Subject/Title: **ASI COLOR MONO II TEST**		Doc#: 6004-450 CLSI
Effective Date: 12/12	Supersedes Revision/Date: 08/11	Revision: 12/12
Supersedes Procedure #	Prepared by: ASI	Date Adopted:

Review Date:		
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Signature:		

For In Vitro Diagnostic Use

Catalog Number	**Kit Size**
450025	25 Tests
450050	50 Tests
450100	100 Tests
4501000	1000 Tests

CPT Code: 86308

1 **INTENDED USE:** The **ASI Color Mono II Test** is an agglutination test for the qualitative and semiquantitative detection of heterophil antibodies, in human serum, associated with infectious mononucleosis (IM). No initial dilution of patient samples is required for this test. These materials are intended to be acquired, possessed and used by healthcare professionals.

2 **SUMMARY AND EXPLANATION:** Infectious mononucleosis involves the reticuloendothelial tissue and is believed to be caused by the Epstein- Barr virus. It is generally limited to and affects children and young adults. Infectious mononucleosis may be confused on a symptomatic basis with other diseases.[1]

Detectable levels of unique heterophil antibodies are produced in patients with infectious mononucleosis.[2] The antibody of IM was shown by Paul and Bunnell to agglutinate sheep and horse erythrocytes and Bunnell subsequently attempted to use this observation as a basis of screening.[2,3] A specific test was not developed until Davidsohn modified the procedure by introducing differential absorption steps to eliminate Forssman and serum sickness antibody confusion.[4,5,6,7] The **ASI Color Mono II Test**, based on the Davidsohn test procedure, is accepted as the classic reference method in detecting infectious mononucleosis and may be used on patients less than 18 years of age.

Absorption procedures have been eliminated in the **ASI Color Mono II Test**. Specifically formulated dyed horse erythrocyte reagent has been rendered nonreactive with Forssman heterophil antibodies and insensitive to serum sickness heterophil antibodies at levels normally found in the general population.

3 **PRINCIPLE OF THE PROCEDURE:** The **ASI Color Mono II Test** is based on the reaction between IM antibodies in the sample to be tested and dyed , color-enhanced horse erythrocytes. A visible agglutination takes place with horse erythrocytes when IM heterophil antibodies are present. Lack of agglutination indicates the absence of IM heterophil antibody in the test sample.

4 REAGENTS

4.1 DYED, COLOR-ENHANCED HORSE ERYTHROCYTE REAGENT: Contains a suspension of dyed horse erythrocytes in buffer with 0.1% sodium azide as preservative.

4.2 CONTROLS (REACTIVE, NONREACTIVE) – Human serum or defibrinated plasma (liquid), with 0.1% sodium azide as a preservative.

5 WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

5.1 **ASI Color Mono II Test Reagent** and **Controls** contain sodium azide. Azides in contact with lead and copper plumbing may react to form highly explosive metal azides. When disposing of reagents containing azide, flush down the drain with large quantities of water to prevent azide buildup.

5.2 **ASI Color Mono II Test Controls** contain human serum or plasma which has been tested at the donor level for HBsAg and for HIV-1, HIV-2 and HCV antibodies and found to be nonreactive. As no known test offers complete assurance that infectious agents are absent, the controls should be considered potentially infectious and universal precautions should be used. The CDC/NIH Health Manual "Biosafety in Microbiological and Biomedical Laboratories" describes how these materials should be handled in accordance with Good Laboratory Practice.

5.3 Reagents must be well mixed before use.

5.4 Do no pipet by mouth.

5.5 Do not smoke, eat, drink or apply cosmetics in areas where plasma/serum samples are handled.

5.6 Any cuts, abrasions or other skin lesions should be suitably protected.

6 HANDLING AND PROCEDURAL NOTES

6.1 In order to obtain reliable and consistent results, the instructions in the package insert must be strictly followed. Do not modify the handling and storage conditions for reagents or samples.

6.2 Do not use past the expiration date indicated on the kit.

6.3 Do not interchange components of one kit with those of another kit.

7 STORAGE INSTRUCTIONS

Store all reagents at 2-8^{o} C in an upright position when not in use. Do not freeze reagents. Pipets and cards do not require refrigeration.

8 INDICATIONS OF DETERIORATION

8.1 Turbidity or precipitation in controls is indicative of deterioration and the component should not be used.

8.2 Bacterial contamination of reagents or specimens may cause false positive results.

9 SPECIMEN COLLECTION AND STORAGE

9.1 Use serum samples and plasma specimens containing EDTA (an anticoagulant). Plasma specimens should be from tubes which have been collected with adequate volume to provide the appropriate proportions of specimen to anticoagulant.

9.2 Separate the serum or plasma as soon as possible after collection and store at 2 - 8° C until testing can be performed.

9.3 If testing is not to be carried out within 72 hours, the serum and plasma should be stored at –20° C or below until testing.

It is not necessary to inactivate the serum or plasma by heat before testing.

10 PERFORMANCE OF THE TEST

	25 Tests	50 Tests	100 Tests	1000 Tests
DYED, COLOR- ENHANCED HORSE ERYTHROCYTE REAGENT	1.0 ml	2.0 ml	2 x 2.0 ml	20 x 2.0 ml
REACTIVE CONTROL	0.5 ml	1.0 ml	1.0 ml	10 x 1.0 ml
NONREACTIVE CONTROL	0.5 ml	1.0 ml	1.0 ml	10 x 1.0 ml
0.05 ml Disposable Stirrer Pipets	25	50	100	1000
Test Card (6-well)	5	9	17	170

11 ADDITIONAL MATERIALS REQUIRED

11.1 Test tubes 12 x 75 mm (dilutions)
11.2 Timer
11.3 Volumetric pipets for dilutions
11.4 Saline (0.85% NaCI solution)
11.5 High intensity light of lamp

12 TEST PROCEDURE

12.1 PREPARATION FOR THE ASSAY

12.1.1 Allow all reagents and samples to warm to room temperature (20-30° C) before use. Remove reagents from foam holders. Do not heat reagents in a water bath.

