

ENGLISH



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AESKUSLIDES®
THE IFA PRODUCT LINE



INSTRUCTION MANUAL

AESKUSLIDES ANA HEp-2

Standard Ref.	Description	Tests
51.100.US	ANA HEp-2 (12 wells)	120

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1. INTENDED USE

AESKUSLIDES ANA HEp-2 is an indirect fluorescent antibody assay utilizing HEp-2 tissue culture cells as a substrate for the qualitative and/or semi-quantitative determination of antinuclear antibodies (ANA) in human serum. **AESKUSLIDES ANA HEp-2 Test System** is intended for use as aid in the diagnosis of systemic rheumatic diseases in conjunction with other clinical and laboratory findings.

2. CLINICAL APPLICATION

Anti-nuclear 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 auto antigens, a single mixture of auto antigens from a cell lysate, or a combination of the two. 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 time² and the method of choice for ANA screening and semi-quantitation.

The antigen of the **AESKUSLIDES ANA HEp-2** 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).¹⁶ **AESKUSLIDES ANA HEp-2** utilizes the indirect fluorescent antibody assay method first described by Weller and Coons,¹⁷ and applied to ANA detection by Friou,¹⁸ and Holman and Kunkel.¹⁹ 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 complex 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.



3. KIT PROCEDURE

Please refer to **Assay Procedure** listed in Common Instructions, Section 13 page 19 for detailed instructions. The following details shall be used for the ANA HEp-2 kits:

- Suggested Screening titer/dilution ratio: 1:40 (10µl serum + 390µl 1x Sample buffer). It is recommended that each laboratory establish its own screening dilution based on its own population and instrumentation used.
- Semi-quantitation:

The significance of a positive screening result should be confirmed by repeating the test with dilutions of the serum. Each laboratory should establish its own titering protocol; however, the following fourfold serial titration is suggested:

- a. Prepare a 1:40 dilution of each patient's serum by adding 10µl serum to 390µl of 1x Sample Buffer
- b. Add 300µl 1x Sample Buffer to tubes #2, #3, #4 and #5
- c. Using a 100µl pipette, transfer 100µl from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 100µl 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. This will give a fourfold titration with the following dilutions:

Tube #1 = 1:40

Tube #2 = 1:160

Tube #3 = 1:640

Tube #4 = 1:2560

Tube #5 = 1:10240

NOTE: The Positive and Negative Controls are intended to be used undiluted. However, if performing a semi-quantitative test, the Positive Control should be diluted as suggested above; assuming a dilution of 1:40 is already made in bottle.

4. QUALITY CONTROL

4.1 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 the **AESKUSLIDES** negative control serum as provided with the **AESKUSLIDES ANA HEp-2** test system, the cells should exhibit less than 1+ fluorescence and appear reddish-orange.

Positive Control

Using the positive ANA pattern control serum as provided with the **AESKUSLIDES ANA HEp-2** test system, the cells should exhibit a homogeneous staining pattern with a fluorescent intensity of 3+ to 4+.

Each control must demonstrate the expected 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 repeated 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.

4.2 Sensitivity Control

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

5. READING OF TEST RESULTS

5.1 Fluorescent Intensity Grading

Fluorescent intensity may be semi-quantitated by following the guidelines established by the Centers for Disease Control, Atlanta, Georgia.²⁰

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.

5.2 Negative

A serum dilution is considered negative for antinuclear antibodies if the cells exhibit less than 1+ fluorescence and lack a clearly discernible pattern. Cells will appear reddish-orange due to the Evans Blue counterstain.

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.²¹
- Intense non-nuclear staining may be observed in some sera containing Anti-Mitochondrial, Anti-Smooth Muscle or other cytoplasmic antibodies.



5.3 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.

5.4 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



6. INTERPRETATION

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

- Peripheral (shaggy, rim, membranous)
- Homogenous (diffuse, solid)
- Speckled (including ACA)
- Nucleolar

Other patterns less frequently seen include Spindle and Ribosomal RNP.

6.1 Peripheral

Smooth staining primarily around the outer region of the nucleus with weaker staining in the center. Not all cells within a well may appear peripheral; some may appear homogeneous. The chromosome region of mitotic cells will exhibit a bright positive staining pattern.

