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AESKUSLIDES®

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INSTRUCTION MANUAL

***AESKUSLIDES®* ANA HEp-2**

Ref 51.1xx

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INSTRUCTION MANUAL

ANA HEp-2

Standard Ref.	Description	Tests
51.100	**ANA HEp-2** (12 wells)	120
51.101	**ANA HEp-2** (12 wells)	120
51.100.Bulk5	**ANA HEp-2** (12 wells) Bulk Kit	600
51.101.Bulk5	**ANA HEp-2** (12 wells) Bulk Kit	600
51.180	**ANA HEp-2** (18 wells)	180
51.181	**ANA HEp-2** (18 wells)	180
51.180.Bulk5	**ANA HEp-2** (18 wells) Bulk Kit	900
51.181.Bulk5	**ANA HEp-2** (18 wells) Bulk Kit	900

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ANA HEp-2

1. INTENDED USE

***AESKUSLIDES*® ANA HEp-2** is an indirect immunofluorescence assay to detect nuclear and/or cytoplasmic autoantibodies in human serum.

The assay is a tool in the differential diagnosis of systemic rheumatic diseases like systemic lupus erythematosus (SLE), mixed connective tissue diseases (MCTD), scleroderma, Sjögren's syndrome (SS), polymyositis and dermatomyositis.

2. CLINICAL APPLICATION

Anti-nuclear antibodies (ANA) directed against various nuclear and cytoplasmic antigens occur in high frequency in systemic rheumatic diseases and thus are an important tool for the differential diagnosis. For instance, SS-A (Ro) and SS-B (La) antibodies are associated with Sjögren's syndrome (SS), and anti-dsDNA (anti-nDNA), anti-SM, anti-histone, and anti-nucleosomes are associated with SLE (systemic lupus erythematosus); anti-RNP antibodies with mixed connective tissue diseases (MCTD) and SLE, anti-Scl-70 antibodies with scleroderma (progressive systemic sclerosis [PSS]), anti-Jo-1 antibodies with polymyositis and dermatomyositis and anti-centromere antibodies with CREST syndrome. For detailed information, see Section 4 below.

Indirect immunofluorescence test (IFT) on eukaryotic cells, e.g., HEp2, has been the established method for detecting ANAs. Although the IFT is a sensitive test, it is laborious when testing large numbers of patient samples and is subject to errors from human interpretation and variability in fluorescent microscope. Single antibody specificities must be determined by more specific testing using, for example, ELISAs or Immunoblots, employing the specific target antigens for a simple and reliable differentiation of ANAs.

Antigen Characterization: HEp-2 cells

Cross–reactivity: Cross-reactivities are unknown.

The detection of antibodies is based on the principle of indirect immunofluorescence assay (IIFA). Glass microscope slides are coated with tissue sections or cells (HEp-2 cells (ANA), Granulocytes (ANCA) or *Crithidia luciliae* (nDNA)). If the patient`s serum contains specific antibodies, they will bind during the first incubation. After removing unbound material by washing steps, bound antibodies are detected by fluorescein-conjugated anti-human immunoglobulins during the second incubation. A specific green fluorescent staining of antigen-antibody-complex can be visualized with a fluorescent microscope.

3. KIT PROCEDURE

For detailed instructions, please refer to the Assay Procedure in Common Instructions, Section 12. The following details shall be used for the ANA HEp-2 kits:

- Counter staining time: 30 to 90 seconds
- Recommended Screening titer:
  - 1:80 for adult samples
  - 1:40 for pediatric samples

4. INTERPRETATION

HEp-2 is a human epithelium cell cultivated from the tissue of a patient suffering from carcinoma of the larynx. The substrate offers homogenous cell growth and no extracellular excipient. For the evaluation of samples, the mitotic cells are of great importance as nuclear patterns may be defined more exactly.

The appropriate end titer is that in which the patient serum shows a simple positive fluorescence.

The evaluation should always be performed with the positive and negative control. Weak fluorescence of the cell nuclei with titers between 1:40 (children) and 1:80 (adults) or vagueness with respect to the clinical results should be checked via the controls. In such a case, the samples should be collected approximately every 6 weeks and tested similarly.[1]

4.1. Nuclear membrane patterns

4.1.1. Smooth nuclear membrane pattern

A smooth homogenous ring-like fluorescence of the nuclear membrane in interphase cells. Some samples with strong antibodies may give an impression of whole nuclear staining. A similar pattern is seen in telophase cells. In metaphase cells the fluorescence is diffusely localized in the cytoplasm, and chromosomal material is unstained.

4.1.2. Punctate nuclear membrane pattern

A discontinuous punctate fluorescence along the nuclear membrane. On focusing through the nucleus, the punctate staining can be seen on the surface of the entire nucleus.

