INFECTION MONONUCLEOSIS LATTEX TEST

(A single latex agglutination format for the specific and semi-quantitative detection of the infectious mononucleosis heterophile antibody)

INTENDED USE
The IM Test provides a simple test format to detect the heterophile antibody to infectious mononucleosis (IM). The latex detection reagent can be used with serum or plasma (also from fingerstick samples) for the test. Due to the presence of the bovine monoclonal antibodies to the latex particle, there is no need to perform a Davidson’s ‘Differential’ absorption test to insure the specificity of a positive result.

SUMMARY AND EXPLANATION
The IM heterophile antibodies appear especially during acute illness in about 85 to 90% of adolescents and young adults with classical IM. The IM antibody is detected in a smaller number of young children with the classical disease. Since the majority of young children do not develop the full-blown syndrome in the course of primary infection, they should, in general, not be expected to develop IM antibodies. The IM antibody titer usually achieves diagnostic significant levels by the end of the first week of illness. In this regard it behaves much like IgM specific Epstein-Barr Virus (the etiological agent for IM) antibodies. IM antibodies can persist for some periods of time.

The first report that described the presence of heterophile antibodies in patient sera with infectious mononucleosis was by Paul and Brunner. The method they described used sheep red cells as the particles for agglutination. Due to the presence of other antigens on the sheep red cell, it became necessary to absorb “heterophile” antigen-containing specimens with substances from animal organs to remove non-IM antigens for sheep or horse red cells. In this fashion, the absorbed serum specimens which retained agglutination capabilities were considered specific for the IM heterophile antibody. Alternatively, when boiled bovine red cells were used for absorption they would selectively remove only the IM heterophile antibody. As a result, the specificity of the original agglutination observation was confirmed since this process would remove only the IM heterophile antibody. In contrast, when boiled bovine red cells were used to detect the heterophile antibody, there was no need to perform the absorption step. Bovine red cells do not contain antigens that react with non-IM antigens. Furthermore, it has been demonstrated that the antigen from bovine red cells exhibits greater potency to specifically inhibit IM agglutination than antigen from either horse or sheep red cells. Thus, when horse or sheep cells are used in an antibody detection test system, specificity of a positive result must always be confirmed. This is due to the presumptive nature of the test when horse or sheep red cells are used as the particles to detect IM antibody.

PRINCIPLE OF THE TEST
Latex particles used in the Aludek IM are sensitized with a bovine red cell-monoclonal antigen. Due to the use of bovine source there is no need to perform differential absorptions to verify the specificity of test results. When agglutination is observed a diagnosis of IM is highly probable. The presence of infectious mononucleosis antibody in serum or plasma at detectable levels will interact with the sensitized particles to produce visible agglutination which is a positive result.

REAGENTS AND MATERIALS SUPPLIED
Store all reagents at 2-8°C
Bring all reagents to Room Temperature before use.
Do not freeze any reagents

The AKUDEX IM Test for Infectious Mononucleosis comprises the following reagents:
1. Latex Reagent: Latex particles sensitized with bovine red cell membrane substance suspended in a buffer Preservative: 0.1% sodium azide.
2. Positive Control: (Human): Preservative: 0.1% sodium azide.
3. Negative Control: (Human): Preservative: 0.1% sodium azide. Do not dilute the controls.
4. Glass/disposable slide(s)
5. Dispensing Pipettes/Syringes: Pipettes to disperse 30μL specimens to the slide rings and for mixing the latex and specimen combination before rotation.
6. Directions for test use.

WARNING
For In Vitro Diagnostic Use only.

PRECAUTIONS
ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIAL INFECTIOUS. The human plasma or serum which is used to manufacture the controls was found negative to HIV II & antibody to HCV and HBsAg using FDA licensed test methods. Because no test method can offer complete assurance that all infectious agents are absent, all specimens of human origin should be considered potentially infectious and handle with care.

