ALPHADIA S.A. / N.V.
DIAGNOSTIC PRODUCTS

IFA ANCA TEST SYSTEM

For *In Vitro Diagnostic* Use

AD CAN60 60 Tests
AD CAN120 120 Tests

INTENDED USE

The ALPHADIA immunofluorescence assay (IFA) is for the in vitro diagnostic detection of anti-neutrophil cytoplasmic antibodies in human serum using ethanol fixed human neutrophils as substrate. The test is intended as an aid in the diagnosis of Wegener's granulomatosis, Idiopathic Crescentic Glomerulonephritis, Microscopic Polyarteritis, Pulmonary Renal Syndrome.

CLINICAL SIGNIFICANCE

In 1939 Friedrich Wegener described a disease called "rhinogenic granulomatosis". Early in disease granulomas are found that, like the rheumatic nodules, develop independently from the vessels in the connective tissue. During the generalized stage, one will usually find the granuloma in close relation to the vessels in different regions of the body, sometimes in only one, sometimes in all organs. The systemic vasculitis occurs later. The connective tissue diseases are associated with autoantibodies to various non-organ-specific antigens, particularly nuclear antigens.[1] Autoantibodies to neutrophils have been known for a long time. As early as 1964 a granulocyte-specific antinuclear factor was reported. [2] The use of an ethanol fixation technique for granulocyte-specific antinuclear antibodies (GS-ANA) is the standard today. The cytoplasmic staining of granulocytes was first published in 1982 in a report of Australian patients with segmental necrotizing glomerulonephritis.[3] Four more patients were reported with vasculitis and glomerulonephritis.[4] In 1985 van der Woude [5] showed that the antineutrophil cytoplasmic autoantibodies (ANCA) occurred with Wegener's granulomatosis, as a result the interest for these antibodies significantly increased.[6] These antibodies, now termed ANCA, have proven useful for the diagnosis of disease in systemic vasculitis.

In the immunofluorescence assay for anti-neutrophil cytoplasmic antibodies, staining patterns that are different from the granular cytoplasmic pattern produced by the antibodies to the 29 kD serine protease have been recognized; in particular, a perinuclear pattern (P-ANCA) has been observed. This pattern is produced from antibodies against 2 other myeloid lysosomal enzymes - myeloperoxidase (MPO) and human leukocyte elastase. P-ANCA antibodies have been identified by Falk and Jennette [7,8] in patients with either idiopathic or vasculitis-associated crescentic glomerulonephritis. The presence of antibodies to P-ANCA or C-ANCA have proven to be highly specific and sensitive for this group of disorders, and useful in the clinical analysis of patients suspected of having vasculitis.[9-12]

ANCA antibody detection by IFA has been a useful aid in the assessment of patient diagnosis, and to a certain extent their prognosis and response to therapy. Further identification and confirmation by enzyme immunoassay for both MPO and PR3 is suggested.[13] Simultaneous testing for MPO and PR3 antibodies are recommended for best patient assessment. [6]

PRINCIPLE OF THE TEST

Diluted patient sera are incubated, in assigned slide wells, and antibodies against ANCA present in the specimen bind to the human granulocytes ethanol fixed to the slide. Unbound antibodies are washed off and a second incubation with anti-human IgG conjugated to fluorescein isothiocyanate (FITC) follows. Unbound conjugate is washed off and the bound conjugate pattern is read visually using a fluorescent microscope.

KIT COMPONENTS

ITEM	CONTENTS	QUANTITY
1.	ANCA (Human Granulocytes) Substrate 6 Well Slide Nr AD SCAN16	10 x 1 ea.
2.	FITC Antihuman IgG (H&L) Conjugate w/Evans Blue. nr AD CGEA25	1 x 2.5 ml
3.	C-ANCA Positive Control nr AD PCCA	1 x 1.0 ml
4.	ANCA Negative control nr AD NCA	1 x 1.0 ml
5.	Mounting Medium nr AD AMM3	1 x 3.0 ml
6.	PBS Buffer (Powder) nr AD PBS1	2 x 1.0 L
7.	6 Well Blotters	10 each
8.	Coverslips (22 X 70 mm)	1 x 12 each

NOTE: Liquid reagents contain the preservative sodium azide (< 0.1%)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Test tube rack or microtiter system.
2. Micro-pipettor of 2 - 20 µL range.
3. Graduated glass pipettes.
4. 1 L. graduated cylinder.
5. Staining dish.
6. Moist chamber.
7. Clean containers for diluted buffers.
8. Distilled or deionized water.
9. Fluorescent Microscope.
10. Lint free paper towel.
11. Timer (60 min. range).

MATERIALS AVAILABLE UPON REQUEST

1. ANCA (Human Granulocytes) Substrate Slides FORMALIN FIXED for confirmation of P-ANCA patterns.
2. P-ANCA Positive Control (Cat.# AD PCPA) liquid ready-to-use.

Call Customer Service department for availability of these products.

PRECAUTIONS

Caution: All blood products should be treated as potentially infectious. Human source materials from which this product was derived were found to be non-reactive for Hepatitis-B surface antigen (HBsAg) and Human Immuno- deficiency Virus (HIV) antibody when tested in accordance with current FDA required tests. No known test methods can offer total assurance products derived from human blood will not transmit HIV, Hepatitis or other potentially infectious agents. Therefore, these agents and all patients' specimens should be handled at Biosafety Level 2 as recommended for any potential infectious human serum or blood specimen in the CDC/NIH manual - "Biosafety in Microbiology and Biomedical Laboratories", 1984 or latest edition.

2. For in vitro diagnostic use only.
3. Do not use reagents past their expiration date.
4. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
5. All reagents must be at room temperature (21-26 C) before running the assay. Temperature WILL affect the results of the assay.
6. Once the procedure has started, do not allow the antigen in the wells to dry out. This may result in false negative test results, or artifacts.
7. Use only distilled or deionized water and clean glassware.
8. Use separate pipette tips for each sample, control and reagent to avoid cross contamination.
9. ANCA Negative and Positive Controls must be run with each assay.
10. No assurance is given that these reagents are free of microbial or fungal contamination.
11. Incubation times and temperatures other than those specified may give erroneous results.
12. Use separate pipette tips for each sample and reagent to avoid cross contamination.
13. Reagents should be inspected for evidence of bacterial or fungal contamination.
14. Do not reuse substrate slide.

STABILITY AND STORAGE

1. The kits and components should be stored at 2 - 8 C.
2. Rehydrated PBS buffer remains stable for 60 days at 2 - 8 C.

SERUM COLLECTION

A whole blood sample should be collected by qualified personnel using approved aseptic venipuncture techniques. Obtain and/or clarify serum samples containing visible particulate matter by centrifugation. The samples may be stored at 2 - 8°C if testing is to be done within 5 days. If stored longer, they should be frozen at -20°C or lower. Do not use a frost-free freezer which may allow the specimens to go through freeze-thaw cycles that may denature the IgG antibody and cause spurious results. Do not use hyperlipemic, hemolytic, heat treated or contaminated samples.

