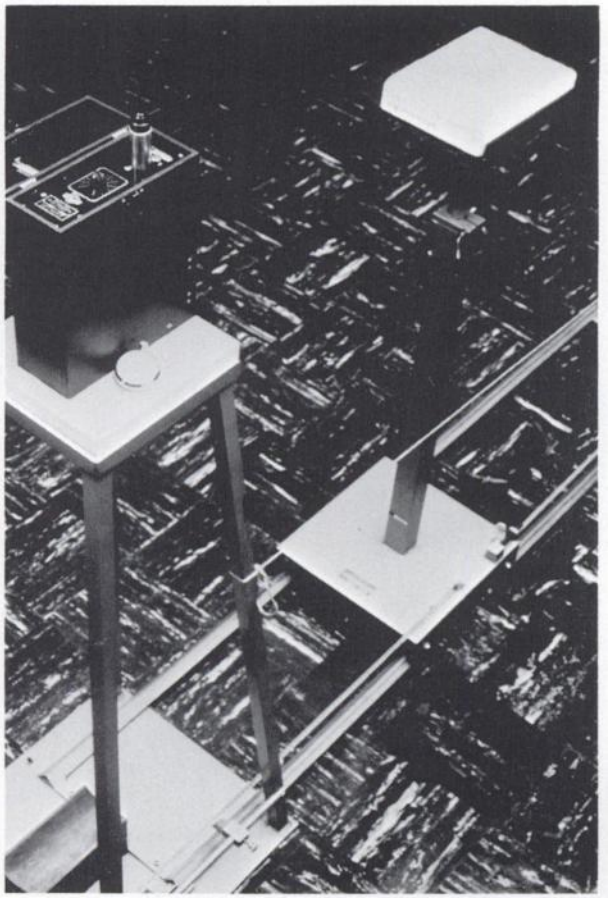

FIG. 1. Electroscope on calibration range is used for assay of high-activity gamma-emitting radionuclides.

appropriate graphs are drawn. For any isotope for which gamma-ray energy and percent occurrence are known, one can assay the nuclide by measuring a known volume of the solution into a 3.5-ml glass vial, adjusting the total volume to 1 ml and determining the net counting rate in the 10-channel photopeak region. The net counting rate is divided by the sensitivity of the instrument for photons of the appropriate energy at the distance at which the sample was counted. Conversion to microcurie activity is made from a knowledge of the fractional occurrence of the gamma ray and any dilution factor that was used.

For photons in the 0.070–0.25-Mev energy range, a similar calibration procedure is followed at 5 kev/channel; the only difference is that the sum of 7 channels is used. Useful standards for the construction of a calibration curve are ^{197}Hg, ^{203}Hg and ^{57}Co. For photon energies of 0.025–0.125 Mev, the sum of 10 channels is used with the instrument calibrated at 1 kev/channel. ^{125}I, ^{203}Hg and ^{57}Co are used as standards.

Instrument calibration is checked daily by counting a flame-sealed ampoule containing ^{137}Cs solution at a fixed distance from the external crystals and a ^{137}Cs rod in the well system. Intercomparisons of all gamma-counting systems are within ± 3.5%.

Liquid-scintillation counting system. A Packard Tri-Carb Model 314 EX liquid-scintillation spectrometer has been used routinely to assay pure beta emitters such as tritium, ^{14}C, ^{35}S and ^{32}P. Accepted liquid-scintillation-counting procedures are employed. These include (1) using NBS standards when they are available or standards from more than one commercial supplier for intercomparison; (2) using the channels-ratio method of determining the effect of quenching on counting efficiency (*3,4*) and (3) counting appropriate standards and blanks before an assay.