A similar pattern is seen in telophase. In metaphase, the fluorescence is diffusely localized throughout the cytoplasm. Some samples with strong antibodies may give an impression of whole nuclear staining.

[1] Wiik A. Scand J Rheumatol 2005; 34:260-8.

4.2. Nuclear patterns

4.2.1. Homogeneous nuclear pattern

A uniform diffuse fluorescence covering the entire nucleoplasm sometimes accentuated in the nuclear periphery, can be associated with different antibodies, mainly directed to chromosomal components (DNA, histones). In some cases, a more intense staining of the inner edge of the nucleus (nuclear rim) can be seen.

Some samples may show an additional appearance of peripheral nucleolar staining. Nucleolar staining is variable. In metaphase, a homogeneous or peripheral chromatin staining is seen.

Two slightly different staining patterns may be distinguished:

- Homogeneous nuclear pattern with homogeneous nucleolar staining.
- Homogeneous nuclear pattern with negative nucleoli.

4.2.2. Speckled nuclear pattern

There are many different speckled nuclear patterns, but they can be difficult to distinguish. Seven patterns can be distinguished with reasonable accuracy. In six of these patterns, the metaphase chromosomes are negative, and in the centromere pattern, they are positive.

4.2.3. Large speckled nuclear pattern (nuclear matrix)

Variable large speckles throughout the nucleoplasm, earlier named “nuclear matrix” staining, most likely caused by staining of interchromatin granules. Nucleoli are negative. Metaphase and telophase cytoplasm show diffusely localized speckles and negative chromatin plates.

4.2.4. Coarse speckled nuclear pattern

Densely distributed, variously sized speckles, generally associated with larger speckles, throughout the nucleoplasm of interphase cells. Nucleoli are negative. Metaphase and telophase cytoplasm contain speckles with condensation around the chromatin plate which is negative. This pattern is most associated with staining of spliceosomes.

4.2.5. Fine speckled nuclear pattern

Fine speckled staining in a uniform distribution is sometimes very dense, so an almost homogeneous pattern is obtained. Nucleoli may be positive (especially with anti-SSB/La antibodies) or negative. The cytoplasm of metaphase cells shows fine speckles and condensation around the chromatin plates, which are negative themselves. Nucleoli of telophase cells may be positive, sometimes being stained stronger than interphase cells.

4.2.6. Grainy Scl-70-like/Topo I-like pattern

A uniform fine grainy staining of the nucleoplasm and the chromosomal areas of metaphase and telophase cells, often with accentuated positive nucleoli. The staining may look homogeneous at lower magnifications. This staining is not only seen with anti-Scl-70 positive sera.

4.2.7. Pleomorphic speckled nuclear pattern

Nuclei of proliferating (S-Phase) cells show a variety of speckles, from fine to very coarse irregular speckles of the nucleoplasm (10-50 % of the cells, depending on the cell preparation). In some cells, the nucleoli are stained as well. Resting (G-Phase) and metaphase cells are negative. This pattern is also known as “anti-PCNA” pattern (anti-proliferating cell nuclear antigen), which can be seen in association with other patterns.

4.2.8. Centromere pattern

Rather, uniform discrete speckles located throughout the entire nucleus (40-60 speckles per nucleus). Telophase and metaphase cells always show these speckles in the condensed chromosomal material. This pattern is produced by antibodies to the kinetochores of chromosomes, which may recognize several centromere proteins (CENP-A, B, C, D, E).

4.2.9. Multiple nuclear dots (NSp-1)

5-10 dots per nucleus staining nuclear substructures, often named PML bodies (because of their pronounced expression in Promyelocytic Leukemia), chromosomes of metaphase and telophase cells are negative.

4.2.10. Few nuclear dots (Coiled body)

2-6 dots per nucleus located in the nucleoplasm, often near nucleoli. No staining of metaphase or telophase chromatin plates.

4.3. Nucleolar patterns

4.3.1. Homogeneous nucleolar pattern

Shows a diffuse uniform fluorescence of the entire nucleoli and no staining of chromosomes.

4.3.2. Clumpy nucleolar pattern

Brightly clustered larger granules corresponding to decoration of the fibrillar centers of the nucleoli as well as the coiled bodies. In mitotic cells, the metaphase and telophase plate appear to have a fluorescent irregular “fan-like” edge.

4.3.3. Punctate nucleolar pattern

Small discrete grains mainly in the center of the nucleoli. In metaphase cells, 2-5 discrete speckles are seen within the chromatin body, corresponding to the nucleolar organizing regions.

4.4. Spindle apparatus patterns

4.4.1. Centriole (centrosome) spindle pattern

The metaphase spindle displays a discrete fluorescent spot at each spindle pole lying perpendicular to one another. One or two bright spots closely adjacent to the nucleus are seen in the cytoplasm of interphase cells.

