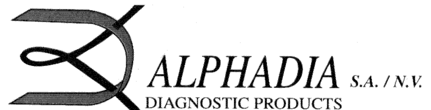

GB

IFA ANA-HEp-2 - IgG

1. INTENDED USE

Immunofluorescence assay for the detection of IgG Antinuclear antibodies in human serum and plasma with HEp-2 cells

REF			IVD	
REF	VH0050	∇ 50	IVD	kit
REF	VH0100	∇ 100	IVD	kit
REF	VH0100C	∇ 100	IVD	kit
REF	VH0250	∇ 250	IVD	kit
REF	VH0300	∇ 300	IVD	kit
REF	VH0300S	∇ 300	IVD	kit
REF	VH0500	∇ 500	IVD	kit
REF	VH0500C	∇ 500	IVD	kit

2. PRINCIPLE OF THE ASSAY

The detection of antibodies is based on the principle of an Indirect Immunofluorescence Assay (IFA). The slides are coated with HEp-2 cells (inactivated). Any specific antibodies present in the patient's sample are bound during the first incubation. After removing unbound material by washing, the presence of specific antibodies is detected using Anti-Human -IgG conjugate during the second incubation.
Excess FITC conjugate is then removed. The formation of a stable three-part complex consisting of fluorescein antibody bound to human antibody, which is bound to antigen can be visualized with the aid of a fluorescence microscope.

3. DIAGNOSTIC RELEVANCE AND INTERPRETATION OF RESULTS

According to the staining of **nucleus and cytoplasm of HEp-2** cells different patterns have been described:

Homogenous staining of nucleus

1. Homogeneous (DNA, histones)
Staining of the entire nucleus, with or without apparent masking of the nucleoli.. The chromosome region of mitotic cells exhibits a bright positive staining pattern.
Disease association: High titres are suggestive of SLE, histone antibodies alone have a high association with drug-induced lupus.

Homogenous/ Peripheral (DNA, histones)
Staining primarily around the outer region of the nucleus with weaker staining in the centre. The chromosome region of mitotic cells shows a positive staining pattern.
Disease association: High titres are suggestive of active SLE.

2. Speckled

Fluorescent aggregates throughout the nucleus present as varying fluorescence of fine to coarse speckles depending on the type of antibody present. The chromosome region of mitotic cells is usually negative.

Sm and nRNP antibodies usually present as coarse speckles, nucleoli often left unstained, chromosome region of mitotic cells negative.
SS-A and SS-B antibodies present as small uniform speckles in a uniform distribution with the chromosome region of mitotic cells negative.
Scl-70 antibodies are indicated as fine speckles with positive staining of the chromosome region and nucleoli of mitotic cells.
PCNA antibodies present as varying fluorescence of fine to coarse speckles in approximately 30-60% of cells.
Disease association: These antibodies are frequently present in patients with SLE, MTCD, Scleroderma, Sjörgen syndrome and Poliomyositis.

3. Centromere (centromere proteins of chromosomes)

Dotted speckles throughout the nucleus, the number corresponds to a multiple of the normal chromosome number. The staining pattern of the mitotic cells shows the configuration of the chromosomes (e.g. pairs of dots arranging themselves in an equatorial plane during metaphase).
Disease association: Centromere antibodies are considered as marker of CREST syndrome, infrequently found in diffuse scleroderma and Raynaud's disease.

4. Nucleolar (PMScl, RNA polymerase 1, fibrillarin)

Fluorescent staining of the nucleoli within the nucleus, sharply separated from the unstained nucleoplasm. The nucleolar fluorescence can be speckled, homogenous or clumpy.
Disease association: High titres are highly specific for PSS and overlap with polymyositis, with lower titres found in SLE, Sjögren's syndrome, Raynaud's disease.

5. Anti-spindle antibodies (spindel apparatus in cells undergoing mitosis)

The centrosomes in mitotic cells are connected with a network of threads.
Disease association: Less frequent in some autoimmune and nonautoimmune diseases (RA, SLE, PBC, Carpal Tunnel Syndrome).