PREPARATION OF REAGENTS AND SAMPLES

1. Bring **all** reagents to room temperature before use. Remove the amount of slides to be used for the day's testing. Remove the slide from the foil pouch by tearing at notch. Carefully remove slide from the pouch and avoid touching the well areas. The slide is ready-to-use.
2. Prepare PBS buffer by pouring the contents of the package into a container and add deionized or distilled water to bring up to 1.0 L final volume. Mix thoroughly. Store 1X solution at 2 - 8°C.
3. Positive and Negative Controls: are ready-to-use, **Do not dilute further.** The Positive Control may be further diluted to the 1+ titer suggested on the vial label.
4. FITC Conjugate is ready-to-use liquid. The conjugate is stable at 2-8°C until the labeled expiration date.
5. Patient's Sera: **Screening**: Dilute each patient serum 1:20 (1+19) with PBS. Prepare dilutions (10 µL + 0.20 ml) in test tubes. **Titration:** Set up doubling dilutions of serum starting at 1:20 (i.e. 1:20, 1:40, 1:80, 1:160, 1:320, etc.) Mix all dilutions by vortexing on low speed.
6. Assign each well of the slide the appropriate sample.

ASSAY PROCEDURE

1. Dispense one drop (20-30 µL) of each diluted patient serum or Control over the appropriate slide well. Do not touch the surface of the slide.
2. Place the slide into a moist chamber for 20 minutes at room temperature (22-26°C).
3. Remove the slide(s) from the moist chamber and allow the serum to run off onto a lint-free absorbent paper towel. Using a wash bottle, gently rinse the remaining sera from the slide using an indirect stream of PBS buffer above the wells, while the slide is tipped, to avoid cross-reactivity between wells. Care should be taken to avoid aiming the stream directly on to the wells.
4. Place the slide(s) into a staining dish containing PBS buffer and incubate for 5 min. Incubate for a second 5 minutes in fresh PBS.
   Note: Caution should be taken to not extend incubation of the wash times. The substrate will be affected and poor morphology will result.
6. Place a blotter on the lab table with absorbent side up. Remove the slide(s) from the PBS and invert so that substrate side faces absorbent side of the blotter. Line up wells to blotter holes. Place the slide on top of the blotter. Do not allow the substrate to dry. Wipe the back of the slide with lint-free paper towel. Apply sufficient pressure while wiping to absorb buffer.
7. Remove the slide from the blotter and place substrate side up on the bench.
8. Deliver 1 drop (20-30 µL) of Conjugate per well, and incubate in a moist chamber for 20 minutes at room temperature.
9. Wash slide as described in steps 3-7.
10. Place 4-5 drops of Mounting Medium randomly on the slide.
11. Apply a 22 X 70 mm coverslip to the slide.
    Examine the reactions on the slide under a fluorescent microscope.
    Note: To maintain fluorescence, store the mounted slide in a moist chamber placed in a dark refrigerator. Read and record reactions.

INTERPRETATION OF RESULTS

Positive = C-ANCA is identified as a positive result when there is intense positive granular staining of the **cytoplasm** that extends to the border of the human granulocyte substrate displaying a 1+ or greater fluorescence and there is absence of nuclear staining.
P-ANCA exhibits intense positive **perinuclear** staining of the multi-lobed nucleus with a poorly defined cell border. A 1+ or greater fluorescence is considered a positive result.
Differentiation between C-ANCA, P-ANCA and possible cross-reactivity with GS-ANA (Granular Specific ANA) should be made on HEp2 cells and formalin fixed ANCA slides.

Negative = A serum is considered negative for ANCA if there is an absence or minimal staining of the cytoplasm, or a low level of uniform (not granular) staining of the cytoplasm. The fluorescence is less than 1+ at 1:20 dilution. The multi-lobed nucleus should also be negative.

QUALITY CONTROL

A positive and negative control serum is supplied with the kit. The controls verify test performance, test integrity and operator reliability. Good laboratory practice dictates running the positive and negative control each time the kit is used.
The Negative Control should result in little or no fluorescence. If this Control shows bright fluorescence, either the Control, antigen, conjugate or technique may be at fault.

The Positive Control should result in bright 3+ to 4+ fluorescence. If this Control shows little or no fluorescence, either the Control, antigen, conjugate or technique may be at fault. Note: This control may be used at the 1+ titer suggested on the label.
In addition to positive and negative controls, a PBS control well should be run to establish that the conjugate is free from non-specific staining of the substrate. If the antigen shows bright fluorescence in the PBS control, repeat assay using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.
A titratable positive control should be run when patient samples are being titered. Either an in-house control of known titer or the Positive Control included in the kit may be run. The 1+ titer of the Positive Control (C-ANCA) is included on the vial label. The Control should titer to +/- 1 two-fold serial dilution in the assay. A P-ANCA control (Cat.# A003L or in-house) of known titer should also be run for quality control of the P-ANCA pattern.

LIMITATIONS

1. The antibody titer obtained from individual samples do not necessarily correlate with disease severity and should not be reported as such. Antibodies from different patients may have different avidities. Paired sera run at the same time under the same conditions will give a better indication of the disease process. However, the result of an assay is not diagnostic proof of the presence or absence of vasculitic disease. Immunosuppressive therapy or treatment should not be started based on a single positive result. ELISA should be used for confirmation.
2. The results obtained from this assay are intended to be an aid to diagnosis only. Each physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures. Low positive samples should be evaluated in light of clinical symptoms and results of testing for other autoimmune antibodies, such as SLE, GBM, etc. Confirmation of results is advised by redrawing and retesting the patient.
3. Positive results obtained from this assay should be confirmed by EIA since ANCA antigens contain additional specificities such as cathepsin, lactoferrin and elastase. Due to sequence similarities some cross-reactivity is possible. In addition, a small number of SLE patients, especially SLE with neurologic disease, will have antibodies to myeloid elastase, cathepsin and lactoferrin.[14,15] In certain diseases (UC and/or PSC), a similar ANCA pattern has been identified but does not react with PR3 or MPO in ELISA.[16-18]
4. Only if test instructions are rigidly followed will optimum results be achieved.
5. Use fresh serum or samples frozen and thawed only once. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.
6. Patients should be screened on ANA HEp2 substrate to avoid confusion. Granulocyte-specific antinuclear antibodies (GS-ANA) also called neutrophil nuclear antibodies (ANNA), are a class of specific antinuclear antibodies that react with neutrophil nuclei in a homogenous pattern, but not with the other substrates used for ANA detection (e.g., rat liver or HEp2 cells). The exact non-histone antigen(s) of GS-ANA have not yet been identified.

