AESKU.BION
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ANA (HEp-2)

ANTIGEN SUBSTRATE SLIDE

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NOTE: Changes highlighted

PRODUCT AVAILABILITY

The following Antinuclear Antibody Antigen Substrate Slides are available individually from Bion Enterprises, Ltd. (Bion):

Antigen Substrate Slide	Code No.	Code No.
Antinuclear Antibody (HEp-2)	AN-1012	AN-1006
Σ Number of Tests	12-Wells	6-Wells

(REF = Code Number)

INTENDED USE

The Bion ANA (HEp-2) ANTIGEN SUBSTRATE SLIDES may be used as the antigenic substrate in indirect fluorescent antibody assays for the qualitative and/or semi-quantitative determination of antinuclear antibodies in human serum. Bion ANA (HEp-2) ANTIGEN SUBSTRATE SLIDES are intended for use as an aid in the diagnosis of certain autoimmune diseases.

SUMMARY AND EXPLANATION

Antinuclear antibodies (ANA) are a group of autoantibodies characterized by specificity for numerous antigenic determinants of cell nuclei. While the role of ANA's in the pathogenesis of autoimmune disease is controversial, they are quite useful as disease markers, primarily for diagnostic screening and also to monitor the course of connective tissue diseases.[1,2,3]

Because of the high correlation of positive antinuclear antibodies with SLE a negative ANA essentially rules out this disease.[4] Although antibodies specific to DNA have a high correlation with SLE,[5] antibodies to a number of other nuclear antigens appear to be of diagnostic and/or prognostic significance in diseases such as Progressive Systemic Sclerosis,[6,7] Mixed Connective Tissue Disease,[8] Sjogren's Syndrome,[9] and Polymyositis;[10] making ANA testing useful not only for SLE, but as a general screening tool for connective tissue diseases.[11]

Among the methodologies available to detect ANA's are EIA, ELISA, Dot Blot and the Indirect Fluorescent Antibody (IFA) technique. The antigen for the first three methods can either be a spectrum of clinically significant, specific autoantigens, a single mixture of autoantigens from a cell lysate, or a combination of the two. These methods are not as sensitive as IFA, nor can they detect the variety of autoantibodies. They also do not have the pattern recognition quality of the IFA. The IFA test is sensitive, screens for a wide variety of known and unknown autoantibodies and, through pattern recognition, offers insights into the probable identity of the antigen and associated autoimmune disorder. It is the dominant methodology in clinical laboratories at this time[2] and the method of choice for ANA screening and semi-quantitation.

The antigen of the Bion ANA substrate is a human epithelial cell (HEp-2) line established by Moore, Sabachensky and Toolan.[12] HEp-2 cells have been shown to have greater sensitivity than tissue sections and yield sharper pattern recognition.[13] The presence of mitotic figures aids in differential pattern recognition as well as detecting previously unreported nuclear antibodies.[14,15] Antinuclear antibodies can be found in all major immunoglobulin classes (IgG, IgA or IgM), therefore, antihuman gammaglobulin conjugate that detects all classes is recommended for use in routine ANA testing.[16]

PRINCIPLE OF THE IFA PROCEDURE

The Bion ANA (HEp-2) ANTIGEN SUBSTRATE SLIDES may be utilized in the indirect fluorescent antibody assay method first described by Weller and Coons[17] and further developed by Riggs, et al.[18] The procedure is carried out in two basic reaction steps:

Step 1 - Human serum is reacted with the antigen substrate. Antibodies, if present, will bind to the antigen forming stable antigen-antibody complexes. If no antibodies are present, the complexes will not be formed and serum components will be washed away.

Step 2 - Fluorescein labeled antihuman antibody is added to the reaction site, which binds with the complexes formed in step one. This results in a positive reaction of bright apple-green fluorescence when viewed with a properly equipped fluorescence microscope. If no complexes are formed in step one, the fluorescein labeled antibody will be washed away, exhibiting a negative result.

REAGENTS

The Bion ANA ANTIGEN SUBSTRATE SLIDES are individually foil-wrapped slides of 6 or 12 wells with HEp-2 tissue culture cells fixed onto each well.

