HAMA ELISA

For the quantitative determination of human anti-mouse antibody in human serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 24-HAMHU-E01
Size: 96 wells
Version: ALPCO 6/15/2010
I. INTENDED USE
The human anti-mouse antibody (HAMA) ELISA test kit is for research use to determine IgG in isotypic HAMA in human serum. This kit is for research use only. It is not use in diagnostic procedures.

II. INTRODUCTION
Therapeutic use of murine monoclonal antibodies (IgG) or their fragments either unmodified or conjugated to drugs, toxins, or radionuclides is becoming increasingly popular (1-4). In patients, multiple injections of murine monoclonal IgG may induce immune response directed against the same IgG and produce significant levels of HAMA in serum. Circulating levels of HAMA can bind to the injected IgG and reduce the efficacy of the antibody therapy (5-7). HAMA also can cause anaphylactic complications to subsequent administration of murine monoclonal IgG (8). In addition, HAMA have been reported to give false positive results in two site immunometric assays which utilize murine monoclonal IgG (9-12).

In some cases, pre-existing HAMA reactivity have been detected without the administration of murine monoclonal IgG in approximately 9% of a normal population. Such responses may be due to polyclonal rheumatoid factors, other heterophilic antibodies or dietary or other exposure (13-15).

Using simple and ready to use strip microplate ELISA, serum HAMA can be assayed quantitatively and results obtained in approximately 2 hours.

III. PRINCIPLE
The HAMA ELISA is a one step, direct bridging assay. Microplate wells which have been pre-coated with IgG are incubated with calibrators or sample and enzyme conjugate. After incubation the wells are washed and incubated with chromogen. substrate solution. The enzyme reaction is terminated by the addition of stop solution and the color developed is read in an ELISA colorimetric analyzer. The measured absorbance is directly proportional to the concentration of HAMA bound to the microplate wells. The concentration of HAMA in serum is determined from a calibration curve constructed with anti-mouse IgG.

IV. WARNING AND PRECAUTIONS
*All materials are for research use only and not for internal or external use in animals or humans. *Some of the reagents in this kit contain merthiolate. Merthiolate may be toxic if ingested. Care should be taken to avoid ingestion. *Handle all compounds and all samples as if capable of transmitting hepatitis and acquired immunodeficiency syndrome. This kit contains components of human origin. When tested by approved methods, the components were found negative for hepatitis B surface antigen and for HIV antibody. However, no known tests can guarantee that such material does not contain the causative agent of viral hepatitis or acquired immunodeficiency syndrome.

V. STORAGE AND STABILITY
Store the test kit in a refrigerator (2-8°C) and protect from moisture. Do not freeze. Reagents are stable until the expiration date shown on the kit and component labels.

VI. SAMPLE COLLECTION
Collect blood by venipuncture into plain tubes avoiding hemolysis. Separate the serum from the cells by centrifugation. Avoid grossly lipemic samples. The samples should be stored at 2-8°C soon after collection. Samples may be stored frozen (-20°C or lower) for longer periods of time. Prepare aliquots of the samples before freezing to avoid repeated freeze and thaw cycles. Do not thaw frozen samples in a hot water bath, thaw at room temperature and mix by gentle swirling or inversion.
VII. TEST PROCEDURE

• Materials supplied:
  Materials supplied in this kit are sufficient for 96 determinations.

Calibrators: Catalog No.s 14-11, 14-12, 14-13, 14-14, and 14-15; serum/buffer matrix containing anti-mouse IgG. Concentration of the calibrators is indicated on the vials (approximately 37-300 ng/ml). Zero calibrator 10 ml, other calibrators 1.0 ml per vial. Merthiolate (0.01%) added as a preservative.

Enzyme conjugate reagent: Catalog No. 14-30; buffered reagent containing IgG horseradish peroxidase enzyme conjugate. Merthiolate (0.01%) added as a preservative. 5 ml.

Antibody coated microplate (96 wells): Catalog No. 14-40; 96 wells microplate containing IgG adsorbed, packaged in a zip-lock bag. This plate consists of twelve polystyrene removable strips mounted in a frame. Each strip included eight wells. 1 plate.

Chromogen substrate solution: Catalog No. 13-60; buffered reagent containing 3, 3’, 5, 5’ tetramethylbenzidine and peroxide. Merthiolate (0.01%) added as a preservative. 10 ml.