Note: A very thin distinct line around the nucleus is not a peripheral ANA, but a nuclear membrane antibody. It differs in that the chromosome region within the mitotic cells is negative.

Nuclear Antigens: nDNA or Histones

Disease Association: High titers to nDNA are suggestive of active SLE. Lower titers are suggestive of SLE or other connective tissue diseases.²²

6.2 Homogenous

Diffuse staining of the entire nucleus, with or without apparent masking of the nucleoli. Some specimens may exhibit moderately large irregular areas of more intense staining; or the pattern may appear granular, especially as the antibody reaches its endpoint. The chromosome region of mitotic cells will exhibit a bright positive staining pattern.

Nuclear Antigens: nDNA, DNP or Histones.

Disease Association: High titers are suggestive of SLE, while low titers may be found in SLE, and Rheumatoid Arthritis.⁵

- a. Antibodies to DNP have the same specificity as the LE Cell Factor.¹¹
- b. Antibodies to Histone alone have a high association with drug-induced lupus.¹¹

6.3 Speckled

Fluorescent aggregates throughout the nucleus which can be very fine to very coarse depending on the type of antibody present. More than one type of speckle may be seen in any one specimen. The chromosome region of mitotic cells is usually negative.

- a. Sm and nRNP antibodies usually present as a coarse speckle with the chromosome region of mitotic cells negative.
- b. SS-A/Ro and SS-B/La antibodies present as small uniform speckles in a uniform distribution with the chromosome region of mitotic cells negative.
- c. Fine dense speckles with positive staining of the nucleoli and chromosome region of mitotic cells may indicate a Scl-70 antibody.



- d. Varying fluorescence of fine to coarse speckles, in approximately 30-60% of cells with positive or negative staining of the mitotic cells, may represent a PCNA (Proliferating Cell Nuclear Antigen) antibody.

Nuclear Antigens: Sm, nRNP, SS-A/Ro, SS-B/La, Scl-70 (DNA-Topoisomerase 1) or PCNA (Cyclin).

Disease Association:

- a. Sm antibodies are highly specific for SLE and appear to be a “marker” for this disease.²³
- b. nRNP along with other types of ANA's have been found in SLE, Rheumatoid Arthritis (RA), and Progressive Systemic Sclerosis (Scleroderma). High levels of nRNP antibodies alone are characteristic of Mixed Connective Tissue Disease (MCTD).²³
- c. SS-A/Ro and SS-B/La antibodies are frequently present in patients with Sjogren's Syndrome without associated RA. Both may be found less frequently in patients with SLE. SS-A/Ro antibodies are found in a high percentage of infants with congenital heart block, neonatal lupus or both.²⁴
- d. Scl-70 antibodies appear to be a “marker” for Progressive Systemic Sclerosis (Scleroderma).²³
- e. Anti-PCNA has been found in a small percentage of patients with SLE.¹⁵

6.4 Anti-Centromere Antibody (ACA)

Discrete uniform speckles throughout the nucleus, the number of which corresponds to a multiple of the normal chromosome number. The staining pattern of the mitotic cells will follow that of the chromosomes, with pairs of dots arranging themselves in an equatorial plane during metaphase and then moving towards their respective centrosomes during anaphase.

Note: An antibody closely resembling the ACA is the Pseudo Centromere (or NSp-1) Antibody. This antibody can be differentiated from the ACA in that the chromosome region of mitotic cells does not stain.²⁵

Nuclear Antigen: Centromere/kinetochore portion of the chromosome.

Disease Association: This antibody is considered a “marker” for the CREST variant of Progressive Systemic Sclerosis.²⁶ It is infrequently found in diffuse Scleroderma and Raynaud's disease.²⁷

6.5 Nucleolar

Fluorescent staining of the nucleoli within the nucleus, sharply separated from the unstained nucleoplasm. The nucleolar fluorescence may be homogeneous, speckled or clumpy and is frequently accompanied by a speckled pattern.

Nuclear Antigens: PM/Scl (PM-1), RNA Polymerase 1 or Fibrillarin (U3).²⁸

Disease Association: High titers are highly specific for Progressive Systemic Sclerosis (PSS), with lower titers found in PSS, SLE, Sjogren's Syndrome and Raynaud's disease.^{29,30}



6.6 Anti-Spindle Antibody

A network of threads connecting the centrosomes to each other in the mitotic cells.

