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LAMS - 603

This is copy 9 of 12 copies

August 14, 1947

This document contains 10 pages

# DETERMINATION OF PLUTONIUM IN HUMAN URINE


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## Abstract

A detailed account is given of the collection, ashing and analysis of a 24-hour urine sample for plutonium. This is an account of the method currently in use at the Los Alamos Laboratories for the diagnosis of exposure of personnel to plutonium.

The statistical method for evaluating results is also given.




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## Determination of Plutonium in Human Urine

### I. Collection of Sample

A 24-hour urine sample is collected in a 3 liter pyrex Erlenmeyer flask. Extreme care is taken to avoid contamination of the sample during collection. The funnel and flask for collecting the sample are retained in a special plywood box with a hinged lid. The individual on whom the test is to be made is instructed to remain at home or in the hospital during the 24-hour period over which he is to collect the sample. He is instructed to bathe carefully and remain in pajamas throughout the day. He is requested to put on white cotton gloves each time he voids to minimize the possibility of contaminating the sample by epithelial cells from the hands. At each voiding, the lid to the special sample box is raised and the urine collected in such a way that the funnel and collection flask are never touched by the individual. After urination the lid of the box is carefully closed to protect the sample from dust and contamination. At the end of the 24-hour collection period, the sample is transported to the laboratory before it is removed from the special plywood box.

### II. Analysis of a 24-hour Urine Sample

#### A. Evaporation and Ashing of Sample

1. Add directly to the urine sample in the collection flask about 200 ml. conc. $HNO_3$, 10-20 drops n-octyl alcohol and 20-30 grains of 20 mesh carborundum and place on a high temperature hot plate controlled by a variac.

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2. Begin evaporation slowly at low temperature. Raise temperature progressively at 5-10 minute intervals until danger of bumping and frothing is over and sample is boiling vigorously.

3. To facilitate evaporation blow a gentle steady stream of filtered air directly into the flask as soon as boiling begins.

4. Evaporate sample until the level of liquid in flask is about 1/2 inch. (about 150 ml.). Do not approach dryness !

5. Transfer from flask to 500 ml. porcelain casserole and quantitatively rinse flask twice with hot conc. $HNO_3$. Transfer washings to casserole.

6. Cover casserole with speedy-vap coverglass and evaporate down at medium heat until sample begins to puff up. Watch sample carefully that it does not foam and froth over.

7. Continue to evaporate sample to dryness by careful heating. If reaction tends to become too violent, alternate periods of heating and cooling. If the sample is heated too rapidly it may ignite at this point.

8. Take casserole off of hot plate and while still hot carefully wash down salts with conc. $HNO_3$ adding the acid dropwise from a dropper.

9. Reheat casserole to evaporate the $HNO_3$ and repeat step 8 until salts are almost completely white with the exception of a few aggregates of carbon.

10. When salts are white remove speedy-vap and ignite casserole, over a Fisher burner. Heat the edges of casserole first by inclining it on a ring and rotating it in the flame. Finally ignite until all traces of carbon are gone.



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11. Cool casserole about 10 minutes and wash walls with a fine stream of 2 N HCl. The HCl treatment should begin before the fusion mass has completely cooled. If the fused mass is allowed to solidify completely chipping of the glazed surface of the casserole may result. Also the salts go into solution less readily.

12. Continue to wash down walls and speedy-vap with 2 N HCl until total volume of acid is about 75-80 ml. Scrub casserole and speedy-vap with policeman and place casserole back on hot plate at medium heat and reflux until most of the salts are in solution. If salts do not go into solution scrub sides of casserole with policeman, add more acid and reheat. The total volume of solution should not exceed 100 ml.

B. Extraction and Analysis

1. Transfer hot solution and any undissolved salts from casserole to 250 ml. centrifuge bottle and centrifuge at 1500 r.p.m. for 15 minutes.

2. Carefully decant supernatant into second centrifuge bottle. Wash the precipitate in the first centrifuge bottle with 10-15 ml. of hot concentrated HCl that was heated in the original casserole. Stir up the precipitate and heat centrifuge bottle on hot plate for few minutes. Centrifuge for 10 minutes and transfer supernatant to second centrifuge bottle. Wash original casserole and precipitate in first centrifuge bottle with 10-15 ml of hot distilled water. Transfer wash to second centrifuge bottle after centrifuging for 10 minutes. Discard any remaining precipitate.

3. To the acid solution (about 125 ml. total volume) add concentrated $NH_4OH$ until a permanent precipitate forms. Add 10-15 ml. of concentrated $NH_4OH$ in excess.

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4. Centrifuge for 15-20 minutes at 1500 r.p.m. Decant and discard the supernatant. Dissolve precipitate in 25-50 ml. of 4N HCl. Wash down the walls of the centrifuge bottle during the addition of the acid.

5. Transfer solution quantitatively to 250 ml. pear shaped separatory funnel. Rinse centrifuge bottle twice with 5 ml. portions of 2N HCl. To the solution in separatory funnel add about 1 mg. of ferric iron as a solution of $FeCl_3$.

6. Neutralize with concentrated $NH_4OH$ using methyl violet as an indicator. The end point should be the point where the indicator is an emerald green (bright green). If a precipitate should form add 2N HCl until precipitate just dissolves.