4.4.2. Spindle pole pattern (NuMA)

Staining only of the pole area of the spindle in metaphase cells (triangular or banana-shaped pattern). Spindle fibers are negative, with a more linear pattern in telophase cells. A fine-speckled nuclear pattern with negative nucleoli is mostly seen in resting and telophase cells, but these may also be negative.

4.4.3. Spindle fiber pattern

Staining of the whole spindle fiber apparatus from pole to the chromatin plate in metaphase cells. No staining of chromosomes in resting or dividing cells.

4.4.4. Midbody pattern (MSA-2)

In prophase and metaphase cells, the fluorescence is localized in the chromosomal region as fine streaks perpendicular to the edge of the metaphase plate. In telophase, the staining is restricted to the cleavage furrow and the narrow connecting midbody between cells, completing cytokinesis. S and G2 phase cells are stained with discrete or patchy nuclear speckles. The pattern is due to antibodies to the MSA-2 antigen (Mitotic Spindle Antigen 2).

4.4.5. CENP-F pattern (MSA-3)

The most characteristic feature is seen in metaphase cells, where the pattern is constituted by two sets of dense large granules surrounding the chromosomal metaphase plate as a grip, often looking like a zipper. These granules are part of the centromere. The surrounding cytoplasm is diffusely stained. In prophase cells, a dense punctate decoration of the chromosomes is seen. A very fine dense nuclear speckled pattern may be seen in a few interphase cells. The pattern is due to antibodies to the MSA-3 antigen (Mitotic Spindle Antigen3), which may be identical to the centromere protein (CENP-F).

4.5. Cytoplasmic fluorescence patterns

4.5.1. Fine speckled cytoplasmic pattern

Fine granules dispersed throughout the cytoplasm, becoming more evident towards the periphery of the cell, sometimes producing a stardust-like appearance. No staining of nuclei or nucleoli. This pattern is characteristic of antibodies to tRNA synthetases. Chromosomal material in metaphase cells is negative.

4.5.2. Diffuse cytoplasmic pattern

A very fine dense granular to homogeneous staining or cloudy pattern covering part or the whole cytoplasm is observed. No staining of nuclei, but nucleoli may be homogeneously stained if antibodies are directed to ribosomal RNA proteins, precursors of which are found in nucleoli. Chromosomal material in metaphase cells is negative.

4.5.3. Mitochondrial like pattern

Larger irregular granules extending from the nucleus throughout the cytoplasm in a reticular network. The main recognized antigen cluster, M2, comprises four major mitochondrial inner membrane proteins. Nuclei and Nucleoli are negative, but strongly positive sera may indicate speckled nuclear staining.

4.5.4. Lysosomal like pattern

Irregular intermediate to large organelles distributed throughout the cytoplasm can be due to antibodies directed to organelles like lysosomes or peroxisomes -no staining of nuclei or nucleoli, but diffuse staining of the cytoplasm of dividing cells.

4.5.5. Golgi like pattern

Staining of a polar organelle adjacent to the nucleus and composed of irregular large granules. Nuclei and nucleoli are negative. Diffuse staining of the cytoplasm of dividing cells with accentuation around chromosomal material.

4.5.6. Actin like pattern

Stress fibers lying in a simple focal plane just underneath the plasma membrane is stained. Nuclei, nucleoli, and metaphase chromosomes are negative. Actin cables span the cell's whole length close to the plasma membrane. Also, dendrite-like extensions on the cell membrane may be seen.

4.5.7. Vimentin like pattern

Abundant fine fibers extending through the whole cytoplasm, staining a radial fiber network as well as aggregated bundles around the nuclei in a coiled form. Nuclei and nucleoli are negative.

4.6. Negative

4.6.1. Negative (example1)

Virtually no green fluorescence of cytoplasm or nuclei can be seen. Single and often irregular green spots appearing in the field represent conjugate aggregates. Cellular structures may look brownish (e.g., Nucleoli), especially in old slide preparations or having been stored with anti-fading agents. Cell debris may give rise to artifactual greenish fluorescent spots.

4.6.2. Negative (example2)

Very faint greenish fluorescence of nuclei, cytoplasm, or both reaching an intensity below that of a weak positive sample. This can best be judged if a weak positive ANA serum is included as a sensitivity control in each daily run, with a clear-out ANA-negative control serum and a clear-cut ANA-positive control serum.

4.7. Undeterminable

Any other staining pattern related to subcellular organelles or structures that have not been included in this classification taxonomy.

4.8. Annex

Laboratories using cryostat sections of animal tissues, i.e., rodent kidney or liver, as ANA substrate commonly miss fluorescence patterns such as the pleomorphic speckled nuclear pattern, centromere pattern, multiple nuclear dots, coiled body nuclear dots, some nucleolar patterns, and most cytoplasmic patterns. Patterns relating to the spindle apparatus are similarly not seen. The mitochondrial-like pattern can be seen on many tissues as a coarse speckled pattern, formerly detected on animal kidney sections. The smooth nuclear membrane pattern is often seen very clearly on rat liver. The cut-off value for positive ANA or HEp-2 cells is commonly set above 1:80, whereas on tissue sections, it is commonly set at a lower dilution.