Nuclear Antigen: Spindle apparatus in cells undergoing mitosis.

Disease Association: There may be some association with Carpal Tunnel Syndrome.¹⁴

6.7 Cytoplasmic Fluorescence

a. Ribosomal RNP (rRNP)

Diffuse granular fluorescence throughout the cytoplasm. May be confirmed by placing the specimen on a stomach section, where it will stain the chief cells. Frequently accompanied by a nucleolar pattern.

Nuclear Antigen: Ribosomal or cytoplasmic RNP.

Disease Association: This antibody has been found in a small percentage of cases of SLE.³¹

b. Non-ANA Cytoplasmic Fluorescence

The two most common autoantibodies are:

1. Anti-Mitochondrial Antibody (AMA) – discrete speckles throughout the cytoplasm in a fibrous network, with denser speckling in the perinuclear region. May be confirmed by testing on appropriate tissue substrate.
2. Anti-Smooth Muscle Antibody (ASMA) – fluorescent strands in the cytoplasm in a spidery network, with fibrils extending from the cell membrane. May be confirmed by testing on appropriate tissue substrate.

6.8 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

Specific pattern controls are available from the Center for Disease Control (limited availability) and other manufacturers.

7. LIMITATIONS OF PROCEDURE

1. ANA serological test results should be used in conjunction with information available from the clinical evaluation and other diagnostic information.
2. Two to ten percent of a normal adult population may have low titer antinuclear antibodies.³²
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.³³
5. 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.
6. 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.^{22,34}
7. SLE patients undergoing steroid therapy or in remission may have a negative ANA.¹¹
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. Test results on specimens from immunosuppressed patients and pregnant women may be difficult to interpret.
10. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed or assay procedure used.³⁵
11. 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.
12. Cell culture substrate slides may exhibit nonspecific fluorescence due to contamination of serum or conjugate, or PBS rinse-wash solutions with bacteria or fungi.
13. 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 reference ranges based on its own population and instrumentation used.
15. Samples containing interfering substances such as hemoglobin due to hemolysis of sample, triglycerides, bilirubin, high titers of heterophiles and/or rheumatoid factor should be interpreted with caution as a low positive sample may lose positivity or a negative sample may become weakly positive in samples containing one or more of these substances.

8. EXPECTED VALUE

The following chart represents 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
Controls:	
20-60 years	2.0%
70-80 years	3.5%
SLE	95.0%
RA	40.0%

Clinical Diagnosis	% Positive
Vasculitides	20.0%
Childhood SLE	64.0%
JRA:	
Systemic	14.0%
Polyarticular	6.0%

9. SPECIFIC PERFORMANCE CHARACTERISTICS

One hundred and thirty eight frozen samples were tested on two lots of reagents from other company and on two lots AESKU slides. The 118 clinical samples had been stored for several years and represented patients being evaluated for rheumatic disease. Additionally included were twenty health fair samples. The health fair samples represented a population with no known clinical symptoms of rheumatic disease. Comparison of two kits was to demonstrate comparability between two independent companies' ANA HEp-2 systems, including pattern consistency.

Table 1: Confidence Intervals Combined Data Set:

		Predicate		
		Positive	Negative	Total
AESKUSLIDES ANA HEp-2	Positive	116	0	116
	Negative	0	22	22
	Total	116	22	138

Positive % Agreement = $116/116 = 100\%$ (95th% CI: 96.8 - 100%)

Negative % Agreement = $22/22 = 100\%$ (95th% CI: 85.1 - 100%)

Table 2: Combined Data Set Pattern Comparison Predicate vs Aesku

Pattern	n	Predicate	AESKUSLIDES
Homogenous	32	32	32
Speckled	82	82	82
Nucleolar	20	20	20
Centromere	6	6	6
Peripheral	3	3	3
Nuclear Membrane	1	1	1

N =144

Clinical samples represented a full spectrum of autoimmune diseases, including SLE, Scleroderma, Scleroderma -CREST, Sjogren's, MCTD, Anti-phospholipid Syndrome, Rheumatoid Arthritis, Polymyositis/dermatomyositis, drug induced SLE, etc.