STORAGE AND STABILITY

The Bion ANA ANTIGEN SUBSTRATE SLIDES are stable in sealed foil pouches at 8°C or lower until labeled expiration date.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of in vitro diagnsotics may perform the test. IVD
2. Substrate slides are for single use only and must not be used more than once.
3. Use with non-Bion reagents could result in erroneous results.
4. Care should be taken when handling substrate slides due to sharp edges.
5. Do not remove slides from pouches until ready for testing. Do not use if the pouch has been punctured, as indicated by a flat pouch.
6. Antigen substrate slides should be brought to room temperature (20-25°C) prior to use.
7. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
8. Refrigeration (2-8°C) of the antigen substrate slides immediately upon arrival will insure stability until labeled expiration date.
9. Antigen substrate slides should not be used beyond the stated expiration date.
10. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
11. Incubation times or temperatures other than those specified may give erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of detergents.
13. Care should be taken to avoid splashing and generation of aerosols.
14. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera is freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
15. Patient samples, as well as all materials coming into contact with them, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories", 1984 Edition. Never pipette by mouth. Avoid contact with skin and mucous membranes.

SPECIMEN COLLECTION

Blood should be collected fasting or at least one hour after meals to avoid lipemic serum, as excess lipids may produce a "film" over the substrate. Aseptically collect 5-8 ml of blood by venipuncture. Allow the blood to clot at room temperature (20-25°C) before separating serum to avoid hemolysis which could interfere with test results. Specimens should be stored refrigerated at 2-8°C and tested within one week of collection. Long term storage should be at -20°C in aliquots to avoid repeated freezing and thawing. Do not store in self-defrosting freezer.

Avoid using contaminated sera as they may contain proteolytic enzymes which will digest the substrate. It is unnecessary to heat inactivate serum specimens prior to testing; however, sera that have been heat inactivated may be used.

When testing paired samples to look for evidence of recent infection, the acute specimen should be obtained as soon as possible after onset of illness and the convalescent specimen obtained 7-14 days later. Acute and convalescent specimens must be tested simultaneously, in the same assay, looking for a significant change in antibody titer between the paired sera. If the first specimen is obtained too late during the course of the infection, a significant rise in the antibody titer may not be detected.

PROCEDURE

Detailed descriptions of indirect immunofluorescence techniques may be found in the references listed in the bibliography.[19,20,21]

MATERIALS PROVIDED

Bion ANA (HEp-2) ANTIGEN SUBSTRATE SLIDES.
Lot Number provided on label. LOT

MATERIALS AVAILABLE FROM Bion

1. Fluorescent Antibody Conjugate with 0.001% Evans Blue counterstain
2. ANA Positive (homogeneous pattern) Human Control Serum
3. ANA Negative Human Control Serum
4. Phosphate Buffered Saline (PBS)
5. Mounting Medium
6. IFA Diluent
7. Other Positive Human Control Sera - Speckled Control, Nucleolar Control, Anti-Centromere Control (ACA), Ribonucleoprotein Control (RNP), Sjogren's Syndrome A Control (SS-A), Sjogren's Syndrome B Control (SS-B) and Scleroderma-70 Control (Scl-70).

MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable test tubes (12 x 75 mm or comparable) and rack
2. Disposable serological pipettes
3. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl with disposable pipette tips
4. Pasteur pipettes and bulbs
5. Moist chambers
6. Plastic squeeze wash bottle
7. Coplin jars or staining dishes with slide racks
8. 24 x 60 mm #1 coverslips
9. Felt tip marking pen
10. Fluorescence microscope equipped with a mercury or tungsten-halogen light source, a 390-490 nm excitation filter and 515-520 nm barrier filter, and optics to give a total magnification of 400X. The excitation wavelength of FITC is 490 nm and the emission wavelength is 520 nm.

TEST PROCEDURE

1. **SPECIMEN PREPARATION**

Screening:

Each laboratory should establish its own protocol for the preparation of serum screening dilutions. Most indirect fluorescent antibody staining procedures utilize a 1:40 dilution of each patient's serum which is prepared by adding 0.05 ml (50 µl) of patient's serum to 1.95 ml of PBS or IFA diluent.

Semi-quantitation:

Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titering protocol. The selection of either twofold or fourfold dilution procedures depends upon the experience level and training of the individual(s) reading the fluorescent antibody assay.

