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INSTRUCTION MANUAL

AESKUSLIDES® nDNA

Ref 53.100

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INSTRUCTION MANUAL

nDNA (*Crithidia luciliae*)

Standard Ref.	Description	Tests
53.100	**nDNA** (10 wells)	100

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nDNA (*Crithidia luciliae*)

1. INTENDED USE

***AESKUSLIDES*® nDNA (*Crithidia luciliae*)** is an indirect immunofluorescence assay to detect IgG antibodies to native double helix DNA in human serum.

The test-kit is for professional use only in laboratories.

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 (ds) DNA are regarded as being specific for systemic lupus erythematosus (SLE) and have been observed in approximately 50-80 % of the patients. Antibodies against dsDNA are found during active phases of SLE. The amount of serum concentration is positively correlated with the severity of the disease. Thus, detection of these autoantibodies is important for the diagnosis and the clinical monitoring of SLE. Consequently, it has been established as 1 of 11 ACR-criteria of SLE. Most patients with SLE display IgG class antibodies against dsDNA. These autoantibodies are associated with lupus nephritis Approximately 30 % of the SLE patients develop IgA class anti-dsDNA antibodies, additionally. There have been suggestions that the presence of these IgA class anti-dsDNA antibodies may define a certain subset of SLE patients. Indeed, studies demonstrated the association of this subclass with certain parameters of the disease activity, such as elevated erythrocyte sedimentation rate, or the consumption of complement component C3, as well as the clinical parameters of cutaneous vasculitis, acral necrosis and erythema. While no association was found for nephritis and arthritis.

IgM class anti-dsDNA antibodies were found in 52 % of the sera from patients with SLE. In contrast to IgG and IgA class autoantibodies, the subclass IgM antibodies do not correlate with disease activity. However, a highly significant negative correlation between IgM anti-dsDNA antibodies and lupus nephritis, including its laboratory parameters was demonstrated. Therefore, IgM class anti-dsDNA antibodies may indicate a subset of lupus patients being protected against the risk of developing nephritis.

Antigen Characterisation: 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 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 the aid of a fluorescent microscope.

3. KIT PROCEDURE

Please refer to the Assay Procedure listed in Common Instructions, Section 12, for detailed instructions. The following details shall be used for the nDNA kits:

- Counter staining time: 30-90 seconds
- Recommended Screening titer:
  - 1:10

4. INTERPRETATION

Crithidia luciliae contains a giant mitochondrion, also known as the kinetoplast which contains only dsDNA.

In the presence of antibodies against nDNA a homogenous fluorescence of the kinetoplast is visible or rather of the nucleus and the kinetoplast which is situated between the nucleus and the basal body near the flagellum.

The evaluation should always be performed with the positive and negative controls.

The sample must be assessed as nDNA negative if fluorescence of the basal body occurs near the flagellum origin or the nucleus only and no specific nDNA antibodies exist.

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

a.s.o.

Immunofluorescence shows a characteristic double spot pattern in the presence of anti-ds-DNA whilst only the nucleus is fluorescent with non-dsDNA nuclear antibodies. (dsDNA is an important autoantigenin SLE with a specificityof 95 %).

In SLE patients' antibodies to a variety of nuclear antigens may be observed. The strongest correlation to this disease is shown by Sm-antibody (glycoprotein) which appears as a speckled nuclear pattern in HEp-2 cells and nDNA-antibody (a peripheral or homogenous pattern in HEp-2 cells).

Antibodies to native double helix DNA 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.[1]

[1] Storch WB; Immunfluoreszenz-Fibel 2nd Edition; Blackwell Wissenschaftsverlag 1997

5. SPECIFIC PERFORMANCE CHARACTERISTICS

5.1. Sensitivity/specificity

***AESKUSLIDES*® nDNA** was compared to another commercially available indirect immunofluorescence test system using *Crithidia luciliae.* 297 sera from patients with SLE, and 479 sera from patients with other diseases (non-healthy controls) have been analyzed manually and evaluated by two readers. A cutoff dilution of 1:10 was used to determine positive samples. Clinical samples represented a full spectrum of autoimmune diseases.

