 **nDNA Test System**

**IVD**

**REF**

 **FA1001**

**Rx Only**

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| --- | --- |
| Institute Name | Date |
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**PRINCIPLE OF THE ASSAY**

The ZEUS IFA nDNA Test System is a pre-standardized indirect fluorescent antibody assay for the qualitative and semi-quantitative determination of nDNA antibodies in patient and control sera. The reaction occurs in two steps:

1. Step one; If nDNA antibodies are present, a reaction between nDNA antibodies and the kinetoplast of the *C. luciliae* substrate takes place in the first step.
2. Step two; goat anti-human immunoglobulin labeled with fluorescein isothiocyanate (FITC) is added to the substrate. If the patient’s sera contains nDNA antibody, a positive apple-green fluorescent antigen-antibody reaction will be observed when the Slides are examined with the fluorescence microscope. A positive reaction is recognized as an intense staining reaction in the small kinetoplasts of the *C. luciliae*.

**TEST SYSTEM COMPONENTS**

**Materials Provided:**

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. SAVe Diluent® contains Sodium Azide (<0.1% w/v) as a preservative.**

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| ● ● ● | 1. | C. *luciliae* Substrate Slides: Ten, 10-well Slides with blotter. |
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| **CONJ** | 2. | Conjugate: Goat anti-human immunoglobulin labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, amber-capped, bottle. Ready to use. |
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| **CONTROL** | **+** | 3. | Positive Control (Human Serum): Will produce positive apple-green staining of the kinetoplast in the *C. luciliae* organisms. One, 0.5mL, red-capped, vial. Ready to use. |
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| **CONTROL** | **-** | 4. | Negative Control (Human Serum): Will produce no detectable nDNA staining. One, 0.5mL, green-capped, vial. Ready to use. |
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| **DIL** | **SPE** | 5. | SAVe Diluent®: One, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.** |
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| **BUF** | **PBS** | 6. | Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters. |
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| **MNTMED** | 7. | Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dripper tipped vials. |

**NOTES:**

1. **The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems, as long as the product numbers are identical: SAVe Diluent® (Product #: FA005CC), Mounting Media (Product #: FA0009S), and PBS (Product #: 0008S).**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

**PRECAUTIONS**

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
4. The Controls are **potentially bio-hazardous 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, these products should be handled at the Bio-safety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual “Biosafety in Microbiological and Biomedical Laboratories”: current edition; and OSHA’s Standard for Bloodborne Pathogens (20).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to their original containers immediately and follow storage requirements.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate. Do not allow the wells to dry out between incubations.
7. The SAVe Diluent**®**, Conjugate, and Controls contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper

azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may by toxic if ingested.

1. Dilution or adulteration of these reagents may generate erroneous results.
2. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
3. Avoid microbial contamination of reagents. Incorrect results may occur.
4. Cross contamination of reagents and/or samples could cause erroneous results.
5. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
6. Avoid splashing or generation of aerosols.
7. Do not expose reagents to strong light during storage or incubation.
8. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
9. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
10. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
11. Do not apply pressure to slide envelope. This may damage the substrate.
12. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
13. Unopened/opened components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
14. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
15. Do not allow slides to dry during the procedure. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, Aq113 x 100mm or comparable.
4. Test tube racks.
5. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing Slides between incubation steps.
6. Cover slips, 24 x 60mm, thickness No. 1.
7. Distilled or deionized water.
8. Properly equipped fluorescence microscope.
9. 1 Liter Graduated Cylinder.
10. Laboratory timer to monitor incubation steps.
11. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).

The following filter systems, or their equivalent, have been found to be satisfactory for routine use with transmitted or incident light darkfield 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 (34, 35). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If delay in testing 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 (37).

**STORAGE CONDITIONS**

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

**ASSAY PROCEDURE**

1. Remove Slides from refrigerated storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove Slides. **Do not apply pressure to flat sides of protective envelope.**
2. Identify each well with the appropriate patient sera and Controls. N**OTE: The Controls are intended to be used undiluted**. Prepare a 1:10 dilution (e.g.: 10µL of serum + 90µL of SAVe Diluent® or PBS) of each patient serum. **The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the Diluent.**

**Dilution Options:**

* 1. Users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in SAVe Diluent**®** or PBS. When evaluated by ZEUS Scientific, an endpoint dilution is established and printed on the Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected end-point titer for each lot of Positive Control.
	2. When titrating patient specimens, initial, and all subsequent dilutions should be prepared in SAVe Diluent**®** or PBS only.
1. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
2. Incubate Slides at room temperature (20 - 25°C) for 30 minutes.
3. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
4. Wash Slides for two, 5 minute intervals, changing PBS between washes.
5. Remove Slides from PBS one at a time. Invert Slide and key wells to holes in blotters provided. Blot Slide by wiping the reverse side with an absorbent wipe. CAUTION: Position the blotter and Slide on a hard, flat surface. Blotting on paper towels may destroy the Slide matrix. **Do not allow the Slides to dry during the test procedure**.
6. Add 20µL of Conjugate to each well.
7. Repeat steps 4 through 7.
8. Apply 3 - 5 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope.

**NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.**

**QUALITY CONTROL**

1. Every time the assay is run, a Positive Control, a Negative Control and a Buffer Control must be included.
2. It is recommended that one read the Positive and Negative Controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If Controls do not appear as described, results are invalid.
3. Negative Control - characterized by the absence of fluorescent staining of the kinetoplast. Staining of the nucleus only and/or staining of the basal body should be interpreted as a negative test.
4. Positive Control - characterized by any apple-green fluorescent staining of the kinetoplast. Staining of the basal body **in conjunction with** the kinetoplast should be considered a positive result.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

**NOTES:**

1. **The intensity of the observed fluorescence may vary with the microscope and filter system used.**
2. **The kinetoplast is generally located closer to the basal body than the nucleus; however, because of the fluid nature of the endoplasm, the location of the kinetoplast may vary from cell to cell (36).**
3. **Read only single, well-defined organisms in each field. Not all organisms will appear optimal; morphology may vary between organisms because of fixation, their stages of growth, and/or their orientation on the Slide as they dried (36).**

**INTERPRETATION OF RESULTS**

1. Titers less than 1:10 are considered negative.
2. Positive Test: Any observed apple-green staining of the small kinetoplast of the *C. luciliae* substrate organism, at a 1:10 dilution based on a 1+ to 4+ scale. 1+ is considered a weak reaction, and 4+ a strong reaction. All sera positive at 1:10 should be titered to endpoint dilution. This is accomplished by making a 1:10, 1:20, 1:40, etc., serial dilution of all positives. The endpoint is the highest dilution that produces a positive reaction.
3. Staining of both the small kinetoplast and the adjacent larger *C. luciliae* nucleus simultaneously should be interpreted as a positive test.
4. Polar staining at the base of the flagella is not significant.
5. Staining of the nucleus only should not be interpreted as a positive test.

**NOTE: A prozone phenomenon may occur at low patient sera dilutions. It is suggested that retesting of the patient specimen be performed at a higher dilution (i.e., 1:40 or 1:80) when this phenomenon is suspected.**

**LIMITATIONS OF THE ASSAY**

1. The ZEUS IFA nDNA Test System is a diagnostic aid. It is therefore imperative that the nDNA antibody results be interpreted in light of the patient’s clinical condition by a medical authority.
2. SLE patients undergoing steroid therapy may have negative test results (5, 8, and 9).
3. Some drugs, particularly hydralazine, may induce nDNA antibody production (5, 6, and 8).

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