Stop solution: Catalog No. 12-80; 1 N stop solution. 10 ml.

Wash buffer: Catalog No. 14-100; 10X concentrated wash buffer containing detergent. Merthiolate (0.1%) added as a preservative. 50 ml.

• Materials required but not supplied:
  ELISA microplate analyzer capable of reading at 450 nm
  Refrigerator (2-8°C)
  Distilled or deionized water
  25, 50, and 100 µl precision pipets, preferably a re-pipetter with disposable tips
  Volumetric measuring cylinder, 10-50 ml
  Dispenser (wash bottle), 100-1,000 ml
  Pasteur Pipets and a bulb
  Test tubes for sample dilution

• Assay Procedure:
  All components must be at ambient temperature before use.
  1. Prepare a plate with the desired number of strips to assay calibrators and samples in duplicate. Keep remaining strips sealed in the bag, protected from moisture.
  2. Prepare a 1:2 dilution of all samples with calibrator 1 (0 ng/ml).
  3. Prepare a 1:10 dilution of the desired volume of 10X concentrated wash buffer with distilled or deionized water.
  4. It is preferable that the chromogen substrate solution not be pipetted directly from the vial because the solution can be easily contaminated with carry-over antibody HRP to the vial. It is therefore suggested that the desired quantity is transferred to a clean test tube and pipetted from there. Discard any remaining solution.
  5. Pipette 50 µl enzyme conjugate to each well.
  6. Add 50 µl of each calibrator and sample to the appropriate wells.
  7. Mix well by a slow shaking of the wells for a few seconds, and then incubate for 60 minutes at room temperature.
  8. After the incubation, aspirate and wash the wells 5 times with diluted wash buffer.
  9. Add 100 µl of chromogen substrate reagent to each well.
  10. Mix well by a slow shaking of the wells for a few seconds, and then incubate for 30 minutes at room temperature.
  11. Add 100 µl of stop solution to each well to stop the color development. Mix well by a slow
shaking of the wells for a few seconds.

12. Determine the absorbance of all the wells in an ELISA colorimetric analyzer at 450 nm against air blank (reference wavelength approximately 630 nm).

**Calculation of Results:**

1. Calculate the mean absorbance value.
2. Subtract the calibrator 1 (0 ng/ml) mean absorbance from the mean absorbance of the other calibrators and samples.
3. Using a linear-linear graph, plot the absorbance on the vertical axis (y-axis) against the concentrations (ng/ml) on the horizontal axis (x-axis) for each of the calibrators and draw a smooth curve approximating the path of the four points. HAMA concentration for the samples may then be estimated from the graph by interpolation. The values are multiplied by 2 (dilution factor) to achieve the final HAMA result.

**Typical standard curve data:**

Representative standard curve data is shown below. These data are intended for illustration only and should not be used to calculate results from another assay.

<table>
<thead>
<tr>
<th>Calibrator No.</th>
<th>Absorbance (ng/ml)</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.060</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.273</td>
<td>37.5</td>
</tr>
<tr>
<td>3</td>
<td>0.446</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>0.731</td>
<td>150</td>
</tr>
<tr>
<td>5</td>
<td>1.403</td>
<td>300</td>
</tr>
</tbody>
</table>

**VIII. LIMITATIONS**

1. In approximately 9% of a normal population, pre-existing HAMA reactivity has been detected.
2. In some groups of individuals, the presence of potentially cross-reactive heterophilic antibodies may give a positive HAMA response.
3. Samples with values greater than 300 ng/ml should be diluted with calibrator 1 (0 ng/ml) and assayed again.
4. Do not use heat treated samples.
5. Slight hemolysis and or lipemia do not interfere with the assay.
6. Avoid repeated freezing and thawing of the samples.

**IX. EXPECTED VALUES**

A reference range study was conducted in 120 human serum samples using the HAMA ELISA. The following results were obtained.

Mean: 51.1 ng/ml        Std. Dev. + 38.09 ng/ml
Range: 0 - 188 ng/ml

The reference range limits suggested by this study should be regarded as guidelines only. Each laboratory should establish its own reference values to conform to the characteristics of the population that is being tested.

**X. REFERENCES**


