Simple Open Fusion for an Iodine Screen Test

Eugene S. Baginski and Bennie Zak

A procedure is described for the screening of serum samples for contamination with iodine. It is extremely rapid, obviates any cross-contamination problem, and is not influenced by the organic compounds of raw serum or filtrates. Studies indicate that the open-combustion technic is a reliable screening system for the handling of large numbers of samples.

Several tests have been proposed for determining the gross contamination sometimes encountered in serums submitted for protein-bound iodine analysis (1–5). These screens are all based on the use of the coupled Ce(IV-As(III)) reaction as a detection device. However, ceric ions in strong acid solution represent a powerful oxidation system for many organic compounds (6). There is, therefore, a good possibility that these compounds are capable of reducing Ce(IV) to give the appearance of the presence of iodine. It is impossible to differentiate simple Ce(IV) reduction from the iodine catalyzed Ce(IV) and As (III) reaction by measurement of loss of Ce(IV) color alone. Our own experiences with several of these preliminary semiquantitative contamination-exclusion tests has indicated that too many samples were eliminated which subsequent analysis showed to be uncontaminated. We attempted to develop a simple procedure which would yield a reliable screen test with an open-alkaline-combustion technic employing ordinary burners and porcelain crucibles. This extremely rapid process could be used to destroy the organic precipitate containing the iodine without apparent loss of the latter in the conversion to high melting potassium iodide. An important feature of such a system would be the ability to screen large numbers of samples without cross

From the Departments of Pathology, Sinai Hospital, Wayne State University College of Medicine, and Detroit Receiving Hospital, Detroit, Mich. Supported in part by a Grant-in-Aid from the Receiving Hospital Research Corporation. Received for publication Sept. 16, 1961.
contamination. It has been possible to accomplish this, and the results of these experiments will be described here.

Experimental

Reagents

Sodium Hydroxide (0.5N)
Zinc Sulfate Stock Solution. Dissolve 100 gm. of zinc sulfate heptahydrate in a 1-L. volumetric flask and dilute to the mark with water. Standardize against the 0.5N NaOH solution and adjust so that 11 ml. of NaOH is required to neutralize 10 ml. of the zinc solution (7).
Zinc Sulfate Working Solution. Make up 1 volume of the zinc sulfate stock with 7 volumes of distilled water.

Sodium Arsenite. Dissolve 14.34 gm. of reagent-grade sodium arsenite in about 900 ml. of concentrated sulfuric acid, cool, and dilute to the mark with water.

Ceric Ammonium Nitrate (0.02N). Dissolve 10.96 gm. of ceric ammonium nitrate in about 500 ml. of distilled water, add 50 ml. of concentrated H₂SO₄, and dilute to 1 L. with water.

Potassium Hydroxide (4N)

Procedure

Manual, Sample

Pipet 1.0 ml. of serum into a crucible (coors No. 170, size 4) containing 8.0 ml. of working zinc sulfate solution and mix well. Add 1.0 ml. of 0.5N NaOH, mix again, and centrifuge the mixture for 5 min. at a moderate speed before decanting the supernatant fluid. Add 1.0 ml. of 4N KOH and mix thoroughly until the precipitate dissolves. Dry the crucible in an oven set at 100°, a step which only takes about 30 min. because of the geometry of the crucible. Place the crucible in a slanted position on a triangle wire above a Fisher Burner and keep the flame at low temperature until the residue becomes black (the residue usually burns with a flame at this point). Increase the flame of the burner to its maximum and rotate the crucible if necessary until all the content becomes yellow and no more carbon is visible (about 3–5 min.). Turn the flame off and let the crucible cool a little before placing it on an asbestos pad to come to room temperature. Pipet 6.0 ml. of As(III) solution into the residue and mix until there is no further effervescence. Centrifuge at high speed for 5 min. Take out a 5.0-ml. aliquot by carefully tipping the crucible on its side in order to avoid pipeting the precipitate and transfer the aliquot into a test tube. Place the tube
in a water bath at 35° for 10 min. Add 1.0 ml. of Ce(IV) solution to each tube and then observe the tubes after 20 min. of incubation. Ordinarily, when a sample is grossly contaminated, it decolorizes almost on contact with the Ce(IV). The other contaminated samples are colorless at various intervals up to 20 min., usually much less. These samples are then removed from the day's run.

Discussion and Results

The original stated purpose of this investigation was to develop a reliable screening system. Three phases of study were felt to be necessary to show reliability, and these phases could be listed as follows:

1. The analysis of known contaminated samples as well as clean samples which were artificially contaminated
2. The study of the residual melt to prove that the catalysis was due to iodine
3. Tracer studies.

Contaminated Samples

Throughout a period of 18 months' study during which over 1300 samples were analyzed, some samples were found to be grossly contaminated. In almost every instance, previous histories showed that these patients had received large amounts of iodine in some form. Samples were selected from patients who had recently received this kind of medication, and again they were found to be grossly contaminated. Then, a series of serums were set up where an excess of iodate was added prior to drying and ashing. The effect of the reaction is described as follows:

Addition of 100 μg. per 1/100 ml.

Five samples were processed, and the absorbance readings ranged from 0.07 to 0.10 after 9 min., 0.01–0.02 after 13 min. and completely colorless after 17 min.

Addition of 200 μg. per 1/100 ml.

Ten samples were analyzed. After 5 minutes the readings ranged from 0.05–0.08, and after 10 min., a complete decolorization was spectrophotometrically apparent.

Residual Melt Experimentation

Several serum samples were ashed in duplicate using the open-fusion technic. After the fusion was complete and the samples were
cooled for subsequent handling, acid arsenic was added to both melts to effect solution. A trace of mercury salt was added to one of each of the duplicates, and then the Ce(IV)-As(III) reaction was carried out. One aliquot of each set retained its catalytic activity while the sample containing mercury was even less decolorized than a reagent blank. This experiment showed good indirect evidence that the catalytic activity was due to iodine since this would be mercury inhibited.

If mercury had been present in the samples before fusion, it would have been lost because of the high temperature of the fusion process. No mercury inhibition has been encountered in this process in the more than 2 years during which this entire study was carried out.

Tracer Study

Twelve serum samples were treated with precipitating agents. A drop of albumin containing radioactive iodine and 1 ml. of 4N KOH were added. A count was made with a scintillation counter after oven-drying the samples. The samples were then ashed with Fisher Burners for 5 min. and recounted. The results showed two samples with a final count indicating complete recovery, whereas the remaining samples with one exception (63%) ranged between 80% and 96%. We have no explanation for this situation and cannot solve it in terms of the possibilities of variable geometry, loss, or some other phenomenon. However, these recoveries would be more than adequate for a screen test as described.

Addendum

Since this work was completed, we have successfully experimented with electrothermal Bunsen burners in place of Fisher burners.

References