Certain staining patterns are commonly recognized together in a single serum, i.e., multiple nuclear dots together with mitochondrial-like pattern (both characteristic of Primary biliary cholangitis) and pleomorphic speckled nuclear pattern together with homogeneous nuclear, coarse, or fine-speckled nuclear pattern (both characteristic of systemic lupus erythematosus). Also, the centromere pattern is commonly seen together with the mitochondrial-like pattern.

It should be remembered that some laboratories and kits use FITC-conjugates directed towards all Ig classes. The present glossary is based on findings with an IgG-specific conjugate.

5. SPECIFIC PERFORMANCE CHARACTERISTICS

One hundred and thirty-eight frozen samples were tested on two lots of reagents from another company and two lots of ***AESKUSLIDES***®. 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. The comparison of the two kits was to demonstrate comparability between two independent companies' ANA HEp-2 systems, including pattern consistency.

a. 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 %)

b. 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[2]

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.

5.1. Reproducibility and Precision

Three different LOTS of ***AESKUSLIDES***® were tested with 15 serum samples, covering the complete set of patterns. These samples were diluted from 1:40 up to 1:10240, and each dilution was analyzed by two independent readers on all three LOTS. Acceptance criteria was a deviation of +/- 1 fluorescence intensity. The acceptance criteria were met for all samples, all dilutions, all LOTS, and all independent readers- more detailed data upon request.

[2] The discrepancy between N=138 from Table 1 and N=144 from Table 2 has its origin in the fact that some samples have no pattern at all (e.g. sample #5), and some samples have two patterns (e.g. sample #2). Table 2 only refers to the number of patterns.

6. DATA INTERPRETATION SHEET

ANA HEp-2

Date:	LOT:
Slide No.:	Operator:

Well No.	ID	Dilution factor	F.I.	Chromosomes (mitosis)	Nucleoplasm	Nucleolus	Cytoplasm	Autoantibodies	Remarks
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									

7. STANDARD KIT CONTENTS

		SLIDES				CONJUGATE			POSITIVE CONTROL		
Kit Ref.	**Kit Description**	**Ref.**	**Wells**	**Coated with**	**Quantity**	**Ref.**	**Description**	**Quantity**	**Ref.**	**Description**	**Quantity**
51.100	**ANA HEp-2** (12 wells)	**S51.100**	12	ANA-HEp-2 Cells	10	**C51.100**	IgG Capped blue: slightly blue-colored solution. Containing: BSA, Fluorescein (FITC) labeled Anti-human Antibody	1x 4.8 ml	**PC51.100**	ANA pattern control (Homogeneous) Capped red: colorless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	1x 0.5 ml
51.101	**ANA HEp-2** (12 wells)					**C51.101**	IgG Capped blue: slightly blue-colored solution. Containing: BSA, Fluorescein (FITC) labelled Anti-human Antibody Containing: BSA, Fluorescein (FITC) labeled Anti-human Antibody (H+L)		**PC51.101**	ANA pattern control (Homogeneous) Capped red: colorless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	1x 0.5 ml
51.100. Bulk5	**ANA HEp-2** (12 wells) **Bulk kit**				50	**C51.100. Bulk**	IgG Capped blue: slightly blue-colored solution. Containing: BSA, Fluorescein (FITC) labeled Anti-human Antibody	2x 10 ml	**PC51.100**	ANA pattern control (Homogeneous) Capped red: colorless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	1x 0.5 ml
51.101. Bulk5	**ANA HEp-2** (12 wells) **Bulk kit**					**C51.101. Bulk**	IgG Capped blue: slightly blue-colored solution. Containing: BSA, Fluorescein (FITC) labelled Anti-human Antibody Containing: BSA, Fluorescein (FITC) labeled Anti-human Antibody (H+L)		**PC51.101**	ANA pattern control (Homogeneous) Capped red: colorless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	1x 0.5 ml

