CE Mark.bmp **ANA HEp-2 Test System**

**IVD**

1920 Tests.bmp960 Tests.bmp1440 Tests.bmp**Q240181 Q240241 Q240961**

**Rx Only**

**REF**

|  |  |
| --- | --- |
| Institute Name | Date |
|  |  |

**PRINCIPLE OF THE ASSAY**

The ZEUS ANA HEp-2 Test System is designed to detect the presence of circulating ANA in human sera. The system employs tissue cell culture substrate and goat anti-human immunoglobulin adjusted for optimum use and free of nonspecific background staining. The reaction occurs in two steps:

1. During the first (sample) incubation, any ANA present in the patient sample may bind to the cell substrate, forming an antigen-antibody complex. Other serum components are subsequently washed away.
2. During the second (conjugate) incubation, anti-human immunoglobulin labeled with FITC is allowed to react with any human immunoglobulin that bound to the substrate during the sample incubation. This will form a stable antigen-antibody-conjugate complex at the location where the initial patient antibody bound to the cell substrate. Excess conjugate is subsequently washed away.
3. The results of the assay can be visualized using a properly equipped fluorescent microscope. Any positive reactions will appear as apple-green fluorescent staining within the cell. If the sample had no specific ANA, there will be no distinct nuclear staining of the cell.

**KIT COMPONENTS**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Component** | | |  | **96 Tests.bmp** | **480 Tests.bmp** | **480 Tests.bmp** | **Description** |
|  | |  |  |  |  |  |  |
| ● ● ● | | |  | 10 | 80 | 80 | Substrate Slides: ANA HEp-2 cell culture substrate slides containing a layer of HEp-2 cells that were grown on the slide and subsequently stabilized. Slides are packaged in a poly-foil pouch with desiccant and blotter. Well configurations vary depending on kit product number. | |
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| **CONJ** | | |  | 5 | 6 | 6 | Conjugate: Dripper-tipped bottle containing goat anti-human immunoglobulin labeled with fluorescein isothiocyanate (FITC) and counterstain. Ready to use, 7 mL. | |
|  | |  |  |  |  |  |  | |
| **CONTROL** | | **+** |  | 1 | 3 | 3 | Homogeneous Positive Control: Dripper-tipped bottle containing human ANA-positive control serum producing 4+ homogeneous nuclear staining with the substrate cells. Ready to use, 1.0mL. | |
|  | |  |  |  |  |  |  | |
|  | |  |  | 1 | 1 | 1 | Speckled Positive Control: Dripper-tipped bottle containing human ANA-positive control serum producing 4+ speckled nuclear staining with the substrate cells. Ready to use, 1.0mL. | |
|  | |  |  |  |  |  |  | |
|  | |  |  | 1 | 1 | 1 | Nucleolar Positive Control: Dripper-tipped bottle containing human ANA-positive control serum producing 4+ nucleolar staining with the substrate. Ready to use, 1.0mL. | |
|  | |  |  |  |  |  |  | |
|  | |  |  | 1 | 1 | 1 | Centromere Positive Control: Dripper-tipped bottle containing human ANA-positive control serum producing 4+ centromere staining with the substrate cells. Ready to use, 1.0mL. | |
|  | |  |  |  |  |  |  | |
| **CONTROL** | | **-** |  | 1 | 3 | 3 | Negative Control: Dripper-tipped bottle containing human ANA-negative control serum. Ready to use, 1.0mL. | |
|  | |  |  |  |  |  |  | |
| **MRC** | | |  | 1 | 1 | 1 | Minimally Reactive Control: Dripper-tipped bottle containing human ANA-positive control serum producing a 1+ homogeneous nuclear staining with the substrate cells. Ready to use, 1.0mL. | |
|  | |  |  |  |  |  |  | |
| **BUF** | **PBS** | |  | 10 | 20 | 20 | PBS: Phosphate-buffered-saline, pH 7.2 ± 0.2. | |
|  | |  |  |  |  |  |  | |
| **MNTMED** | | |  | 5 | 6 | 6 | Mounting Media: Phosphate buffered glycerol, 3mL. | |

**NOTES: Mounting Media (Product Number: FA0009S), and PBS (Product Number: 0008S) are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems. Test System also contains a Component Label containing lot specific information inside the Test System box.**

