

**AccuDiag™**  
**ANA HEp-2**  
**IFA Kit**

**REF 231005D**



Test	Ana HEp-2 IFA Kit
Method	Indirect Fluorescent Antibody Method
Principle	Qualitative & Semi Quantitative
Sample	10 µL
Total Time	~ 80 min.
Shelf Life	12 Months from the manufacturing date
Sensitivity	100 %
Specificity	100 %

**INTENDED USE**

The Diagnostic Automation, Inc. ANA HEp-2 Test System is a pre-standardized assay designed for the qualitative and semi-quantitative detection of antinuclear antibodies. The test is intended to aid in determining SLE and differentiating clinically similar connective tissue disorders, and is for In Vitro diagnostic use.

**SUMMARY AND EXPLANATION**

The indirect fluorescent antibody (IFA) technique method has been used extensively for detecting the presence of ANA in the sera of patients with systemic lupus erythematosus (SLE), and other clinically similar connective tissue disorders. In addition, ANA may be associated with numerous drug-induced lupus syndromes which clinically mimic the spontaneous form of SLE. The IFA technique was adapted to ANA testing by several investigators following the basic methods originally described by Coons. ANA are primarily composed of IgG antibodies; however, IgA and IgM ANA may also be detected. It is now recognized that many sources of nuclear material may be employed as a substrate for ANA testing. Although most of the original ANA research was performed using rat or mouse liver or kidney tissue section substrate, the use of human or animal embryonic tissue cell culture substrates has provided a reliable and easy to interpret alternative substrate for ANA testing. The HEp-2 cell line is a recommended substrate for detecting centromere antibody, which is highly indicative of the CREST variant of progressive systemic sclerosis. There are several different patterns of nuclear and cytoplasmic immunofluorescence. These various patterns and the basis for them are as follows:

**Homogeneous -**

Homogeneous or diffuse staining patterns of the nucleus is consistent with autoantibodies to native DNA (nDNA) histones and/or deoxyribonucleoprotein (DNP). The chromosomes of the mitotic cells (dividing cells) are important indicators of a homogeneous pattern because they will stain as irregularly shaped masses with more intensely stained outer edges.

**Speckled Patterns -** The speckled pattern is the most commonly observed ANA pattern. A uniform “true speckled” pattern may be seen with centromere antibodies in cells not in division. A clumpy speckled pattern may be seen with antibodies to n-RNP, Sm, and SSB/La.

1. Fine speckled pattern, chromosome-negative: Numerous small and uniform points of fluorescence uniformly scattered throughout the nucleus. The nucleoli will generally appear unstained. The mitotic cells may demonstrate a few speckles in their cytoplasm, but the chromosomes will be negative.
2. Course speckled pattern, chromosome-negative: Medium-sized points of fluorescence will be scattered throughout the nuclei with distinct nuclear margins. Larger-sized points of fluorescence may also be observed; however, they are too numerous and variable in size to be identified as a nucleolar pattern. The chromosomes in the mitotic cells will be negative.
3. Discrete speckled, chromosome (centromere specificity) positive: The chromosomes will be positive in mitotic cells; in fact, the discrete speckles will only be clustered in the chromosome mass clearly demonstrating the various stages of mitosis. The centromere pattern has been recognized to be associated with the CREST syndrome, which is a milder variant of progressive systemic sclerosis (PSS). The centromere pattern will demonstrate discrete and uniform points of fluorescent speckles scattered throughout the nucleus. Mitotic cells will be positive, demonstrating a clustering of the centromeres in the chromosomes in different arrangements according to the mitotic stage. Harmon, et al demonstrated that serum samples containing highly monospecific anti-SSA/Ro gave an IF-ANA test pattern of discrete nuclear speckles on a wide variety of human cells and tumor nuclei. Such serum samples with monospecific anti-SSA/Ro produced very little cytoplasmic staining of substrate cells. A distinct, large, variable speckled pattern of 3 to 10 large speckles in the nucleus has been described. These patients with large, variable speckles have undifferentiated rheumatic disease syndromes with IgM antihistone H-3 antibody.

