

ENGLISH

 AESKU.DIAGNOSTICS THE DIAGNOSTIC TOOL THAT WORKS	Product Ref.	51.1xx.US
	Product Desc.	ANA HEp-2-Gamma
	Manual Rev. No.	005US: 2025-01-20

AESKUSLIDES®
THE DIAGNOSTIC TOOL THAT WORKS



INSTRUCTION MANUAL

AESKUSLIDES® ANA HEp-2-Gamma

Catalog/Reference No.	Description	Tests
51.101.US	ANA HEp-2-Gamma (12 wells)	120
51.101.US.BULK5	ANA HEp-2-Gamma Bulk Kit (12 wells) x5	600
51.181.US	ANA-HEp-2-Gamma (18 wells)	180
51.181.US.BULK5	ANA-HEp-2-Gamma Bulk Kit (18 wells) x5	900

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AESKUSLIDES® ANA HEp-2-Gamma

1 INTENDED USE

AESKUSLIDES® ANA HEp-2-Gamma is an indirect fluorescent antibody assay utilizing HEp-2 cell coated slides as a substrate for the qualitative and/or semi-quantitative determination of antinuclear antibodies (ANA) in human serum **by manual microscopy or with the HELIOS® Automated IFA System.**

This *in vitro* diagnostic assay is used as an aid in the diagnosis of systemic rheumatic diseases in conjunction with other clinical and laboratory findings.

All suggested results obtained with the HELIOS® Automated IFA System must be confirmed by trained personnel.

2 CLINICAL APPLICATION

Anti-nuclear antibodies (ANA) directed against a variety of 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 the 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 are associated 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. [1]

The detection of antibodies is based on the principle of indirect immunofluorescence assay (IIFA). Glass microscope slides are coated with tissue sections or cells (with this assay, HEp-2 cells (ANA)). 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 seen with the aid of a fluorescent microscope. [2]

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3 MATERIALS

3.1 MATERIALS PROVIDED

Catalog/ Reference	Reagent	Quantity				Volume	Description	Ready to use
		51.101.US	51.101.US. BULK5	51.181.US	51.181.US.BULK5			
S51.100	Slides	10	50	-	-	-	-	YES
	Wells	12		-		-	Coated with ANA HEp-2 Cells	YES
S51.180	Slides	-	-	10	50	-	-	YES
	Wells	-	-	18		-	Coated with ANA HEp-2 Cells	YES
C51.101.US	CONJUGATE	1 x	-	-	-	4 ml	Containing: BSA, Fluorescein (FITC) labelled goat anti-human IgG (Gamma Chain) Capped blue: slightly blue colored solution.	YES
C51.101.US.BULK	CONJUGATE	-	2 x	1 x	3 x	10 ml	Containing: BSA, Fluorescein (FITC) labelled goat anti-human IgG (Gamma Chain) Capped blue: slightly blue colored solution.	YES
PC51.101.US	Positive Control	1 x		1 x		0.5 ml	ANA pattern control (Homogeneous)	YES
							Capped red: colorless solution.	
							Containing: Human serum (diluted), sodium azide <0.1 % (preservative)	
NCIFA	Negative Control	1 x		1 x		0.5 ml	Capped green: colourless solution.	YES
							Containing: Human serum (diluted), sodium azide <0.1 % (preservative)	
*EBIFA (optional)	Evans Blue 0.2 %	1 x		1 x		1.5 ml	Capped white: Blue coloured solution	NO
							Containing: PBS, Evans Blue.	
							Dilute the Evans Blue 0.2 % 1:3000 in 1x WBIFA	

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Catalog/ Reference	Reagent	Quantity				Volume	Description	Ready to use
		51.101.US	51.101. US. Bulk5	51.181.US	51.181. US.Bulk5			
MMIFA	Mounting Medium	1 x	-	1 x	-	8 ml	Validated for use with the HELIOS®	YES
							Capped white: colourless solution	
							Containing: PBS, Glycerin.	
MMIFA.BULK	Mounting Medium	-	3 x	-	3 x	12 ml	Validated for use with the HELIOS®	YES
							Capped white: colourless solution	
							Containing: PBS, Glycerin.	
WBIFA	Wash buffer (10x)	1 x	3 x	1 x	3 x	100 ml	Capped white: colourless solution	NO
							Dilute the concentrated buffer 1:10 in distilled water (e.g.: 100 ml + 900 ml). Containing: BSA, PBS, sodium azide (preservative).	
SBIFA	Sample buffer (1x)	1 x 70 ml	3 x 70 ml	1 x 100 ml	3 x 100 ml	-	Capped white: colourless solution	YES
							for the dilution of patient sera	
							Containing: BSA, PBS, sodium azide (preservative).	