12.1.2 All reagents are ready for use as supplied. Gently mix the reagents before use to ensure homogeneity.

12.1.3 Gently shake the Erythrocyte Reagent before each use to ensure homogeneity.

13 ASSAY PROTOCOL – QUALITATIVE

13.1 Using the stirrer pipets, deliver one free-falling drop (0.05 ml) of each sample onto a separate circle on the test card. Use a fresh pipet for each sample. When using the stirrer pipet, keep it in a vertical position to ensure accurate delivery. Repeat by adding one free-falling drop of REACTIVE or NONREACTIVE CONTROL from the dropper vials supplied. Note the location of each sample by using the numbers located below and to the left of each circle.

13.2 Shake the vial of Dyed, Color-Enhanced Horse Erythrocytes gently to uniformly mix the suspension. Add one free-falling drop of reagent to each control and sample.

13.3 Using the flat end of the stirrer pipets, mix each sample and reagent and spread over the entire circle.

13.4 Gently rotate the card for 2 minutes. Observe for agglutination under high intensity light. All test results should be compared to both REACTIVE and NONREACTIVE CONTROLS.

14 ASSAY PROTOCOL – SEMIQUANTITATIVE

14.1 For each test specimen to be titrated, label 5 test tubes (12 x 75mm).

14.2 To each tube add 0.2 ml physiological saline.

14.3 To tube No. 1 add 0.2 ml of undiluted test sample.

14.4 Serially make two-fold dilutions by mixing contents of tube No. 1 with a pipet and transferring 0.2 ml to tube No. 2. Repeat serial transfers for each tube. For the five tubes, the dilution range is from 1:2 to 1:32. If required, additional sample dilutions can be added.

14.5 Proceed with step 2 as in the qualitative procedure.

15 QUALITY CONTROL

15.1 Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control Procedures. Controls with graded reactivity should be included. If control samples do not yield the expected response, the assay should be considered invalid and the assay repeated. If the repeat assay does not elicit the expected results for the control samples, discontinue use of the kit and contact ASI Technical Support at 800.654.0146.

16 INTERPRETATION OF RESULTS

16.1 INTERPRETATION OF RESULTS – QUALITATIVE

16.1.1 REACTIVE: Any degree of agglutination or rimming within the test area as compared to the nonreactive control.

16.1.2 NONREACTIVE: Smooth or finely granular suspension with no visible agglutination .

16.2 INTERPRETATION OF RESULTS – SEMIQUANTITATIVE

16.2.1 The highest dilution in which visible agglutination occurs is considered the endpoint titer. The sensitivity of the **ASI Color Mono II Test** is adjusted such that positive reactions will occur with serum or plasma samples that have guinea pig kidney absorbed Davidsohn sheep cell titers of 1:28. A guinea pig kidney absorbed Davidsohn sheep cell titer can be approximated by multiplying the observed titer by 28.

17 LIMITATIONS OF THE PROCEDURE

17.1 In accord with all diagnostic methods, a final diagnosis should not be made on the results of a single test, but should be based on a correlation of test results with other clinical findings.

17.2 Contaminated, lipemic, or grossly hemolyzed sera should not be used because of the possibility of nonspecific results.

17.3 Reaction times longer than specified might cause false positive results due to a drying effect.

17.4 Due to possible prozone effects, the strength of agglutination in the screening test is not indicative of the IM heterophil antibody titer.

17.5 False negative results have been reported. Some of these may represent cases of IM which persistently remain sero-negative for the IM heterophil antibody.[8,9] However, some false negative results have been shown to be due to a delayed IM heterophil antibody response.[10]

17.6 IM heterophil antibody titers have been shown to persist in some cases for months and years after clinical symptoms have subsided.[11,12,13,14] Conversely, IM heterophil antibodies have been detected prior to the onset of clinical symptoms.[15] Thus, caution should be exercised in the interpretation of test results.

17.7 Patients with exceptionally high levels of the serum sickness heterophil antibody may test falsely positive for the IM heterophil. These patients are generally found only in countries where "horse serum is used prophylactically".[15]

17.8 The IM heterophil has been associated with several diseases other than IM.[8,11,16,17,18] These include leukemia, Burkitt's lymphoma, pancreatic carcinoma, viral hepatitis, cytomegalovirus infections and others. In these cases, it is difficult to disprove the possibility of concurrent disease states.[19]

18 EXPECTED RESULTS AND PERFORMANCE CHARACTERISTICS

Detectable levels of the IM heterophil antibody can usually be expected to occur between the sixth and tenth day following the onset of symptoms. The level usually increases through the second or third week of illness and, thereafter, can be expected to persist with gradual decline over a 12-month period. Positive results should be seen in approximately 98% of all IM cases.[18] False negative and false positive rates of 2% and 6-13% respectively, are to be expected.[18] In a clinical study involving more than 500 tests, the **ASI Color Mono II Test** showed 100% correlation with another commercial product.

19 BIBLIOGRAPHY

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