The following fourfold serial titration is suggested for testing:

a. Prepare a 1:40 dilution of each patient's serum by adding 0.05 ml (50 µl) of patient's serum to 1.95 ml of PBS or IFA diluent in tube #1

b. Add 0.3 ml PBS to tubes #2, #3, #4, and #5.
Note: DO NOT use IFA diluent for serial dilutions.

c. Using a 100 µl pipette, transfer 0.1 ml (100 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.1 ml (100 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer.

Following is a suggested twofold titration:

a. Prepare a 1:40 dilution of each patient's serum by adding 0.05 ml (50 µl) of patient's serum to 1.95 ml of PBS in tube #1.

b. Add 0.2 ml PBS to tubes #2, #3, #4, and #5.
Note: DO NOT use IFa diluent for serial dilutions.

c. Using a 200 µl pipette, transfer 0.2 ml (200 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.2 ml (200 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer.

These titrations will have the following dilutions:

Fourfold	Twofold
Tube #1 = 1:40	Tube #1 = 1:40
Tube #2 = 1:160	Tube #2 = 1:80
Tube #3 = 1:640	Tube #3 = 1:160
Tube #4 = 1:2560	Tube #4 = 1:320
Tube #5 = 1:10,240	Tube #5 = 1:640

2. **SLIDE PREPARATION**

Remove reagents and as many substrate slides as are required from the refrigerator or freezer and allow to equilibrate to room temperature (20-25°C) for at least five minutes. Remove slides from sealed foil pouches being careful not to touch the antigen surface. Identify each slide using a felt tip marking pen.

3. **SPECIMEN APPLICATION**

Using separate Pasteur pipettes, apply one drop (20-30 µl) of the positive control, one drop (20-30 µl) of the negative control, and one drop (20-30 µl) of each patient serum dilution to individual wells of the slide. Do not touch the antigen surface with the pipette while dropping. Do not allow the drops to mix, as cross contamination of samples between wells could cause erroneous results.

4. **INCUBATION 1**

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. THE ANTIGEN MUST NOT BE ALLOWED TO DRY DURING ANY OF THE FOLLOWING STEPS. Nonspecific binding may occur if the reagent is allowed to dry on the slide.

5. **RINSE 1**

Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. Do not focus the PBS stream directly onto the wells. To prevent cross contamination tilt slide first toward wells 1-6 and, running a PBS stream along the midline of the slide, allow the PBS to run off the top edge of the slide. Then, tilt the slide toward wells 7-12 and repeat this procedure, allowing the PBS to run off the bottom edge of the slide. For six well slides, tilt slide down and run the PBS stream across the slide above the wells, allowing the PBS to run off the bottom edge of the slide.

6. **WASH 1**

Place the slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

7. **CONJUGATE APPLICATION**

Remove slides from the wash one at a time, shake off excess PBS, dry around outside edges if necessary, and return each slide to the moist chamber. Apply one drop of fluorescent anti-human antibody conjugate with counterstain (diluted to its predetermined proper working dilution) to each well of each slide, making sure that each well is completely covered.

8. **INCUBATION 2**

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. Protect slides from excessive light.

9. **RINSE 2**

Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. As suggested in step 5., do not focus PBS stream directly onto the wells.

10. **WASH 2**

Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

11. **COVERSLIP**

Remove slides one at a time from last PBS wash, shake off excess PBS and immediately add two to four drops of mounting medium across the slide. Tilt slide and rest the edge of the coverslip against the bottom of the slide allowing the mounting medium to form a continuous bead between the coverslip and slide. Gently lower the coverslip from the bottom of the slide to the top, being careful to avoid air bubbles. Drain excess mounting medium by holding the edge of the slide against absorbent paper. Wipe off back of slide.

12. **READ**

Examine stained slides as soon as possible using a properly equipped fluorescence microscope. It is recommended that slides be examined on the same day they are stained. If any delay is anticipated, store slides in the refrigerator (2-8°C) away from direct light and read the following day. Do not allow mounting medium to dry between slide and coverslip. If drying should occur, add additional mounting medium or recoverslip slide.