This class of antibodies is said to be associated with active rheumatoid arthritis (RA), especially when associated with vasculitis and/or neutropenia in frequencies up to 75%. No reactivity to myeloperoxidase or proteinase -3 is detected in these patients. An atypical P-ANCA pattern associated with inflammatory bowel disease (IBD) is not completely defined. It is detected in 59-84% of ulcerative colitis, only 10-20% of Crohn disease, and also in 65-84% of primary sclerosing cholanitis with and without IBD.[23,24] These IBD-related P-ANCA are not associated with antibodies to PR3 or to MPO. Atypical ANCA patterns are considered "suspect" and further testing by EIA is recommended. Testing serum samples for their reactivity with HEp2 cells and formalin fixed slides helps to differentiate atypical pattern antibody specificities such as GS-ANA, elastase or lactoferrin from P-ANCA for confirmation of a positive result.

EXPECTED RESULTS

Published clinical studies have demonstrated that < 1% of patients who had a suspected diagnosis of vasculitis and/or glomerulonephritis exhibited a positive C- or P-ANCA pattern. Anti-PR3 and anti-MPO antibodies are highly sensitive and specific, therefore the combination of IFA and EIA testing is suggested. Combined testing by IFA and EIA for ANCA antibodies will result in the best specificity and sensitivity.

The presence of C-ANCA antibodies is associated with many different vasculitic disease states with classic Wegener's resulting in a sensitivity range of >90%. Classic or extended Wegener's has been characterized by granulomatomous inflammation of the respiratory tract, necrotizing crescentic glomerulonephritis and systemic vasculitis. Patients with more benign disease such as pauci-immune necrotizing glomerulonephritis are detected approximately 40-50% of the time. In patients with limited Wegener's without renal involvement the rate of detection is 67-86%.[19-22]

It should be noted that not all ANCA positive sera will react with PR3 or MPO antigens. Depending on the methods and antigens used in the test, the sensitivity range is approximately 70-100%. Positive ANCA sera may contain additional antibodies that are not present in the PR3 antigen or MPO antigen.[19-22] Published studies have demonstrated a high percentage of P-ANCA antibodies in various systemic vasculitic and glomerulonephritis disease states. Positive ANCA results obtained in patients with other types of vasculitides (sometimes in the form of microscopic polyarteritis, Churg-Strauss syndrome, and polyangitis) typically give a P-ANCA pattern and are usually due to antibodies to MPO. Some studies have shown up to 92% positive incidence of anti-MPO in Systemic Small Vessel Vasculitis, polyarteritis, primary Glomerulonephritis and Rapidly Progressive Glomerulonephritis confrirmed patients. In a group with systemic necrotizing vasculitis of the polyarteritis group, 50% of the patients had MPO antibodies. In another study, 50 of 424 sera with a perinuclear staining pattern were positive for MPO antibodies as tested by ELISA.[10,11,17,18]

An agreement at the 1st International Workshop on ANCA in 1988 recommended that sera should be screened for ANCA antibodies at a 1:20 dilution for immunofluorescent assays. Based on this recommendation, this IFA was established using a 1:20 serum dilution. As confirmation, a series of 100 normal blood donors (male and female, ages 20-65) from the East coast were tested and found negative in this assay using the 1:20 serum dilution.

The following table shows the incidence of ANCA antibodies found in the various known disease state and normal sera obtained from the clinical studies performed in the evaluation of this ANCA Antibody IFA kit:

Disease State	Total Tested	No. C-ANCA Negative	No. C-ANCA Positive	No. P-ANCA Negative	No. P-ANCA Positive
Wegener's	44	6	38	39	5
ICGN	41	31	10	13	28
PAN	4	1	3	4	0
PRS	8	6	2	3	5
No DX	36	20	16	23	13
MPA	21	19	2	2	19
CGN	7	4	3	3	4
UC	24	20	4	14	10
SLE	17	16	1	14	3
All other Autoimmune	30	28	2	25	5
All other Vasculitic	12	8	4	8	4
Normals	198	195	3	196	2

ICGN = Idiopathic Crescentic Glomerulonephritis; PAN = Polyarteritis Nodosa; PRS = Pulmonary Renal Syndrome; No DX = No Diagnosis; MPA = Microscopic Polyarteritis; CGN = Crescentic Glomerulonephritis; UC = Ulcerative Colitis; SLE = Systemic Lupus Erthymatosis

The overall clinical sensitivity/specificity/agreement between the ALPHADIA ANCA IFA results and clinical diagnosis of Idiopathic Crescentic Glomerulonephritis, Pulmonary Renal Syndrome, Microscopic Polyarteritis in this study is as follows.

ICGN, MPA and PRS diagnosed

		+	-
ALPHADIA	+	50	51
P-ANCA	-	16	325

Sensitivity = 75.7% Specificity = 86.4% Agreement = 84.8%

The overall clinical sensitivity/specificity/agreement between the ALPHADIA ANCA IFA results and **clinical diagnosis** of Wegener's Granulomatosis in this study is as follows.

Wegener's diagnosed

		+	-
ALPHADIA	+	38	52
C-ANCA	-	6	346

Sensitivity = 86.3% Specificity = 86.9% Agreement = 86.9%

PERFORMANCE CHARACTERISTICS

Comparison A study was performed using 244 patient samples obtained from a laboratory specializing in autoimmune diseases and 198 normal blood donor samples. These samples were assayed in the ALPHADIA ANCA IFA kit using the C-ANCA pattern results compared to an ELISA anti-PR3 kit. The results yielded an 86.0% sensitivity, 97.1% specificity, and 94.8% agreement to the ELISA procedure. The results are summarized as follows:

Reference EIA (PR3)

		+	-
ALPHADIA	+	80	10
ANCA IFA	-	13	339

Relative Sensitivity = 86.0%
Relative Specificity = 97.1%
Relative Agreement = 94.8%

These same samples were assayed with the ALPHADIA ANCA IFA kit using the P-ANCA pattern interpreted results compared to an ELISA anti-MPO kit. The results yielded a 98.7% sensitivity, 93.1% specificity, and 94.1% agreement to the ELISA procedure. The results are summarized as follows:

Reference EIA (MPO)

		+	-
ALPHADIA	**+**	**76**	**25**
P-ANCA	**-**	**1**	**340**

Relative Sensitivity = 98.7% Relative Specificity = 93.1%
Relative Agreement = 94.1%

Cross Reactivity
A study was performed using 50 autoimmune disease characterized samples obtained from an outside laboratory. The samples were run on the ALPHADIA ANCA IFA. Only one patient exhibited a positive C-ANCA pattern on IFA, for which the diagnosis indicated SLE autoimmune disease for this patient. The other SLE known patients did not demonstrate a positive reaction on the C-ANCA IFA. The nature of the SLE related disease can cause a number of anomalous antibody responses, which are unknown, and may demonstrate a slightly positive result. Eight samples exhibited a positive P-ANCA pattern in IFA. Three of the samples were SLE, but the other diagnoses were mixed.