**PRECAUTIONS**

1. For *in vitro* diagnostic use.
2. Reagents may contain a preservative that may be toxic if ingested.
3. Non-nuclear staining of the cell substrate may be observed with some human sera.
4. Do not apply pressure to slide envelope. This may damage the substrate.
5. Reagents from other sources or manufacturers should not be used. Follow test procedure carefully.
6. Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, suspect bacterial contamination.
7. DO NOT freeze and thaw reagents more than once. Repeated freezing and thawing destroys antibody activity.
8. Controls are **POTENTIALLY BIOHAZARDOUS MATERIALS**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg. and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/National Institutes of Health manual “Biosafety in Microbiological and Biomedical Laboratories”: current edition; and OSHA’s Standard for Bloodborne Pathogens (39).
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Pipets capable of delivering 10 to 200µL. 6. Small serological, Pasteur, capillary or automatic pipettes.
2. Disposable reagent reservoirs. 7. Small test tubes, 13 x 100mm or a 96 well sample dilution plate for preparing sample dilutions.
3. Cover slips, thickness No. 1. 8. Large Staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides.
4. Moist chamber. 9. Properly equipped fluorescence microscope assembly with the proper stage for the slide configuration used.
5. Distilled or deionized water.

The following filter systems or their equivalent have been found to be satisfactory for routine use with transmitted or incident light dark-field assemblies:

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| **TRANSMITTED LIGHT** | | | | | |
| Light Source: Mercury vapor 200W or 50W | | | | | |
| Excitation Filter | | Barrier Filter | | Red Suppression Filter | |
| KP490 | | K510 or K530 | | BG38 | |
| BG12 | | K510 or K530 | | BG38 | |
| FITC | | K520 | | BG38 | |
| Light Source: Tungsten – Halogen 100W | | | | | |
| KP490 | | K510 or K530 | | BG38 | |
| **INCIDENT LIGHT** | | | | | |
| Light Source: Mercury Vapor 200, 100, 50 W | | | | | |
| Excitation Filter | Dichroic Mirror | | Barrier Filter | | Red Suppression Filter |
| KP500 | TK510 | | K510 or K530 | | BG38 |
| FITC | TK510 | | K530 | | BG38 |
| Light Source: Tungsten – Halogen 50 and 100 W | | | | | |
| KP500 | TK510 | | K510 or K530 | | BG38 |
| FITC | TK510 | | K530 | | BG38 |

**SPECIMEN COLLECTION**

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (32, 33). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for up to 8 hours. Store sera between 2 - 8°C if testing is not performed within 8 hours. If a delay in testing (48 hours or more) is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (40).

**STORAGE CONDITIONS**

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| --- | --- |
| storage2-8.bmp | Unopened Test System. |
| Mounting Media, Conjugate, Slides, Positive Control and Negative Control. |
| Rehydrated PBS (Stable for 30 days). |
| storage2-25.bmp | Phosphate-buffered-saline (PBS) Packets. |

**ASSAY PROCEDURE**

**Preparation of Reagents:**

1. Phosphate-buffered-saline (PBS). Empty contents of one buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved.
2. Human ANA positive controls. Use as packaged. Do not dilute.
3. Human ANA minimally reactive positive control. Use as packaged. Do not dilute
4. Human ANA negative control. Use as packaged. Do not dilute.
5. Goat anti-human immunoglobulin FITC labeled conjugate. Use as packaged. Do not dilute.

**NOTE: The Controls are intended to be used undiluted.** As an option, users may titrate the Positive Control(s) to endpoint. In such cases, the Control(s) should be diluted two-fold in PBS. When evaluated at ZEUS, an endpoint dilution is established and printed on the homogeneous Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own mean titer for each lot of Control.

