MONO-LEX™ System

INTENDED USE
Remel MONO-LEX™ System is a 2-minute latex agglutination test for the specific detection of infectious mononucleosis heterophile antibody, for qualitative and semi-quantitative procedures, to aid in the diagnosis of infectious mononucleosis. It provides a simple test format to detect the heterophile (Paul-Bunnell) antibody uniquely associated with infectious mononucleosis (IM). The MONO-LEX™ System can be used with serum or plasma (also from fingerstick samples). Due to the presence of the bovine mononucleosis antigen on the latex particle, there is no need to perform a Davidson’s “Differential” absorption test to ensure the specificity of a positive result.

SUMMARY AND EXPLANATION
The IM heterophile antibodies appear, especially during acute illness, in about 80 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 heterophile antibodies. The IM antibody titers usually achieve significant diagnostic 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 period of time or reappear many years later.

The first report that described the presence of heterophile antibodies in patient sera with infectious mononucleosis was by Paul and Bunnell. They described a method using sheep red blood cells as particles for agglutination. Due to the presence of other antigens on the sheep red cell, it became necessary to absorb “heterophile” antibody-containing specimens with substances from animal organs to remove non-IM agglutinins for sheep or horse red cells. In this fashion, the absorbed serum specimens which retained agglutination capability 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 IM heterophile antibody, there was no need to perform a differential absorption step. Bovine red cells do not contain antigens that react with non-IM agglutinins. 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 red cells are used in an IM antibody detecting-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
Latex particles used in MONO-LEX™ are sensitized with a bovine red cell-mononucleosis antigen. Due to the use of a bovine source, there is no need to perform differential absorptions to verify the specificity of the 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 aggregation, which is a positive result.

REAGENTS AND MATERIALS SUPPLIED
Store reagents at 2-8°C. Do not freeze. Do not dilute the Latex or Controls or interchange them with other MONO-LEX™ kit lot numbers.

- **MONO-LEX™ Latex Reagent**: Latex particles sensitized with bovine red cell membrane substance suspended in buffer
  Contains 0.1% sodium azide as a preservative
- **MONO-LEX™ Positive Control**: Human serum containing specific mononucleosis heterophile antibody, diluted in buffer
  Contains 0.1% sodium azide as a preservative
- **MONO-LEX™ Negative Control**: Normal human serum diluted in buffer
  Contains 0.1% sodium azide as a preservative
- **Disposable black slides**
- **Pipetstirs**: To deliver and mix specimens – 50 µl
- **Instructions for Use (IFU)**

*Included with 100 Tests/Kit only (REF R421021)

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Clinical rotator capable of 100 rpm, (2) Lancets for fingerstick procedure, (3) Capillary tubes to draw blood from punctured fingertips, (4) High intensity lamp, (5) Graduated pipettes or micropipettors for serial dilution, (6) Calibrated pipette to deliver 50 µl (use with REF R421022) (7) 0.85% Saline for diluting specimens, (8) 12 x 75 mm Tubes, (9) Timing device, (10) Plastic mixing sticks.

PRECAUTIONS
This product is For In Vitro Diagnostic Use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimen containers after use. Directions should be read and followed carefully.

Safety Precautions
1. Potential Biohazardous Material: The human serum used to manufacture the controls, has been shown to be nonreactive for the presence of hepatitis B surface antigen (HbsAg) and antibodies to HIV and HCV using FDA-licensed test methods. This does not ensure the absence of these (or other) infectious agents. Handle these reagents as if they were potentially infectious. Information on handling human sera is provided in the CDC/NIH manual, Biosafety in Microbiological and Biomedical Laboratories.
2. The Latex Reagent, Negative Control, and Positive Control contain 0.1% sodium azide, which is harmful if swallowed. Contact of sodium azide with acids liberates toxic gases. It is toxic to the aquatic environment and may cause long-term effects. It may react with copper and lead plumbing to form highly explosive metal azides. Upon disposal of reagents into a sink, flush with large amounts of water to prevent azide buildup.
3. Refer to the Material Safety Data Sheet for detailed information on reagent chemicals.

PRODUCT DETERIORATION
This product should not be used if (1) the appearance of the reagents has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

The Latex Reagent should appear as a milky suspension of particles. If nonspecific clumping is observed which is not dispersed by normal resuspension procedures, do not use the reagent.

The Positive and Negative Control reagents should be clear and particulate-free. If turbidity is observed, do not use the control(s).

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT
Serum or plasma is the only acceptable sample to be used for testing with the MONO-LEX™ System. Serum should be removed from centrifuged, fully clotted whole blood and transferred to a clean, labeled tube. If plasma is needed for other tests, use heparin, EDTA, or CPDA-1 as the anticoagulant during whole blood collection. The resulting plasma should be transferred from the centrifuged blood tube to a clean, labeled tube. It is preferable to test samples on the day of collection.

If testing is not performed on the day of collection, store the serum or plasma in a sealed, labeled tube at 2-8°C for up to one week. A preservative, such as sodium azide (0.1%) or thimerosal (0.1%), may be added (final concentrations). If longer periods of storage are used, sample(s) should be frozen at or below -20°C. Avoid repeated freeze-thaw cycles of specimen(s).

Serum or plasma can be heated for 30 minutes at 56°C if desired. Clarify the heated specimen before testing by centrifugation. It is good practice to centrifuge specimens which have been stored before testing.

