EQUIPMENT AND GLASSWARE

A. General Equipment

1. Centrifuge with tachometer.
2. Racks, test tube
3. Water bath, 37° and 56° C.
4. Kahn shaking machine (275 - 285 oscillations per minute, with 1 1/2 inch stroke)

B. Glassware

1. Test tubes, 13 x 100 mm (O.D.)
2. Pipettes, Serological, deliver to the tip
   1.0 ml. graduated in hundredths
   5.0 ml. graduated in tenths.
3. Flasks, Erlenmeyer, with inserted V shaped ridge producing two semicircular compartments
   125 ml.
   250 ml.
4. Flasks, Erlenmeyer, glass stoppered
   500 ml.
   1 liter
5. Graduated cylinders
   100 ml.
   250 ml.
   1 liter
6. Filter funnels, fluted, 200 mm.

HINTON TEST

REAGENTS

1. Hinton Indicator. (See page_____, "Preparation of Hinton Indicator")
2. 5% saline solution.
   a. Weigh 5 gms. of previously dried sodium chloride (A.C.S.) and 22.2 mgs. salicylic acid for each 100 ml. of saline solution.
   b. Add sodium chloride and salicylic acid to 100 ml. of freshly distilled water and heat solution in an autoclave at 15 pounds pressure for 15 minutes.
   c. Store salt solution in glass-stoppered bottles at room temperature. This solution may be used for three weeks.
3. 50% solution of glycerine.
   a. Mix equal volumes of Baker and Adamson's Glycerine (Reagent) and distilled water. Store in refrigerator. This solution keeps indefinitely.

PREPARATION OF SERUM

1. Remove serums from clots by centrifuging and pipetting or decanting.
2. Heat the serums in the 56° C. water bath for 30 minutes. Serums should not be heated before the day of testing. If it is necessary to retest a specimen, use serum freshly separated from the clot if available.
3. Recentrifuge any specimen in which visible particles have formed during heating.

PREPARATION OF GLYCERINATED HINTON INDICATOR

1. Pipette one part of Hinton Indicator into one compartment of an Erlenmeyer flask having a molded, inverted V-ridge.

   (Note: Not less than 1 ml. or more than 5 ml. of Hinton Indicator should be mixed at one time.)

2. Pipette 0.8 part of 5% sodium chloride solution into the other compartment of the Erlenmeyer flask. Care should be used in pipetting the salt solution into the flask in order to avoid premature mixing of these solutions.

3. Stopper flask and mix by shaking the flask very rapidly from side to side for one minute.

4. Let the mixture stand exactly five minutes.

5. Add 13.2 parts of 5 per cent salt solution and shake thoroughly.

6. Add 15 parts of 50 per cent glycerol solution and shake thoroughly until the suspension is homogenous.

7. Store in a glass-stoppered bottle or flask in the refrigerator. This suspension, referred to as glycerinated-indicator solution, remains usable for at least three weeks.

STANDARD HINTON TEST WITH SERUM

1. Arrange test tubes (100 x 13 mm. outside diameter) in suitable racks so that there is one tube for each serum to be tested and for positive serum, negative serum, and saline controls. Number tubes to correspond to the identifying number of serums.

2. Pipette 0.5 ml. of each heated serum into its corresponding tube.

   (Note: Occasionally very strongly-positive serums will elicit a negative reaction when 0.5 ml. of serum is employed as the testing quantity. When this type of reaction is

suspected, 0.1 ml. serum should also be tested in addition to the 0.5 ml. quantity of serum.)

3. Pipette 0.5 ml. of the glycerinated Hinton Indicator into each serum tube.

   (Note: Flask containing glycerinated Hinton Indicator should be shaken when taken from refrigerator. Remove quantity of glycerinated indicator needed and return flask to cold storage immediately.)

4. Shake rack of tubes by hand vigorously to thoroughly mix the contents of each tube.
5. Shake rack of tubes on a Kahn shaking machine for 5 minutes.
6. Remove the rack from the shaking machine and place in 37° C. water bath for 16 hours.

   (Note: The water bath must not be covered during this period.)

READING AND REPORTING

1. Remove each tube from the rack carefully without disturbing contents.
2. Hold the tube upright in front of a shaded cylindrical flourescent bulb.
3. Note the clarity of the fluid, and the presence or absence of white flakes or white coarse granules at the meniscus.
4. Slant tube at an angle of 45°, shake gently, and observe presence or absence of flocculation and record positive or negative findings.