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

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3.2 MATERIALS NOT PROVIDED

Catalog / Reference	Name	Description
IOS-1000.US	HELIOS® Automated IFA System	The HELIOS® Automated IFA System is an automated system for immunofluorescence processing with image capturing with an integrated fluorescence microscope and software. The HELIOS® is a further development of the HELMED® IFA processor. The HELIOS® will automatically perform: sample dilution, transfer of controls and diluted sample to wells, incubations, washing steps, conjugate addition, mounting medium addition, image acquisition, positive/negative pre- classification, estimate the End Point Titer, and assign pattern interpretation. For routine laboratory use by professional users under controlled environmental conditions for more information, refer to the HELIOS® Automated IFA System Instruction Manual Catalogue #20140055. All suggested results obtained with the HELIOS® Automated IFA System must be confirmed by trained personnel.

The **HELIOS® Automated IFA System** can also be referred to as the **HELIOS® Automated IFA System**. The **HELIOS® Automated IFA System** is designed for routine laboratory use by professional users under controlled environmental conditions and under compliance with installation and maintenance requirements. The system performs immunofluorescence assays from patient samples, for use as aid in the diagnosis of various autoimmune diseases in conjunction with other clinical and laboratory findings.

4 STORAGE AND SHELF LIFE

Store all reagents at 2-8 °C / 35.6-46.4 °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.

Once prepared, reconstituted solutions are stable for 1 week at 2-8 °C / 35.6-46.4 °F.

Ready to use reagents (sample diluent, conjugate, controls and mounting medium) should be used within the expiration date. Once opened, these reagents are stable for up to 7 months.

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5 PRECAUTIONS OF USE / HEALTH HAZARD INFORMATION

1. **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:
2. 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.
3. This kit contains potentially hazardous components. Though kit reagents are not classified as being irritants to eyes and skin we recommend avoiding contact with eyes and skin and wearing disposable gloves.
4. All human source material used for some reagents of this kit (e.g. controls) have 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.
5. The kit contains material of animal origin (BSA, Immunoglobulin) as stated in the table of kit components, handle according to national requirements.
 6. Do not pipette by mouth. Do not smoke, eat or drink when manipulating the kit.
7. Do not mix or substitute reagents from different lot numbers. This may lead to variations in the results.
8. Keep all bottles capped/closed after use to avoid bacterial contamination.
9. Always pipette all solutions with new sterile pipetting tips.
10. Never expose components to temperatures higher than 37 °C / 98.6 °F (except MMIFA).
11. Never let the slide wells dry out during the entire procedure.
12. Never freeze the slides.
13. Each laboratory should establish its own in-house controls upon its own techniques, controls, equipment and patient population according to its established procedures.
14. A 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.
15. 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. [22]
16. 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. [23]
17. SLE patients undergoing steroid therapy or in remission may have a negative ANA. [24]
18. Positive test results may not be valid in persons who have received blood transfusions or various blood products within the past several months. [25]
19. Test results on specimens from immunosuppressed patients and pregnant women may be difficult to interpret. [26,27,28]

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6 KIT PROCEDURE

6.1 Sample Collection and Handling

Use freshly collected serum samples. Blood withdrawal must follow national requirements. Collect blood samples aseptically.

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

Sera with particles should be cleared by low-speed centrifugation ($<1000 \times g$). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used during the first 8 h and then stored tightly closed at 2-8 °C / 35,6-46,4 °F up to 48 h, or frozen at -20 °C / -4 °F for longer periods. Avoid repeated freezing and thawing. [3,19]

6.2 Screening and Titrations

6.2.1 Suggested Screening dilution ratio

Suggested screening dilution ratio:

- 1:40 (e.g. 10 µl serum + 390 µl 1x Sample buffer) or
- 1:80 (e.g. 10 µl serum + 790 µl 1x Sample buffer)

It is recommended that each laboratory establish its own screening dilution based on its own patient population and instrumentation used.

6.2.2 Titrations / Serial dilutions (Manual)

After screening the samples for positive/negative ANA, a titration of positive samples is recommended. The highest dilution in which staining intensity just appears positive, is expressed as an endpoint titer.

Each laboratory should establish its own manual titrating protocol; however, here is a fourfold serial titration example:

- Prepare a 1:40 dilution of each patient's serum by adding 10 µl serum to 390 µl of 1x Sample/Wash Buffer.
- Add 300 µl 1x Sample/Wash Buffer to tubes #2, #3, #4 and #5.

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c. Using a 100 µl pipette, transfer 100 µl from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 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. 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 the contents of the bottle have a dilution of 1:40.

6.2.3 Titrations / Serial dilutions (HELIOS® Automated IFA System)

Additional titrations can be performed on the **HELIOS® Automated IFA System** by creating a worklist and selecting the desired titrations. See **HELIOS® Automated IFA System** Instruction Manual for more detailed instructions.

6.3 Reagent Preparation

Allow all components to reach room temperature (20-26 °C / 68–78.8 °F) before use and mix well (do not shake or introduce air bubbles).

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.

6.3.1 Manual Assay Reagent Preparation

1. Dilute the concentrated Wash buffer 1:10 with distilled water.
2. Dilute patient sera (1:40 or 1:80 dilution, depending on the screening dilution chosen) with sample buffer.
3. Controls are ready to use.