**Nucleolar Pattern -** The nucleolar pattern demonstrates a homogeneous or speckled staining of the nucleolus. This pattern is often associated with a dull, homogeneous fluorescence in the rest of the nucleus. The chromosomes in the mitotic cells will be negative. The nucleolar pattern suggests autoantibodies to 4 - 6S RNA. The nucleolar fluorescence will appear as homogeneous, clumped, or speckled, depending on the antigen to which the autoantibody reacts. Antinucleolar antibodies occur primarily in the sera of patients with scleroderma, systemic lupus erythematosus, Sjögren’s syndrome, or Raynaud’s phenomenon.

**Peripheral (Rim) -** The nuclei stain predominantly at their periphery. The chromosomes of the mitotic cells stain as irregularly shaped masses with more intensely stained outer edges. This pattern is often seen with autoantibodies to nDNA. If the chromosomes of the mitotic cells are negative, then the pattern would be suggestive of autoantibodies to the nuclear membrane and not to nDNA, and not reported as a peripheral pattern (see nuclear membrane interpretation below).

**Additional Patterns**

1. **Spindle fiber pattern, chromosome-positive:** The spindle fiber pattern is unique to cells undergoing mitosis where only the spindle apparatus fluoresces. This pattern has a “spider web” appearance extending from the centriole to the centromeres. The pattern is suggestive of autoantibodies to the microtubules and its significance is unclear; however, an association between the spindle fiber pattern and carpal tunnel syndrome has been suggested.
2. **Midbody pattern:** The midbody pattern is a densely staining region near the cleavage furrow of telophase cells, that is, in the area where the two daughter cells separate. The clinical significance of the pattern is unknown; however, the pattern has been recognized in selected patients with systemic sclerosis.
3. **Centriole pattern:** The centriole pattern is characterized by two distinct points of fluorescence in the nucleus of the mitotic cells or one distinct point of fluorescence in the resting cell. The significance of this pattern is not known; however, it has been observed in PSS.
4. **Proliferating cell nuclear antigen (PCNA) pattern:** The proliferating cell nuclear antigen pattern is observed as a fine to course nuclear speckling in 30 - 60% of the cells in interphase, and a negative staining of the chromosome region of mitotic cells. The PCNA is very specific for patients with SLE but

not detected in other connective tissue disease disorders. It has been reported that SLE patients with the PCNA pattern have a higher incidence of diffuse glomerulonephritis.

5. **Antinuclear membrane (nuclear laminae):** The antinuclear membrane pattern appears as a rim around the nucleus and resembles a rim pattern; however, it is distinguished from the rim pattern by the fact that the metaphase chromosome stage is negative. This autoantibody is important to report because it is recognized to be associated with autoimmune liver disease.

**Cytoplasmic Patterns**

1. **Mitochondrial (AMA) pattern:** The pattern will characteristically have numerous cytoplasmic speckles with the highest concentration in the perinuclear area. The pattern can be observed in interphase and mitotic cells. The clinical significance of AMA is most frequently an association with primary biliary cirrhosis, especially when the AMA is a high titer.
2. **Golgi apparatus pattern:** The golgi apparatus pattern is characterized by positive cytoplasmic staining that is concentrated on only one side of the perinuclear region. The clinical significance is uncertain, but reports in the literature have suggested an association with SLE and Sjögren’s Syndrome.
3. **Lysosomal pattern:** The lysosomal pattern is observed as a few discrete speckles sparsely spaced throughout the cytoplasm. The pattern is observed in the cytoplasm of interphase and mitotic cells. The clinical significance is unknown.
4. **Ribosomal pattern:** The ribosomal pattern is characterized by numerous cytoplasmic speckles with the highest concentration around the nucleus. It is distinguished from the mitochondrial pattern because of the smaller specks and higher density. The significance of the pattern is unknown.
5. **Cytoskeletal pattern:** The cytoskeletal pattern is characterized by a distinct “spider web” or fibrous appearance throughout the cell. It has been reported to be associated with autoimmune liver disease (anti-smooth muscle).