Table 3: Analytical sensitivity and linearity of CDC reference panel was equivalent between and AESKU kits and other company's device.

Sample I.D.	Dilution	Other Lot #1 Reader #1	Other Lot #1 Reader #2	AESKU Lot #1 Reader #1	AESKU Lot #1 Reader #2	Reactivity/ Specificity	Pattern
CDC #1	40	4	4	4	4	Anti-native DNA	Homogeneous
"	80	4	4	4	4	"	"
"	160	3-4	3	4	3-4	"	"
"	320	3	2-3	3	3	"	"
"	640	2	2	2-3	2-3	"	"
"	1280	1	1	2	2	"	"
"	2560	±	±	1	1	"	"
"	5120	-	-	±	±	"	"
"	10240	-	-	-	-	"	"
CDC #4	40	4	4	4	4	Anti-nuclear RNP	Speckled
"	80	4	4	4	4	(U-1 RNP)	"
"	160	4	3-4	4	3-4	"	"
"	320	3-4	3-4	3-4	3-4	"	"
"	640	2-3	2-3	2-3	3	"	"
"	1280	1-2	2	2	2-3	"	"
"	2560	±	1	1-2	1-2	"	"
"	5120	-	±	±	±	"	"
"	10240	-	-	-	-	"	"
CDC #6	40	3-4	4	3-4	4	Anti-Fibrillarin	Nucleolar
"	80	2-3	3	3	3	(U3-RNP)	"
"	160	2	2	2	2	"	"
"	320	±	±	±	1	"	"
"	640	-	-	-	±	"	"
"	1280	-	-	-	-	"	"
"	2560	-	-	-	-	"	"
"	5120	-	-	-	-	"	"
"	10240	-	-	-	-	"	"



Sample I.D.	Dilution	Other Lot #1 Reader #1	Other Lot #1 Reader #2	AESKU Lot #1 Reader #1	AESKU Lot #1 Reader #2	Reactivity/ Specificity	Pattern
CDC #7	40	3	3	3	3-4	Anti-SS-A/Ro	Speckled
"	80	2	2	2	2	"	"
"	160	±	±	±	1	"	"
"	320	-	-	-	-	"	"
"	640	-	-	-	-	"	"
"	1280	-	-	-	-	"	"
"	2560	-	-	-	-	"	"
"	5120	-	-	-	-	"	"
"	10240	-	-	-	-	"	"
CDC #2	40	4	4	4	4	Anti SS-B/La	Speckled
"	80	3-4	3-4	3-4	3-4	"	"
"	160	3	3	3	2-3	"	"
"	320	2-3	2-3	2-3	2	"	"
"	640	2	2	1-2	1-2	"	"
"	1280	1-2	1-2	1	1	"	"
"	2560	1	1	±	±	"	"
"	5120	-	-	-	-	"	"
"	10240	-	-	-	-	"	"
CDC #3	40	4	4	4	4	Speckled	S
"	80	3	4	3-4	4	(U1-RNP, SSA/Ro, SSB/La)	"
"	160	3	3	3	3	"	"
"	320	2-3	2-3	3	2-3	"	"
"	640	2	2	2	2	"	"
"	1280	1	1	1	1	"	"
"	2560	±	±	±	±	"	"
"	5120	-	-	-	-	"	"
"	10240	-	-	-	-	"	"
CDC #5	40	4	4	4	4	anti-Sm	S
"	80	4	4	4	4	"	"
"	160	4	4	4	4	"	"
"	320	3-4	3	3-4	3-4	"	"
"	640	3	2-3	3-4	3	"	"
"	1280	2-3	2-3	3	2-3	"	"
"	2560	2-3	2	2-3	2	"	"
"	5120	1-2	1-2	2	1-2	"	"
"	10240	1	1	1	1	"	"