6.3.2 Reagent Preparation for use with the HELIOS® Automated IFA System

1. Dilute the concentrated Wash buffer 1:10 with distilled water.
2. Controls are ready to use.
3. The samples will be automatically diluted by the **HELIOS® Automated IFA System**.

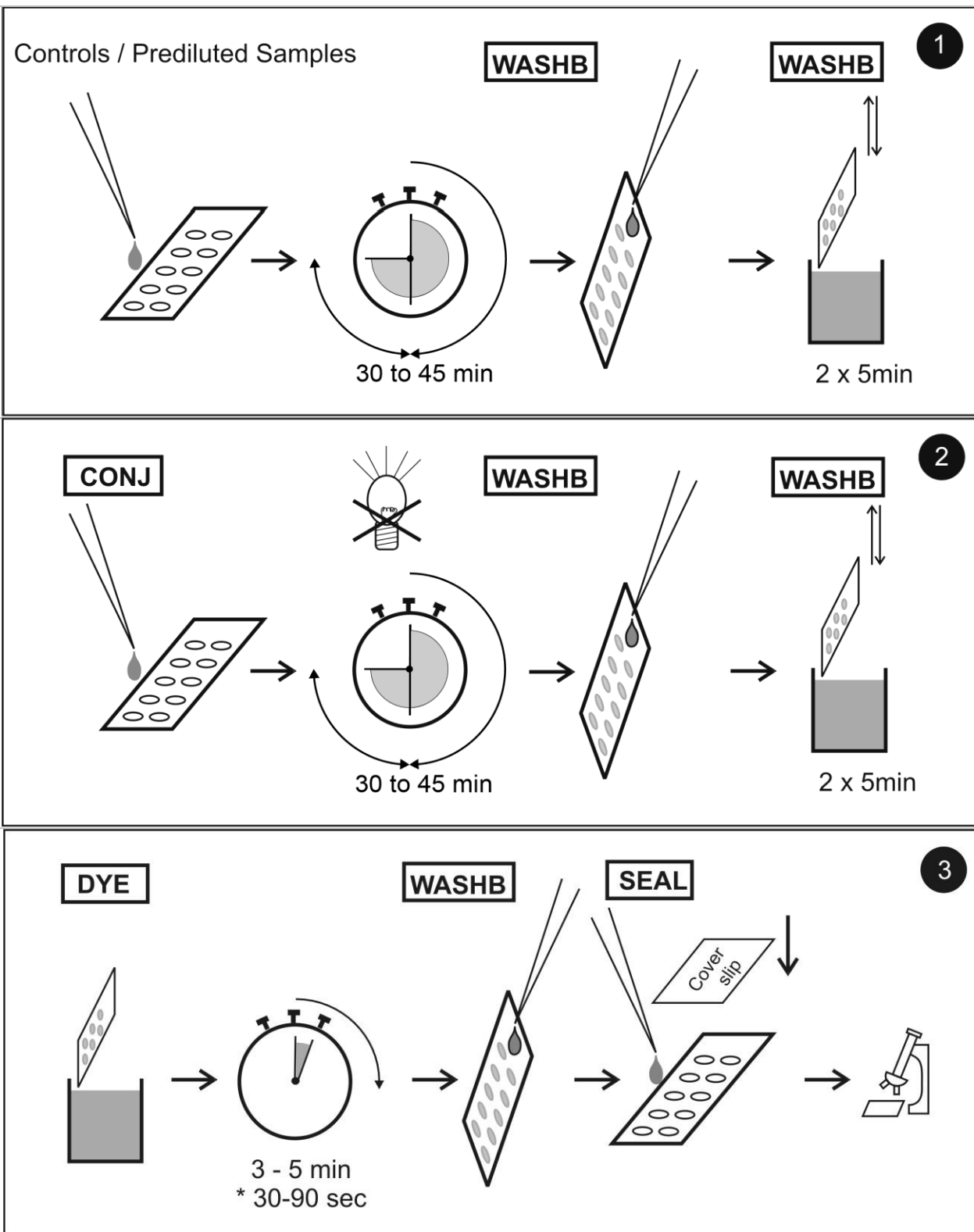
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6.4 Workflow

6.4.1 Manual Workflow

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. 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 serum. It is important to use as much test material as is necessary to cover the well completely. However, avoid serum overlapping between wells as this may cause incorrect results. Incubate slide(s) 30 minutes (not exceeding 45 minutes) at room temperature in the moist incubator tray. Use consistent incubation times for the samples.
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 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) 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 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 lead to damage to the substrate. Do not blot or dry the slide in any manner or allow 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 incubator tray and cover each well with an adequate volume of conjugate and make sure that the well is covered completely. Incubate slide(s) for 30 minutes (not exceeding 45 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) 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 counterstain into the staining dish and incubate the slides in it. Evans Blue covers unspecific background fluorescence. Counter staining time: 30 to 90 seconds Remove slide(s) after the incubation time 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 volume of mounting medium along midline of each slide. Carefully place 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). If not read immediately, cover (protect from light) and store the slide(s) at 2-8 °C / 35.6-46.4 °F.