Staining of cytoplasm

Fibrous or granular fluorescence in the cytoplasm.
Ribosomal RNP
Diffuse granular fluorescence throughout the cytoplasm (confirmation on appropriate tissue sections is recommended).
Jo-1 (PL-7, PL-12)
Fine speckles usually with low fluorescent intensity concentrated in the perinuclear region.
Mitochondrial
Fine speckles throughout the cytoplasm in a fibrous network (confirmation on appropriate tissue sections is recommended).
Cytoskeleton
Fluorescent strands in the cytoplasm with fibrils extending from the cell membrane, caused by antibodies to actin (smooth muscle antibodies) and other components of the cytoskeleton (e.g. tubulin, vimentin), confirmation on appropriate tissue sections is recommended.
Disease association: These fluorescence patterns has been found by different autoimmune and nonautoimmune diseases.

4. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

In tests to determine interlot and intralot reproducibility, the controls showed no discrepancies in staining patterns and staining intensity.

5. Bib

1. R.L. Humbel: Detection of antinuclear antibodies by immunofluorescence Manual of Biological Markers of Diseases Kluwer Acadecmic Publishers Dordrecht A1: 1-6 1993
2. M.J. Fritzler and E.M. Tan: Antinuclear Antibodies and the Connective Tissue Diseases Laboratory Diagnostic Procedures in the Rheumatic Diseases 1985
3. R.L. Humbel: Autoanticorps et Maladies Auto-immunes Elsevier 2e edition Elsevier 1997
4. Wulf B. Storch: Immunfluoreszenzfibel 2. Auflage Blackwell Wissenschaftsverlag1997

6. KIT COMPONENTS

Number and volume of the kit components are indicated on kit label.

1. SLIDES SLIDES
slides coated with cell substrates

10x5 wells (VH0050); 10x10 wells (VH0100, VH0100C); 50x5 wells (VH0250)); 30x10 wells (VH0300); 25x12 wells (VH0300S); 50x10 wells (VH0500, VH0500C)

2. FITC ANTI-HUMAN CONJUGATE CONJ FITC IgG
ready-to-use anti-human IgG conjugate
preservative: <0,1% sodium azide.

2 ml (VH0050); 3 ml (VH0100); 2x3 ml (VH0100C); 3x3 ml (VH0250; VH0300; VH0300S)); 1x 15 ml (VH0500); 2x13.5 nl (VH0500C)

3. NEGATIVE CONTROL CONTROL -
human serum, ready to use
preservative: <0,1% sodium azide.

0,5 ml (VH0050); 1 ml (VH0100, VH0100C); 2,5 ml (VH0250), 3 ml (VH0300, VH0300S); 2x 2,5 ml (VH0500, VH0500C)

4. POSITIVE CONTROL CONTROL + hom
human serum (ANA homogenous type), ready to use
preservative: <0,1% sodium azide.
The titre is reported on the label

0,5 ml (VH0050); 1 ml (VH0100, VH0100C); 2,5 ml (VH0250), 3 ml (VH0300, VH0300S); 2x 2,5 ml (VH0500, VH0500C)

5. EVANS BLUE EVBL
ready to use

3 ml (VH050, VH0100, VH0100C); 3 x 3 ml (VH0250, VH0300, VH0300S); 5x 3 ml (VH0500, VH0500C)

6. MOUNTING MEDIUM MM
ready to use

3 ml (VH0050, VH0100, VH0100C); 3 x 3 ml (VH0250, VH0300, VH0300S); 1x 15 ml (VH0500, VH0500C)

7. PBS-BUFFER BUF PBS
powder

2x (VH0050, VH0100, VH0100C); 5x (VH0250); 6x (VH0300, VH0300S); 10x (VH0500, VH0500C)

The safety data sheet (MSDS) is available upon request.

7. STORAGE AND STABILITY

Store all reagents at 2-8°C. Protect them from intense light and do not freeze. The expiration date of each component is indicated on the respective vial label. Do not use reagents beyond the expiration date.
The diluted BUF PBS is stable up to 4 weeks when stored at 2-8°C. Use only SLIDES with an intact vacuum packaging.

8. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- distilled water
- graduated cylinder
- coplin jars or staining dishes
- plastic squeeze bottle
- test tubes for sample dilution
- cover slips (24x60 mm)
- volumetric pipette
- moist chamber
- timer
- fluorescence microscope with FITC system,
  (excitation wavelength 490 nm, emission wavelength 510 nm)

9. WARNINGS OR PRECAUTIONS

SAFETY PRECAUTIONS

The IFA test is for IVD use only.