Sample I.D.	Dilution	Other Lot #1 Reader #1	Other Lot #1 Reader #2	AESKU Lot #1 Reader #1	AESKU Lot #1 Reader #2	Reactivity/ Specificity	Pattern
CDC #8	40	4	4	4	4	anti-Centromere	ACA
"	80	4	3-4	4	3-4	"	"
"	160	3-4	3	4	3	"	"
"	640	2	2	2	2	"	"
"	1280	1	1	1	1	"	"
"	2560	±	±	±	±	"	"
"	5120	-	-	-	-	"	"
"	10240	-	-	-	-	"	"
CDC #9	40	4	4	4	4	anti-Scl-70	S
"	80	3	3-4	3-4	3-4	"	"
"	160	3	3	3	3	"	"
"	320	2-3	2-3	2-3	2	"	"
"	640	2	2	2	1-2	"	"
"	1280	1	±	±	±	"	"
"	2560	±	-	-	-	"	"
"	5120	-	-	-	-	"	"
"	10240	-	-	-	-	"	"
CDC #10	40	2-3	2-3	2-3	2-3	anti-Jo-1	Cyto. Speck.
"	80	2	2	2	2	"	"
"	160	±	±	±	±	"	"
"	320	-	-	-	-	"	"
"	640	-	-	-	-	"	"
"	1280	-	-	-	-	"	"
"	2560	-	-	-	-	"	"
"	5120	-	-	-	-	"	"
"	10240	-	-	-	-	"	"
CDC #11	40	4	4	4	4	anti-PM/SCL (PM-1)	N
"	80	3-4	4	3-4	4	"	"
"	160	3-4	4	3	3	"	"
"	320	3	3	2-3	2-3	"	"
"	640	2-3	2	2	2	"	"
"	1280	1-2	1-2	1-2	1	"	"
"	2560	±	±	±	±	"	"
"	5120	-	-	-	-	"	"
"	10240	-	-	-	-	"	"



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ANA HEp-2

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12. STANDARD KIT CONTENTS

12.1 STANDARD KITS

		SLIDES (10x in each kit)			CONJUGATE (4 ml vial)			POSITIVE CONTROL (1x 0.5ml vial in each kit)	
Kit Ref.	Kit Description	Ref.	Wells	Coated with	Quantity	Ref.	Description	Ref.	Description
51.100.US	ANA HEp-2 (12 wells)	s51.100	12	ANA HEp-2 Cells	1x	C51.100	Containing: BSA, Fluorescein (FITC) labelled goat anti-human IgG (H+L Chain), Evans Blue. Capped blue: slightly blue colored solution.	PC51.100	ANA pattern control (Homogeneous) Capped red: colorless solution. Containing: Human serum (diluted), Sodium Azide <0.1% (preservative)

NOTE: The contents of the remaining components of the kits i.e. Common reagents (buffers, Neg. Ctrl, Mounting Medium etc.) are described below in section 13. COMMON REAGENTS CONTENTS as they are identical in all of the AESKUSLIDES references.



AESKUSLIDES COMMON MANUAL

The following instructions are common throughout our entire **AESKUSLIDES** product portfolio. For specific details regarding each product reference please refer to the specific reference manual also included in this kit.

13. COMMON REAGENTS CONTENTS

a. Common Reagents

The following reagents are common for the entire **AESKUSLIDES** product line. Quantities are per kit:

Ref.	Reagent	Quantity / Volume		Description	Ready to use
NCIFA	Negative Control	1x	0.5ml	Capped green: colourless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	YES
MMIFA	Mounting Medium	1x	8ml	Validated for use with the HELMED® Capped white: colourless solution Containing: PBS, Glycerin.	YES
WBIFA	Washbuffer (10x)	1x	100ml	Capped white: colourless solution Dilute the concentrated buffer 1:10 in distilled water (e.g.: 100 ml + 900 ml). Containing: BSA, PBS, sodium azide (preservative).	NO
SBIFA	Samplebuffer (1x)	1x	100ml	Capped white: colourless solution for the dilution of patient sera Containing: BSA, PBS, sodium azide (preservative).	YES

b. Materials required but not provided

1. Distilled water – CAP Type one or equivalent
2. Disposable test tubes and rack
3. One liter screw capped container
4. One liter volumetric flask or one liter graduated cylinder
5. Calibrated pipettes to deliver 10µl to 100µl, with disposable pipette tips
6. Disposable serological pipettes
7. Timer
8. Moist chambers
9. Plastic squeeze wash bottle
10. Coplin jars or staining dishes with slide racks
11. Permanent black felt tip marking pen
12. Coverslips, No.1, 24 x 60 mm
13. Fluorescence microscope equipped with a mercury or tungsten-halogen light source, a 390-490nm excitation filter and 515-520nm barrier filter, and optics to give a total magnification of 400X. The excitation wavelength of FITC is 490nm and the emission wavelength is 520nm

In case that the product information, including the labeling, is defective or incorrect please contact the manufacturer or the distributor of the test kit.