Reproducibility
Studies were performed to demonstrate the Intra-Run, Inter Day and Inter-Lot reproducibility of the ALPHADIA ANCA IFA Test kit. The reproducibility studies were performed using the screening dilution of 1:20 for five sera; 3 positive sera samples (one high, one mid-range and one low) and two negative sera samples. The fluorescence intensities were interpreted on a graded scale of 1+ to 4+. The Intra-Run study used the sera in replicates of 6 wells each. The same 5 samples were run on three lots of kits over 5 days for Inter-Day and Inter-Lot Reproducibility. The negative samples remained negative in all wells throughout the testing period. All of the readings for the three positive sera demonstrated a consistent fluorescence intensity of 1+2, 2+3, and 4+ respectively.

Component	AD PBS1 PBS Powder Packets AD AMM3 Mounting Medium	**Precautionary Statement Prevention:** P264 Wash thoroughly after handling.
Pictogram		P280 Wear protective gloves and clothing. **Response:** P305+P351+P338 IF IN EYES:Rinse cautiously with water for several minutes. Remove contact lenses if easy to do. Continue rinsing.
Signal Word	**WARNING**	
Hazard Statement	H319 Causes serious eye irritation	P337+P313 If irritation persists, get medical advice/attention.

BIBLIOGRAPHY

1. Wegener, F., *The histopathological definition of Wegener's granulomatosis*, In: APMIS Suppl. 19, 98, , p 13, 14, 1990
2. Faber, V., Elling, P., Norup, G., et.al., *An antinuclear factor specific for leucocytes*, Lancet 2:344-345, 1964
3. Davies, D.J., Moran, J.E., Niall, J.F, et.al., *Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus etiology,* Br. Med.J., 285:606, 1982
4. Hall, J.B., Wadham, BMCN, Wood, C.F.,et.al., *Vasculitis and glomerulonephritis: a subgroup with an antineutrophil cytoplasmic antibody*, Aust. NZ J. Med., 14:277-278, 1984
5. Van der Woude, F.J., Rasmussen, N., Lobatto, S., et al: *Autoantibodies against neutrophils and monocytes: Tool for diagnosis and marker of disease activity in Wegener's granulomatosis,* Lancet, 2: 425-429, 1985
6. Weislander, J., *How are antineutrophil cytoplasmic autoantibodies detected?*, Am. J. Kidney Diseases, Vol XVIII, No. 2 (Aug.), pp 154-158, 1991
7. Falk, R.J., Jennette, C.J., *Antineutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis*, N. Engl. J. Med., 318: 1651-1657, 1988
8. Falk, R.J., Hogan, S.L., Wilkman, A.S., Terrell, S., Lauritzen, S., Charles, L.A. and Jennette, J.C.: *Myeloperoxidase specific anti-neutrophil cytoplasmic autoantibodies (MPO-ANCA),* In: Neth J Med, , 36: pp. 121 - 125, 1990
9. Daha, M.R. and Falk, R.J.: *Anti-Myeloperoxidase antibodies and clinical associations,* In: Neth J Med, 1990, 36: pp. 152-153.
10. Cohen Tervaert, J.W., et.al., *Association of autoantibodies to myeloperoxidase with different forms of vasculitis*, Arthritis and Rheumatism, Vol. 33, No. 8 (Aug.) pp. 1264-1272, 1990
11. Goldschmeding, R., et.al., *Different immunological specificities and disease associations of c-ANCA and p-ANCA*, Neth. J. of Med., 36: 114-116, 1990
12. Wiik, A., *Current classification and definition of autoantibodies to neutrophil granulocytes*, APMIS, Suppl. 19, 98: 24-25, 1990
13. Saxena, R. & Weislander, J., *Differential diagnosis of reno-pulmonary syndrome using enzyme-linked immunosorbent assay*, Europ. Clinical Lab., Apr. , 1991
14. Flesch, B.K., et.al., *Anti-elastase, cathepsin G, and lactoferrin antibodies in sera with c-ANCA or atypical fluorescence staining pattern (Abstract),* The 3rd International workshop on ANCA, Wash. D.C., 8, 1990
15. Lucena-Fernandes, M.F., et.al., *Atypical ANCA in rheumatic diseases (Abstract)*, The 3rd International workshop on ANCA, Wash. D.C., 8, 1990
16. Hardarson, S., et.al., *Antineutrophil cytoplasmic antibody in inflammatory bowel and hepatobiliary diseases*, Clin. Micro. and Immunol., Vol. 99, No. 3: 277-281, 1992
17. Goeken, J.A., *ANCA in inflammatory bowel and liver diseases: new evidence for an autoimmune etiology?*, Clinical Immunol. Newsletter, Vol. 13, No. 9/10, 1993
18. Bygren, P., Rasmussen, N., Isaksson, B., & Weislander, J., *Anti-neutrophil cytoplasm antibodies, anti-GBM antibodies and anti-dsDNA antibodies in glomerulonephritis*, European J. of Clin. Investig., 22, pp 783-792, 1992
19. Gross, W.L., , 5th International ANCA Workshop, September, 1993
19. Kallenberg, C.G., et.al., Amer. J. Medicine, 93, pp 675-681, 1992
20. Falk, R.J., et.al., , Amer. J. Kidney Dis., Vol. XVIII, No. 2 (Aug.) pp 145-147, 1991
22. Nolle, B., et.al., , Annals Intern. Med. III, pp 28-40, 1989
23. Duerr, R.H., Targan, S.R., Landers, C.J., et.al., *Neutrophil cytoplasmic antibodies: a link between primary sclerosing cholangitis andf ulcerative colitis*, Gastroenterology, 100, pp 1385-91, 1991
24. Duerr, R.H., Targan, S.R., Landers, C.J., et.al., *Anti-neutrophil cytoplasmic antibodies in ulcerative colitis, Comparison with other colitides/diarrheal illnesses,* Gastroenterology, 100, pp 1590-96, 1991

ALPHADIA sa/nv
DIAGNOSTIC PRODUCTS
Avenue Vésale 26
B1300 WAVRE
BELGIUM
TEL : 32 (0) 10 24 26 49
FAX : 32 (0) 10 24 55 99
contact@alphadia.be

CE

EC	REP	Emergo Europe Prinsessegracht 20 2514 AP The Hague The Netherlands

CE

Français

ALPHADIA S.A. / N.V.
DIAGNOSTIC PRODUCTS

IFA ANCA TEST SYSTEM

Réservé *au diagnostic in vitro.*

AD CAN60 60 Tests
AD CAN120 120 Tests

Intitulé du test:
Détection des anticorps anti-c-ANCA par IFI

Application:
Test d'immunofluorescence indirecte pour la détection des anticorps anti-cytoplasmiques des polynucléaires neutrophiles dans le sérum de patients.