DATA

The data presented in this paper have been restricted to include only those per-unit-volume or

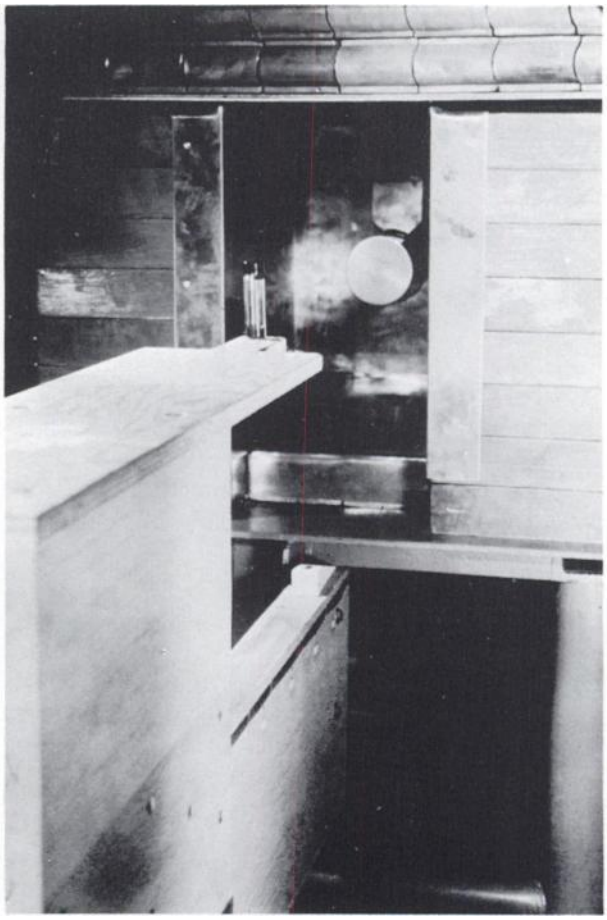

FIG. 2. Sample in 3.5-ml glass vial is positioned at fixed and reproducible distance from a 3 × 3-in. NaI(Tl) crystal in a graded lead shield.

gross assays* performed on liquid solutions or suspensions during the 1962–1966 period. When both per-unit-volume and gross assays were done on a shipment, only the per-unit-volume assay is included in the study. Assays performed after any manipulation of the material by the NIH personnel are not included, the only exception being the simple withdrawal of liquid for accurate measurement of volume. The special problems associated with the radioactive gases have been treated elsewhere (*5,6*), and assays of gases are excluded from this statistical study. Assays of generator eluates—^{99m}Tc and ^{132}I—are not included because of the lack of supplier's assay for comparison purposes.

The NIH assay methods are considered to be accurate to ± 10%. If the supplier's assay is within ± 10% of the NIH assay and if there is no significant contamination, the material is acceptable for use in patients. If, however, these criteria are not met, the supplier is contacted to determine the reason for the discrepancy. In no case has the divergence between the NIH confirmed assay and the supplier's assay been attributed to an error in the NIH assay.

The assays reported in this study have been grouped according to the calendar year in which they were performed. Divergence between the NIH assay and the supplier's assay was expressed as a ratio using the NIH assay value as a base. The assays were then separated into divergence intervals, e.g., ≤ 0.10, 0.11–0.20, etc. The number of assays in each interval was then expressed as a percentage of the total number of assays for that year.

An examination of the yearly figures (Table 1) shows that the percentage of assays in the ≤ 0.10 interval was at a maximum 83.5% in 1962, remained fairly constant at 76–78% from 1963 through 1965 and dropped to 70.6% in 1966. There was a corresponding increase in the percentage of assays exceeding ± 10%, particularly in the assays exceeding ± 20%. Several factors are believed to have contributed to this trend:

1. The use of the more recently accepted isotopes, such as ^{197}Hg, ^{75}Se and ^{87m}Sr, has increased markedly. Suitable standards are not easily obtainable, especially for ^{197}Hg† and ^{75}Se. From the time the first shipment of ^{197}Hg-labeled compounds were received in January 1965 the use increased until at least two shipments per week were received at the NIH in 1966. The assays of this nuclide varied quite markedly from the supplier's estimate (*7*).

2. In 1963, syringes each containing approximately 5 μc ^{131}I-labeled human serum albumin were received. A large percentage of these syringes showed discrepancies in excess of ± 10%.

3. During the 1964 calendar year, the NIH began performing gross assays as well as per-unit-volume assays on gamma emitters. As a result, some errors in the supplier's per-unit-volume assay were discovered which previously could have gone unnoticed if the supplier's volume measurements were incorrect.