Figure 1. Diagram of Manual Workflow



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6.4.2 HELIOS® Workflow

For more detailed instructions, refer to **HELIOS® Automated IFA System Instruction Manual** (Catalogue #20140055).

No.	Step description
1.	Activate the HELIOS® Automated IFA System software by double-clicking 
2.	Select Instrument. Select 
3.	Load samples into the sample racks.
4.	Remove required slide(s) from pouch(es) and label them. Do not touch the wells.
5.	Load reagents and slide(s) onto the HELIOS® Automated IFA System.
6.	Create a new worklist or open an existing worklist.
7.	Select test: AESKU_HELIOS_ANAHEp2_12w
8.	Select Screening mode and choose “Finish”.
9.	If the “Scan Sample Barcodes When Creating New Worklist” and “Scan Rack Layout When Creating New Worklist” has been pre-selected, the HELIOS® Automated IFA System will scan the racks and read the sample barcodes. Otherwise, manually enter the Sample ID’s in the worklist.
10.	Select Start. If “Start Loading Wizard Before Run” has been selected, the Reagent Wizard will provide prompts to double-check the Reagent placement. If it has not been pre-selected, the run will start right away.
11.	The HELIOS® Automated IFA System automatically processes the slides. The HELIOS® Automated IFA System image capturing starts automatically after the mounting medium has been added.
12.	HELIOS® Automated IFA System Result confirmation: Perform classification as described in the HELIOS® Automated IFA System Instruction Manual . All suggested results obtained with the HELIOS® Automated IFA System must be confirmed by trained personnel.

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7 QUALITY CONTROL

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

Positive Control

- Viewed with a manual microscope: Using the positive ANA pattern control serum as provided with the **AESKUSLIDES® ANA HEp-2-Gamma** test system, the cells should exhibit a homogeneous staining pattern with a fluorescent intensity of 3+.
- Viewed with the **HELIOS® Automated IFA System**: Using the positive ANA pattern control serum as provided with the **AESKUSLIDES® ANA HEp-2-Gamma** test system, the cells should exhibit a homogeneous staining pattern with a strong fluorescent intensity when compared to the negative control and the **HELIOS® Automated IFA System** Image Capturing module should pre-classify the Positive Control as positive.

Negative Control:

- Viewed with a manual microscope: Using the **AESKUSLIDES®** negative control serum as provided with the **AESKUSLIDES® ANA HEp-2-Gamma** test system, the cells should exhibit less than 1+ fluorescence and appear green.
- Viewed with the **HELIOS® Automated IFA System**: Using the **AESKUSLIDES®** negative control serum as provided with the **AESKUSLIDES® ANA HEp-2-Gamma** test system, the cells should appear green but not fluorescent when compared with the positive control and the **HELIOS® Automated IFA System** Image Capturing module should pre-classify the Negative Control as negative.

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.

Optional Titer Control

A certificate of analysis (CoA) included in each **AESKUSLIDES® ANA HEp-2-Gamma** kit for the Positive Control (PC) provides with it a calculated endpoint titer. As an additional (optional) control, the positive control can be used to test substrate sensitivity, check technique, conjugate quality and the microscope optical system by evaluating a set of PC dilutions.

To perform this optional sensitivity test, dilute the PC fourfold (+/-) with sample diluent. Include in the run one fourfold dilution above and one fourfold dilution below the PC endpoint titer.

Acceptance criteria is as follows:

The final endpoint titer must be within +/- one dilution range around the CoA endpoint titer. If the diluted controls fail to show the proper reaction upon repeat testing, do not report the test results.

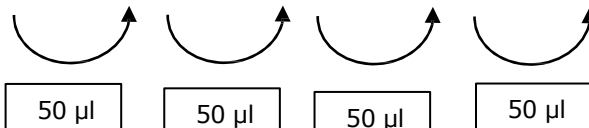
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Example dilution scheme:

Values indicated on the CoA:

- PC pre-diluted at 1/40
- Calculated Endpoint Titer: 1/320

Dilution	1/80	1/160	1 /320	1/640	1/1280
Sample diluent volume	50 µl	50 µl	50 µl	50 µl	50 µl
PC volume	50 µl	-	-	-	-



If the results are positive at one of the expected value dilutions (1/160, 1/320, or 1/640), the run passes the Optional Titer Control test. If the results are negative at all of the expected value dilutions, then Optional Titer Control test fails. Do not report test results.

Adapt the example schema to the endpoint titer value provided in the CoA (EPT values may differ between lots).

8 RESULTS

A positive screening result should be confirmed by repeating the test with additional dilutions/titrations of the serum (see sections 5.2.2 Serial Titrations (Manual) and 5.2.3 Titrations/Serial Dilutions (**HELIOS® Automated IFA System**)).

The specificity of the antibody can be further tested by running identification tests for antinuclear antibodies using defined antigens (ie. ELISA, Blot, or Mutiplex).

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8.1 Positive/Negative Confirmation (Manual Microscope)

Fluorescence Intensity Grading

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

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

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.