		SLIDES				CONJUGATE			POSITIVE CONTROL		
Kit Ref.	**Kit Description**	**Ref.**	**Wells**	**Coated with**	**Quantity**	**Ref.**	**Description**	**Quantity**	**Ref.**	**Description**	**Quantity**
51.180	**ANA HEp-2** (18 wells)	**S51.180**	18	ANA-HEp-2 Cells	10	**C51.100. Bulk**	IgG Capped blue: slightly blue-colored solution. Containing: BSA, Fluorescein (FITC) labelled Anti-human Antibody	1x 10 ml	**PC51.100**	ANA pattern control (Homogeneous) Capped red: colorless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	1x 0.5 ml
51.181	**ANA HEp-2** (18 wells)					**C51.101. Bulk**	IgG Capped blue: slightly blue-colored solution. Containing: BSA, Fluorescein (FITC) labeled Anti-human Antibody Containing: BSA, Fluorescein (FITC) labeled Anti-human Antibody (H+L)		**PC51.101**	ANA pattern control (Homogeneous) Capped red: colorless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	1x 0.5 ml
51.180. Bulk5	**ANA HEp-2** (18 wells) **Bulk kit**				50	**C51.100. Bulk**	IgG Capped blue: slightly blue-colored solution. Containing: BSA, Fluorescein (FITC) labeled Anti-human Antibody	3x 10 ml	**PC51.100**	ANA pattern control (Homogeneous) Capped red: colorless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	1x 0.5 ml
51.181. Bulk5	**ANA HEp-2** (18 wells) **Bulk kit**					**C51.101. Bulk**	IgG Capped blue: slightly blue-colored solution. Containing: BSA, Fluorescein (FITC) labeled Anti-human Antibody Containing: BSA, Fluorescein (FITC) labelled Anti-human Antibody (H+L)		**PC51.101**	ANA pattern control (Homogeneous) Capped red: colorless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	1x 0.5 ml

NOTE: The contents of the remaining components of the kits i.e. Common reagents (Neg. Ctrl, Mounting Medium etc.) are described below in section 8 COMMON REAGENTS CONTENTS.

8. COMMON REAGENTS CONTENTS

8.1. Common Reagents

Kit Ref.	Quantity	Ref.	Reagent	Volume	Description	Ready to use
All kits	1x	**NCIFA**	Negative Control	0.5 ml	Capped green: colorless solution. Containing: Human serum (diluted), sodium azide <0.1 % (preservative)	YES
All kits	1x	*** EBIFA**	Evans Blue 0.2 %	1.5 ml	Capped white: Blue-colored solution. Containing: PBS, Evans Blue. Dilute the Evans Blue 0.2 % 1:3000 in 1x WBIFA	NO
51.100; 51.101; 51.180; 51.181	1x	**MMIFA**	Mounting Medium	8 ml	Validated for use with the HELMED® or HELIOS® Capped white: colorless solution Containing: PBS, Glycerin.	YES
51.100.Bulk5; 51.101.Bulk5; 51.180.Bulk5; 51.181.Bulk5	3x	**MMIFA.BULK**	Mounting Medium	12 ml		
51.100; 51.101; 51.180; 51.181	1x	**WBIFA**	Washbuffer (10x)	100 ml	Capped white: colourless solution. Dilute the concentrated buffer 1:10 in distilled water (e.g.: 100 ml + 900 ml). Containing: PBS, sodium azide (preservative).	NO
51.100.Bulk5; 51.101.Bulk5; 51.180.Bulk5; 51.181.Bulk5	3x					
51.100; 51.101	1x	**SBIFA**	Sample buffer (1x)	70 ml	Capped white: colourless solution for the dilution of patient sera Containing: PBS, BSA, sodium azide (preservative).	YES
51.100.Bulk5; 51.101.Bulk5	3x					
51.180; 51.181	1x			100 ml		
51.180.Bulk5; 51.181.Bulk5	3x					

Quantities are per kit. (*) must be ordered separately.

8.2. Materials required but not provided

1. Distilled water
2. Test tubes for sample dilution
3. Measuring flask
4. Volumetric pipette
5. Timer
6. Fluorescence microscope with FITC system, (490 nm excitation filter, 510 nm barrier filter)
7. Incubator tray
8. Staining dish
9. Pipetting tips
10. Cover slips (24 x 60 mm)
11. Squeeze wash bottle

If the product information, including the labeling, is defective or incorrect, please get in touch with the manufacturer or the test kit supplier.

9. STORAGE AND SHELF LIFE

Store all reagents at 2-8 °C / 35.6-46.4 °F, protected from direct 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 slides at 2-8 °C / 35.6-46.4 °F in their original containers. Once prepared, reconstituted solutions are stable for at least 1 week at 2-8 °C / 35.6-46.4 °F.

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

10. PRECAUTIONS OF USE

c. 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. Although this product is not considered particularly toxic or dangerous in conditions of intended use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified as irritating to eyes and skin, we recommend avoiding contact with eyes and skin and wearing disposable gloves.

All human source material used for some reagents of this kit (controls, e.g.) has been tested by approved methods and found negative for HBsAg, Hepatitis C, and HIV. However, no test can guarantee the absence of viral agents in such material completely. Thus, handle kit controls and patient samples as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin (BSA, Immunoglobulin), as stated in the table of contents, handle according to national requirements.

d. 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. Keep all flasks sealed after use to avoid bacterial contamination.
4. Always pipette all solutions with new sterile pipetting tips.
5. Never expose components to higher temperatures than 37 °C / 98.6 °F (except MMIFA).
6. Never let the slide wells dry out during the whole procedure.
7. Never freeze the slides.