Principe:
La principale réaction du test implique des anticorps circulant dans le sérum du patient qui s'attachent à leurs antigènes homologues. Ceci se produit pendant la période d'incubation alors que le sérum recouvre la surface de l'antigène. Une seconde réaction suit alors une période de rinçage qui élimine tous les anticorps humains libres. Le réactif utilisé lors de la seconde réaction est un conjugué anti-globuline humaine marquée à la fluorescéine. La surface de l'antigène est ensuite soigneusement rincée pour éliminer l'excès de conjugué libre, et visualisée sous un microscope à fluorescence adaptée.

Matériel fourni:
Conservation et stabilité des composants
1. Lames d'antigènes ANCA (conserver entre 2 et 8 °C). No AD SCAN16
2. Contrôle positif C-ANCA (conserver entre 2 et 8 °C). No AD PCCA
3. Contrôle négatif ANCA (conserver entre 2 et 8 °C). No AD NCA
4. Conjugué ITCF anti-IgG H&L avec bleu d'Evans 2.5 ml (conserver entre 2 et 8 °C). No AD CGEA25
5. Le liquide de montage pour ANCA No AD AMM3 est stable lorsqu'il est conservé entre 2 et 8 °C.
6. Le tampon PBS réhydraté reste stable pendant 60 jours entre 2 et 8 °C.

Matériel supplémentaire requis mais non fourni:
Portoir de tubes à essai ou plaques de microtitration.
Micro-pipette de 2 à 20 µL.
Pipettes graduées en verre.
Éprouvette cylindrique graduée de 1 litre.
Bac de coloration.
Chambre humide.
Récipients propres pour tampons dilués.
Eau distillée ou déionisée.
Microscope à fluorescence.
Serviette en papier non pelucheuse.
Minuterie (réglable sur 60 min.)

Préparation des réactifs:
1. Sachet de tampon No AD PBS1. Réhydrater le tampon avec 1 litre d'eau distillée stérile.

Prélèvement des échantillons:
Les échantillons de sang doivent être prélevés dans des conditions aseptisées. Une hémolyse est évitée par une séparation rapide du sérum du caillot. Le sérum doit être conservé entre 2 et 8 °C en cas d'analyse prévue dans un délai de quelques jours. On peut garder le sérum pendant 3 à 6 mois en le conservant à une température maximale de -20 °C. Éviter les sérums lipémiques et fortement hémolytiques. Lorsque les échantillons sont expédiés à température ambiante, l'ajout d'un agent conservateur tel que 0,01 % (thimérosal) ou 0,095 % d' azide de sodium est fortement conseillé.

Instructions du test:
Test de screening: diluer les sérums du test au 1:20 avec du PBS. Préparer des dilutions (10 µL + 0,20 ml) dans des tubes à essai. Titrage: préparer des dilutions sérielles du sérum à partir de 1:20 (à savoir, 1:20, 1:40, 1:80, 1:160, 1:320, etc.). Mélanger toutes les dilutions en vortexant à faible vitesse.
1. Une fois les lames parvenues à température ambiante, ouvrir le conditionnement des lames en le déchirant à l'encoche. Retirer la lame avec soin et éviter de toucher les parties où se situent les antigènes. La lame est maintenant prête à l'emploi.
2. Déposer une goutte de sérum dilué (20 à 30 µl) et des contrôles sur les puits contenant les antigènes.
3. Placer la lame contenant le sérum du patient et les contrôles dans une chambre humide à température ambiante pendant 20 minutes (environ 24 °C).
4. Enlever la lame de la chambre humide et pour permettre au sérum de s'écouler sur un morceau de serviette en papier. À l'aide d'une pissette, rincer délicatement le reste de sérum de la lame en prenant soin de détourner le jet de rinçage du puits.
5. Laver dans du PBS pendant cinq minutes. Répéter l'opération en utilisant du PBS frais.
6. Placer un buvard sur la table de laboratoire avec le côté absorbant tourné vers le haut. Retirer la lame du PBS et la retourner de manière à placer le côté frottis en face du côté absorbant du papier buvard. Absorber le tampon entre les puits à l'aide du buvard. Placer la lame sur le buvard. Ne pas laisser les frottis sécher. Essuyer le dos de la lame avec une serviette en papier sèche et non pelucheuse. Exercer suffisamment de pression sur la lame tout en l'essuyant pour absorber le tampon.
7. Déposer 1 goutte (20 à 30 µl) de conjugué dans chaque puits. Répéter les étapes 3 à 6.
8. Déposer 4 à 5 gouttes de liquide de montage sur la lame.
9. Déposer une lamelle couvre-objet de 22 x 70 mm. Examiner la lame sous un microscope à fluorescence.
Remarque: pour maintenir la fluorescence, conserver la lame montée dans une chambre humide placée à l'obscurité dans un réfrigérateur.

Contrôle de qualité:
1. Des contrôles de sérums positif et négatif doivent être inclus dans chaque série pour confirmer la reproductibilité, sensibilité et spécificité du mode opératoire du test.
2. Le contrôle de sérum négatif devrait faire apparaître peu (1+) ou pas de fluorescence. La mise en évidence d'une fluorescence vive par ce contrôle peut résulter du contrôle, de l'antigène, du conjugué ou de la technique.
3. Le contrôle de sérum positif devrait être montrer une fluorescence vive de 3+ à 4+. La mise en évidence d'une fluorescence faible ou inexistante par ce contrôle peut résulter du contrôle, de l'antigène, du conjugué ou de la technique.
4. En plus des contrôles de sérums positif et négatif, effectuer un contrôle PBS pour s'assurer que le conjugué ne provoque pas de coloration non spécifique du substrat antigénique. Si l'antigène montre une fluorescence vive avec le contrôle PBS, répéter l'opération à l'aide d'un nouveau conjugué. Une fluorescence de l'antigène peut résulter d'une dégradation du conjugué ou de l'antigène.