Example of Endpoint Extrapolation (below):

1:40 = 3+
 1:160 = 2+
 1:640 = +/-
 1:2560 = Neg

Report: 320

Report all titers and patterns seen, extrapolating the titer where necessary. Example of Titer and Pattern (below):

1:40 = 4+ Homogeneous
 1:160 = 3+ Homogeneous
 1:640 = 2+ Homogeneous and 3+ Speckled
 1:2560 = 1+ Homogeneous and 2+ Speckled 1:10240 =
 +/- Speckled

Report: 2560 Homogeneous and 5120 Speckled

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Negative Results

1. A serum dilution is considered negative for antinuclear antibodies if the cells exhibit less than 1+ fluorescence and lacks a clearly discernible pattern.
2. A sample is considered negative for antinuclear antibodies if it exhibits less than 1+ fluorescence at the chosen screening dilution (1:40 or 1:80) and all greater dilutions, or if the fluorescence observed is not a discernible ANA pattern.
3. Negative samples may exhibit fluorescent staining slightly greater than the Negative Control, but less than 1+.
4. 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.
5. Intense non-nuclear staining may be observed in some sera containing Anti-Mitochondrial type 2, Anti-Smooth Muscle or other cytoplasmic antibodies [21].

Positive Results

1. 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.
2. 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 the chosen screening dilution (1:40 or 1:80) or greater.
3. Multiple antinuclear antibodies may be present in a given specimen; one masking the other. Serially diluting the specimen will aid in distinguishing these patterns.
4. Report all titers and patterns seen.

8.2 User confirmation of HELIOS® results

The **HELIOS® Automated IFA System** Result Confirmation module facilitates software-based documentation of positive/negative identification. For user classification purposes, the images are available for each well along with the pattern suggestion. **All suggested results obtained with the HELIOS® Automated IFA System must be confirmed by trained personnel.**

For further details on how to complete results confirmation, refer to the **HELIOS® Automated IFA System** Instruction Manual.

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8.3 Pattern Interpretation

Seven major staining patterns have been validated with the **AESKUSLIDES® ANA-HEp-2-Gamma** Assay and/or in combination with the **HELIOS® Automated IFA System**.

1. Homogenous
2. Speckled
3. Anti-Centromere-Antibody
4. Nucleolar
5. Nuclear membrane
6. Nuclear dots
7. Cytoplasmic

8.4 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 following table presents an example of homogenous staining:

Homogeneous				
Interphase	Nucleoli	Mitosis	Antigen	Disease Associations
Uniform diffuse staining of the nucleoplasm (intensity can vary) in some cases, a staining reinforcement of nuclear rim may occur [4,5]	Mostly positive. Sera may show a peripheral nucleolar staining	In all phases, homogeneous or peripheral chromatin staining	dsDNA, nucleosomes, histones	Systemic Lupus Erythematosus (high and low titers), Drug-induced Lupus, Rheumatoid Arthritis (low titers)

8.4.1 Speckled

Fluorescent aggregates throughout the nucleous, which can be very fine to very coarse depending on the type of antibody present. More than one type of speckled pattern may be seen in any one specimen. The chromosome region of mitotic cells is usually negative. The following table provides examples of speckled staining:

Speckled				
Interphase	Nucleoli	Mitosis	Antigen	Disease Associations
Large Speckled (Nuclear Matrix): Variable large speckles arranged as a network.	Negative	No staining of the condensed chromatin in mitotic cells.	Heterogeneous ribonuclear proteins (hnRNP)	Mixed Connective Tissue Disease. Can also occur in Rheumatoid Arthritis, Systemic Lupus Erythematosus and Systemic Sclerosis.
Coarse Speckled: Dense intermediate sized speckles, together with large speckles throughout the nucleoplasm. [4,5]	Negative	Cells in mitosis without staining of the condensed chromosomal region.	Ribonucleoproteins Sm (from U2 to U6 without U3) and RNP (U1)	Systemic Lupus Erythematosus, Mixed Connective Tissue Disease
Fine Speckled SSA/SSB: Fine to discrete speckled staining of the nuclei in a uniform distribution. [4,5, 16,17]	Mostly negative. Staining of a few nucleoli may be observed in presence of anti-SSB/La and anti-Ku antibodies.	Metaphase cells show fluorescence in the cytoplasm, with condensation around the chromatin plate which itself is negative.	Nuclear proteins: SSA/Ro, Ro60, Ro52, SSB/La, Ku (70 kDa and 80 kDa heterodimer) and Mi-2 (240kDa)	Systemic Lupus Erythematosus, Neonatal Lupus, Cutaneous Lupus Erythematosus, Sjögren's Syndrome, Systemic Sclerosis, Dermatomyositis
Dense Fine Speckled (DFS70): Dense fine speckles uniformly distributed throughout the nucleus. [4,7]	Negative	Metaphase and telophase cells show a strong speckled fluorescence of the chromosomes.	LEDGF-p75 (lens epithelium-derived growth factor 75) initially termed DFS70 (70 kDa protein)	No known Disease Association
Fine Speckled/Homogeneous (Scl-70): Intense fine speckled staining (may appear homogeneous) covering the entire nucleoplasm. [4,155]	May or may not be positive.	Metaphase and telophase cells show staining of the chromatin plate with 2-5 discrete dots (nuclear organizing region).	DNA topoisomerase 1	Diffuse Systemic Sclerosis

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8.4.2 Anti-Centromere Antibody (ACA) [4,5,8,15]

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.