Each laboratory should establish its own in-house controls upon its own techniques, controls, equipment, and patient population according to its 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 the values of the controls do not meet the criteria, the test is invalid and must be repeated. The following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions, and washing methods. If the tested items show aberrant values or any deviation or the validation criteria are not met without a justified cause, please contact our local representative.

11. SAMPLE COLLECTION, HANDLING AND STORAGE

Preparation of samples: use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Collect blood samples aseptically.

Lipemic, icteric, hemolyzed, or microbially contaminated specimens may cause interference.

Sera with particles should be cleared by low-speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry, and empty tubes. After separation, the serum samples should be used during the first 8h, respectively stored tightly closed at 2-8 °C / 35.6-46.4 °F up to 2 weeks or frozen at -20 °C / -4 °F for longer periods. Avoid repeated freezing and thawing.

12. ASSAY PROCEDURE

e. Preparation prior to pipetting

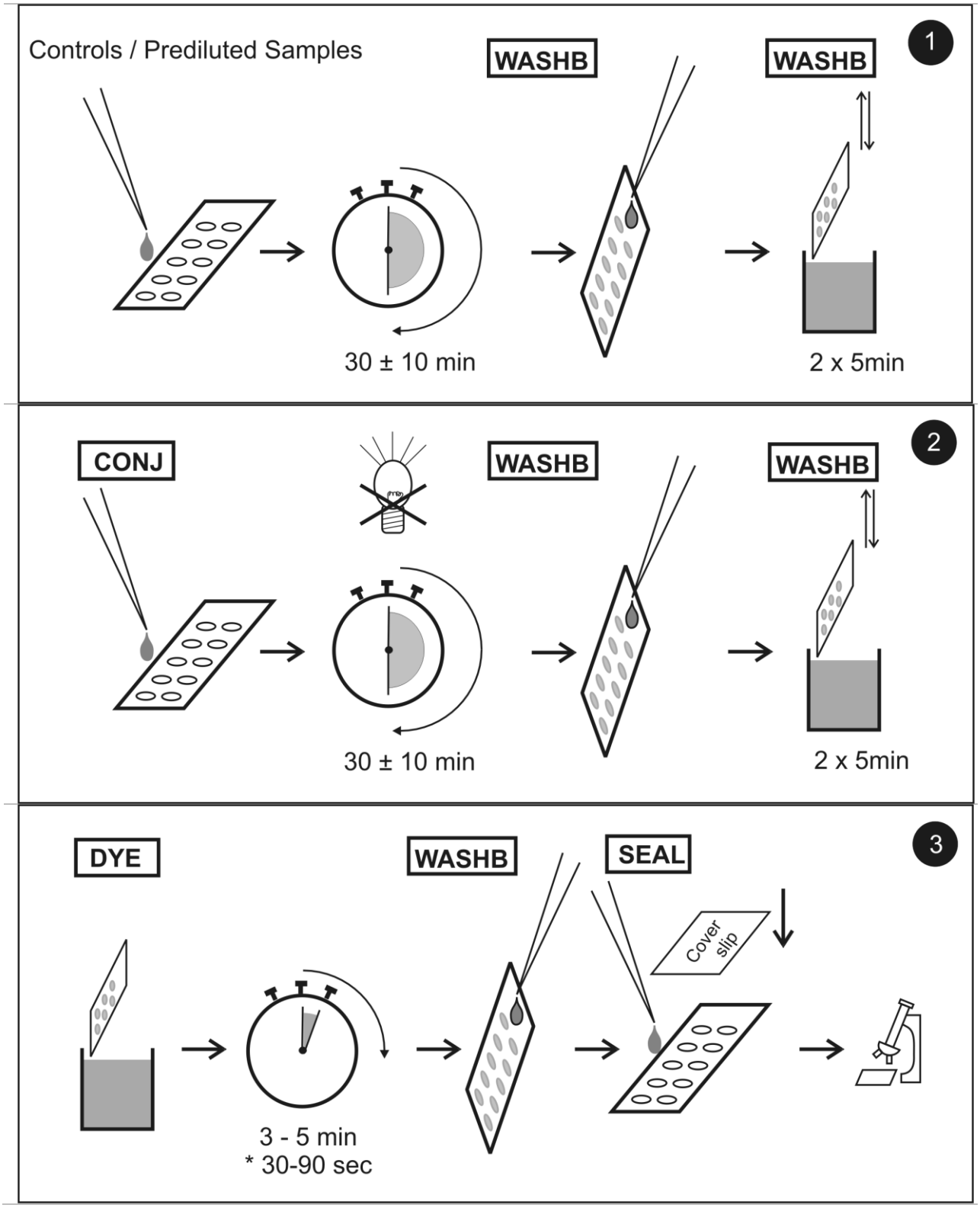
Allow all components to reach room temperature (20 – 26 °C / 68 – 78.8 °F) before use, mix well, and follow the recommended incubation scheme for optimum the performance.

