# EQUIPMENT AND GLASSWARE

A. General Equipment

1. Centrifuge with tachometer.
2. Racks, test tube.
3. Water baths, one 37°C and one 56°C.
4. Kahn shaking machine (275 - 285 oscillations per minute, with 1 1/2 inch stroke), or other shaker. A Cenco Meinze laboratory shaker is satisfactory for small laboratories.
5. Interval timer.
6. Maximum and minimum thermometer.

B. Glassware

1. Test tubes, 100 x 11 mm. O.D. (manufactured by Kimble and described as 45060/873) *
2. Pipettes, Serological, deliver to the tip.
   1.0 ml. graduated in hundredths.
   5.0 ml. graduated in tenths.
   10.0 ml. graduated in tenths.
3. Flasks, Erlenmeyer, with inverted V-shaped ridge in bottom that produces two semicircular compartments (so-called Hinton flasks).
   125 ml.
   250 ml.
4. Flasks, Erlenmeyer, glass-stoppered.
   500 ml.
   1 liter
5. Graduated cylinders.
   100 ml.
   250 ml.
   500 ml.
   1 liter
6. Filter funnels, fluted, 200 mm.

*Tubes 100 x 13 mm. O.D. are not as desirable as the small ones because with the weakly-positive serums the reactions are much more difficult to read.

# HINTON TEST

REAGENTS

1. Hinton Indicator. (See page ____, "Preparation of Hinton Indicator").
2. 5% sodium chloride solution.
   a. Weigh 5 gms. of previously dried sodium chloride (A.C.S.).
   b. Add sodium chloride 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. 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 a Hinton flask.

   (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 flask. Care should be used in pipetting the salt solution into the flask in order to avoid premature mixing of these solutions.
3. 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% salt solution and shake thoroughly.
6. Add 15 parts of 50% 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 MINTON TEST WITH SERUM

1. Arrange test tubes (100 x 11½ mm. O.D.) in suitable racks so that there is one tube for each serum to be tested and for positive and negative serum 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 Minton Indicator into each serum tube.

   (Note: Flask containing glycerinated Minton 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 on shaking machine for 5 minutes.
5. 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. The bath should be equipped with a maximum and minimum thermometer, and the temperature should not fall below 34°C. nor rise above 39°C. for reliable tests.)

READING AND REPORTING

1. Place a shaded cylindrical fluorescent electric bulb 18 or more inches long in front of a darkened background. The bulb should be about 10 - 14 inches above the level of the eyes and 5 - 7 inches in front of them.
2. Remove each tube from the rack carefully without disturbing contents.
3. Hold the tube at a 45° angle in front of the eyes and on the level with them.
4. Look for clarification of the fluid and for presence or absence of a ring of white flakes or white coarse granules at the meniscus.
5. Lift the tube about 10 inches above the eyes, tilt it so that it is almost parallel with the bulb, and, while slightly agitating the tube, look through it towards the darkened background to determine the 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 flocculation when tubes are gently shaken.

   Negative: Those reactions demonstrating absence of ring or

band of flocculos, and no flocculation or granularity when tubes are gently shaken. Occasionally a non-granular scum at the meniscus may be caused by faulty technique. Hemolyzed serums bacterially contaminated frequently produce a whitish ring which is strongly adherent to tube.

6. Centrifuge all tubes in which clear-cut negative or positive readings cannot be made at 3000 r.p.m. for 5 minutes.

7. Remove tubes from centrifuge and read as described in No. 5, 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.

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

RAPID MINTON TEST WITH SERUM

1. Heat fresh serum for 3 minutes at 60°C.
2. Arrange test tubes (100 x 11½ mm. O.D.) in suitable racks so that there is one tube for each serum to be tested and for positive and negative serum controls.
3. Pipette 0.5 ml. of serum to be tested into properly numbered test tube.
4. Add 0.5 ml. of glycerinated indicator to each serum tube and shake on shaking machine for 10 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 10 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.

(Note: Despite very close agreement between the results by the Standard Technique and the Rapid Technique, it is best to place the tubes in the refrigerator until such a time as they can be placed in a water bath for sixteen hours and then read as Standard tests.)

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% 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 gm. of cholesterol (Merck's C.P.) to every 100 ml. of alcoholic extract.
12. Warm stock indicator (cholesterolized alcoholic extract) in a 37°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, M.N.: Standardization of Kinton Indicator; Ven. Dis. Inform., 23:263-265, 1942.

INDICATORS PREPARED FROM CARDIOLIPIN

Stock Hinton Indicators have been prepared from cardiolipin and lecithin manufactured according to the direction of Pangborn, and the tests performed with these indicators thus far have been more accurate than those performed with indicators made from extracts of beef heart. Our experience has been confined to the use of three batches of lecithin and cardiolipin prepared in Dr. Pangborn's laboratory and one batch prepared in the Lederle Laboratories. Until cardiolipin and lecithin are obtainable commercially of specified concentration and potency, it will be impossible to give a brief description of how to adjust the amounts of these substances for optimum results. The stock indicator prepared from cardiolipin, lecithin, and cholesterol is diluted exactly as it is if a dilute indicator were being prepared from the standard stock Hinton indicator.