1. Do not mix lot specific reagents, such as SLIDES, controls and CONJ FITC from different kit lots. BUF PBS, MM and EVBL can be used for all IFA tests.
2. Seal all bottles properly after use in order to avoid bacterial contamination. All samples and kit components should be considered potentially infectious. All control samples have been tested for Hepatitis Bs antigen, anti-HIV I and II, anti-HCV (CE/FDA) and found to be negative.
3. The coated SLIDES are inactivated. However, normal laboratory precautions should be maintained when handling with infectious material. Do not pipette by mouth.
4. Avoid contact with skin and mucous membranes when handling reagents, which contain preservatives (see kit contents). Wash thoroughly with water in case of contact and possibly look up a doctor.
5. Controls containing sodium azide may react with lead and copper plumbing, building up explosive metal acids. Flush with sufficient water when disposing of reagents.
6. For disposal the legal regulations have to be followed.

10. SPECIMEN COLLECTION AND STORAGE

1. Only qualified and well-trained employees should carry out the assay procedure.
2. The instruction for use describes the applicable test method. In case of modification or applications others than the intended use, or the use of automatic processors, the user has to validate the procedure and take the responsibility for it.
3. Microbial contaminated specimen may cause interference.
4. Lipaemic, haemolytic or icteric samples should only be tested with reservations although in our testing no negative influence has been found.
5. Suitable specimens are serum or plasma (heparinized, EDTA) samples obtained by standard laboratory techniques.
6. The samples should not be heat-inactivated since non-specific results may occur.
7. Patient samples should be stored at 2-8°C.
   For long-term storage -20°C or lower is recommended.
   Avoid repeated freeze-thaw cycles.
8. **Note:** Diluted patient samples must be used on the same day.

11. REAGENT PREPARATION

Bring all reagents to room temperature prior to use!

Prepare BUF PBS: Completely dissolve one vial of BUF PBS in 1 litre of distilled water. Reconstituted buffer solution should have a pH of 7,3 to 7,6.

Dilution of samples: Dilute patient sample with BUF PBS according to test demands (screening titre).

Controls are ready to use.

12. PIPETTING AND INCUBATION STEPS

Screening Titre : 1:80 or 1:160

Note! Do not allow wells to dry at any time during the test procedure.

1. Take required SLIDES out of the foil packets shortly before use and identify SLIDES using a permanent marking pen. Do not touch the wells.

2. **First incubation:**
   Pipette 1 drop of each control and 20 – 50 µl of each diluted sample onto the respective wells (covered completely) being careful not to touch cell substrate with pipette tip. The controls should be carried out for every testrun. Place SLIDES into a well-closed moist chamber to prevent drying.

   Incubate SLIDES 30 minutes at room temperature.

3. **First wash step:**
   Rinse the SLIDES gently with BUF PBS, using a squeeze wash bottle. Do not focus the buffer stream directly onto the wells. To prevent cross contaminations avoid rinsing from one well across other wells. For ten-well slides run PBS-stream from the midline of the slides successive along both rows to the edge of the SLIDES. Soak the SLIDES three times for **5 minutes** with BUF PBS in a coplin jar or staining dish with BUF PBS.

4. **Second incubation:**
   After the washing procedure, shake of excess BUF PBS and place SLIDES back into moist chamber. Immediately add 1 drop of CONJ FITC to each well.

   Incubate SLIDES 30 minutes at room temperature in the dark.

5. **Second wash step:**
   see **First wash step**

6. **Counterstain:**
   Add 5 drops EVBL to 100 ml BUF PBS solution in a staining dish and stain SLIDES for 5 minutes. EVBL covers unspecific background fluorescence.

7. Remove SLIDES from the staining dish, rinse briefly with BUF PBS, shake off the excess BUF PBS and apply 2-3 drops of MM across the slides. Gently lower the coverslip from the bottom to the top of the slides, avoid air bubbles.

8. Read SLIDES within 30 minutes at 400-800x total magnification, using a fluorescence microscope (FITC filtercombination). Avoid longer exposition of one field of vision to minimize bleaching of FITC fluorescence.