While the more exotic isotopes were finding increasing favor during the study period, significant quantities of the more commonly used isotopes were purchased. The U.S. Atomic Energy Commission (*8*) has authorized physicians to possess and use limited quantities of ^{131}I, ^{125}I, ^{60}Co, ^{58}Co and ^{51}Cr in prepackaged individual doses of specific diagnostic radiopharmaceuticals. Because the NIH re-

TABLE 1. PERCENTAGE OF ASSAYS IN EACH DIVERGENCE INTERVAL DURING 5-YR STUDY PERIOD

Divergence interval	Calendar year 1962 (%)	1963 (%)	1964 (%)	1965 (%)	1966 (%)
≤ 0.10	83.50	76.62	76.09	77.86	70.62
0.11–0.20	14.25	21.83	15.68	14.88	21.87
0.21–0.30	1.00	0.57	5.39	3.82	4.68
0.31–0.40	0.50	0.19	1.79	2.81	1.87
0.41–0.50	0.50	0.76	1.02	0.00	0.62
> 0.50	0.25	0.00	0.00	0.60	0.31
Total No. of assays included	400	522	389	497	640

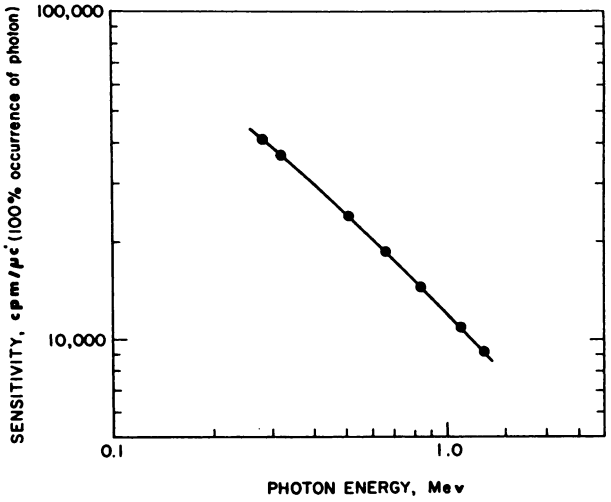

FIG. 3. Typical calibration curve for multichannel analyzer.

* Gross assay is the determination of total amount of activity in the container as received from a supplier.

† A ^{197}Hg solution standard became available from the National Bureau of Standards in April 1967.

ceived very limited quantities of any of the prepackaged individual doses, it was decided to examine the assays of two nuclides, ^{131}I and ^{51}Cr, in all chemical forms and in all types of shipments, both individual doses and bulk materials. The assay of these two commonly used isotopes should not be in doubt after so many years of use. Together these two nuclides accounted for 76% of all assays included in the study. On the average 78.7% of all ^{51}Cr assays were within $\pm$ 10% while 84.5% of the ^{131}I assays were in the same divergence interval. Assays for both of these nuclides showed some slight improvement in agreement with time, particularly during the last 3 years of the study. In the cases of both ^{131}I and ^{51}Cr, the agreement is better than the average of all assays, where 76% were in the ≤ 0.10 interval.

The discrepancies encountered during the study period can be broadly classified as to cause: incorrect shipment, erroneous assay date, wrong volume, incorrect supplier assay, inconsistent labels, uncertainty in decay scheme and contamination. The following are given as examples.

Incorrect shipment. A typical example of this type of discrepancy involved an NIH order which called for the delivery of 75 mc of ^{35}S. When the package reached the NIH, the packing slip was correct, but the material was a 1-mc shipment intended for a local college. The college, in turn, received the 75 mc intended for the NIH together with a packing slip for a 1-mc shipment.

Erroneous assay date. This type of error is particularly serious with short half-life radionuclides. Within a 6-week period, the NIH received two shipments of colloidal ^{198}Au suspensions whose assay dates were incorrect by 2 and 3 days, respectively. When notified of the apparent errors, the supplier confirmed the true assay dates and stated he would notify his other ^{198}Au customers of the error.