Interprétation du titre:
Le titre est la dilution de sérum de patients la plus élevée qui montre une faible (1+) fluorescence en cas de coloration granuleuse positive généralisée du cytoplasme du polynucléaire humain et en l'absence de coloration nucléaire.
Inférieur à 1:20 : Normal, négatif
Supérieur à 1:20 : Positif
La sensibilité, la spécificité, la concordance clinique des résultats des ANCA par IFI et le diagnostic clinique de la glomérulonéphrite idiopathique (GI), du syndrome de Goodpasture (SG), et de la polyarthrite rhumatoïde (PR) de cette étude est la suivante:

GI, SG et PR diagnostiqués

		+	-
ALPHADIA	+	50	51
P-ANCA	-	16	325

Sensibilité = 75,7 %
Spécificité = 86,4 %
Concordance = 84,8 %
La sensibilité, la spécificité, la concordance clinique des résultats des ANCA par IFI et le diagnostic clinique de la granulomatose de Wegener de cette étude est la suivante:

Granulomatose de Wegener diagnostiquée

		+	-
ALPHADIA C-ANCA	+	38	52
	-	6	346

Sensibilité = 86,3 %
Spécificité = 86,9 %
Concordance = 86,9 %

Caractéristiques de performance:
Comparaison
Une étude a été effectuée sur 244 échantillons de patients obtenus d'un laboratoire spécialisé en maladies auto-immunes et sur 198 échantillons de donneurs de sang sains. Ces échantillons ont été analysés avec le kit ALPHADIA de screening des ANCA par IFI en utilisant les résultats des C-ANCA comparés à un kit ELISA anti-PR3. Les résultats sont récapitulés de la manière suivante:

EIA de référence (PR3)

		+	-
ALPHADIA ANCA IFA	+	80	10
	-	13	339

Sensibilité = 86,0 %
Spécificité = 97.1 %
Concordance = 94.8 %
Ces mêmes échantillons ont été testés avec le kit ALPHADIA ANCA IFA, et les résultats de l'interprétation des P-ANCA ont été comparés avec un kit ELISA MPO.
Le résumé des résultats est présenté ci-dessous:

EIA de référence (MPO)

		+	-
ALPHADIA P-ANCA IFA	+	76	25
	-	1	340

Sensibilité = 98,7%
Spécificité = 93,1%
Concordance = 94,1%

Limites de la procédure:
Aucun diagnostic ne doit reposer sur un seul test sérologique, divers facteurs hôtes devant être pris en compte. Ce test est réservé au diagnostic in vitro

Précautions:
1. Le HBsAg et le HTLV-III/LAV ont été testés par dosage radioimmunologique pour tous les composants d'origine humaine, par une méthode approuvée par la FDA, et se sont avérés négatifs. Ceci ne garantit toutefois pas l'absence de HBsAg ou de HTLV-III/LAV. Tous les composants d'origine humaine doivent être manipulés avec les précautions appropriées.
2. Les contrôles et le conjugué contiennent de l'azide de sodium (0,095%).
3. Ne pas utiliser les composants après leur date de péremption.
4. Suivre les instructions de la méthode exactement comme elles figurent dans cette notice afin de garantir des résultats valables.
5. Réservé au diagnostic in vitro.
6. Manipuler les lames par les bords car une pression appliquée directement sur les puits contenant les antigènes peut altérer l'antigène.
7. Une fois la procédure lancée, ne pas laisser sécher les antigènes des puits. Ceci peut entraîner l'obtention de résultats faussement négatifs ou l'apparition d'artéfacts superflus.
8. Utiliser des tips pour chaque échantillon et réactif pour éviter une contamination croisée.
9. Les réactifs doivent être inspectés afin d'éliminer une éventuelle contamination bactérienne ou contamination fongique.
10. Ne pas réutiliser les lames.

:

Composant	AD PBS1 sachet poudre PBS AD AMM3 Liquide de montage	**Déclaration de précaution** **Prévention:** P264 Se laver les mains soigneusement après manipulation. P280 Porter des gants de protection/des vêtements de protection/un équipement de protection des yeux/ du visage. **Réponse:** P305+P351+P338 EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer. P337+P313 Si l'irritation oculaire persiste: consulter un médecin.
Pictogramme		
Mot de signal	**ATTENTION**	
Mention de danger	H319 Provoque une sévère irritation des yeux.	

ALPHADIA sa/nv
DIAGNOSTIC PRODUCTS
Avenue Vésale 26
B1300 WAVRE
BELGIUM
TEL : 32 (0) 10 24 26 49
FAX : 32 (0) 10 24 55 99
contact@alphadia.be

CE

EC	**REP**

Emergo Europe
Prinsessegracht 20
2514 AP The Hague
The Netherlands

CE

Deutsch

ALPHADIA S.A. / N.V.
DIAGNOSTIC PRODUCTS

IFA ANCA TEST SYSTEM

Die Tests sind für die *diagnostische Verwendung in vitro* bestimmt.

AD CAN60 60 Tests
AD CAN120 120 Tests

Testtitel:
IFT-Test für C-ANCA-Antikörper

Verwendungszweck:
Indirekter Immunofluoreszenztest zur Erkennung von antineutrophilen cytoplasmatischen Antikörpern im Patientenserum

Prinzip:
Die primäre Testreaktion erfasst im Serum des Patienten zirkulierende Antikörper, die sich an ihre homologen Antigene anlagern. Dies findet in der Inkubationszeit statt, während das Serum die Antigenoberfläche bedeckt. Auf einen Auswaschvorgang, in dem alle ungebundenen humanen Antikörper entfernt werden, folgt eine sekundäre Reaktion. Das in der sekundären Reaktion verwendete Reagens ist ein fluoresceinmarkiertes Antihumanglobulinkonjugat. Die Antigenoberfläche wird danach vollständig von ungebundenem Konjugat freigespült und unter einem geeigneten Fluoreszenzmikroskop betrachtet.

Bereitgestellte Materialien:
Lagerung und Stabilität von Komponenten
1. ANCA Antigenobjektträger beschichtet mit humanen Granulozyten (bei 2-8 °C lagern)
2. C-ANCA Positivkontrolle (bei 2-8 °C lagern)
3. ANCA Negativkontrolle (bei 2-8 °C lagern)
4. ANCA FITC IgG H&L Konjugat mit Evans-Blau 2.5 ml (bei 2-8 °C lagern) Nr AD CGEA25
5. ANCA Einbettungsmittel Nr. AD AMM3 lässt sich bei 2-8 °C stabil lagern.
6. Rehydratisierter PBS-Puffer bleibt 60 Tage lang bei 2-8 °C stabil.

Weitere erforderliche, aber nicht bereitgestellte Materialien:
Reagenzglasgestell oder Mikrotitersystem
Mikropipette mit einem Bereich von 2 - 20 µL
Glaspipetten mit graduierter Maßangabe
1-Liter-Messzylinder
Färbeschale
Feuchtkammer
Saubere Behälter für Pufferverdünnungen
Destilliertes oder entionisiertes Wasser
Fluoreszenzmikroskop
Nichtfaserndes Papiertuch
Laborwecker (Bereich 60 min)

Reagensvorbereitung:
1. Pufferpackung Nr. AD PBS1. Puffer mit 1 Liter sterilem destilliertem Wasser rehydratisieren.

Probennahme:
Serologische Proben sollten unter aseptischen Bedingungen genommen werden. Hämolyse wird durch umgehende Trennung des Serums vom Koagulat vermieden. Serum sollte bei 2-8 °C gelagert werden, wenn es innerhalb weniger Tage analysiert werden soll. Serum lässt sich bei -20 °C oder darunter 3 bis 6 Monate lang lagern. Lipämisches und stark hämolytisches Serum sollte vermieden werden. Wenn Proben bei Raumtemperatur bereitgestellt werden, wird die Zugabe eines Konservierungsmittels wie 0,01% (Thimerosal) oder 0,095% Natriumazid sehr empfohlen.