Nuclear Antigen: Centromere/kinetochore portion of the chromosome.

Centromere				
Interphase	Nucleoli	Mitosis	Antigen	Disease Associations
23-46 speckles distributed throughout the entire nucleus.	Negative	“Block” of closely associated speckles found in the condensed nuclear chromatin of metaphase, anaphase and telophase cells.	CENP-A, B, C, D, E and even CENP-G and H	Limited Systemic Sclerosis – CREST (Calcinosis, Raynaud’s Syndrome, Esophageal dysmotility, Sclerodactyly, Telangiectasia), Primary Biliary Cholangitis, Raynaud’s Syndrome and in a minority of patients Sjögren’s Syndrome

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8.4.3 Nucleolar

Fluorescent staining of the nucleoli within the nucleus sharply separated from the unstained nucleoplasm. The nucleolar fluorescence may be homogeneous, speckled or clumpy. Frequently accompanied by a speckled pattern. The following table provides some examples of nucleolar staining:

Nucleolar				
Interphase	Nucleoli	Mitosis	Antigen	Disease Associations
Nucleolar Homogeneous PM-Scl: Homogeneous fluorescence of the nucleoli, with weaker fine granular reaction of the nucleoplasm. [4,5, 12,133,14]	Positive	Region of condensed chromosomes not stained. May have fine granular staining outside of chromosomes.	PM-Scl 75 and PM-Scl 100	Polymyositis/Systemic Sclerosis Overlap syndrome, Myositis, Systemic Sclerosis
Nucleolar Homogeneous Th/To: Homogeneous fluorescence of the nucleoli, and granular nucleoplasm granular. [4,5]	Positive	Region of condensed chromosomes not stained. Fine granular staining outside of chromosomes may occur	Rpp25 (component of Th/To complex)	Systemic Sclerosis limited cutaneous form
Nucleolar Clumpy Fibrillarin: Granular fluorescence of nucleoli. Nucleoplasm is dark although the coiled bodies may be stained as few nuclear dots. [4,5]	Positive	Metaphase and telophase plates show reticular staining.	U3-nRNP fibrillarin	Systemic Sclerosis (high specificity)
Nucleolar Speckled/Punctated RNA polymerase 1/NOR: Speckled nucleolar fluorescence of the nucleoli, frequently associated with fine speckled staining of nucleoplasm. [4,5]	Positive	Region of condensed chromosomes not stained. Few dots corresponding to the nucleolar organizing regions (NOR) may fluoresce. Fine granular to smooth fluorescence, outside of chromosomes, may occur	RNA polymerase 1/NOR	Systemic Sclerosis particularly associated with diffuse cutaneous form

8.4.4 Nuclear membrane

Nuclear membrane				
Interphase	Nucleoli	Mitosis	Antigen	Disease Associations
Smooth Nuclear Membrane: Fine, linear fluorescence and staining of folds in nuclear membrane with light homogeneous staining of the nucleus. [4,5,10,11]	Negative	Metaphase: Chromatin plate negative. Post telophase: Nuclear membrane reconstitution. Newly formed nuclei are surrounded by a fine membranous fluorescence.	Lamins A, B1, B2, and C. Specificities against lamin associated proteins (LAP 1A and LAP 2) have been also identified.	Systemic Lupus Erythematosus, Systemic Sclerosis, Chronic Active Hepatitis
Punctate Nuclear Membrane (Nuclear pore complex): Discontinuous punctate (granular/speckled) staining along the nuclear membrane. Enhanced fluorescence when two nuclear membranes touch. [4,8,10]	Negative	Metaphase cells show a diffuse staining throughout the cytoplasm, without chromosomal staining.	(Nuclear pore complex) Gp210, nucleoporin p62	Primary Biliary Cholangitis

8.4.5 Nuclear dots

Nuclear dots				
Interphase	Nucleoli	Mitosis	Antigen	Disease Associations
Multiple Nuclear Dots: Five to twenty differently sized dots which are spread over the cell nucleus. [4,8,10,11]	Negative	Chromosomes of metaphase or telophase cells are negative.	Sp-100 protein, PML (promyelocytic leukemia protein)	Primary Biliary Cholangitis
Few Nuclear Dots: Two to six bright dots in the nucleoplasm, often adjacent to the nucleoli. [4,5]	Negative	No staining of metaphase or telophase chromatin plates.	Coiled bodies 80kDa (nuclear protein designated p80 coilin)	No clear clinical features have been associated with anti-p80 coilin autoantibodies.