13. SUGGESTIONS FOR TROUBLESHOOTING

In case that the IFA instructions are followed strictly, the reagents are handled with care and the samples and reagents are pipetted carefully, the following kinds of errors can be avoided to a large extend.

ERROR	POSSIBLE CAUSES
Cross contamination	-Too much test material -Fluid remaining between the wells, should be carefully dried around outside edges if necessary
Too few cells or organisms	-Cell lysis following prolonged contact with deionised water (observe the wash procedure) -Buffer squirted directly on the substrate in the well (observe the wash procedure) -Proteolytic enzymes have attacked the substrate (inactivate sample) -In covering the monolayer was disturbed
Inhomogenous fluorescence	-sample dried in the well, fluorescence stronger at the edge (moist environment) -sample does not cover the test well (air bubbles/ sample at the hole well) -Buffer crystals on the SLIDES (wash) -Microscope incorrectly adjusted (check the adjustment of the microscope) -Unsuitable immersion oil
Unclear picture	-too much MM or air pockets -Microscope is dirty
Little or no fluorescence	-Bacterial contamination of the sample or CONJ FITC (check storage) -Microscope not adjusted -pH-value (7,3 – 7,6) of BUF PBS too low -CONJ FITC exposed to light (store CONJ FITC protected from the light)
Background fluorescence	-SLIDES dried in a hot air stream (do not use a hairdryer and do not let the wells dry) -Lipaemic, haemolytic or bacterial contamination sample (use only fresh sample) -Marking the SLIDES with a wax pencil produces a film on the SLIDES (use a waterproof glass marker)

14. VALIDITY OF THE ASSAYS

All controls should be carried out with every test run.
The fluorescence intensity of the CONTROL+ is shown in the Quality Control Certificate. If controls give invalid levels then results from test samples are invalid too and retesting is required.

15. READING OF THE RESULTS

Fluorescence intensity:

The fluorescence intensity may be semi-quantified following our introduction:

3+ bis 4+ = maximal fluorescence, brilliant yellow-green
2+ = less brilliant yellow-green fluorescence
1+ = definite but dull yellow-green fluorescence
Equivocal – (+) = very dim yellow-green fluorescence

The degree of intensity is not of clinically relevance and has only limited value as an indicator of titre. Differences in microscope optics, filters and light source may result in differences of +1 or more in intensity.

Negative result
A serum dilution (screening titre) is considered negative for ANA if the HEp-2 cells exhibit less than 1+ fluorescence and lack a clearly discernable pattern. Cells will appear reddish-orange due to Evans blue counterstain.

Positive result
A serum dilution (screening titre) is considered positive for ANA if the fluorescent staining is at an intensity of 1+ or greater with a clearly discernable pattern of fluorescence.

A. SEMIQUANTITATIVE TITRE

For the semi-quantification of the test results the CONTROL- and the CONTROL+ must be tested with every test run. The fluorescent intensity of the sample can then be compared visually, or with the aid of an automatic processor, with the fluorescent intensity of the CONTROL+.

B. QUANTITATIVE TITRE

For quantification of the test results, the samples must be diluted to the endpoint titre. The endpoint titre is determined as the last dilution in which a **equivocal – (+)** reaction is visible.

Sample titration:

If the fluorescent intensity of the sample is **<** the fluorescent intensity of the Positive control, please dilute the sample to the endpoint titre of the Positive control (the titre is reported on the label)

If the fluorescent intensity of the sample is ≥ the fluorescent intensity of the Positive control, please dilute the sample higher than the endpoint titre of the Positive control (the titre is reported on the label)

