ENGLISH

AESKUSLIDES®
THE IFA PRODUCT LINE

INSTRUCTION MANUAL

nDNA (Crithidia luciliae)

Standard Ref.	Description	Tests
53.100.US	**nDNA** (Crithidia luciliae) (10 wells)	100
53.100.US.Bulk5	**nDNA** (Crithidia luciliae) (10 wells) bulk kit x5	500
53.100.US.Bulk10	**nDNA** (Crithidia luciliae) (10 wells) bulk kit x10	1000
53.120.US	**nDNA** (Crithidia luciliae) (12 wells)	120

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nDNA (Crithidia luciliae)

1 INTENDED USE

***AESKUSLIDES*® nDNA (Crithidia luciliae)** is an indirect immunofluorescence assay utilizing Crithidia luciliae coated slides as a substrate for the qualitative and/or semi-quantitative determination of antibodies to native double stranded DNA (dsDNA) in human serum. This in vitro diagnostic assay is used as an aid for the diagnosis of Systemic Lupus Erythematosus (SLE) in conjunction with other clinical and laboratory findings. The assay can be processed manually and analyzed at the microscope or processed and analyzed with **HELIOS® Automated IFA System**.

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

2 CLINICAL APPLICATION

Antibodies binding to DNA belong to the group of anti-nuclear Antibodies (ANA) that have been observed in several autoimmune diseases. Antibodies reacting with native double-stranded DNA (nDNA) are regarded as being specific for systemic lupus erythematosus (SLE) and have been observed in approximately 50-80% of the patients. Antibodies against double-stranded DNA (dsDNA) are found during active phases of SLE. Autoantibodies tend to disappear during disease remission and upon immunosuppressive treatment. The amount of the serum concentration is positively correlated with the severity of the disease [1]. Thus, detection of these autoantibodies is important for the diagnosis and the clinical monitoring of SLE. Diagnosis is made based on the American College of Rheumatology (ACR) classification criteria. Autoantibodies to dsDNA have been established as 1 of 11 ACR-criteria for the diagnosis of SLE. However, absence of dsDNA antibodies does not rule out SLE in any case. Only if 4 of 11 criteria are met, the diagnosis SLE can be confirmed with a certainty of 80-90% [2].

For the detection of antibodies against dsDNA in human serum variable methods have been established, e.g. enzyme-linked immunosorbent assay (ELISA), radioimmuno assay (RIA), immunofluorescence assay. The immunofluorescence test uses Crithidia luciliae cells as antigenic substrate. The cells possess a giant mitochondrium containing of highly condensed, circular dsDNA. This DNA, known as kinetoplast, is reported to be free of histones and other nuclear antigens, like single-stranded DNA [3,4]. In comparison to other assays, it is a simple and specific method for detecting anti-dsDNA antibodies [5].

Antigen Characterization: mitochondrial DNA from Crithidia luciliae (monoflagelate protozoa)

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 *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 IgG during the second incubation. A specific green fluorescent staining of antigen-antibody-complex can be visualized with the aid of a fluorescent microscope.