8.4.6 Cytoplasmic Fluorescence

Cytoplasmic Fluorescence [4,5,6,8,10,11]				
Interphase	Nucleoli	Mitosis	Antigen	Disease Associations
Fine Dense Granular to Homogeneous Rib-P Cytoplasm shows a very fine dense granular to homogeneous staining with vacuoles. No staining of the nucleus. [4,5]	Homogeneously stained (rRNA proteins) or negative.	Chromosomal material in metaphase cells is negative.	Phosphorilated proteins (P proteins): P0 (35 kD), P1 (19 kD) and P2 (17 kD)	Systemic Lupus Erythematosus
Mitochondria Type 2 AMA-M2: Fluorescence of large irregular granules organized as a network of filaments extending around nucleus and throughout the cytoplasm. No staining of nucleus. [4,8,10,11]	Negative	Chromosomal material in metaphase cells is negative.	Pyruvate dehydrogenase complex (PDC). The PDC-E2 antigen is the main antibody target in PBC.	Primary Biliary Cholangitis, less frequent in Systemic Sclerosis, CREST, Systemic Lupus Erythematosus and Sjögren's Syndrome
F-Actin Staining of straight cytoplasmic cables (microfilaments) running the length of the cell. No staining of the nucleus. [4,5,6,8,10]	Negative	Chromosomal material in metaphase cells is negative.	Filamentous (polymerized) actin (F-actin)	Chronic Autoimmune Hepatitis type 1

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9 LIMITATIONS OF PROCEDURE

1. All results must be confirmed by a trained operator.
2. ANA serological test results should be used in conjunction with information available from the clinical evaluation and other diagnostic information.
3. Two to ten percent of a normal adult population have antinuclear antibodies [19].
4. 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 in normal young individuals [19,20].
5. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed or assay procedure used.
6. 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.
7. 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.
8. 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.

Special Controls

1. For Prescription Use Only.
2. Only for use with reagents that are indicated for use with the assay.
3. The assay is for use by a trained operator in a clinical laboratory setting.
4. All software-aided results must be confirmed by the trained operator.
5. For use only by manual microscopy or with **HELIOS® Automated IFA System**.

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10 EXPECTED VALUES

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

To determine the prevalence of ANA in a panel of 151 sera from healthy donors tested with **AESKUSLIDES® ANA HEp-2-Gamma**. Each serum was tested at a dilution of 1:40 and 1:80. Slides were processed manually according to the IFU and subsequently analyzed at the microscope by two independent readers.

Normal Range Study AeskusLIDES® ANA HEp-2-Gamma								
number of samples	1:40				1:80			
	Reader 1		Reader 2		Reader 1		Reader 2	
	n	%	n	%	n	%	n	%
negative	109	71.7	104	68.4	120	78.9	119	78.3
positive	43	28.3	48	31.6	32	21.1	33	21.7
* Densely Fine Speckled (DFS) 70	9	5.9	11	7.2	9	5.9	11	7.2
anti-mitochondrial antibodies (AMA)	11	7.2	12	7.9	6	3.9	6	3.9
cytoplasmic	2	1.3	4	2.6	0	0.0	0	0.0
speckled	12	7.9	12	7.9	11	7.2	8	5.3
nucleolar	3	2.0	3	2.0	1	0.7	3	2.0
homogeneous	3	2.0	3	2.0	2	1.3	2	1.3
undefined positive	3	2.0	3	2.0	3	2.0	3	2.0
Total	152	100.0	152	100.0	152	100.0	152	100.0

*The presence of DFS 70 (Densely Fine Speckled 70) has no known clinical significance [18].

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11 SPECIFIC PERFORMANCE CHARACTERISTICS

Inter-Assay Precision	HELIOS®		HELIOS® w/ User Confirmation		Manual <i>AESKUSLIDES</i> ® ANA HEp-2-Gamma	
% Agreement (95% CI)	1:40 Dilution	1:80 Dilution	1:40 Dilution	1:80 Dilution	1:40 Dilution	1:80 Dilution
Positive Agreement	100 (99.6 - 100)	99.2 (98.4 - 99.6)	100 (99.6 - 100)	100 (99.6 - 100)	100 (98.1 - 100)	100 (98.1 - 100)
Negative Agreement	99.6 (98.6 - 99.9)	100 (99.2 - 100)	100 (99.2 - 100)	100 (99.2 - 100)	100 (96.3 - 100)	100 (96.3 - 100)
Overall Agreement	99.9 (99.5 - 100)	99.5 (99.0 - 99.7)	100 (99.7 - 100)	100 (99.7 - 100)	100 (98.7 - 100)	100 (98.7 - 100)
Total Pattern Agreement	98.8 (97.9 - 99.3)	99.1 (98.3 - 99.5)	100 (99.6 - 100)	100 (99.6 - 100)	100 (98.1 - 100)	100 (98.1 - 100)