Wrong volume. The NIH gross assays differ significantly from the supplier's assay in a number of instances in which the activity per unit volume is high. These are shipments in which the total volume is only a fraction of a milliliter and the concentration may be more than 100 mc/ml. In such cases, the small total volume prohibits the performance of a per-unit-volume assay. Such an assay is done after dilution or after a labeling process, e.g., iodination of protein; however, no effort is made to correlate this per-unit-volume assay with the supplier's original estimate. When only the gross assay data is available, it may be suggested that the reason for the discrepancy is based on a general disagreement on the assay of the isotope involved. This cause of discrepancy was ruled out when the suppliers and the NIH compared assay results on identical solutions of lower activity, and no significant discrepancies were found. It can only be assumed that the original measurements of small volume of liquid were in error.

This type of error can be of significance to the clinician who intends to use the material for carefully calculated therapeutic results. For example, the NIH ordered 25 mc of ^{131}I-labeled sodium iodide and actually received 41 mc. Had this material been used for a therapy without strict attention to the gross assay as well as to the per-unit-volume assay, the patient would have received 1.6 times the calculated dose.

Incorrect supplier assay. Examples of this type of error involve such varied compounds as: (a) diisopropylfluorophosphate labeled with ^{32}P which gave an assay 65% higher than the supplier's label would indicate, (b) tritiated thymidine which showed values which were 70–75% of the supplier's estimate, (c) cyanocobalamin-^{57}Co and chromic chloride labeled with ^{51}Cr, each of which showed only one-half the activity as given on the label. In each case the appropriate supplier was notified and confirmed his error.

Inconsistent labels. A shipment of diagnostic ^{131}I-labeled sodium iodide capsules was received at the NIH. The outer container bore a label reading "47 μCi/capsule," while the label on the bottle actually containing the capsules read "26 μCi/capsule." The latter label was correct. In the same shipment, each bottle was supposed to contain 12 capsules. One held 11 capsules while another held 13.

Uncertainty in decay schemes. One of the problems associated with the assay of ^{197}Hg lay in the uncertainty in its decay scheme which led to varying interpretations of a millicurie of this nuclide. This meant that if a physician normally administered 1 mc of company X's material, he might have had to give more or less than 1 mc of company Y's material to get the same counting rate.

In an effort to alleviate this problem, the various manufacturers met during the November 1966 Oak Ridge Institute of Nuclear Studies symposium on radioactive pharmaceuticals. Agreement was reached to use a value of 93.5 photons in the 67–80-kev energy range per 100 disintegrations of ^{197}Hg. This photon yield was based on the best available information at that time. It is highly desirable that similar conferences recommend arbitrary values for those nuclides whose exact decay schemes are uncertain. These arbitrary values should be accepted by all concerned until more reliable information becomes available.

Contamination. Radioactive impurities associated with the isotopes used in medicine may be either

expected or unexpected. For example, before the availability of enriched mercury targets, contamination of 3–6% ^{203}Hg was expected in ^{197}Hg products. In one case, the ^{197}Hg product was unacceptable because of the presence of 16.7% ^{203}Hg. Three days later when the material was to be used, the contamination would have been 34.6% of the desired ^{197}Hg activity. The supplier was notified and replaced the shipment with one having only 6.1% ^{203}Hg.

^{47}Ca decays to ^{47}Sc, an expected contaminant. In a shipment received in mid-1964, a second contaminant was observed and identified as ^{169}Yb using radiochemical separation and spectrum analysis. The supplier confirmed the presence of ^{169}Yb in an amount of about 20% of the desired ^{47}Ca activity and replaced the material.

CONCLUSION

Numerous types of error are possible. On a routine basis the NIH experience indicates that errors exceeding ± 10% will occur in about 23 of every 100 shipments. However, the magnitude of gross error in excess of ± 20% is relatively small—5 of every 100 shipments. The frequency of error is of significance to the radiotherapist in whose work a 50% overdose can possibly prove fatal and to the user who demands accuracy of ± 10%. The user whose requirements are less stringent should be aware of the possibility of error by the supplier and should take this into account when he obtains anomalous data.

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