1. Preparation of the Wash Buffer: Dilute the concentrated buffer 1:10 with distilled water.
2. Dilution of samples: Dilute patient sera (for screening titer, refer to the ***Kit Procedure*** section above according to the product reference you are using) with 1x Sample buffer. These vary between HEp-2, nDNA, rLKS, EMA etc. kits.
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.
5. In the case of increased viscosity and/or opacity, the Mounting Medium reagent (MMIFA) should be heated in a water bath for 15 minutes at 56 °C / 132.8 °F and then allowed to reach room temperature (20 – 26 °C / 68 – 78.8 °F) before use. After this procedure, the heated reagent can be used for up to 21 consecutive days, provided it is stored at 2-8°C / 35.6-46.4 °F, as specified on the reagent label and in the instructions for use.

f. 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.
2.	**Preparation of incubator tray**: Place a small volume of deionized or distilled water in an incubator tray and place slide(s) on supports in the incubator tray. Incubate slide(s) 30 minutes ± 10 minutes at room temperature in the moist incubator tray. Use consistent incubation times for the conjugate. **First incubation**: Pipette an adequate volume of each diluted serum and controls (ready to use) into the appropriate wells; avoid direct contact of the pipette with the 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 completely cover the wells. But avoid a running between the wells because this can lead to incorrect results.
3.	**Washing**: After incubation, remove slides from the 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 the slide first towards one row and carefully run a stream of wash buffer along the midline of the slide, allowing the wash buffer to run off the lower edge of the slide. Then, tilt the slide towards the other row and repeat this procedure, allowing the wash buffer to run off what is now the lower edge of the slide. Wash slide(s) for 10 minutes with wash buffer in a slide staining dish. Avoid direct contact of solid items with the substrate. For optimal results, change the buffer solution once after 5 minutes. Lift slide(s) out of the staining dish and carefully remove excess washing buffer. NOTE: It is important that slide wells do not dry out during the procedure, as this may damage to the substrate. Please do not blot or dry the slide in any manner or allow the slide to sit without fluorescent antibody reagent for longer than a few seconds.
4.	**Second incubation**: After the washing procedure, return the slide immediately to the incubator tray, cover each well with an adequate volume of FITC-conjugate, and ensure that the wells are covered completely. Incubate slide(s) 30 minutes± 10 minutes at room temperature in the dark.
5.	**Washing**: After incubation, remove slides from the incubator tray and rinse briefly with wash buffer using a squeeze wash bottle. Do not squirt buffer directly on the wells. Wash slide(s) for 10 minutes with wash buffer in a slide staining dish. For optimal results, change the buffer solution once after 5 minutes.
6.	***Optional counterstain**: Dilute counterstain (Evans Blue) 1:3000 in Wash buffer and mix well. Tilt the counterstain into the staining dish and incubate the slides in it. Refer to the ***Kit Procedure*** section above according to the product reference that you are using for incubation time details. Evans Blue covers unspecific background fluorescence. Remove slide(s) after incubation and rinse briefly with washing buffer. Remove excess washing buffer. Please do not blot or dry the slide in any manner.
7.	**Mounting Medium**: Add an adequate mounting medium volume along each slide's midline. Carefully place the coverslip in position, avoiding air bubbles.
8.	**Reading**: Read slide(s) immediately at 400 - 800 x total magnification with a fluorescent microscope. (490 nm excitation filter, 510 nm barrier filter).

g. Workflow


Controls / Prediluted Samples
WASHB
WASHB
1
30 ± 10 min
2 x 5min
CONJ
WASHB
WASHB
2
30 ± 10 min
2 x 5min
DYE
WASHB
SEAL
3
Cover slip
3 - 5 min
* 30-90 sec

13. 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 2-8 °C / 35.6 -46.4 °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.