The reagent in this kit contains sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large amount of water to prevent this reaction build-up. Use Aludek IM Lates Kitis in accordance with supplied instructions. Dropper Bulbs in this kit contain natural rubber.

STABILITY OF REAGENTS
The AKUDEX IM Lates Reagent should appear as a milky suspension of particles. If non-specific clumpings is observed which is not dispersed by normal resuspension procedures, do not use the reagent. The control reagents should be clear, particle-free solutions. If turbidity is observed, do not use the control(s). The positive control must agglutinate the latex reagent. If either no agglutination using the positive control or agglutination with the negative control is observed, a loss of reagent stability may have occurred. The kit should be discarded upon it’s expiration date.

MATERIAL REQUIRED BUT NOT PROVIDED
Clinical rotator capable of 100 rpm, lanes for fingerstick procedure; capillary tubes for use to draw blood from punctured fingertips; high-intensity lamp; graduated pipettes for serial dilution of specimens; saline solution for diluting specimens, 12×75 tubes; and a timing device.

SPECIMEN COLLECTION AND HANDLING
Whole blood can be collected by venepuncture or fingerstick methods; before puncture, make a sterile area on the skin site. To obtain serum from venupuncture, place the harvested blood in a clean tube and allow the clotting process to occur. It can be removed and placed in another clean tube which was appropriately identified. Serum can be tested immediately. If serum is not tested immediately, the tube should be sealed and stored at 2-8°C for up to one week. Sodium azide can be added to the final concentration of 0.1%. If serum specimens are to be kept longer, they should be stored frozen at or below -20°C. To obtain serum or plasma from a fingerpucture puncture, the use of an appropriate sized non-heparinized capillary tube is necessary. Using a sterile capillary tube collect the blood from the puncture site and place the tube in a vertical position to allow clot formation to occur. Score the interface where the serum or plasma joins the formed elements and break the tube at this site. If 30μl of serum or plasma is available in the severed section of the tube, you can place this volume on the slide and proceed with the test procedure. Alternatively, the specimen can be placed in a small tube and stored as above. If plasma is needed for other tests, use heparin, EDTA or CPDA-1 as the anti-coagulant during whole blood collection. Use the resulting plasma as the patient specimen in the test procedure. If specimens present with gross hemolysis, floating materials/turbidity, inadequate volumes, or a large volume of sedimented debris, another sample should be obtained for the test procedure as contamination or improper handling of the specimen may have occurred.

STORAGE OF SPECIMENS
1. If batching of specimens for testing is desired, store the serum or plasma samples in a sealed marked tube at 2-8°C for up to one week. A preservative such as sodium azide (0.1%) or thimersol (0.01%) should be added (final concentration).
2. If longer periods of storage are used, the samples should be frozen at or below -20°C. Avoid repeated thaw-freeze cycles of the specimen.
3. It is a good practice to centrifuge stored specimens before performing the test procedure.

PROCEDURE
SUMMARY. The test procedure requires that a drop of latex be placed in a ring or well. Using the supplied pipette, place a falling drop of the patient specimen in a specified ring(s) on the slide. The combination(s) is mixed and spread with the paddle end of the pipette in the ring. The slide is rotated at 100rpm for 2 minutes. Immediately afterward, each ring is observed under a bright light for any agglutination. If any agglutination is observed, a positive result must be recorded for that specimen.

Quality Control of the Latex Control Reagents.
1. Place a drop of resuspended latex reagent in two separately identified rings on the slide.
2. Place a drop of the positive control and a drop of the negative control reagents in the identified rings on the slide.
3. Mix and spread the combination in the area inside the rings.
4. Immediately after the two minute rotation observe the two rings for any agglutination.
5. The positive control ring must provide obvious agglutination while the negative control ring must not produce any agglutination and the latex appears as a milky suspension of particles.
6. This Quality Control Procedure should be performed as often as the needs of the laboratory dictate.