Regarding diagnostic relevance and interpretation of results see **page 1**

16. Symbole nach IVD/ symbols used with IVD devices/ Symbole / Símbolos/ Simboli/ Simbolos/ Symboly

SLIDES	Objektträger/ slides/ lames/ portaobjetos/ vetrini/ lâminas
BUF PBS	PBS-Puffer/ PBS-buffer/ Tampon PBS/ Buffer PBS / tampone PBS/ Tampão PBS
CONJ FITC	FITC-Konjugat/ FITC conjugate/ Conjugue FITC/ conjugado FITC / FITC Coniugato/ Conjugado FITC
CONTROL+	Positive Kontrolle/ positive control/ Contrôle positif/ control positivo/ controllo positivo/ controle positivo
CONTROL-	Negative Kontrolle/ negative control/ Contrôle négatif/ control negativo/ controllo negativo/ controle negativo
MM	Einschlussmedium/ Mounting medium/ Liquide de Montage/ Medio de Montaje/ Mezo di montaggio/ Meio de Montagem
Bib	Literatur/ Literature/ Littérature/ Bibliografia/ Bibliografía/ Literatura/
LOT	Charge/ lot/ Lot/ lote/ carcia/ lote
IVD	In-vitro-Diagnostikum/ in vitro diagnostic/ Diagnostic in vitro / diagnóstico In-vitro/
REF	Artikel Nr./ reference or order number/ Référence ou numéro de commande/ referencia o número de pedido/ codice di riferimento o di commissione/ referência ou número de encomenda
∇ 100	100 Bestimmungen/ tests/ testś / determinazioni/ testes
	Gebrauchsanweisung beachten/ consult instructions for use/ consulter le mode d'emploi/consultar las instrucciones de uso/ consultare le istruzioni per l'uso/ consultar instruçõesde uso
	Temperaturgrenzen/ temperature limitation/ Limites de température/ Limites de temperatura/ Limiti di temperatura/ Limites de temperatura/
	Verfallsdatum:/ expiry date/ date d'expiration/ Fecha de caducidad/ Data di decadenza/ Limite de validade/ Datum Expirace
	Hergestellt von/ manufactured from/ fabriqué par/ elaborado por/ fabbricato da/ produzido por

ALPHADIA, Avenue Vésale 26, B-1300 Wavre,
BELGIUM Tel.: +32 10 24 26 49 Fax: +32 10 24 55 99
email: contact@alphadia.be

CE

Symbole nach IVD/ symbols used with IVD devices/ Symbole / Símbolos / Simboli / Simbolos IVD

Symbol	Meaning
SLIDE	Objektträger/ slides/ lames/ portaobjetos/ vetrini/ lâminas
BUF PBS	PBS-Puffer/ PBS-buffer/ Tampon PBS/ Buffer PBS / tampone PBS/ Tampão PBS
CONJ FITC	FITC-Konjugat/ FITC conjugate/ Conjugue ITCF/conjugado FITC / FITC Coniugato/ Conjugado FITC
CONTROL +	Positive Kontrolle/ positive control/ Contrôle positif/ control positivo/ controllo positivo/ controle positivo
CONTROL -	Negative Kontrolle/ negative control/ Contrôle négatif/ control negativo/ controllo negativo/ controle negativo
EVBL	Evans Blue/ Evans Blue/ Bleu Evans/ Azul de Evans/ Blue di Evan/ Azul de Evans
MM	Einschlussmedium/ Mounting medium/ Liquide de Montage/ Medio de Montaje/ Mezo di montaggio/ Meio de Montagem
SPE DIL	Probenverdünnungspuffer/ dilution buffer/ Tampon de dilution/ reactivo compensador/ soluzione tampone/ estabilizador de diluição
LOT	Charge/ lot/ Lot/ lote/ carcia/ lote
IVD	In-vitro-Diagnostikum/ in vitro diagnostic/ Diagnostic in vitro/ diagnóstico in-vitro/ In-vitro diagnostic/ diagnóstico In-vitro
REF	Artikel Nr./ reference or order number/ Référence ou numéro de commande/ referencia o número de pedido/ codice di riferimento o di commissione/ referência ou número de encomenda
RTU	gebrauchsfertig/ ready to use/ Prêt à l'emploi/ liso para su uso/ pronto per l'uso/ pronto para usar
100	100 Bestimmungen/ tests/ testś/ / determinazioni/ testes
	Gebrauchsinformation beachten/ consult instructions for use/ consulter le mode d'emploi/ consultar las instrucciones de uso/ consultare le istruzioni per l'uso/ consultar instruções de uso
	Temperaturgrenzen/ temperature limitation/ Limites de température/ Limites de temperatura/ Limiti di temperatura/ Limites de temperatura
	Verfallsdatum:/ expiry date/ date d'expiration/ Fecha de caducidad/ Data di decadenza/ Limite de validade
	Hergestellt von/ manufactured from/ fabriqué par/ elaborado por/ fabbricato da/ produzido por

D IFA TESTDURCHFÜHRUNG

Patientenserum mit BUF PBS entsprechend dem angegebenen Suchtiter verdünnen.
Kontrollen sind RTU!
Benötigte Anzahl SLIDE entnehmen und beschriften. SLIDE nur an den Rändern anfassen.
1. / 2. Inkubation in einer feuchten Kammer.