14. STORAGE AND SHELF LIFE

Store all reagents at 2°C-8°C/35-46°F, protected from intense light. The expiration date of each component is indicated on the respective label. Do not use reagents beyond the expiration date.

Store all reagents and the slides at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 week at 2-8°C/35-46°F.

Reagents and the slides shall be used within the expiry date indicated on each component, only.

15. PRECAUTIONS OF USE

15.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Refer to the following for maximum safety:

Recommendations and precautions

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.

Sera used to prepare positive and negative controls have been tested by an FDA approved method and found to be negative (were not repeatedly reactive) for the presence of Hepatitis B surface Antigen (HBsAg) and antibodies to Hepatitis C (HCV Ab) and HIV 1 & 2. However, because no test method can offer complete assurance of the absence to these or other infectious agents, these reagents 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.

The preservatives used in conjugates, controls and sample buffer and wash buffer are toxic if ingested. Azides may react with copper or lead plumbing to form explosive metal azides. When disposing, flush drains with water to minimize buildup of azide and metal compounds.

Care should be taken to avoid splashing and generation of aerosols.

15.2 General directions for use

1. Do not pipette by mouth. Do not smoke, eat or drink when manipulating the kit.
2. Do not mix or substitute reagents from different lot numbers. This may lead to variations in the results.
3. Reusable glassware must be washed and thoroughly rinsed free of detergents.
4. Always pipette all solutions with new sterile pipetting tips.
5. Never expose components to higher temperature than 37°C / 98.6°F.
6. Never let the slide wells dry out during the whole procedure.



15.3 NORMAL VALUES

Each laboratory should establish their own normal control ranges dependent on their own techniques, controls, equipment and patient population according to their established procedures.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated.

In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated. The following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods. If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without a justified cause please contact our local representative.

16. SAMPLE COLLECTION, HANDLING AND STORAGE

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. Previously frozen specimens after thawing should be thoroughly mixed prior testing. It is recommended that sera freeze thawed no more than one time. Do not store in self-defrosting freezer.

Lipemic, icteric, hemolyzed or microbial contaminated specimen may cause interference. 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 a significant change in antibody titer, the two specimens must be tested simultaneously in the same assay.

17. ASSAY PROCEDURE

a. Preparation prior to pipetting

Allow all components to reach room temperature (20 - 26°C / 64 - 78.8°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

1. Preparation of the Wash Buffer: **Dilute the concentrated buffer 1:10 with distilled water and let reconstitute for 15 minutes at 37°C / 98.6°F.**
2. Dilution of samples: Dilute patient sera (for screening titer and semi-quantitation refer to **Kit Procedure** section above) according to the product reference that you are using with 1x Sample buffer.
3. Controls are ready to use.



4. Prepare a protocol: Data interpretation sheets are available in the **Kit Procedure** section according to the product reference that you are using.