   Positive: Those reactions demonstrating white flakes or white coarse granules at the meniscus, and definite floccula-

tion when tubes are gently shaken.

Negative: Those reactions demonstrating absence or ring or band of floccules, frequently a non-granular scum at the meniscus, and no flocculation or granularity when tubes are gently shaken. Hemolyzed serums bacterially contaminated frequently produce a whitish ring which is strongly adherent to tube.

5. Centrifuge all tubes in which clear-cut negative or positive readings cannot be made at 2000 r.p.m. for 10 minutes.

6. Remove tubes from centrifuge and read as described in No. 4, recording only doubtful and negative findings as follows:

Doubtful: Those reactions demonstrating coarse granulation at the meniscus and definite flocculation when tubes are gently shaken.

Negative: All specimens failing to react as described under "Doubtful" above.

7. Report those specimens as "unsatisfactory" which are hemolyzed and bacterially contaminated, unless the reaction is strongly positive.

RAPID HINTON TEST WITH SERUM

(Note: This procedure should only be employed when there is urgent need for an emergency report. Whenever possible, the result of the rapid method should be checked by the standard method.)

1. Heat fresh serum for 3 minutes at 60° C.

2. Arrange test tubes (100 x 13 mm. outside diameter) in suitable racks so that there is one tube for each serum to be tested and for a positive serum, negative serum, and saline control.

4. Add 0.5 ml. of glycerinated indicator to each serum tube and shake on Kahn shaking machine for 5 minutes.
5. Remove rack of tubes from shaking machine and place in the 37° C. water bath for 20 minutes.
6. Remove tubes from water bath and centrifuge tubes at 2000 r.p.m. for ten minutes.
7. Remove tubes from centrifuge without agitating contents.
8. Read each tube as described under "Reading and Reporting".
9. Report in accordance with the following outline:

Positive: Those reactions demonstrating plainly visible flakes at the meniscus, and well-marked flocculation when tubes are gently shaken.

Negative: Those reactions demonstrating absence of ring or band of floccules at the meniscus, and no flocculation when tubes are gently shaken.

Unsatisfactory: Those specimens hemolyzed or bacterially contaminated, unless the reaction is strongly positive.

PREPARATION OF STOCK INDICATOR

A cholesterolized alcoholic extract of beef heart muscle is employed. Bacto beef heart, prepared by the Difco Laboratories, Detroit, Michigan, may be used.

1. Weigh 100 grams of beef heart powder and transfer to a one liter glass stoppered Erlenmeyer flask.
2. Add 400 ml. pure anesthesia ether and shake thoroughly by hand for 10 minutes.
3. Allow powder to settle, then pour off ether through filter paper, discarding this and all other ether extractions.

4. Scrape moist powder from filter paper and return to original flask.

   (Note: Do not allow main portion of extracted tissue to dry between extractions.)

5. Add 400 ml. pure anesthesia ether to the moist powder and repeat extractions as before.

6. Repeat ether extractions for a total of five extractions.

7. Remove powder from flask, dry thoroughly, and weigh.

8. Place dry powder in a glass stoppered bottle with 95 per cent ethyl alcohol, using 5 ml. of alcohol for each gram of powder.

9. Extract for three days at room temperature, and shake contents of flask vigorously by hand 3 times each day.

10. Filter alcoholic extract through fat free filter paper into a clean glass stoppered flask.

11. Add 0.4 ml. of cholesterol (Pfanstiehl, ash free, C. P.) to every 100 ml. of alcoholic extract.

12. Warm stock indicator (cholesterolized alcoholic extract) in a 56° C. water bath until the cholesterol is in solution.

13. Stock indicator should be tested against an indicator found to be wholly satisfactory as determined by both clinical and serologic means.

    (Note: Occasionally, adjustment of the lipoidal concentration is necessary for the preparation of a satisfactory stock indicator. A procedure has been described whereby lipoidal concentration can be changed as may be required.[1])

1. Harris, Ad; Rosenberg, A.; Bossak, H.N.: Standardization of Hinton Indicator; Ven. Dis. Inform., 23:263-265, 1942.