**ANA Negative**

Autoantibody to SSA/Ro is present in high frequency in a clinical subset of lupus called Subacute Cutaneous Lupus Erythematosus (SCLE). Many patients with SCLE have been falsely labeled as having “ANA-negative” lupus. Many of these so-called “ANA-negative” LE patients will demonstrate a positive IF-ANA on substrate of HEp-2 cells containing the SSA/Ro antigen. Anti-SSA/Ro antibodies may be present in the absence of traditional ANAs, with SLE seen in persons genetically deficient in C4 and occasionally other complementary deficiencies. In addition, C4 deficiency may be associated with increased susceptibility to development of SLE upon treatment with hydralazine. These patients, if female, are likely to deliver infants with congenital heart block or lupus dermatitis. Although the level of ANA may not correlate with the clinical course of a particular autoimmune disease state, the various patterns of nuclear staining may be associated with specific disease states.

The following table summarizes the various auto-antibodies noted above with respect to disease association:

Antibody	Disease State	Relative Frequency of Antibody Detection %
Anti-Jo-1	Myositis	25-44 % (18)
Anti-Sm	SLE	30*
Anti-RNP	MCTD, SLE	100** and > 40, respectively
Anti-SSA/Ro	SLE, Sjögren’s	15 and 30 – 40, respectively
Anti-SSBLA	SLE, Sjögren’s	15 and 60 – 70, respectively
Anti-Scl-70	Systemic sclerosis	20-28*
*Highly Specific **Highly Specific when present alone at high titer		

**TEST PRINCIPLE**

The Diagnostic Automation, Inc. ANA HEp-2 IFA Test System is designed to detect the presence of circulating ANA in human sera. The assay 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. The first involves the sample incubation where 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. The second is the Conjugate incubation where the 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. 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.

**SPECIMEN COLLECTION AND PREPARATION**

1. DAI 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 should be used in this assay. 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 that 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 (38).

**MATERIALS AND COMPONENTS**

**Materials provided with the test kits**

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

1. **Ana HEp-2 substrate slides:** Ten, 12-well Slides with absorbent blotter and desiccant pouch.
2. **Conjugate:** Goat anti-human immunoglobulin labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5 mL, clear capped bottle. Ready to use.
3. **Positive Control (Human Serum):** Will produce positive apple-green homogeneous, staining of the cell nucleus. One, 0.5mL, red-capped, vial. Ready to use.
4. **Negative Control (Human Serum):** Will produce no detectable staining of the nucleus. One, 0.5mL, green-capped, vial. Ready to use.
5. **Sample Diluent:** Two, 30 mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. **Note: The Sample Diluent will change color when combined with serum.**
6. **Phosphate-buffered-saline (PBS):** pH 7.2 ± 0.2. To prepare PBS, 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.
7. **Mounting media (Buffered Glycerol):** Two, 3.0 mL, white-capped, dropper tipped vials.

**Note: Kit also contains:**

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.



**Materials required but not provided**

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 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, 24x60mm, 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:*

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

**ASSAY 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. **DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.**
2. Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted.** Prepare a 1:40 dilution (e.g.: 10µL of serum + 390 µl of Sample Diluent or PBS) of each patient serum. **The Sample Diluent will undergo a color change confirming that the specimen has been combined with the Diluent.**  
**Dilution Options:**
  - a. As an option, users may prepare initial sample dilutions using PBS. 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 Sample Diluent or PBS. When evaluated by DAI, and 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.
  - b. When titrating patient specimens, initial dilutions should be prepared in Sample Diluent, PBS and all subsequent dilutions should be prepared in the Sample Diluent or PBS only.
3. With suitable dispenser (listed above), dispense 20-40µL of each Control and each diluted patient sera in the appropriate wells.

4. Incubate Slides at room temperature (20 - 25°C) 20-30 minutes.
5. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
6. Wash Slides two, additional time, changing PBS between washes. Slides may soak during each wash for up to five minutes. **NOTE: For those using automated washers, set the washer to wash each well three times with a soak of zero to five minutes.**
7. 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.**
8. Add 20-40 µL of Conjugate to each well.
9. Repeat steps 4 through 7.
10. Apply 3 - 5 drops of Mounting Media to each Slide (between the wells) and coverslip. Mounting Media must be added within two hours of completing the last wash cycle. Examine Slides immediately with an appropriate fluorescence microscope.