14. REGULATORY SYMBOLS

IVD	- Diagnosi in vitro	- For in vitro diagnostic use
	- Pour diagnostic in vitro	- Para uso diagnóstico in vitro
	- In-vitro-Diagnostikum	- In Vitro Διαγνωστικό μέσο
	- Para uso Diagnóstico in vitro	
REF	- Numero d'ordine	- Cataloge number
	- Référence Catalogue	- Numéro de catálogo
	- Katalognummer	- Αριθμός παραγγελίας
	- Número de catálogo	
LOT	- Descrizione lotto	- Lot
	- Lot	- Lote
	- Chargen Bezeichnung	- Χαρακτηρισμός παρτίδας
	- Lote	
UDI	- Identificatore univoco del dispositivo	- Unique device identifier
	- Identifiant unique de l'appareil	- Identificador único del dispositivo
	- eindeutige Produktidentifizierung	- Μοναδικό αναγνωριστικό συσκευής
	- Identificador único do dispositivo	
CE	- Conformità europea	- EC Declaration of Conformity
	- Déclaration CE de Conformité	- Declaración CE de Conformidad
	- Europäische Konformität	- Ευρωπαϊκή συμφωνία
	- Declaração CE de conformidade	
eIFU WWW.AESKU.COM	- Rispettare le istruzioni elettroniche per l'uso	- See electronical instructions for use
	- Voir les instructions d'utilisation électronique	- Siga las instrucciones electrónicas de uso
	- Elektronsiche Gebrauchsanweisung beachten	- Ακολουθήστε τις ηλεκτρονικές οδηγίες χρήσης
	- Seguir as instruções electrónicas de utilização	
	- Da utilizzarsi entro	- Use by
	- Utilise avant le	- Utilizar antes de
	- Verwendbar bis	- Χρήση μέχρι
	- Utilizar antes de	
+2°C +8°C	- Conservare a 2-8°C (35.6-46.4°F)	- Store at 2-8°C (35.6-46.4°F)
	- Conserver à 2-8°C (35.6-46.4°F)	- Conservar a 2-8°C (35.6-46.4°F)
	- Lagerung bei 2-8°C (35.6-46.4°F)	- Φυλάσσεται στους 2-8°C (35.6-46.4°F)
	- Conservar entre 2-8°C (35.6-46.4°F)	
	- Prodotto da	- Manufactured by
	- Fabriqué par	- Fabricado por
	- Hergestellt von	- Κατασκευάζεται από
	- Fabricado por	
DYE	- Colorante Blue-Evans	- Evans-Blue Dye
	- coloration au Bleu Evans	- Colorante Azul de Evans
	- Evans-Blue Färbelösung	- Evans Blue
	- Evans Blue	
CONTROL +	- Controllo positivo	- Positive Control
	- Contrôle Positif	- Control Positivo
	- Positiv Kontrolle	- Θετικός ορός ελέγχου
	- Controlo positivo	
CONTROL −	- Controllo negativo	- Negative Control
	- Contrôle Négatif	- Control Negativo
	- Negativ Kontrolle	- Αρνητικός ορός ελέγχου
	- Controlo negativo	
SEAL	- Mezzi di montaggio	- Mounting media
	- milieu de montage	- Medio de montaje
	- Mounting Medium	- Μέσο μονιμοποίησης
	- Meio de montagem	
CONJ	- Coniugato	- Conjugate
	- Conjugé	- Conjugado
	- Konjugat	- Σύζευγμα
	- Conjugado	
	- Vetrino per microscopio	- Microscope slide
	- lame de microscope	- Portaobjetos
	- Objektträger	- Αντικειμενοφόρο πλακίδιο
	- Lâmina	
WASHB 10x	- Tampone di lavaggio	- Wash Buffer
	- Tampon de Lavage	- Solución de lavado
	- Waschpuffer	- Ρυθμιστικό διάλυμα πλύσης
	- Solucão de lavagem	
SB 1x	- Tampone di campione	- Sample Buffer
	- Tampon de Echantillons	- Solución de muestras
	- Probenpuffer	- Ρυθμιστικό διάλυμα δειγμάτων
	- Solução para amostras	

	- Numero di determinazioni	- Number of determnations
	- Nombre de déterminations	- Número de determinaciones
	- Anzahl der Prüfungen	- Αριθμός προσδιορισμών
	- Número de determinações	
	- Non riutilizzare	- Do not reuse
	- Ne pas réutiliser	- No reutilizar
	- Nicht wiederverwenden	- Μην επαναχρησιμοποιείτε
	- Não reutilizar	
	- Proteggere dall'esposizione alla luce	- Protect from exposure to light
	- Protéger de l'exposition à la lumière	- Proteger de la exposición a la luz
	- Vor Sonnenlicht schützen	- Προστασία από την έκθεση στο φως
	- Proteger da exposição à luz	
	- Conservare all'asciutto	- Store dry
	- Stocker au sec	- Almacenar en seco
	- Trocken aufbewahren	- Αποθηκεύστε ξηρά
	- Armazenar em local seco	
	- Non utilizzare se la confezione è danneggiata e consultare le istruzioni per l'uso	- Do not use if package is damaged and consult instructions for use
	- Ne pas utiliser si l'emballage est endommagé et consulter le mode d'emploi.	- No utilizar si el envase está dañado y consultar las instrucciones de uso
	- Nicht verwenden, wenn die Verpackung beschädigt ist, und Gebrauchsanweisung beachten	- Μην χρησιμοποιείτε εάν η συσκευασία έχει υποστεί ζημιά και συμβουλευτείτε τις οδηγίες χρήσης.
	- Não utilizar se a embalagem estiver danificada e consultar as instruções de utilização	