7. Add 2 ml. of freshly prepared aqueous 6% cupferron solution. Shake separatory funnel for at least 1 minute.

8. Add 3/4 ml. of $CHCl_3$ and extract for about 1 minute. Invert funnel and relieve pressure being careful not to lose any of the material. Draw off $CHCl_3$ into 40 ml. graduated conical centrifuge cone.

9. Continue extraction with 3 ml. portions of $CHCl_3$ until chloroform layer is colorless (about 5 extractions).

10. Evaporate off $CHCl_3$ in an oil or water bath at 65-70°C. (avoid superheating).

11. To the residue in centrifuge cone add 3/4 ml. of conc. $HNO_3$ and 1 ml. 72% $HClO_4$. Wash down the walls of cone during addition. Mix well by rotating cone.

12. Place cone in a controlled wax bath and gradually increase temperature to 150°C. Swirl the cones occasionally. Continue to heat at 150°C. until foaming has subsided.

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13. After foaming has subsided continue to elevate temperature to 190-200°. Allow perchloric acid solution to reflux at this temperature for about 1 1/2 hours. Clean walls of cone by inclining and rotating carefully. The volume of the acid should have been evaporated down to 1/2 ml. or less.

14. The acid solution should be colorless when cooled down. Dilute to exactly 4 ml. with distilled water. Add 2 drops of 20% hydroxylamine hydrochloride solution and let stand for about 1 hour.

15. Add 200 micrograms of $La^{+++}$ in about 0.1 ml. of $La(NO_3)_3$ solution. Mix. Add 1 ml. of conc. HF and swirl the cone for a few minutes. Centrifuge for 10 minutes at 1800 r.p.m. Decant and discard the supernatant. Invert cone on piece of filter paper to drain. Wash precipitate with 2 ml. of 0.1 N HF. Break up the precipitate and wash down walls of cone by swirling. Centrifuge as before. Discard supernatant and invert cone to drain.

16. Transfer precipitate quantitatively to stainless steel plate. Transfer is accomplished by slurrying the precipitate in about 2 drops of distilled water and drawing the slurry up into a capillary syringe pipette.

17. Evaporate water on hot plate at sufficiently low temperature to avoid spattering. Ignite the stainless steel plate lightly for about 1 minute in the flame of a Fisher burner to burn off organic matter.

18. Count for two one-hour periods in a low background alpha counter.

## III. General Considerations and Interpretation of Results

The analysis of 150 24-hour urine samples from persons never exposed to plutonium gave an average blank value of 0.67 ± 0.38 c/m. There is a great probability that this positive blank actually represents a naturally occurring cupferron extractable alpha activity of the body. The standard practice at this laboratory is to subtract a blank value of 1 c/m from each result. The final result is interpreted according to the following formula:

$$PE = 0.67\sqrt{\frac{S + 2B}{T} + 2\left(\frac{N}{0.67}\right)^2}$$

where PE = probable error

T = 30 minutes - counting time

B = mean background of counter (0.35 c/m)

N = mean deviation of blank (+0.62 c/m --an early value)

S = c/m in sample (corr. for counter background and ave. blank)

Table 1.

STATISTICAL SIGNIFICANCE OF RESULTS

| Sample c/m | Probable Error (c/m) with Probability of Occurrence of a Specific Error | | | | |
|---|---|---|---|---|---|
| | 1 in 2 | 1 in 4 | 1 in 10 | 5 in 100 | 1 in 100 |
| 1 | 0.89 | 1.52 | 2.17 | 2.58 | 3.40 |
| 2 | 0.90 | 1.54 | 2.19 | 2.61 | 3.43 |
| 3 | 0.91 | 1.55 | 2.21 | 2.64 | 3.47 |
| 4 | 0.92 | 1.57 | 2.24 | 2.67 | 3.50 |
| 5 | 0.93 | 1.59 | 2.26 | 2.70 | 3.55 |
| 7 | 0.96 | 1.64 | 2.34 | 2.80 | 3.66 |
| 9 | 0.97 | 1.65 | 2.36 | 2.82 | 3.70 |
| 12 | 0.98 | 1.67 | 2.39 | 2.85 | 3.74 |
| 15 | 1.00 | 1.71 | 2.44 | 2.90 | 3.81 |
| 20 | 1.03 | 1.76 | 2.51 | 2.99 | 3.93 |

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Table I was compiled by applying the above formula and above values to sample counts ranging from 1 to 20 c/m. Results of urine determinations may be evaluated by referring to the table, i.e., if a sample has a net count of 1 c/m (after subtracting the average counter background and a blank value of 1 c/m) there is one chance in two that it is an error by as much as 0.89 c/m and one chance in one hundred that it is an error by as much as 3.4 c/m.

At this laboratory 1 microgram of plutonium is considered the maximum safe body tolerance. If the urinary excretion rate is taken as 0.01% of the body content per 24-hour period and the activity of plutonium taken as 70,000 c/m/$\gamma$ then a tolerance dose is considered to be the excretion of $1 \times 10^{-4} \times 7 \times 10^{-4} = 7$ c/m/24-hour sample of urine. When determined by the cupferron procedure and interpreted by the above formula there is one chance in two that the tolerance value is in error by as much as 0.96 c/m, and one chance in one hundred that the error is as great as 3.6 c/m.



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