Intra-Assay Precision	HELIOS®		HELIOS® w/ User Confirmation		Manual <i>AESKUSLIDES</i> ® ANA HEp-2-Gamma	
% Agreement (95% CI)	1:40 Dilution	1:80 Dilution	1:40 Dilution	1:80 Dilution	1:40 Dilution	1:80 Dilution
Positive Agreement	100 (98.2 - 100)	99 (96.6 - 99.7)	100 (99.1 - 100)	100 (99.1 - 100)	100 (99.1 - 100)	100 (99.1 - 100)
Negative Agreement	100 (88.6 - 100)	100 (88.6 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)	100 (94 - 100)
Overall Agreement	100 (98.4 - 100)	99.2 (97 - 99.8)	100 (99.2 - 100)	100 (99.2 - 100)	100 (99.2 - 100)	100 (99.2 - 100)
Total Pattern Agreement	96.7 (93.3 - 98.4)	96.2 (92.7 - 98.1)	100 (99.1 - 100)	100 (99.1 - 100)	100 (99.1 - 100)	100 (99.1 - 100)

Sensitivity/Specificity at 1:40 Dilution

SLE	CTD + AIL	Non-Healthy Controls
% Sensitivity (% 95 CI)	% Sensitivity (% 95 CI)	% Specificity (% 95 CI)
Manual <i>AESKUSLIDES</i> ® ANA HEp-2-Gamma		
86.2 (75.8 - 92.6)	84.9 (79 - 89.4)	57.4 (50 - 64.5)
Manual <i>AESKUSLIDES</i> ® ANA HEp-2 H+L		
82.6 (70 - 90.7)	82.4 (74 - 88.5)	58.4 (50.5 - 65.9)
HELIOS®		
86.4 (76.1 - 92.7)	84.8 (78.8 - 89.3)	58 (50.6 - 65.1)
HELIOS® with User Confirmation		
85.7 (75.4 - 92.3)	84.4 (78.4 - 88.9)	58.2 (50.8 - 65.3)

Sensitivity/Specificity at 1:80 Dilution

SLE	CTD + AIL	Non-Healthy Controls
% Sensitivity (% 95 CI)	% Sensitivity (% 95 CI)	% Specificity (% 95 CI)
Manual <i>AESKUSLIDES</i> ® ANA HEp-2-Gamma		
79.2 (68 - 87.3)	79.2 (72.8 - 84.5)	68.9 (61.6 - 75.3)
Manual <i>AESKUSLIDES</i> ® ANA HEp-2 H+L (Predicate)		
78.2 (65.1 - 87.4)	79.5 (70.9 - 86)	67.7 (60 - 74.6)
HELIOS®		
78.1 (66.7 - 86.3)	78.3 (71.8 - 83.6)	68.3 (61.1 - 74.8)
HELIOS® with User Confirmation		
78.4 (67.1 - 86.6)	78.9 (72.5 - 84.2)	67.4 (60.1 - 74)

The discrepancy seen when comparing the Se/Sp for 1:40 dilution vs. 1:80 dilution is due to the number of low positive (low titer) samples analyzed in this study. At 1:40 dilution, these sera were identified as positive while at 1:80 dilution, these sera were identified as negative. This impacts the sensitivity calculation. Additionally, there is a known inverse relationship between sensitivity and specificity (Lalkhen AG, et. al. 2008). Thus, at 1:80 dilution compared to 1:40 dilution, sensitivity decreases while specificity increases.

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12 TROUBLESHOOTING

12.1 Troubleshooting - Manual

ERROR	POSSIBLE CAUSES	SOLUTION
Low cell density	- Cell lysis following prolonged contact with deionised 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 lense	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

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12.2 Troubleshooting - HELIOS® Automated IFA System

ERROR	POSSIBLE CAUSES	SOLUTION
Low cell density	Cell lysis following prolonged contact with deionised water	- Refer to HELIOS® Automated IFA System User Manual - Perform Weekly maintenance
	Proteolytic enzymes have attacked the substrate	Inactivate serum
Uneven fluorescence	Serum dried in the well, fluorescence stronger at the edge	- Refer to HELIOS® Automated IFA System User Manual - Perform Weekly maintenance
	Serum does not cover the test well	
	Cross-reaction between the wells	
	Microscope incorrectly adjusted	Refer to HELIOS® Automated IFA System User Manual
Diffuse picture	I.F. Microscope is dirty. Possible scratches on the lense	- Clean the microscope according to its instructions - Refer to HELIOS® Automated IFA System User Manual
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	- Refer to HELIOS® Automated IFA System User Manual - Perform Weekly maintenance - Use only fresh sera - Check correct filter / objective

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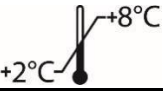
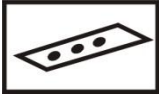
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	Manual Rev. No.	005US: 2025-01-20

14 REGULATORY SYMBOLS

	For in vitro diagnostic use
	Catalogue number
	Lot
	Unique Device Identifier
	EC Declaration of Conformity
	See instructions for use
	Use by
	Store at 2-8 °C (35.6-46.4 °F)
	Manufactured by
	Evans-Blue Dye
	Positive Control
	Negative Control
	Mounting media
	Conjugate
	Microscope slide
	Wash Buffer



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SB 1x	Sample Buffer
Σ xx	XX tests
Rx only	For Prescription Use Only