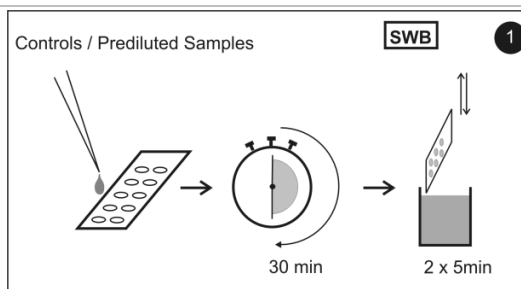
b. Test Procedure

No.	Step description
1.	Remove required slide(s) from pouch(es) and mark them. Do not touch the wells. Do not allow the slides to dry out. Identify each slide using a permanent black marking pen. <u>Note:</u> Deviation from the defined test procedure, such as incubation times or temperatures, may give erroneous results.
2.	First incubation: Pipette an adequate volume of each diluted serum and controls (ready to use) into the appropriate wells; avoid direct contact of pipette with slide surface. Make sure that each well is completely covered with a corresponding serum. It is important to use as much test material as necessary to cover the well completely. But avoid a running between the wells because this may cause incorrect results. Incubate slide(s) 30 minutes at room temperature in a moist incubator tray.
3.	Washing: After incubation remove slides from incubator tray and rinse briefly with Wash buffer using a squeeze wash bottle. Do not squirt buffer directly on the wells. NOTE: To prevent cross contamination tilt slide first toward wells 1-6 and, running Wash buffer stream along the midline of the slide, allow the Wash buffer to run off the top edge of the slide. Then tilt the slide toward wells 7-12, and repeat this procedure, allowing the Wash buffer to run off the bottom edge of the slide. Wash slide(s) 10 minutes with Wash buffer in a slide staining dish. For optimal results to change the buffer solution after 5 minutes, one time. Lift slide(s) from staining dish and carefully remove excess washing buffer. NOTE: It is important that slide wells do not dry out during the procedure or damage to the substrate may occur. Please do not blot or dry the slide in any manner or allow slide to sit without fluorescent antibody reagent for longer than some seconds.
4.	Second incubation: After the washing procedure return slide immediately to incubator tray and cover each well with an adequate volume of FITC-conjugate and make sure that the well is covered completely. Incubate slide(s) 30 minutes at room temperature. Protect from excessive light.
5.	Washing: After incubation remove slides from incubator tray and rinse briefly with Wash buffer using a squeeze wash bottle. Do not squirt buffer directly on the wells. Wash slide(s) 10 minutes with wash buffer in a slide staining dish. For optimal results, change the buffer solution after 5 minutes, one time.
6.	Mounting Medium: Remove slides one at a time from wash buffer, shake off excess wash buffer 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.

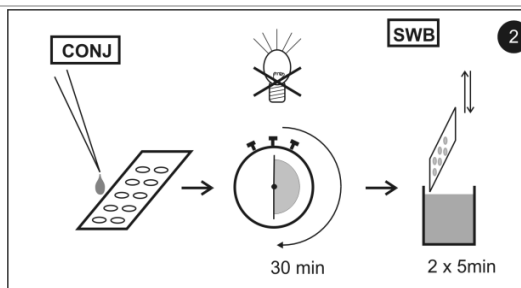
7. **Reading:** Examine stained slides as soon as possible using a properly equipped fluorescence microscope. It is recommended that slides be examined on the same day they are stained. If any delay is anticipated, store slides in the refrigerator (2-8°C) away from direct light and read the following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

c. Workflow

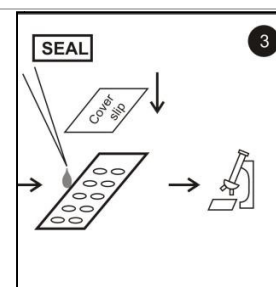
1. Pipette each diluted serum and controls into the appropriate wells.
2. Place a small volume of deionized or distilled water in an incubator tray and place slide(s) on supports in the incubator tray.
3. Incubate slide(s) 30 minutes at room temperature (20-26°C/64-78.8°F).
4. Remove slides from incubator tray and rinse briefly with Wash buffer using a pipette. Wash slides 5 minutes for two times in a staining dish.



5. Cover each well completely with an adequate volume of FITC - conjugate inside the incubator tray.
6. Incubate slide(s) 30 minutes at room temperature (20-26°C/64-78.8°F) in the dark.
7. Remove slides from incubator tray and rinse briefly with washing buffer using a pipette. Wash slides 5 minutes for two times in a staining dish.



8. Add mounting medium along midline of each slide.
9. Read slide(s) immediately at 400 - 800x total magnification with a fluorescent microscope (490nm excitation filter, 510nm barrier filter).