Specimens that are grossly hemolyzed, contain floating material or sediment, are turbid, or are inadequate in volume should not be used. This may occur as the result of improper handling or bacterial contamination, which may cause protein denaturation. A fresh specimen should be obtained.

PROCEDURE
Good Laboratory Practices to Follow:
1. Follow the instructions in the IFU.
2. Allow the reagents to equilibrate to room temperature before use.
3. Resuspend the reagents before dispensing into circles.
4. Use a clean, lint-free, disposable black slide provided with the kit.
5. Use a fresh pipette to deliver each specimen.
6. Do not allow the Latex Reagent vial tip to touch the disposable slide.
7. Follow appropriate microbiological procedures in handling and disposing of the materials used in the performance of the test.
8. Calibrate the clinical rotator to verify that rotations are 100 rpm.
A. Qualitative Testing:
1. Perform Quality Control as outlined, before testing specimens.
2. Resuspend Latex Reagent by inverting vial several times. Hold vial in a vertical position over the disposable black slide. Squeeze the vial to deliver a drop of resuspended Latex Reagent into a separate circle for each specimen to be tested.
3. Using a fresh pipetstir or calibrated pipette, dispense 50 µl of each specimen to be tested into the center of a labeled circle next to or on top of the Latex Reagent.

Note: To use the pipetstir, hold the pipetstir shaft vertically between thumb and forefinger. Squeeze the shaft and immerse the angled tip into the specimen. Release the pressure on the shaft to allow specimen to flow into the pipetstir. Hold pipetstir over the center of a labeled circle on the slide. Squeeze the shaft to deliver a 50 µl drop alongside or on the drop of latex.
4. Using the paddle end of the pipetstir or a plastic mixing stick, mix together each combination and spread over the entire inside area of each circle. Discard pipetstir or plastic mixing stick.
5. Place the slide on a clinical rotator set at 100 rpm and rotate for 2 minutes. Alternatively, the slide may be rotated by hand at a similar rpm and duration.
6. Immediately following the 2-minute rotation, examine each circle for agglutination and record results (see INTERPRETATION). A high intensity tungsten lamp can serve as an aid in this 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 circle. For weak positive results, compare the tested specimen to the Negative Control reaction for interpretive assistance.

B. Semi-Quantitative Testing of Positive Specimens:
1. Obtain and label eight tubes 1 through 8. Add 0.2 ml of 0.85% saline to each tube.
2. Add 0.2 ml of the specimen to tube 1. Using a graduated pipette, mix the contents of tube 1 and transfer 0.2 ml 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 tubes 1 to 8, respectively.
5. Test each dilution following the protocol described in steps 2 through 6 of the Qualitative Testing procedure.

INTERPRETATION

Qualitative Test:
Positive - A positive result occurs when any level of agglutination is observed with the Latex Reagent in combination with a specimen immediately after the rotation step. Thus, the patient specimen is considered to contain specific, infectious mononucleosis heterophile antibody at a detectable level in this case.

Negative - A negative result occurs when no agglutination of the Latex Reagent and the latex particles appear as a milky suspension immediately after the rotation step.

Negative specimens from patients who present with strong evidence of IM, should be retested after dilution at 1:10 in saline.

Semi-Quantitative Test:
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 by multiplying the titer result by 28.

QUALITY CONTROL
All lots of the MONO-LEX™ System have been tested and found to be acceptable. Quality control testing should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

1. Place one drop of resuspended Latex Reagent in each of two labeled circles on a disposable black slide.
2. Place one drop of Positive Control and one drop of Negative Control in the circles.
3. Mix and spread each combination in the area inside each circle.
4. Rotate the slide for 2 minutes at 100 rpm.
5. Immediately after the 2 minute rotation, examine the circles for the presence of agglutination.
6. Expected Results:
   a. The Positive Control must produce obvious agglutination.
   b. The Negative Control must not produce any agglutination; the latex appears as a milky suspension of particles.

LIMITATIONS OF THE PROCEDURE
The MONO-LEX™ System must be part of other clinical and diagnostic results. Other disease states can mimic IM and must be differentiated from it. The laboratory results must be reviewed in light of the patient history by a physician.

EXPECTED VALUES
When the heterophile antibody associated with IM is present in serum or plasma, it can be expected to agglutinate the Latex Reagent. Some patients take a longer period of time to demonstrate IM heterophile antibody than others. In cases where patients present with other diagnostic elements associated with IM and the MONO-LEX™ System is negative, subsequent specimens may be of diagnostic value to the physician.12

PERFORMANCE CHARACTERISTICS
A total of 175 specimens at a University Hospital laboratory and from a Student Health Center laboratory were tested by both the MONO-LEX™ System and another latex system. Any discrepant results by a specimen were examined further after bovine red cell absorption to ensure the specificity of the agglutination reactions. The comparative test results produced the following: the sensitivity and specificity of the MONO-LEX™ System were 100% and 99.4%, respectively; the predictive values for a positive and negative test were 94.7% and 100%, respectively; the efficiency of the MONO-LEX™ System was 99.4%.13

BIBLIOGRAPHY

PACKAGING
REF R421021, MONO-LEX™ System..............................100 Tests/Kit
REF R421022, MONO-LEX™ System...............................1000 Tests/Kit

Symbol Legend

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