**NOTE: If a delay of more than 48 hours 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.**

**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, the ANA results must always be interpreted 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.

**INTERPRETATION ACCORDING TO PATTERN OF NUCLEAR STAINING**

PATTERN	DISEASE MOST FREQUENTLY FOUND IN	Reference
Homogeneous High Titer	SLE	(3,8,9, and 16)
Low Titer	Rheumatoid Arthritis and other diseases	(1)
Centromere	CREST Syndrome variant of PSS	(27)
Speckled	Scleroderma Raynaud's Syndrome Sjögren's Syndrome Mixed Connective Tissue Disease	(34-36)
Nucleolar	Scleroderma	(37)
Peripheral	SLE	(2,8,9 and 16)



### 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 the Controls be read prior to evaluating the test samples. If controls do not appear as described, results may be invalid.
  - a. Negative Control - characterized by the absence of specific fluorescence and a red, or dull green, background staining of all cells due to counterstain.
  - b. Positive Control – (homogeneous pattern) characterized by apple-green fluorescence. The homogeneous 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:

- a. **Non-specific reagent trapping may exist. It is important to adequately wash slides to eliminate false positive results.**
- b. **The intensity of the observed fluorescence may vary with the microscope and filter system used.**
- c. **Non-nuclear staining of the cell substrate may be observed with some human sera.**

### PERFORMANCE CHARACTERISTICS

The Diagnostic Automation, Inc. ANA HEp-2 IFA test system was tested in parallel with a reference procedure employing rat liver substrate. Routine ANA testing was performed by both procedures on 434 patient specimens. Of these 434 sera, 116 were positive by both procedures. The DAI ANA HEp-2 IFA Test System showed 97% agreement with respect to positive and negative results, and 100% with respect to staining pattern. Of the 21 discrepancies in titer, the DAI IFA ANA HEp-2 procedure was one dilution lower in 18 specimens. Five of these 18 specimens that were negative using the DAI ANA HEp-2 IFA procedure were positive at 1:20 by the rat liver reference procedure.

### LIMITATIONS OF PROCEDURE

1. The ANA HEp-2 IFA test is a laboratory diagnostic aid, and by itself is not diagnostic. Positive ANA may be found in apparently healthy people. It is therefore imperative that ANA 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.
3. Many commonly prescribed drugs may induce ANA.
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.

### EXPECTED VALUES

The expected value in the normal population is negative or less than 1:40. However, apparently healthy individuals may contain ANA in their sera. This percentage increases with aging, particularly in the 7<sup>th</sup> decade of life.

### 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.
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 Sample 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 be toxic if ingested. Dilution or adulteration of these reagents may generate erroneous results.
8. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
9. Avoid microbial contamination of reagents. Incorrect results may occur.
10. Cross contamination of reagents and/or samples could cause erroneous results.
11. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
12. Avoid splashing or generation of aerosols.
13. Do not expose reagents to strong light during storage or incubation.
14. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
15. 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.
16. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
17. Do not apply pressure to slide envelope. This may damage the substrate.
18. 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.
19. 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.
20. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
21. 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.

### STORAGE

2-8°C	Unopened Test System
2-8°C	Mounting Media, Conjugate, Diluent, Slides, Positive and Negative Controls
2-8°C	Rehydrated PBS (Stable for 30 days)
2-25°C	Phosphate-buffered-saline (PBS) Packets

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 <b>ISO 13485</b> <b>ISO 9001</b>			
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<b>REF</b> 231005D	<b>IFA – ANA HEp- 2</b>		
<table border="1"> <tr> <td>EC</td> <td>REP</td> </tr> </table>	EC	REP	<b>CEpartner4U, Esdoornlaan 13,</b> <b>3951DB Maarn. The Netherlands.</b> <a href="http://www.cepartner4u.eu">www.cepartner4u.eu</a>
EC	REP		
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