In the comparison for ***AESKUSLIDES*® nDNA** we obtained the following results:

a. Table 1: Confidence Intervals Combined Data Set

		Predicate		
		Positive	Negative	Total
***AESKUSLIDES*® nDNA**	Positive	79	14	93
	Negative	108	575	683
	Total	187	589	776

Sensitivity = 79/187 = 42.2 % (95^{th} % CI: 35.4 - 49.4 %)
Specificity = 575/589 = 97.6 % (95^{th} % CI: 96.1 - 98.6 %)

95 % confidence intervals are score confidence intervals according to Wilson's method[2].

5.2. Reproducibility and Precision

Within-laboratory and between-laboratory studies were performed to demonstrate reproducibility of ***AESKUSLIDES*® nDNA**. 11 serum samples were assayed on ***AESKUSLIDES*® nDNA** on five days, with two runs per day and three replicates per run at three study sites. The sample set, including negative, low, medium, and high positive samples were processed manually, and by **HELIOS® Automated IFA System**, and evaluated by two readers. Positive and negative agreements were ≥ 90 %. Samples showed consistent fluorescence intensities on all wells tested.

[2] Wilson, E. B. (1927). Probable Inference, the Law of Succession, and Statistical Inference. Journal of the American Statistical Association, 22(158), 209–212. https://doi.org/10.2307/2276774

6. DATA INTERPRETATION SHEET

nDNA

Date:	Lot.:
Slide No.:	Operator:

Well No.	ID	Dilution factor	F.I.	Nucleus	Kinetoplast	Basal body	Results	Notes
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								

7. STANDARD KIT CONTENTS

		SLIDES				CONJUGATE			POSITIVE CONTROL		
Kit Ref.	**Kit Description**	**Ref.**	**Wells**	**Coated with**	**Quantity**	**Ref.**	**Description**	**Quantity**	**Ref.**	**Description**	**Quantity**
53.100	**nDNA** (10 wells)	**S53.100**	10	*Crithidia luciliae* cells	10 x	**C53.100**	**IgG** Capped blue: slightly blue coloured solution. Containing: BSA, Fluorescein (FITC) labelled Anti-human Antibody	1 x 5 ml	**PC53.100**	nDNA positive control. Capped red: colourless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	1 x 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 REAGENT CONTENTS.

8. COMMON REAGENTS CONTENTS

b. Common Reagents

Ref.	Reagent	Quantity / Volume		Description	Ready to use
NCIFA	Negative Control	1 x	0.5 ml	Capped green: colorless solution. Containing: Human serum (diluted), sodium azide <0.1 % (preservative)	YES
*** EBIFA**	Evans Blue 0.2 %	1 x	1.5 ml	Capped white: Blue colored solution Containing: PBS, Evans Blue. Dilute the Evans Blue 0.2 % 1:3000 in 1 x WBIFA	NO
MMIFA	Mounting Medium	1 x	8 ml	Validated for use with the HELMED® Capped white: colorless solution Containing: PBS, Glycerin.	YES
WBIFA	Wash buffer (10 x)	1 x	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
SBIFA	Sample buffer (1 x)	1 x	70 ml	Capped white: colorless solution for the dilution of patient sera Containing: BSA, PBS,sodium azide (preservative).	YES

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

c. 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

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

9. 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.**

10. PRECAUTIONS OF USE

d. 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 being irritant 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.