**Test Procedure:**

1. Remove slides from storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove slides containing the HEp-2 cell culture substrate. DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.
2. Prepare patient sera at 1:40 dilution in PBS (for example: 10µL of sample plus 390µL of PBS). If additional serial dilutions are to be tested, prepare subsequent dilutions in reconstituted PBS. After adding patient sample to PBS, mix the sample well by withdrawing and expelling the specimen several times.
3. Identify each well with the appropriate patient sera and controls.
4. If test samples are to be titered, serial dilutions should be made in reconstituted PBS.
5. Using suitable dispenser (capillary, Pasteur, or automatic pipette), dispense one drop or approximately 20µL of patient sera and control sera on the cells in the respective wells. Spread sera over entire area of the wells being careful not to touch substrate with pipette tip.
6. Incubate slides in a moist chamber at room temperature (20 - 25°C) for 20 - 30 minutes.
7. Remove slides from the moist chamber one at a time and gently rinse with a stream of PBS. **DO NOT DIRECT THE STREAM OF PBS INTO THE TEST WELLS. NOTE: To avoid cross-contamination when using the 96-well test system, place slide in palm of hand and grasp edges with fingertips. Quickly invert the slide and, using a “snap” motion, expel excess sera.**
8. Place slides in a staining dish and wash in PBS for 2, 5-minute intervals with one change of PBS. Use a magnetic mixing setup or other means of gentle agitation.
9. Remove slides from PBS **one at a time**. Invert slide and key wells to holes in blotters provided. Blot slide by wiping the bottom side with an absorbent wipe. CAUTION: Position the blotter and slide on a hard, flat surface. Do not invert the slide on top of paper towels. Blotting on paper towels may destroy the substrate. DO NOT ALLOW THE SLIDES TO DRY DURING THE TEST PROCEDURE.
10. Add one drop or approximately 20µL of conjugate to each well.
11. Repeat steps 6 through 9.
12. Apply a suitable number of drops of mounting media (according to the number of wells per slide) to each slide (between the wells) and coverslip.**NOTE:** **Be sure each well has mounting media coverage.**
13. Examine slides immediately with an appropriate fluorescence microscope assembly. NOTE: If delay in examining slides is anticipated, seal coverslip with nail polish and store in refrigerator. It is recommended that slides be examined on the same day of testing.

**QUALITY CONTROL**

1. A Positive Control (4+ homogeneous), a Minimally Positive Control (1+ homogeneous), a Negative Control, and a Buffer Control must be included with each run.
2. It is recommended that the Controls be read prior to evaluating the test samples. If the controls do not appear as described, results may be invalid.
   1. Negative Control - characterized by the absence of specific fluorescence and a red background staining of all cells due to counterstain.
   2. Homogeneous Positive Control - characterized by apple-green fluorescence. The staining pattern is a diffused uniform staining of the entire nucleus.
3. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

**NOTES: Non-specific reagent trapping may exist. It is important to adequately wash slides to eliminate false positive results. The intensity of the observed fluorescence may vary with the microscope and filter system used. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.**

**INTERPRETATION OF RESULTS**

1. The interpretation of the results depends on the pattern observed, the titer of the autoantibody, and the age of the patient. The elderly, especially women, are prone to develop low-titered autoantibodies (<1:80) in the absence of clinical autoimmune disease. In contrast, a 1:20 titer of a significant pattern of autoantibody(s) in a young person may suggest that overt disease may occur later. Experience suggests that a 1:40 dilution is a good dilution to screen for ANA. Low-titer positive results may occur in apparently healthy persons; therefore, always interpret ANA results in light of the patient’s total clinical presentation.
2. Titers less than 1:40 are considered negative.
3. Positive test: A positive reaction is the presence of any pattern of nuclear apple-green staining observed at a 1:40 dilution based on a 1+ to 4+ scale of staining intensity. 1+ is considered a weak reaction and 4+ a strong reaction. All sera positive at 1:40 should be titered to endpoint dilution. This is accomplished by making 1:40, 1:80, 1:160, etc. serial dilutions of all positives. The endpoint titer is the highest dilution that produces a 1+ positive reaction.
4. Homogeneous patterns with peripheral accentuation are frequently found in sera from patients with SLE.

|  |  |  |
| --- | --- | --- |
| **Pattern** | **Disease Most Frequently Found In** | **Reference** |
| Homogeneous High Titer  Homogeneous Low Titer | SLE  Rheumatoid Arthritis and other diseases | (3, 8, 9, 16)  (1) |
| Centromere | CREST Syndrome variant of PSS | (27) |
| Speckled | Scleroderma/Raynaud’s Syndrome/ Mixed connective tissue disease  Sjögren’s Syndrome | (34-36) |
| Nucleolar | Scleroderma | (37) |
| Peripheral | SLE | (2, 8, 9, 16) |

**LIMITATIONS**

1. The ANA test is a laboratory diagnostic aid and by itself is not diagnostic. Positive ANA may be found in apparently healthy individuals. It is therefore imperative that ANA results be interpreted in light of the patients clinical condition by a medical authority.
2. SLE patients undergoing steroid therapy may have negative test results.
3. Many commonly prescribed drugs may induce ANA (6, 7).
4. One autoantibody pattern may partially or completely obscure the diagnostic features of the other. In such instances, it is necessary to titrate the serum.
5. No definitive association between the pattern of nuclear fluorescence and any specific disease state is intended with this product.

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