Cenogenics

Monodex

SUMMARY AND TEST PRINCIPLE

Paul and Bunnel reported that sera from patients with infectious mononucleosis (IM) contain heterophile antibodies which agglutinated sheep and horse erythrocytes[1].

Forssman discovered a second group of heterophile antibodies, unrelated to IM, which were also found to agglutinate sheep and horse erythrocytes[2].

The Forssman type of heterophile antibodies were found in sera of patients with various disease conditions and also in individuals who had been exposed to horse serum[3,4].

While the Forssman heterophile are also absorbed from serum by horse or guinea pig kidney, the IM or heterophile antibodies are not. By contrast, only IM heterophile antibodies are absorbed by beef erythrocytes. This is the basis of the differential serological test introduced by Davidsohn[5,6,7].

MONODEX test incorporates both Paul and Bunnel screening procedures and the Davidsohn differential absorption techniques, thus providing a screening or differential test. Furthermore, the MONODEX uses horse erythrocyte stroma as indicator reagents for enhanced stability.

In the screening test, the serum is allowed to react with the indicator Horse Stroma reagent. In this case, agglutination of the stroma is indicative of a positive reaction. For conformation, positive sera are retested according to the differential procedure. This is accomplished by absorbing the test serum separately with both the Horse Kidney Antigen and the Beef Stroma Antigen. The Horse Kidney will absorb only the Forssman type antibody while the Beef Antigen will absorb only IM heterophile antibodies.

REAGENTS

1. Dyed Horse Stroma (erythrocyte stroma)
2. Horse Kidney Antigen (Absorbent I)
3. Beef Stroma Antigen (Absorbent II)
4. I.M. Positive Control Serum
5. I.M. Negative Control Serum

REAGENT PREPARATION

The reagents in the MONODEX KIT are all ready to use. Prior to use bring all reagents and specimens to room temperature.

Shake reagents before use.

Use new dispenstir for each test sample.

Use new mixing stick for each sample.

Do not interchange kit reagents with those from other kits.

After use, wash glass slide with distilled water. Do not use detergent.

SPECIMEN PREPARATION

Serum or plasma can be used in this test. Inactivation of the serum is not necessary. However, inactivated serum may be used. If the serum or plasma cannot be used within 24 hours after collection, it should be frozen. After thawing, specimen should be mixed thoroughly and clarified by centrifugation if particulate matter is present.

STORAGE

Store at 2°—8° C. Do not freeze.

WARNING

The reagents in this kit contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volume of water to prevent azide buildup.

TEST PROCEDURE

Materials provided:

Dyed Horse Erythrocyte Stroma
Horse Kidney Absorbent I
Beef Stroma Absorbent II
I.M. Positive Control Serum
I.M. Negative Control Serum
Disposable dispenstirs
6-ring glass slide
Disposable mixing sticks

Materials required but not provided:

Isotonic saline (0.85% sodium chloride)

SCREENING TEST

1. Use new dispenstir for each sample. Squeeze dispenstir between the thumb and the forefinger and insert into the sample. Release pressure. This will allow serum to fill the tip.
2. Hold the dispenstir perpendicularly over the circle of the glass slide and squeeze to release one free-failing drop.
3. Shake the container of Horse Stroma, then squeeze a drop on the sample.
4. Using the mixing stick, thoroughly mix the sample and reagent and spread over the entire circle.
5. Rock slide gently for one minute with a slight partial left and right rotation.
6. At the end of one minute, observe for agglutination while holding the slide under a high intensity lamp or fluorescent light.

Agglutination is indicative of a positive test. A blue homogeneous suspension or finely granular pattern is indicative of a negative reaction. (Note: in most cases, agglutination will occur in less than a minute.)

DIFFERENTIAL TESTING

Only positive samples from the screen need to be retested.

1. Using a dispenstir place one drop of the sample onto the first upper circle and one drop onto the circle directly below it. (Each slide can be used for three differential tests.)
2. Shake Horse Kidney Antigen (Absorbent I) container and squeeze one drop over the serum sample of the upper circle.
3. Shake the Beef Stroma Antigen container (Absorbent II) and squeeze one drop over the serum sample of the lower circle.
4. Mix well with mixing stick, using a different part of the stick for the upper and lower circle.
5. Shake Horse Stroma container and squeeze one drop on each circle.
6. Mix well and spread over the entire circle.
7. Rock slide gently for one minute with a slight partial left and right rotation.

8. Read results at the end of one minute under a high intensity or fluorescent light.

RESULTS

Positive result:

If agglutination pattern is stronger on upper circle, the test is positive.

Negative result:

1. If agglutination pattern is stronger in the lower circle, the test is negative for IM. (The serum is Forssman positive.)
2. If no agglutination appears in either circle, the test is negative.
3. If agglutination is equal in both circles, dilute sample 1:10 (0.1 ml in 0.9 ml saline) and repeat test.

SEMI-QUANTITATIVE TITRATION PROCEDURE

The heterophile antibody has been shown to bear no relation to the severity of the disease[8]. However, if quantitative determination of the IM antibody titer is desired, the following procedure may be used.

1. Prepare sample dilutions using 0.85% saline solution as follows:

Tube #	Sample	Dilution
1	0.5 ml of sample + 0.5 ml of saline	1:2
2	0.5 ml of tube #1 + 0.5 ml saline	1:4
3	0.5 ml of tube #2 + 0.5 ml saline	1:8
4	0.5 ml of tube #3 + 0.5 ml saline	1:16
5	0.5 ml of tube #4 + 0.5 ml saline	1:32

2. Test each dilution using only Horse Kidney Absorbent.
3. The highest **dilution** which gives a visible agglutination is taken as the end point and the titer value is the reciprocal of that dilution.

PERFORMANCE AND LIMITATIONS OF THE TEST

The result of MONODEX test, as with other serological procedures, should not be used as a sole diagnostic criterion for the presence or absence of the disease state, but as an aid to diagnosis when other criteria are applied. In some cases, false positive results have been shown to be due to residual IM antibody present after clinical symptoms have subsided. Likewise, it has been shown that false negative results may be due to delayed heterophile antibody response[9].

PERFORMANCE CHARACTERISTICS

With over 500 tests performed MONODEX showed 99% correlation with other commercial tests, which utilize fresh stabilized horse erythrocytes.[10]

QUALITY CONTROL

For positive control, use I.M. Positive Control Serum in lieu of patient's serum. Positive reaction should occur within one minute.

For negative control, use I.M. Negative Control Serum. The negative control will produce no agglutination after one minute. The relative degree of smoothness of the reagents should be considered and incorporated in reading the results.

BIBLIOGRAPHY

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