DO NOT USE THE KIT IF THE EXPECTED RESULTS OF THE CONTROL ARE NOT OBTAINED

Good Laboratory Practices to follow.
1. Use the Test Directions as provided
2. Allow the reagents to achieve room temperature before using.
3. Resuspend the reagents before dispensing them onto the slide.
4. Use a clean, lint-free slide for the test procedure.
5. Use a fresh pipette to deliver each specimen.
6. Do not allow the tip of the latex vial to touch the specimen.
7. Follow appropriate microbiological procedures in handling and dispensing of the materials used in the performance of the test.
8. Calibrate the clinical rotator to insure the 100rpm level.

PROTOCOL TECHNIQUES
A) Qualitative Testing
1. Perform the Quality Control steps as outlined before testing specimens.
2. Shake the IM test reagent gently, expel contents of dropper and refill, then place one drop onto the glass slide.
3. Using dispenser, add one free-falling drop of the undiluted patient serum onto the drop of latex reagent and mix both together with the paddle end of the pipette. Discard the pipette.
4. Place the slide on the clinical rotator at 100rpm for 2 minutes. Alternatively, the slide may be rotated by hand at a similar rpm and duration.
5. Immediately following the timed 2 minute rotation carefully
observe each ring briefly for any agglutination. (see RESULTS section below) and record the results. The use of a high intensity tungsten light can serve as an aid in the observation process. The observations may be assisted by gently rocking the slide once or twice to yield a flow pattern of the reacting materials in the ring. For very weak positive results, compare the tested specimen to the negative control reaction for interpretive assistance.

1. Obtain and mark eight tubes, 1 through 8. Add 0.2 ml of 0.85% saline to the tubes #1 through #8.
2. Add 0.2ml of the patient specimen to tube #1. Using a fresh pipette mix the contents of tube #1 and transfer 0.2ml to tube #2. Do not mix the contents of tube #2 with this pipette.
3. With a fresh pipette, mix the contents of tube #2 and deliver 0.2 ml to tube #3. Do not mix the contents of tube #3 with this pipette.
4. Follow this method to produce serial doubling dilutions of the specimen out to tube #8. The dilutions which have been established are from 1:2 to 1:256 for tube #1 to #8 respectively.
5. Test each dilution following the protocol as described in Steps 2 through 5 of the Qualitative Testing procedure.

RESULTS
Quantitative Test Results
A positive test result occurs when any level of agglutination is observed by the latex reagent in combination with a patient specimen immediately after rotation step. Thus the patient specimen is considered to contain specific mononucleosis heterophile at a detectable level in this case. A negative test result occurs when no agglutination of the latex reagent is observed and the latex particles appear a milky suspension immediately after the rotation step.

Certain patients who present strong evidence of IM and whose IM heterophile test results are negative should always be retested at a 1:10 saline dilution of the specimen.

Semi-Quantitative Test Results
When Positive specimens are examined by serial dilution, the titer is the reciprocal of the last dilution which produces a positive result (agglutination). Although it is no longer necessary to convert the titer as performed above to a classical guinea pig absorbed horse cell titer, it can be obtained after multiplying the resulting titer by 28.

LIMITATION OF PROCEDURE
The result of the IM test must be part of other clinical and diagnostic results. Other disease states can be confused with or mimic infectious mononucleosis and must be differentiated from IM. The laboratory results must be reviewed in light of the patient history by the physician.

EXPECTED VALUES
A positive result has occurred when any degree of agglutination of the latex reagent in combination with a patient specimen is observed immediately after the 2 minute rotation step. If no agglutination is observed, the heterophile antibody associated with infectious mononucleosis is not present at detectable levels. This event is a negative test result. If the heterophile antibody associated with mononucleosis is present in a patient specimen, it is expected to agglutinate the latex reagent. Some patients take a period of time to demonstrate IM heterophile antibody who have presented with most other diagnostic elements associated with infectious mononucleosis. In these cases, subsequent samples may assist the laboratory findings.

BIBLIOGRAPHY