▼

20µL (1 Tropfen) KONTROLLE UND VERDÜNNTE PATIENTENPROBE AUFTRAGEN

▼

1. INKUBATION 30 min. 23+/- 1°C

▼

1. WASCHSCHRITT

▼

Nach 1. Inkubation SLIDE 10min. in einer Küvette mit hergestelltem BUF PBS waschen. Puffer nach 5min. wechseln. Auftragsstellen nicht austrocknen!

▼

1Tropfen CONJ FITC je Testfeld auftragen

▼

2. INKUBATION 30MIN. 23+/- 1°C

▼

2. WASCHSCHRITT (siehe 1. Waschschritt)

▼

Pro **150 ml hergestelltem** BUF PBS in der Küvette 5 Tropfen EVBL zugeben, SLIDE hineinstellen.

▼

GEGENFÄRBUNG MAX. 5 MIN.

▼

SLIDE kurz mit hergestellter BUF PBS abspülen. Zwischen die Testfelder kleine Tropfen MM geben. Deckglas auflegen (Luftblasen vermeiden).

▼

Mit Fluoreszenzmikroskop (FITC-Filterkombination) bei 400-800-facher Vergrößerung beurteilen.

GB IFA ASSAY PROCEDURE

Dilute patient sera with BUF PBS solution to the appropriate dilution for the screening titre.
Controls are RTU!
Remove required SLIDE from pouche, mark them.
Do not touch the wells.
1. / 2. Incubation in a incubator tray.

▼

20µL (1 drop) OF EACH DILUTED SERUM AND CONTROLS INTO THE APPROPRIATE WELLS

▼

1. INCUBATION 30MIN. 23+/- 1°C

▼

1.WASH STEP

▼

After 1. Incubation wash SLIDE 10min. in BUF PBS . Change buffer solution after 5min. Slide wells do not dry out!

▼

Cover each well with 1 drop of CONJ FITC

▼

2. INCUBATION 30MIN. 23+/- 1°C

▼

2.WASH STEP (see 1. WASH STEP)

▼

Per **150 ml** BUF PBS solution, add 3-4 drops EVBL and immerse SLIDE .

▼

COUNTERSTAIN MAX. 5 MIN.

▼

Rinse SLIDE with BUF PBS solution.
Add 2-3 drops of MM along the midline of each slide.Place coverslip in position (avoiding air pockets).

▼

Read SLIDE immediately at 400-800x total magnification with a fluorescent microscope.

(F) TECHNIQUE D´IFI

Amener les sera des patients à la dilution de screening adéquate en les diluant avec le BUF PBS.
Les contrôles sont RTU !
Enlever la SLIDE du sachet.Ne pas toucher les puits.
1. / 2. Les incubations se font en chambre humide.

▼

Déposer 20µl (1 goutte) de chaque serum dilué ou des contrôles dans un puits

▼

1. Incuber 30 minutes, à 23 +/- 1°C

▼

Premier LAVAGE

▼

Après incubation laver la SLIDE 10 min. dans BUF PBS
Changer le BUF PBS après 5 min.
Ne jamais sécher les puits.

▼

Déposer dans chaque puits 1 goutte de CONJ FITC

▼

2. Incuber 30 minutes à 23 +/- 1°C

▼

Second LAVAGE (volr 1. Lavage)

▼

Ajouter **5 gouttes d´EVBL** par **150 ml** BUF PBS dans le dernier bain. Immerger les SLIDE

▼

Contre-colorer max. 5 min.