FLUORESCENT INTENSITY GRADING

Fluorescent intensity may be semi-quantitated by following the guidelines established by the Centers for Disease Control, Atlanta, Georgia:[22]

4+ = Maximal fluorescence; brilliant yellow-green.
3+ = Less brilliant yellow-green fluorescence.
2+ = Definite but dull yellow-green fluorescence.
1+ = Very dim subdued fluorescence.

The degree of fluorescent intensity is not clinically relevant and has only limited value as an indicator of titer. Differences in fluorescence microscope optics, filters and light sources may result in differences of 1+ or more fluorescent intensity when observing the same slide using different microscopes.

QUALITY CONTROL

SPECIFICITY CONTROL

Both a positive and negative antibody control must be included with each run. These controls must be examined prior to reading test samples and should demonstrate the following results:

Negative Control
Using a negative control serum on Bion ANA (HEp-2) SUBSTRATE SLIDES, the cells should exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain.

Positive Control
Using a positive control serum on Bion ANA (HEp-2) SUBSTRATE SLIDES, the cells should exhibit well defined specific fluorescent staining pattern at an intensity of 3+ or greater. The fluorescent staining pattern should be that of the pattern of the positive control used.

Each control must demonstrate the expected type of reaction in order to validate the test. If the controls fail to appear as described above, the test results should not be reported and the test should be repeated. If upon repeat testing the controls still fail to show the proper reaction, do not report test results.

The specificity of the antigen substrate can further be tested by running a panel of various types and patterns of antinuclear antibodies (These are available separately from Bion).

SENSITIVITY CONTROL

A titered control included with each run tests substrate sensitivity, as well as, checks technique, conjugate quality and the microscope optical system. The endpoint titer of this control must be determined and there must not be more than a twofold difference (+/-) in titer from this determined endpoint. Each run should include the endpoint dilution, one twofold or fourfold dilution above and one twofold or fourfold dilution below the endpoint dilution. The more concentrated dilution should be positive and the less concentrated dilution negative. If the control does not behave as described, the test results are invalid and the tests should be repeated. If the control again fails to show the proper reaction upon repeat testing, do not report the test results.

READING OF TEST RESULTS

NEGATIVE

A serum dilution is considered to be negative for antinuclear antibodies if the cells exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain, or if the fluorescence observed is not a discernible ANA pattern.

A sample is considered negative for antinuclear antibodies if it exhibits less than 1+ fluorescence at a serum dilution of 1:40 and all greater dilutions, or if the fluorescence observed is not a discernible ANA pattern.

... Negative samples may exhibit fluorescent staining slightly greater than the negative control, but less than 1+.

... Some sera may show a low degree of nuclear or cytoplasmic fluorescence with no clearly discernible staining pattern. This phenomenon is generally due to heterophile antibodies and should be reported as negative.[23]

... Intense non-nuclear staining may be observed in some sera containing Anti-Mitochondrial, Anti-Smooth Muscle or other cytoplasmic antibodies.

POSITIVE

A serum dilution is considered positive for antinuclear antibodies if the fluorescent staining is at an intensity of 1+ or greater with a clearly discernible pattern of fluorescence.

A sample is considered positive for antinuclear antibodies if it exhibits a characteristic ANA staining pattern with a fluorescent intensity of 1+ or greater at a serum dilution of 1:40 or greater.

... Multiple antinuclear antibodies may be present in a given specimen; one masking the other. Serially diluting the specimen will aid in distinguishing these patterns.

... Report all titers and patterns seen.

TITRATION

If a semi-quantitative titration is performed, the result should be reported as the reciprocal of the last dilution in which 1+ apple-green fluorescent intensity with a clearly discernible staining pattern is detected. When reading fourfold serial dilutions, endpoints can be extrapolated where necessary.

EXAMPLE OF ENDPOINT EXTRAPOLATION:
1:40 = 3+
1:160 = 2+
1:640 = +/-
1:2560 = Neg
The extrapolated endpoint is reported as 320.

Report all titers and patterns seen, extrapolating the titer where necessary.

EXAMPLE:
1:40 = 4+ Peripheral and 3+ Homogeneous
1:160 = 3+ Homogeneous
1:640 = 2+ Homogeneous and 3+ Speckled
1:2560 = 1+ Homogeneous and 2+ Speckled
1:10,240 = +/- Speckled

Report: 40 Peripheral, 2560 Homogeneous and 5120 Speckled.