3 MATERIALS

3.1 MATERIALS PROVIDED

Catalog/ Reference	Reagent	Quantity				Volume	Description	Ready to use
		53.100.US	53.100. US.Bulk5	53.100. US.Bulk10	53.120. US			
S53.100 S53.120.US*	Slides	10	50	100	10	-	10 or 12 wells per slide	YES
	Wells	10			12	-	Coated with Crithidia luciliae cells	
C53.100 C53.100.US*	Conjugate	1x	5x	10x	1x	4 ml	IgG Capped blue: slightly blue colored solution. Containing: BSA, Fluorescein (FITC) labelled Anti-human IgG Antibody	YES
PC53.100 PC53.100.US*	Positive Control	1x				0.5 ml	nDNA positive control. Capped red: colorless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	YES
NCIFA NCIFA.US*	Negative Control	1x				0.5 ml	Capped green: colorless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	YES
EBIFA (optional not included in the kit)	Evans Blue 0.2%	1x				3 ml	Capped white: Blue colored solution Containing: PBS, Evans Blue. Dilute the Evans Blue 0.2% 1:3000 in 1x WBIFA	NO
MMIFA MMIFA.US*	Mounting Medium	1x	3x	6x	1x	8 ml	Validated for use with the HELIOS® Capped white: colorless solution Containing: PBS, Glycerin.	YES
WBIFA WBIFA.US*	Wash Buffer (10x)	1x	3x	6x	1x	100 ml	Capped white: colorless solution Dilute the concentrated buffer 1:10 in distilled water (e.g.: 100 ml + 900 ml). Containing: PBS, sodium azide (preservative).	NO
SBIFA SBIFA.US*	Sample Buffer (1x)	1x	3x	6x	1x	70 ml	Capped white: colorless solution for the dilution of patient sera Containing: BSA, PBS, sodium azide (preservative).	YES

**only applicable for 53.120.US*

3.2 MATERIALS NOT PROVIDED

Catalog/ Reference	Name	Description
IOS-1000	**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® Automated 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. **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®**. The **HELIOS®** 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.

Store all reagents and the 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 within the expiry date indicated on each component, only.

5 PRECAUTIONS OF USE / HEALTH HAZARD INFORMATION

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:

1. 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.

2. 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.

3. 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.

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

5. Do not pipette by mouth. Do not smoke, eat or drink when manipulating the kit.

6. Do not mix or substitute reagents from different lot numbers. This may lead to variations in the results.

7. Keep all bottles capped/closed after use to avoid bacterial contamination.

8. Always pipette all solutions with new sterile pipetting tips.

9. Never expose components to temperatures higher than 37°C / 98.6°F.

10. Never let the slide wells dry out during the entire procedure.

11. Never freeze the slides.

12. Each laboratory should establish its own in house controls upon its own techniques, controls, equipment and patient population according to its established procedures.

13. 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.

14. 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]

15. Positive dsDNA results may be seen in a small percentage of patients with other autoimmune diseases (e.g. Sjögren's Syndrome, Rheumatoid Arthritis) and infectious diseases. [7,8,9]

16. SLE patients undergoing immunosuppressive treatment, corticosteroid therapy, or in remission may be negative for dsDNA antibodies. [1,10]

17. Positive test results for antinuclear antibodies may not be valid in persons who have received blood transfusions or various blood products within the past several months. [11]

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 x 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. [12,13]

6.2 Screening and Titrations

6.2.1 Suggested Screening dilution

Screening dilution 1:10

Crithidia luciliae contains a giant mitochondrion, also known as the kinetoplast which contains only dsDNA. The kinetoplast is situated between the nucleus and the basal body near the flagellum.

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

Examples for dilution:

1:10	10 µL serum	+	90 µL Sample Buffer
1:20	10 µL serum	+	190 µL Sample Buffer
1:40	10 µL serum	+	390 µL Sample Buffer
1:80	10 µL serum	+	790 µL Sample Buffer

6.2.2 Titrations / Serial dilutions (Manual)

After screening the samples for positive/negative dsDNA, 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 titering protocol; however, here is a fourfold serial titration example:

a. Prepare a 1:10 dilution of each patient´s serum by adding 20 µl serum to 180 µl of 1x Sample Buffer.

b. Add 100µl 1x Sample/Wash Buffer to tubes #2, #3, #4, #5, #6 and #7.

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 twofold titration with the following dilutions:

Tube #1 = 1:20

Tube #2 = 1:40

Tube #3 = 1:80

Tube #4 = 1:160

Tube #5 = 1:320

Tube #6 = 1:640

Tube #7 = 1:1280

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 1:10.

6.2.3 Titrations / Serial dilutions (HELIOS®)

Additional titrations can be performed on the **HELIOS®** 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).

6.3.1 Manual Assay Reagent Preparation

1. Dilute the concentrated Wash buffer 1:10 with distilled water.
2. Dilute patient sera 1:10 with sample buffer.
3. Controls are ready to use.