Testanweisung:
Screening: verdünnen Sie Testsera 1:20 mit PBS. Bereiten Sie Verdünnungen (10 µl + 0,20 ml) in geeigneten Reagenzgefäßen vor.
Titrationen: setzen Sie Serumverdünnungen mit jeweils verdoppelter Verdünnung an, beginnend bei 1:20 (d. h. 1:20, 1:40, 1:80, 1:160, 1:320 etc.). Mischen Sie alle Verdünnungen durch vorsichtiges Vortexen bei niedriger Geschwindigkeit.
1. Wenn die Objektträger Raumtemperatur erreicht haben, reißen Sie die Objekttr.gerhülle an der Kerbe auf. Entnehmen Sie den Träger vorsichtig, ohne die Antigenbereiche zu berühren. Der Objektträger ist nun einsatzbereit.
2. Geben Sie jeweils einen Tropfen verdünntes Serum (20 bis 30 µl) und Kontrolle auf die Antigenkavitäten.
3. Legen Sie den Objektträger mit dem Patientenserum und den Kontrollen 20 Minuten lang in eine Feuchtkammer bei Raumtemperatur (ungefähr 24 °C).
4. Nehmen Sie den Objektträger aus der Feuchtigkeitskammer, und lassen Sie das Serum auf ein Stück Papiertuch ablaufen. Spülen Sie verbleibende Sera mit einer Waschflasche vom Objektträger, wobei Sie sorgfältig darauf achten, den Spülstrahl nicht direkt auf die Kavität zu richten.
5. Fünf Minuten in PBS waschen. Wiederholen Sie den Vorgang mit frischem PBS.
6. Legen Sie ein Löschblatt auf den Labortisch, mit der absorbierenden Seite nach oben. Nehmen Sie den Objektträger aus dem PBS und drehen Sie ihn um, so dass die Gewebeseite der absorbierenden Seite des Löschblatts zugewandt ist. Richten Sie die Kavitäten auf die Löschblattlöcher aus. Legen Sie den Objektträger auf die Löschblattoberseite. Das Gewebe darf nicht trocknen. Wischen Sie die Tr.gerrückseite mit einem nichtfasernden Papiertuch ab. Üben Sie beim Abwischen zum Absorbieren des Puffers genügend Druck auf den Objektträger aus.
7. Geben Sie 1 Tropfen (20-30 µl) Konjugat auf jede Antigenkavität. Wiederholen Sie die Schritte 3-6.
8. Geben Sie 4-5 Tropfen Einbettungsmittel auf den Objektträger.
9. Setzen Sie ein Deckglas von 22 x 70 mm auf. Untersuchen Sie den Objektträger unter einem Fluoreszenzmikroskop.
Hinweis: um die Fluoreszenz aufrechtzuerhalten, lagern Sie den präparierten Objektträger in einer Feuchtigkeitskammer in einem dunklen Kühlschrank.

Qualitätskontrolle:
1. Positive und negative Serumkontrollen müssen täglich beim Testen einbezogen werden, um die Reproduzierbarkeit, Empfindlichkeit und Spezifität der Testprozedur zu bestätigen.
2. Die negative Serumkontrolle sollte zu geringer (+) oder ausbleibender Fluoreszenz führen. Sollte sich bei dieser Kontrolle helle Fluoreszenz zeigen, ist die Kontrollprobe, das Antigen, das Konjugat oder die Vorgehensweise möglicherweise fehlerhaft.
3. Die positive Serumkontrolle sollte zu heller Fluoreszenz von 3+ bis 4+ führen. Sollte sich bei dieser Kontrolle geringe oder keine Fluoreszenz zeigen, ist die Kontrollprobe, das Antigen, das Konjugat oder die Vorgehensweise möglicherweise fehlerhaft.
4. Zusätzlich zu positiven und negativen Serumkontrollen sollte eine PBS-Kontrolle durchgeführt werden, um sicherzustellen, dass das Konjugat frei von unspezifischer Färbung des Antigensubstrats ist. Wenn das Antigen bei der PBS-Kontrolle helle Fluoreszenz zeigt, wiederholen Sie mit frischem Konjugat. Wenn das Antigen noch immer fluoresziert, ist das Konjugat oder das Antigen möglicherweise fehlerhaft.

Titer-Interpretation:
Der Titer ist die höchste Verdünnung von Patientenserum, bei der sich schwache Fluoreszenz (1+) zeigt, wenn eindeutig körnige Cytoplasma-Färbung vorliegt, die sich bis zum Rand des humanen Granulocyten-Substrats erstreckt, und keine nucleare Färbung vorhanden ist.
Kleiner als 1:20 Negativ
Größer als 1:20 Positiv

Die gesamte klinische Empfindlichkeit/Spezifität/Übereinstimmung zwischen den ALPHADIA ANCA IFT-Ergebnissen und der klinischen Diagnose von idiopathischer crescentischer Glomerulonephritis, pulmonar-renalem Syndrom, mikroskopischer Polyarteriitis in dieser Studie sind wie folgt:

ICGN, MPA und PRS diagnostiziert

		+	-
ALPHADIA	+	50	51
P-ANCA	-	16	325

Empfindlichkeit = 75,7%
Spezifität = 86,4%
Übereinstimmung = 84,8%

Die gesamte klinische Sensitivität/Spezifität/Übereinstimmung zwischen den ALPHADIA ANCA IFA-Ergebnissen und der klinischen Diagnose von Wegenerscher Granulomatose in dieser Studie sind wie folgt:

Wegenersche Granulomatose diagnostiziert

		+	**-**
ALPHADIA	+	38	52
C-ANCA	-	6	346

Sensitivität = 86,3%
Spezifität = 86,9%
Übereinstimmung = 86,9%

Leistungsmerkmale:
Vergleich Es wurde eine Studie mit 244 Patientenproben aus einem für Autoimmunkrankheiten spezialisierten Labor und 198 normalen Blutspendeproben durchgeführt. Diese Probem wurden mit dem ALPHADIA ANCA IFA-Kit unter Verwendung der C-ANCA-Muster-Ergebnisse untersucht und mit einem ELISA anti-PR3-Kit verglichen. Es ergaben sich 86,0% Sensitivität, 97,1% Spezifität und 94,8% Übereinstimmung mit der ELISA-Methode. Zusammenfassung der Ergebnisse wie folgt:

Referenz EIA (PR3)

		+	**-**
ALPHADIA	+	80	10
ANCA IFA	-	13	339

Relative Sensitivität = 86,0%
Relative Spezifität = 97,1%
Relative Übereinstimmung = 94,8%

Die gleichen Probem wurden mit dem ALPHADIA p-ANCA IFT-Kit nach den Richtlinien für die Bewertung der p-ANCA Muster untersucht und mit einem ELISA anti-MPO-Kit verglichen. Es ergaben sich 98,7% Sensitivität, 93,1% Spezifität und 94,1% Übereinstimmung mit der ELISA-Methode. Zusammenfassung der Ergebnisse wie folgt:

Referenz EIA (MPO)

		+	-
ALPHADIA	+	76	25
P-ANCA	-	1	340

Relative Sensitivität = 98,7%
Relative Spezifität = 93,1%
Relative Übereinstimmung = 94,1%

Grenzen des Testverfahrens: Keine Diagnose sollte auf nur einem einzigen serologischen Testergebnis basieren, da verschiedene Wirtsfaktoren zu berücksichtigen sind.