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

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

f. Preparation prior to pipetting

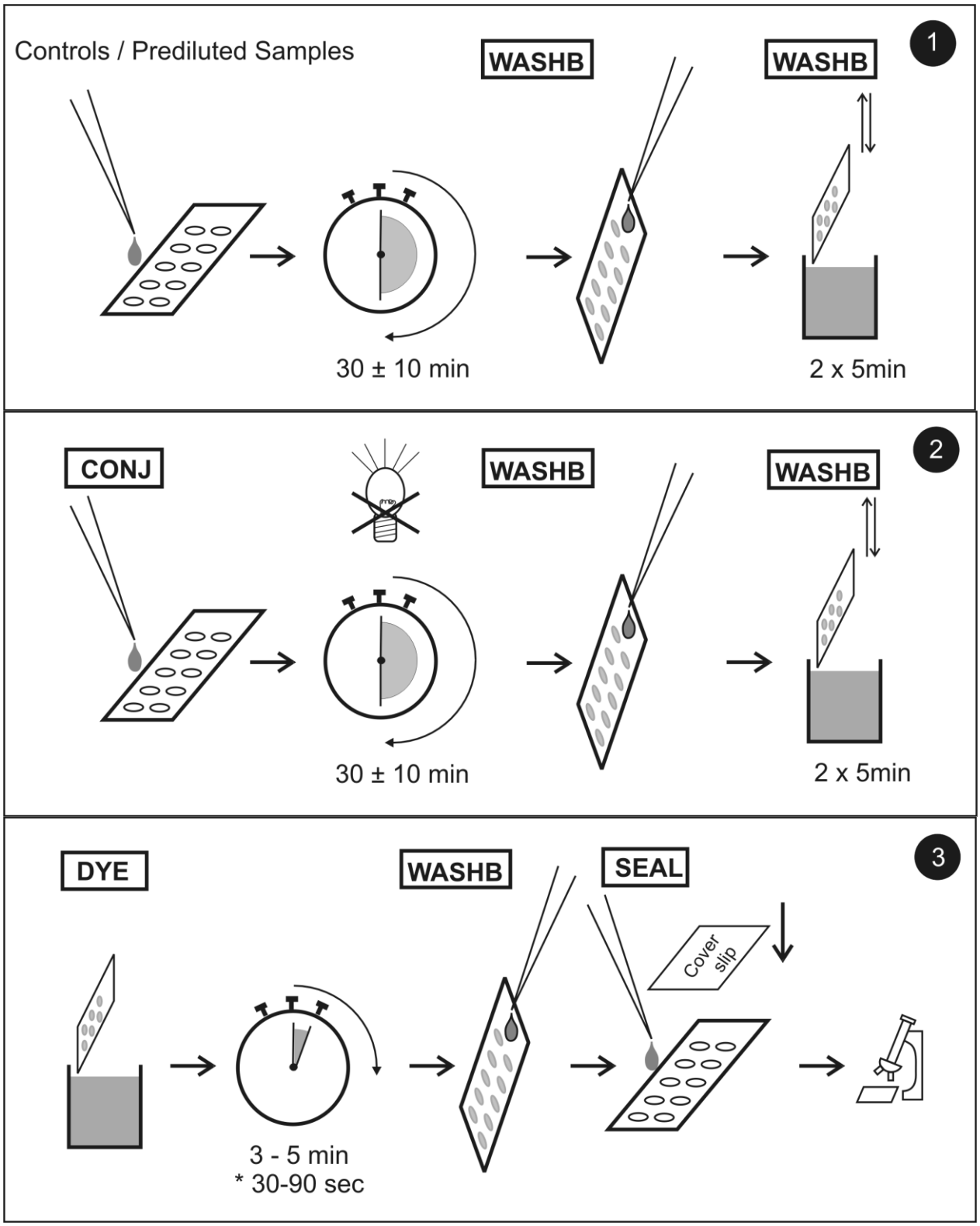
Allow all components to reach room temperature (20-26 °C / 68-78.8 °F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

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 ***Kit Procedure*** section above according to the product reference that 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

g. 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 pipette with slide surface. Make sure that each well is completely covered with a corresponding serum. It is important to use as much test material as necessary to cover the well completely. But avoid a running between the wells because this may cause incorrect results.
3.	**Washing**: After incubation remove slides from incubator tray and rinse briefly with wash buffer using a squeeze wash bottle. Do not squirt buffer directly on the wells. NOTE: To prevent cross contamination tilt slide first 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. Please 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 slide immediately to incubator tray and cover each well with an adequate volume of FITC-conjugate and make sure that the well is covered completely. Incubate slide(s) 30 minutes± 10 minutes at room temperature in the dark.
5.	**Washing**: After incubation remove slides from incubator tray and rinse briefly with wash buffer using a squeeze wash bottle. Do not squirt buffer directly on the wells. Wash slide(s) 10 minutes with wash buffer in a slide staining dish. For optimal results, change the buffer solution 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. Refer to ***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 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).

h. Work flow


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
	- Elektronische 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	