TROUBLESHOOTING

Possible solutions to problems that may occur in immunofluorescent assays are discussed in an accompanying brochure entitled “TROUBLESHOOTING IN IMMUNOFLUORESCENCE”.

INTERPRETATION OF RESULTS

Four major staining patterns which may occur singly or in combinations have been described:

1. Peripheral (shaggy, rim, membranous)
2. Homogeneous (diffuse, solid)
3. Speckled (including ACA)
4. Nucleolar

Other patterns less frequently seen include Spindle and Ribosomal RNP.

CORRELATION OF ANA's WITH IFA STAINING PATTERNS

Antibody Against:	Staining Pattern:	Mitotic Cells:
nDNA	Peripheral & Homogeneous	Positive
Histones	Peripheral & Homogeneous	Positive
DNP	Homogeneous	Positive
Sm	Coarse Speckle	Negative
nRNP	Coarse Speckle	Negative
SS-A	Small Uniform Speckle	Negative
SS-B	Small Uniform Speckle	Negative
Scl-70	Fine Dense Speckle & Nucleolar	Positive
PCNA	Variable Speckle	Negative or Positive
ACA	Discrete Uniform Speckle	Positive Centromeres
PM/Scl	Nucleolar (homogeneous)	Negative
RNA Polymerase 1	Nucleolar (speckled)	Few Discrete Speckles
Fibrillarin	Nucleolar (clumpy)	Positive fibers
Spindle	Spindle Apparatus	
rRNP	Cytoplasmic	Negative

LIMITATIONS OF THE PROCEDURE

1. Antinuclear antibody test results should be used in conjunction with information available from clinical evaluation and other diagnostic information.
2. Two to ten percent of a normal adult population have antinuclear antibodies.[24]
3. Antinuclear antibodies are known to be age and sex related. With increasing age there is an increased incidence of ANA’s; therefore, a positive low titer result may be normal for certain individuals in the absence of other clinical signs and symptoms. Antinuclear antibodies are not usually found, however, in normal young individuals.
4. Some positive reactions have been reported in relatives of patients suffering from a connective tissue disease who may develop such a disease at a later time.[25]
5. Positive ANA results may be seen in a small percentage of patients with Infectious and/or Neoplastic diseases, and also in diseases of drug etiology.[26,27]
6. SLE patients undergoing steroid therapy or in remission may have a negative ANA.[11]
7. Test results of specimens from immunosuppressed patients and pregnant women may be difficult to interpret.

LIMITATIONS OF THE PROCEDURE (continued)

8. Positive test results may not be valid in persons who have received blood transfusions or various blood products within the past several months.
9. Cytoplasmic fluorescence in the majority of the cells may be due to the presence of antimitochondrial antibodies (AMA) often seen in primary biliary cirrhosis.[28]
10. Positive test results from cord blood or neonates should be interpreted with caution. The presence of antinuclear antibodies in cord blood is usually the result of passive transfer from mother to the fetus. A negative test, however, may be useful in excluding a possible autoimmune process.
11. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed or assay procedure used.[30]
12. If both the positive and negative control substrate cells are not visible when viewed using the fluorescence microscope, it may be necessary to replace or realign the light source and check the specific filters.
13. Cell culture substrate slides may exhibit nonspecific fluorescence due to contamination of antibodies or PBS rinse-wash solutions with bacteria or fungi. It is very important that personnel reading the staining results have experience in fluorescence microscopy.
14. In general titers of 1:40 and 1:80 are considered low titers, 1:160 and 1:320 are considered medium titers, and 1:640 and greater are considered high titers. It is recommended that each laboratory establish its own ranges.

EXPECTED VALUES

The following chart presents the incidence of antinuclear antibodies utilizing a HEp-2 cell ANA substrate in patient population studies performed at the Duke University Medical Center Division of Rheumatic and Genetic Disease laboratories over a two year period. This represents a study of over 9,000 control sera and over 4,500 abnormal sera.