18. TROUBLESHOOTING

ERROR	POSSIBLE CAUSES	SOLUTION
Low cell density	Cell lysis following prolonged contact with deionized water Buffer squirted directly on the substrate in the well	Follow the recommended wash procedure
	Proteolytic enzymes have attacked the substrate	Inactivate serum
Uneven fluorescence	Serum dried in the well, fluorescence stronger at the edge	Always incubate in a humid environment
	Serum does not cover the test well	Apply an adequate volume of test material
	Cross-reaction between the wells	Avoid running between the wells in the first incubation
	Marking the slide with a wax pencil produces a film on the slide	Use a standard (non-wax) pencil
	Microscope incorrectly adjusted	Check the adjustment of the UV-lamp
Diffuse picture	Slide incubated in refrigerator without cover	Seal slide with nail polish or paraffin wax
	I.F. Microscope is dirty. Possible scratches on the lens	Clean the microscope according to its instructions
Little or no fluorescence	Conjugate and slides thawed and refrozen	Conjugate and slides stored at 2°C-8°C/ 35 -46°F.
	Controls diluted	Check instructions, use ready to use kit controls
	Bacterial contamination of the sera or conjugate Microscope not adjusted - pH-value of Washing buffer too low (pH value 7.4 ± 0.2)	Check conditions
	- FITC conjugate exposed to light	- Store conjugate protected from the light
Background fluorescence	- Incorrectly washed - Slide dried out - Lipemic, hemolytic sera - Microscope error	- Check the washing instructions - Do not allow the slide to dry out - Use only fresh sera - Check correct filter / objective



AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS

Doc.: **AESKUSLIDES-COMMON INSTRUCTIONS**

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IVD	- Diagnosi in vitro - Pour diagnostic in vitro - In Vitro Diagnostikum - Para uso Diagnóstico in vitro	ro diagnostic use - Para uso diagnóstico in vitro - In Vitro Διαγνωστικό μέσο
REF	* Numero d'ordine * Référence Catalogue * Bestellnummer * Número de catálogo	* Catalogue number * Numéro de catálogo * Αριθμός παραγγελίας
LOT	* Descrizione lotto * Lot * Chargen Bezeichnung * Lote	* Lot * Lote * Χαρακτηρισμός παρτίδας
CE	* Conformità europea * Déclaration CE de Conformité * Europäische Konformität * Declaração CE de Conformidade	* EC Declaration of Conformity * Declaración CE de Conformidad * Ευρωπαϊκή συμφωνία
	* Rispettare le istruzioni per l'uso * Voir les instructions d'utilisation * Gebrauchsanweisung beachten * Ver as instruções de uso	* See instructions for use * Ver las instrucciones de uso * Λάβετε υπόψη τις οδηγίες χρήσης
	* Da utilizzarsi entro * Utilise avant le * Verwendbar bis * Utilizar antes de	* Use by * Utilizar antes de * Χρήση μέχρι
	* Conservare a 2-8 °C * Conserver à 2-8 °C * Lagerung bei 2-8 °C * Conservar entre 2-8 °C	* Store at 2-8 °C (35-46 °F) * Conservar a 2-8 °C * Φυλάσσεται στους 2-8 °C
	* Prodotto da * Fabriqué par * Hergestellt von * Fabricado por	* Manufactured by * Fabricado por * Κατασκευάζεται από
DYE	* Colorante Blue-Evans * coloration au Bleu Evans * Evans-Blue Färbelösung * Evans Blue	* Evans-Blue Dye * Colorante Azul de Evans * Evans Blue
CONTROL +	* Controllo positivo * Contrôle Positif * Positiv Kontrolle * Controllo positivo	* Positive Control * Control Positivo * Θετικός ορός ελέγχου
CONTROL -	* Controllo negativo * Contrôle Négatif * Negativ Kontrolle * Controllo negativo	* Negative Control * Control Negativo * Αρνητικός ορός ελέγχου
SEAL	* Mezzi di montaggio * milieu de montage * Mounting Medium * Meio de montagem	* Mounting media * Medio de montaje * Μέσο μονιμοποίησης
CONJ	* Coniugato * Conjugé * Konjugat * Conjugado	* Conjugate * Conjugado * Σύζευγμα
	* Vetrino per microscopio * lame de microscope * Objektträger * Lâmina	* Microscope slide * Portaobjetos * Αντικειμενοφόρο πλακίδιο
WASHB 10x	* Tampone di lavaggio * Tampon de Lavage * Waschpuffer * Solución de lavado	* Wash Buffer * Solução de lavagem * Ρυθμιστικό διάλυμα πλύσης
SB 1x	* Tampone di campione * Tampon de Echantillons * Probenpuffer * Solución de Muestras	* Sample Buffer * Solução de Muestras * Ρυθμιστικό διάλυμα δειγμάτων
	* XX determinazioni * XX tests * XX Bestimmungen * XX Testes	* XX tests * XX pruebas * XX προσδιορισμοί