6.3.2 Reagent Preparation for use with the HELIOS®

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®**.

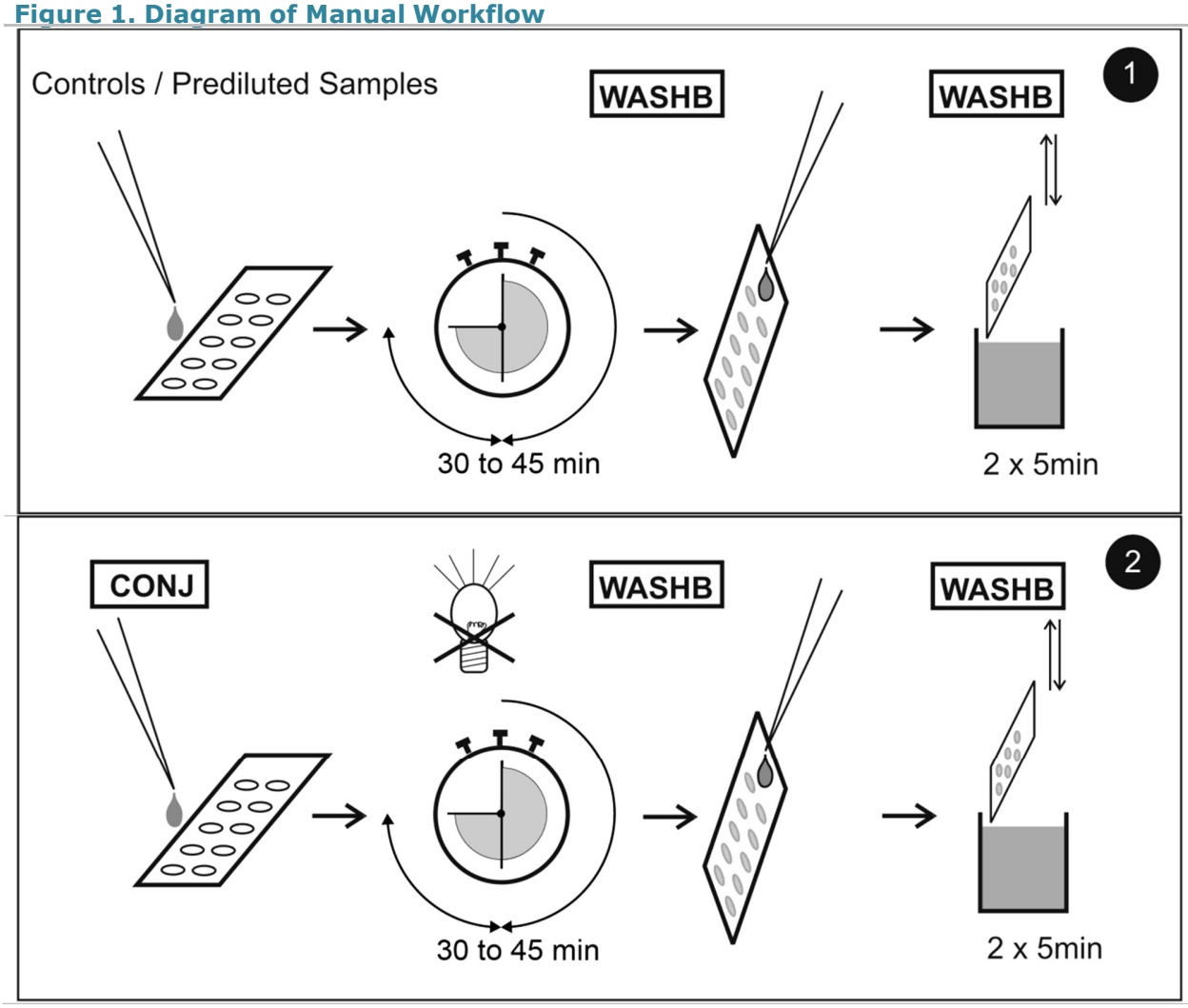
6.4 Workflow

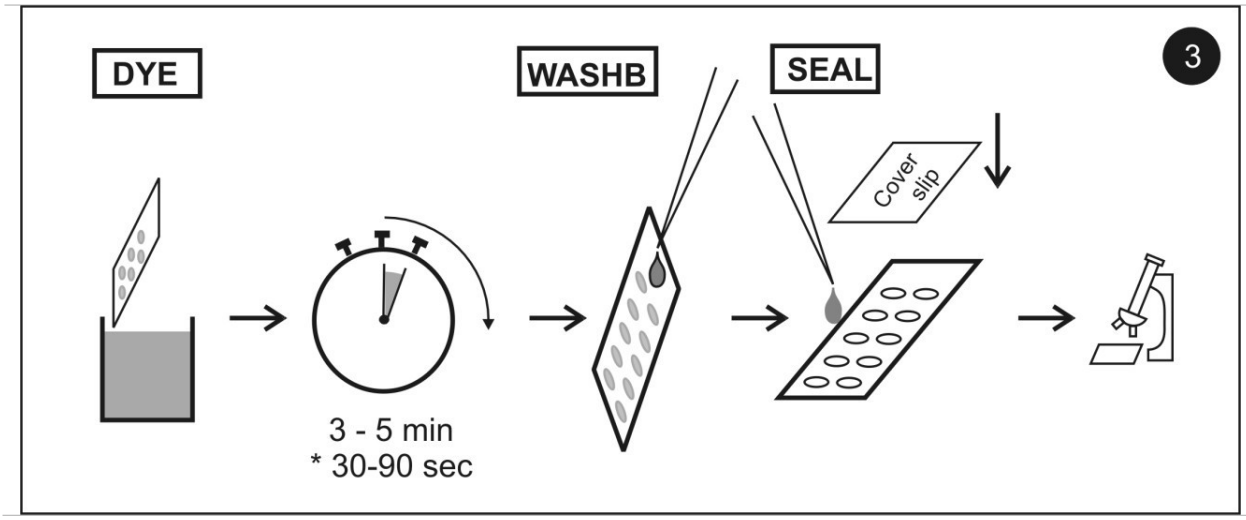
6.4.1 Manual Workflow

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





This manual workflow is applicable for 10 well and 12 well slides.

6.4.2 HELIOS® Workflow

For more detailed instructions, refer to **HELIOS® Automated IFA System** Instruction Manual.

No.	Step description
1.	Activate the **HELIOS®** 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®**.
6.	Create a new worklist or open an existing worklist.
7.	Select test: AESKU_HELIOS_nDNA_10w or AESKU_HELIOS_nDNA_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®** 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®** automatically processes the slides. The **HELIOS®** image capturing starts automatically after the mounting medium has been added.
12.	**HELIOS®** Result confirmation: Perform classification as described in the **HELIOS®** Instruction Manual. All suggested results obtained with the **HELIOS®** must be confirmed by trained personnel.

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

a. Viewed with a manual microscope: Using the positive nDNA control serum as provided with the ***AESKUSLIDES*® nDNA** test system, the cells should exhibit a positive staining of the kinetoplast with a fluorescent intensity of 3+.
b. Viewed with the **HELIOS®**: Using the positive nDNA control serum as provided with the ***AESKUSLIDES*® nDNA** test system, the cells should exhibit a positive staining of the kinetoplast with a strong fluorescent intensity when compared to the negative control. The **HELIOS®** DNA Pattern Plus can be used to pre classify the Positive Control as positive.

Negative Control:

a. Viewed with a manual microscope: Using the ***AESKUSLIDES*®** negative control serum as provided with the ***AESKUSLIDES*® nDNA** test system, the cells should exhibit less than 1+ fluorescence and appear green. No fluorescence staining of the kinetoplast should be visible.
b. Viewed with the **HELIOS®**: Using the ***AESKUSLIDES*®** negative control serum as provided with the ***AESKUSLIDES*® nDNA** test system, the cells should appear green but no fluorescence staining of the kinetoplast should be visible. The **HELIOS®** DNA Pattern Plus can be used to pre-classify the Positive Control as positive.