▼

Rincer les SLIDE avec du tampon BUF PBS
Déposer 2-3 gouttes de MM sur la ligne médiane des lames. Déposer la lamelle couvre-objet en évitant les bulles

▼

Lire les SLIDES immédiatement à 400-800x, au microscope à fluorescence

(E) DESAROLLO TEST IFA

Diluir el suero del paciente con BUF PBS en proporción adecuada para titulación de screening.
Controles están RTU (listos para uso) !
Retirar el numero adecuado de SLIDE de su envuelta, marcarlos. No tocar los pocillos.
Incubaciones 1 y 2 en placa de incubación

▼

20µL (1 gota) de cada suero diluido y controles en los pocillos correspondientes

▼

INCUBACIÓN 1, 30 min, 23°C +/- 1°C

▼

Primer LAVADO

▼

Después del lavado. Incubar el SLIDE 10 min.con BUF PBS]. Cambiar el BUF PBS cada 5 min. No dejar que los pocillos se sequen.

▼

Cubrir cada pocillo con 1 gota de CONJ FITC

▼

INCUBACIÓN 2, 30 min. 23°C +/- 1°C

▼

Segundo LAVADO (Ver lavado 1)

▼

Para **150 ml** BUF PBS anadir 5 gotas EVBL **y sumergir el** SLIDE .

▼

SEGUNDA TINCIÓN MAX. 5 MIN

▼

Aclarar el SLIDE con solución BUF PBS . Anadir 2-3 gotas de MM en el centro del porta. Cubrir con un cubre (evitando las burbujas)

▼

Leer inmediatamente los SLIDE a 400-800x con un microscopio de fluorescencia.

(I) TEST IFA - PROCEDURA

Diluire I sieri dei pazienti con soluzione BUF PBS fino alla diluizione appropriata per il titolo screening. I controlli sono RTU!
Estrarre le SLIDE necessari dall´involucro, siglarli.Non toccare i pozzetti. Eseguire la 1ª e 2ª incubazione in una camera da incubazione

▼

20µL (1 goccia) di ogni siero diluito e dei controlli nei pozzetti

▼

1ª incubazione, 30 minuti a 23 ± 1°C

▼

Prima fase di lavaggio

▼

Dopo la prima incubazione lavare le SLIDE per 10 minuti in BUF PBS. Cambiare il BUF PBS dopo 5 minuti. Non lasciare che i pozzetti si secchino!

▼

Coprire ogni pozzetto con una goccia di CONJ FITC

▼

2ª incubazione, 30 minuti a 23+/- 1°C

▼

Seconda fase di lavaggio (vedere la prima fase)

▼

Ogni **150 ml** di BUF PBS aggiungere 5 gocce di EVBL e immergere i SLIDE .

▼

COLORAZIONE MAX 5 minuti
.

▼

Risciacquare le SLIDE con BUF PBS .
Aggiungere 2-3 gocce di MM lungo la linea mediana di ogni vetrino. Mettere un coprivetrino in posizione (evitare la formazione di bolle d´aria).

▼

Leggere immediatemente le SLIDE con un microscopio a fluorescenza usando un ingrandimento 400-800x.

(P) TÈCNICA IFA

Diluir as amostras com solução BUF PBS na dilução apropriada para a pesquise do titulo.
Controlos estão RTU**!**
Retirar os necessàrios SLIDE da embalagem, marcar.
Não tocar nos poços.
1./2. Incubar num suporte de incubação.

▼

20µL (1 gota) DE CADA AMOSTRA DILUIDA E DOS CONTROLOS NOS POCOS APROPRIADOS

▼

1. INCUBAÇÃO 30MIN. a 23+/-1°C

▼

1. PASSO DE LAVAGEM

▼

Após 1. incubação lavar SLIDE 10min. em BUF PBS
Mudar a solução tempo após 5 min.
Os poços das SLIDE não podem secar.

▼

Cobrir cada poço com 1 gota CONJ FITC

▼

2. INCUBAÇÃO 30MIN. 23+/- 1°C

▼

2. PASSO DE LAVAGEM (Ver 1. passo de lavagem)

▼

Por **150 ml** solução BUF PBS, adicionar 5 gotas EVBL **a imergir** SLIDE .

▼

CONTRASTE MAX. 5 MIN.

▼

Enxaguar SLIDE com solução BUF PBS.
Adicionar 2-3 gotas de MM ao longo da linha média de cada SLIDE. Colocar as lariolas em posição (evitar bolhas de ar)

▼

Ler SLIDE imediatamente a 400-800x da ampliação total com um microscópio de fluorescénola.