Cross-Contaminator Exclusion Test for Dry-Ashing Determination of Protein-Bound Iodine

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Oxidation of organic material as a preliminary to determination of iodine, whether by wet or dry methods, entails loss of iodine if insufficient organic matter is present during the reaction (1, 2, 3). In blood serum, the organic content suffices to prevent this loss when the amount of iodine in the specimen is in the range found in physiological or pathological sera, 0–30 μg./100 ml., as has been repeatedly shown in recovery experiments (4, 5, 6, 7). When the iodine content of serum exceeds 500 μg./100 ml., as is sometimes the case after administration of iodinated radiopaque media, or other contact with iodinated medication, iodine may be volatilized in theashing stage of the analysis, to be reabsorbed in other tubes. Such cross-contamination invalidates the entire series of tests, with loss of analyst's time and delay of reports.

To avoid delays caused by the presence of such cross-contaminators, a simple method of screening them out in advance was desirable. In connection with the quantitative dry-ashing procedure in use in this laboratory (7), the cross-contaminator exclusion test described below has proven satisfactory. It requires only 1 drop of serum, and adds no more than 30 minutes of operating time to a series of 20–40 PBI determinations.

REAGENTS AND APPARATUS

* 4N Sodium carbonate in dropper bottle.
* Approximately 4N hydrochloric acid in dropper bottle.
* Approximately 0.15N arsenious acid in dropper bottle.


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* These items are identical with those used in quantitative PBI procedure (7).
* Approximately 0.065N ceric ammonium sulfate in dropper bottle.
* Muffle furnace, with control for 600° ± 25°.
  Automatic electric Time Switch.¹
  Twin infrared heater with reflectors.²
  Stainless steel test tube rack, 6 rows of 15 x 14 mm. holes.³
  Pyrex test tubes with lip, 75 x 12 mm.

PROCEDURE

1. Place 1 drop (0.05 ml.) of serum and 2 drops of 4N Na₂CO₃ into a test tube. Shake rack. Dry completely under infrared heater.
   The serum is dropped from the pipet used for the quantitative tests.
   Step 1 is set up simultaneously with the start of protein precipitation for the quantitative work. The Fisher Infra-rediator effects good drying in 2 hours if the lamps are adjusted about ½ in. from the tops of the tubes.
2. Put rack into muffle furnace preheated to 600°. Set automatic time switch to shut off at 2½ hours.
   Step 2 is started at about the same time that the material in the quantitative tests goes into the drying oven.
3. To the cold ash, add 2 drops 4N HCl plus 1 drop arsenious acid solution. Shake. Allow to stand ten minutes. Add 1 drop ceric ammonium sulfate solution. Shake. Allow to stand 5 minutes. Read colors over a white background immediately at the end of 5 minutes, looking downward through tubes. Decolorized tubes indicate potential cross-contaminators.⁴
   Step 3 is carried out the morning after Step 2. Reading of cross-contaminator exclusion tests is complete by the time the muffle is preheated to 600° for the quantitative determinations.
   To guard against reagent deterioration, a blank containing Na₂CO₃ only, as well as a normal serum and one to which inorganic iodide equivalent to 400 μg. of iodine per 100 ml. has been added, are included in the qualitative series. The first two should be clear yellow at 5 minutes, the last completely decolorized.

EXPERIMENTAL DATA

When the need for a test to exclude cross-contaminating artefactual sera from the muffle furnace stage of dry ashing PBI determinations

³ Drummond Scientific Company, Philadelphia.
⁴ Specimens decolorising in 5 minutes are withdrawn from the quantitative ashing procedure. The dried proteinate, however, is not discarded. Instead, it is ashed separately and run through the quantitative procedure as usual, as a verification of the qualitative test.
became apparent, a search was made for a simple qualitative procedure. Feigl's (10) spot plate test for iodine appeared most promising, since its reagents were similar to those used in most quantitative procedures for serum iodine. It was accordingly adapted to serum ash. Pyrex test tubes were found to be the best ashing containers, as in quantitative work, since iodine losses occurred during ashing in shallow platewells or micro-crucibles.

RESULTS

Typical results appear in Table 1. Decolorization of cerium within 5 minutes after its addition to the acidified and arsenized ash solution was regularly obtained at a level of 0.2 μg. I₅ per drop (0.05 ml.) of serum, equivalent to 400 μg. I₅/100 ml. This finding was not affected by the source of the added iodine, whether inorganic, as thyroxin, or as arte-factual serum with an unknown iodine compound content. To avoid occasional cross-contamination during the qualitative muffling by sera containing very large amounts of iodine (>9000 μg./100 ml.), the proportion of carbonate to serum was increased to double that in the quantitative procedure. The effectiveness of this measure has led to consideration of the possibility of improvement of quantitative dry-ashing procedures in the same direction, a matter now being studied in our laboratory.

DISCUSSION

Practical methods for the determination of iodine in the fractional microgram amounts present in small serum samples depend on the catalytic acceleration of the reduction of ceric salts by arsenite in acid solutions, first described by Kolthoff and Sandell (8). While with the aid of instrumental analysis (9), this reaction is capable of differentiating increments of inorganic iodide in pure aqueous solution as small as 0.02 μg./100 ml., its sensitivity in qualitative work, in which the distinction

<table>
<thead>
<tr>
<th>Iodine (μg./100 ml.)</th>
<th>I₅ plus reagents unmuffled (min.)</th>
<th>I₅ plus reagents muffled (min.)</th>
<th>I₅ plus serum and reagents muffled (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Y 10</td>
<td>Y 10</td>
<td>Y 10</td>
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<tr>
<td>200</td>
<td>D 3</td>
<td>D 4</td>
<td>D 7</td>
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<tr>
<td>400</td>
<td>D 1</td>
<td>D 3</td>
<td>D 4</td>
</tr>
<tr>
<td>800</td>
<td>D 0.75</td>
<td>D 1.5</td>
<td>D 3</td>
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Y = yellow; D = decolorized.
is between clearly visible yellow and colorlessness, is considerably lower. Feigl's (10) spot plate modification of the Kolthoff-Sandell reaction is read at 30 minutes, for which time an identification limit of 0.05 µg. KI per drop is given, equivalent to 75 µg. iodine per 100 ml. The sensitivity of the serum cross-contaminator exclusion test described above, for which a time limit of 5 minutes has been established for convenient inclusion in the quantitative procedure, and which contains much sodium chloride, is of the order of 400 µg. per 100 ml., or one twenty-thousandth that of increment differentiation under optimum conditions. It is nevertheless sufficient for the purpose. Concentrations of iodine below this level have not, in experience with the method in use in this laboratory, caused cross-contamination. If further analytical experience, or the advent of iodinated medications of cross-contaminating capacity at lower concentration, makes a more sensitive test desirable, one may be readily adapted from the experimental data set forth.

Not all artefactual sera have the same cross-contaminating potential. Some sera having iodine content upward of 5000 µg./100 ml. did not cross-contaminate known control sera included in the series. On the other hand, some artefactual sera with iodine content one tenth the above level have been implicated. It is evident that volatilization of iodine depends to a large extent on the nature of the compound present. Further study to evaluate the analytical characteristics of the various iodinated medications apt to be found in serum may prove valuable.

SUMMARY

A simple qualitative test for iodine in blood serum has been described. It is designed to detect sera which are potential cross-contaminators in dry-ashing PBI procedures. One drop of serum is used. Added operating time in a PBI series of 20–40 determinations is not over 30 minutes.

REFERENCES