Each control must demonstrate the *expected reaction* in order to validate the test. If the *controls fail to appear as desc*ribed 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*® nDNA** kit provides a calculated endpoint titer for the Positive Control (PC). 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 twofold (+/-) with sample diluent. Include in the run one twofold dilution above and one twofold dilution below the PC endpoint titer.

Acceptance criteria is as follows:

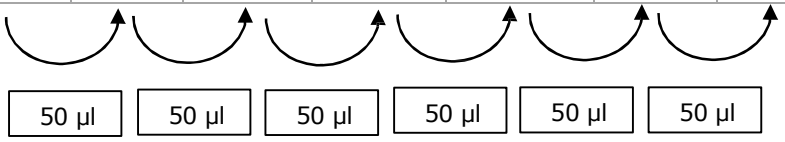
The final endpoint titer must be within +/- one dilution range around the CoA endpoint titer. If the diluted controls fail to show the proper reaction upon repeat testing, do not report the test results.

Example dilution scheme:

Values indicated on the CoA:

- PC pre-diluted at 1/10
- Calculated Endpoint Titer: 1/640

Dilution	1/20	1/40	1 /80	1/160	1/320	**1/640**	1/1280
Sample diluent volume	50 µl	50 µl	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 (1/320, 1/640, or 1/1280), 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

8.1 Interpretation of Results

In the presence of antibodies against dsDNA a fluorescence staining of the kinetoplast is visible. This fluorescence can be evenly dispersed over the whole kinetoplast, or appear as an outer rim of the kinetoplast.
In addition to the kinetoplast, also other cell structures can be stained simultaneously, e.g. the nucleus, the basal body or the flagellum. However, this is of no relevance for the positive/negative classification of the sample.
If the kinetoplast shows no fluorescence, the sample must be assessed as nDNA negative. Even if other cell structures like the nucleus, the basal body or the flagellum are stained, the sample has to be considered as negative.
Negative samples may also exhibit fluorescent staining of the entire cell with greater intensity than the Negative Control, but without staining of the kinetoplast.

The result evaluation should always be performed by comparing the samples with the positive and negative controls.

Sample Evaluation in accordance to staining of different cell-structures:

Result	Kinetoplast	Other cell structures (Nucleus, Basal Body, and/or Flagellum)
Positive	+	-
Positive	+	+
Negative	-	-
Negative	-	+

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®**).

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

8.2 Positive/Negative Confirmation (Manual Microscope)

Fluorescense Intensity Grading

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

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.

If a semi-quantitative titration is performed, the endpoint titer should be reported as the reciprocal of the last dilution in which 1+ apple-green fluorescent intensity with a clearly discernible staining of the kinetoplast is detected.

Example of Endpoint titer determination (below):

1:10 = 4+ Kinetoplast staining
1:20 = 3+ Kinetoplast staining
1:40 = 3+ Kinetoplast staining
1:80 = 2+ Kinetoplast staining
1:160 = 1+ Kinetoplast staining
1:320 = +/- Kinetoplast staining
1:640 = Neg

Report: 1:160

Report all titers and specific fluorescence staining seen.

Negative Results

1. A serum dilution is considered negative for dsDNA antibodies if the fluorescence staining of the kinetoplast is less than 1+.

2. A sample is considered negative for dsDNA antibodies if it exhibits less than 1+ fluorescence staining of the kinetoplast at the chosen screening dilution (1:10) and all greater dilutions.

Positive Results

1. A serum dilution is considered positive for dsDNA antibodies if a fluorescence staining of the kinetoplast is visible with an intensity of 1+.

2. A sample is considered positive for dsDNA antibodies if it exhibits a fluorescence staining of the kinetoplast with a fluorescent intensity of 1+ or greater at the chosen screening dilution (1:10) or greater.