Clinical Diagnosis	% Positive	Clinical Diagnosis	% Positive
Controls:		Vasculitides	20.0%
20-60 years	2.0%	Childhood SLE	64.0%
70-80 years	3.5%	JRA	
SLE	95.0%	Systemic	14.0%
RA	40.0%	Polyarticular	6.0%
MCTD	99.0%	Pauciarticular	
PSS (Diffuse)	85.0%	HLA B27 pos.	0.0%
PSS (CREST variant)	93.0%	HLA B27 neg.	26.0%
PM/DM	25.0%		

SPECIFIC PERFORMANCE CHARACTERISTICS

To investigate the relative specificity and sensitivity of the Bion ANA SUBSTRATE SLIDES, 120 specimens were compared qualitatively and semi-quantitatively with another commercially available IFA HEp-2 ANA substrate slide. All tables represent averaged results from two independent readers.[31]

The relative sensitivity and specificity are summarized in TABLE 1. The one specimen in which there was disagreement was a CDC Reference Serum having high levels of antibodies to SS-A.

TABLE 1

		BION Pos.	BION Neg.	BION Relative Sensitivity	BION Relative Specificity
Other	Pos.	71	0	100%	100%
	Neg.	1	48		

TABLE 2

Spec #	BION	Other	Spec #	BION	Other	Spec #	BION	Other
1	80 H	80 H	25	40 S	40 S	49	320 S	160 S
2	40 H	40 H	26	80 S	80 S	50	640 S	640 S
3	80 H	40 H	27	80 S	40 S	51	640 S	160 S
4	40 H	40 H	28	40 S	40 S	52	160 S	40 S
5	160 H	80 H	29	40 S	40 S	53	2560 S	320 S
6	320 H	160 H	30	80 S	40 S	54	1280 S	640 S
7	1280 H	640 H	31	2560 S	2560 S	55	640 S	640 S
8	5120 H	2560 H	32	10240 S	5120 S	56	2560 S	2560 S
9	5120 H	5120 H	33	2560 S	40 S	57	160 S	<40
10	640 H	320 H	34	640 S	640 S	58	640 S	320 S
11	10240 H	10240 H	35	2560 S	640 S	59	80 S	40 S
12	2560 H	1280 H	36	320 S	320 S	60	40 S	40 S
13	640 H	320 H	37	1280 S	1280 S	61	640 S	320 S
14	1280 H	640 H	38	10240 S	5120 S	62	640 C	640 C
15	640 H	320 H	39	5120 S	40 S	63	10240 C	5120 C
16	640 H	320 H	40	5120 S	640 S	64	320 C	320 C
17	1280 H	1280 H	41	10240 S	5120 S	65	640 C	320 C
18	1280 H	640 H	42	640 S	1280 S	66	1280 N	1280 N
19	640 H	320 H	43	10240 S	5120 S	67	640 N	320 N
20	80 H	40 H	44	160 S	160 S	68	320 N	160 N
21	1280 H	640 H	45	10240 S	10240 S	69	160 N	160 N
22	80 S	80 S	46	5120 S	5120 S	70	80 N	80 N
23	40 S	40 S	47	160 S	160 S	71	320 cR	640 cR
24	320 S	320 S	48	160 S	80 S	72	640S/160H	160 H

TABLE 2 represents the titers and patterns obtained with the 72 positive specimens in TABLE 1. H=Homogenous pattern, S=Speckled pattern, C=Centromere antibody, N=Nucleolar pattern, and cR=cRNP antibody (cytoplasmic pattern).

Interlot precision of the Bion ANA SUBSTRATE SLIDES was evaluated by testing eleven serum specimens (2 negative and 9 positive over a range of titers) on three successive days using three different lot numbers.[31] There was no more than a twofold difference (+/-) in titer between any of the comparison testings, which is within the confidence limits of this methodology.[30]

Intralot precision of the Bion ANA SUBSTRATE SLIDES was evaluated by running nine different types of antinuclear antibodies three times within one run using three different slides from the same lot.[31] Again, there was no more than a twofold difference in titer between any of the comparison testings.

Each laboratory should determine its own performance characteristics using all reagents assembled to perform the IFA test.

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MANUFACTURER

Bion Enterprises, Ltd.
455 State Street, Suite 100
Des Plaines, IL 60016 USA
Phone: +1-847-544-5044
Fax: +1-847-544-5051

EC REP

Qarad, Cipalstraat 3,
B-2440 Geel, Belgium