3. Report all titers and specific fluorescence staining seen.

8.3 User confirmation of HELIOS® results

The **HELIOS®** 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 **HELIOS®** positive/negative result suggestion provided through the DNA Pattern Plus software tool. All suggested results obtained with the **HELIOS®** must be confirmed by trained personnel.

For further details on how to complete result confirmation, refer to the **HELIOS®** Instruction Manual.

9 LIMITATIONS OF PROCEDURE

1. All results must be confirmed by a trained operator.
2. nDNA serological test results should be used in conjunction with information available from the clinical evaluation and other diagnostic information.
3. Anti-dsDNA antibodies are rarely found in normal adult population. [13,14]
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. [13,15,16]
5. Antibodies to dsDNA are highly specific for SLE. Although low levels of nDNA antibody may be observed in other disease states e.g. Sjögren syndrome, Mixed Connective Tissue Disease (MCTD) and dermatomyositis, high titres of nDNA antibody are detected almost exclusively in SLE. [17]
6. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed or assay procedure used.
7. 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.
8. 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.
9. In general titers of 1:10 is considered low titer, 1:20 and 1:40 are considered medium titers, and 1:80 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**.

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.

The prevalence of dsDNA was analyzed with ***AESKUSLIDES*® nDNA** in a panel of 164 sera from healthy donors. Each serum was tested at a dilution of 1:10. Slides were processed manually according to the IFU and subsequently analyzed at the microscope by two independent readers.

Normal Range Study ***AESKUSLIDES*® nDNA**				
Number of Samples	Reader 1		Reader 2	
	n	%	n	%
negative	163	99.4	164	100
positive	1	0.6	0	0.0
Total	164	100.0	164	100.0

11 SPECIFIC PERFORMANCE CHARACTERISTICS

Precision at 1:10 Dilution

Within-Lab Precision of ***AESKUSLIDES*® nDNA** (Crithidia luciliae) processed on **HELIOS®** and manually, based on CLSI Guideline EP12-A2

Sample ID	Total number	HELIOS®		Total number	HELIOS® w/ Reader Confirmation		Manual Performance	
		% Negative	% Positive		% Negative	% Positive	% Negative	% Positive
S1	30	100	0	60	60.0	40.0	15.0	85.0
S2	30	33.3	66.7	60	0	100	0	100
S3	30	56.7	43.3	60	5.0	95.0	0	100
S4	30	46.7	53.3	60	8.3	91.7	0	100
S5	30	0	100	60	0	100	0	100
S6	30	6.7	93.3	60	0	100	0	100
S7	30	0	100	60	0	100	0	100
S8	30	0	100	60	0	100	0	100
S9	30	3.3	96.7	60	0	100	0	100
S10	30	83.3	16.7	60	100	0	98.3	1.7
S11	30	70.0	30.0	60	100	0	100	0

Between-Lab Precision of ***AESKUSLIDES*® nDNA** (Crithidia luciliae) processed on **HELIOS®** and manually.

% Agreement (95% CI)	HELIOS®	HELIOS® w/ Reader Confirmation	Manual Performance
Positive Agreement	88.4 (85.7 - 90.7)	97.9 (96.9 - 98.5)	99.5 (99 - 99.8)
Negative Agreement	77.8 (71.2 - 83.2)	99.4 (98 - 99.8)	99.7 (98.4 - 100)
Overall Agreement	86 (83.5 - 88.3)	98.2 (97.4 - 98.8)	99.6 (99.1 - 99.8)
FI Agreement	n/a	n/a	97.5 (96.6 - 98.1)

Within-Lab Precision of endpoint titer determination on ***AESKUSLIDES*® nDNA** processed on **HELIOS®** and processed manually, based on CLSI Guideline EP12-A2.

Percentage of samples that are +/-1 titer level	Total number	HELIOS® w/ Reader Confirmation	Manual Performance
Site 1	300	95.0 %	96.7 %
Site 2	300	90.7 %	92.7 %
Site 3	300	92.7 %	92.7 %
Combined Sites	900	92.8 %	94.0 %

Between-Lab Precision of endpoint titer determination on ***AESKUSLIDES*® nDNA** processed on **HELIOS®** and processed manually.

% Titer agreement (95% CI)	Site 1 vs. Site 2	Site 1 vs. Site 3	Site 2 vs. Site 3
HELIOS® w/ Reader Confirmation	88.7 (84.6 - 91.8)	80.3 (75.5 - 84.4)	85.7 (81.2 - 89.2)
Manual Performance	94.3 (91.1 - 96.4)	83 (78.3 - 86.8)	86.7 (82.4 - 90.1)

Sensitivity/Specificity at 1:10 Dilution

297 sera from patients with SLE and 479 sera from patients with other diseases have been analyzed on ***AESKUSLIDES*® nDNA** to calculate sensitivity and specificity. The table shows the summarized results from three study sites.

% Sensitivity (95 % CI)	% Specificitiy (95 % CI)
SLE (n=297)	Other Diseases (n=479)
Manual Performance	
20.8 (19 - 22.8)	96.8 (96.1 - 97.4)
HELIOS® w/ Reader Confirmation	
20.6 (18.8 - 22.5)	96.2 (95.4 - 96.8)
HELIOS®	
19.2 (16.7 - 21.9)	84.4 (82.4 - 86.2)

Interference Testing

Sera in the negative, high, medium and low positive range have been spiked with different potentially interfering substances. No interference has been detected with the tested substances at the indicated concentrations:

Interfering Substance	Minimum Concentration tested	Maximum Concentration tested
Bilirubin conjugated	0.1 mg/mL	0.4 mg/mL
Bilirubin unconjugated	0.1 mg/mL	0.4 mg/mL
Hemoglobin	2.5 mg/mL	5.0 mg/mL
Triglycerides	5 mg/mL	20 mg/mL
RF IgM	200 IU/mL	400 IU/mL
Rituximab	0.5 mg/mL	2.0 mg/mL
Methylprednisolone	0.2 mg/mL	0.8 mg/mL
Cyclophosphamide	1.0 mg/mL	4.0 mg/mL
Methotrexate	0.025 mg/mL	0.1 mg/mL
Azathioprine	0.0075 mg/mL	0.03 mg/mL
Belimumab	2 mg/mL	8 mg/ml
Hydroxychloroquine	0.006 mg/mL	0.024 mg/mL
Mycophenolat	0.012 mg/mL	0.048 mg/mL
Ibuprofen	0.5 mg/mL	2 mg/mL
Naproxen	0.5 mg/mL	2 mg/mL

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

12.2 TROUBLESHOOTING - HELIOS®

ERROR	POSSIBLE CAUSES	SOLUTION
Low cell density	- Cell lysis following prolonged contact with deionised water	- Refer to **HELIOS®** 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®** User Manual - Perform Weekly maintenance
	Serum does not cover the test well	
	Cross-reaction between the wells	
	Microscope incorrectly adjusted	- Refer to **HELIOS®** User Manual
Diffuse picture	I.F. Microscope is dirty. Possible scratches on the lense	- Clean the microscope according to its instructions - Refer to **HELIOS®** 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®** User Manual - Perform Weekly maintenance - Use only fresh sera - Check correct filter / objective

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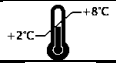
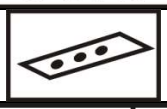
14 REGULATORY SYMBOLS

IVD	For in vitro diagnostic use
REF	Cataloge number
LOT	Lot
UDI	Unique device identifier
CE	EC Declaration of Conformity
	Instructions for use
	Use by
	Store at 2-8°C (35.6-46.4°F)
	Manufactured by
CONTROL +	Positive Control
CONTROL −	Negative Control
SEAL	Mounting media
CONJ	Conjugate
	Microscope slide
WASHB 10x	Wash Buffer
SB 1x	Sample Buffer
	Number of determinations
Rx only	For Prescription Use Only