Vorsichtsmaßnahmen:
1. Alle humanen Bestandteile wurden mit Radioimmuntest auf (HBsAg) und HTLVIII/LAV mit einer FDA-anerkannten Methode negativ getestet. (Nicht wiederholt reaktiv.) Dies gewährleistet jedoch nicht die Abwesenheit von HBsAg oder HTLVIII/LAV. Alle humanen Bestandteile sollten mit angemessener Sorgfalt gehandhabt werden.
2. Den Kontrollproben und dem Konjugat ist sodium azide (0,095%) beigefügt.
3. Verwenden Sie keine Bestandteile über das Verfallsdatum hinaus.
4. Befolgen Sie die methodischen Anweisungen genau wie in dieser Beilage beschrieben, um gültige Ergebnisse zu gewährleisten.
5. Die Tests sind für die diagnostische Verwendung in vitro bestimmt.
6. Fassen Sie die Objektträger an den Kanten an, da direkter Druck auf die Antigenkavitäten das Antigen beschädigen kann.
7. Nach Beginn der Prozedur darf das Antigen in den Kavitäten nicht austrocknen. Dies kann zu falsch negativen Testergebnissen oder unnötigen Artefakten führen.
8. Verwenden Sie getrennte Pipette Tips für jede Probe und Reagenz zu treffen, um 9. Kreuzkontaminationen zu vermeiden.
9. Reagenzien geprüft werden sollen auf Anzeichen von Bakterien- oder Pilzbefall.
10. Nicht wiederverwenden Objektträger.

Komponente	AD PBS1 PBS Pufferpackung AD AMM3 Einbettungsmittel	**Sicherheitshinweis** **Prävention:** P264 Nach Gebrauch … gründlich waschen. P280 Schutzhandschuhe/Schutzkleidung/Augenschutz/Gesichtsschutz tragen. **Antwort:** P305+P351+P338 BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen.. Weiter spülen. P337+P313 Bei anhaltender Augenreizung: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.
Piktogramm		
Signalwort	**ACHTUNG**	
Signalwort	H319 Verursacht schwere Augenreizung.	

ALPHADIA sa/nv
DIAGNOSTIC PRODUCTS
Avenue Vésale 26
B1300 WAVRE
BELGIUM
TEL : 32 (0) 10 24 26 49
FAX : 32 (0) 10 24 55 99
contact@alphadia.be

CE

EC	REP

Emergo Europe
Prinsessegracht 20
2514 AP The Hague
The Netherlands

CE

L'italiano

ALPHADIA S.A. / N.V.
DIAGNOSTIC PRODUCTS

IFA ANCA TEST SYSTEM

Per uso *diagnostico in vitro*.

AD CAN60 60 Tests
AD CAN120 120 Tests

Titolo del test:
Test IFA per anticorpi anti-C-ANCA

Uso previsto:
Test di immunofluorescenza per la ricerca di anticorpi anti-neutrofili citoplasmatici nel siero del paziente

Principi:
La reazione primaria del test implica la circolazione nel siero del paziente di anticorpi che si legano ai loro antigeni omologhi. Ciò si verifica durante il periodo di incubazione, quando il siero ricopre la superficie dell'antigene. Successivamente ad un periodo di risciacquo necessario per la rimozione degli anticorpi umani non legati, avviene una reazione secondaria. Il reagente utilizzato nella reazione secondaria è un coniugato anti-globulina umana marcato con fluoresceina. La superficie dell'antigene viene quindi completamente risciacquata in modo da eliminare il coniugato non legato e quindi osservata con un idoneo microscopio a fluorescenza.

Materiali in dotazione:
Conservazione e stabilità dei componenti
1. Vetrini con antigene ANCA (conservare a una temperatura di 2-8°C).
2. Controllo positivo C-ANCA (conservare a una temperatura di 2-8°C).
3. Controllo negativo ANCA (conservare a una temperatura di 2- 8°C).
4. Coniugato ANCA FITC IgG H&L con Evans Blue 2.5 ml (conservare a una temperatura di 2-8°C).n. AD CGEA25
5. La soluzione di montaggio ANCA n. AD AMM3 rimane stabile se conservata a una temperatura di 2-8°C.
6. Il tampone fosfato reidratato rimane stabile per 60 giorni a una temperatura di 2-8°C.

Materiali aggiuntivi richiesti ma non in dotazione:
Rack di provette o sistema per microtitolazione.
Micropipettatrice da 2-20 µL.
Pipette in vetro graduate.
Cilindro graduato da 1 L.
Vaschetta per colorazione.
Camera umida.
Contenitore pulito per tamponi diluiti.
Acqua distillata o deionizzata.
Microscopio a fluorescenza.
Carta assorbente che non lasci residui.
Timer (range 60 min.).

Preparazione del reagente:
1. Confezione tampone n. AD PBS1. Reidratare il tampone con 1 litro di acqua distillata sterile.

Raccolta dei campioni:
Raccogliere i campioni sierologici in condizioni asettiche. L'emolisi viene evitata separando tempestivamente il siero dal coagulo. Conservare il siero a una temperatura di 2-8°C se questo deve essere analizzato entro pochi giorni. È possibile conservare il siero per un periodo di 3-6 mesi a una temperatura pari o inferiore a -20° C. Evitare il siero lipemico e fortemente emolitico. Se i campioni vengono spediti a temperatura ambiente, si raccomanda l'aggiunta di un conservante quale (timerosal) 0,01% o sodio azide 0,095%.

Istruzioni per il test:
Screening: diluire i sieri da testare 1:20 in tampone fosfato. Preparare diluizioni (10 µL +0,20 ml) in provette per test.
Titolazioni: titolazione: impostare diluizioni di siero al raddoppio a partire da 1:20 (cioè 1:20, 1:40, 1:80, 1:160, 1:320, ecc.). Mescolare tutte le diluizioni in un vortice a bassa velocità.
1. Quando i vetrini raggiungono la temperatura ambiente, strapparne l'involucro in corrispondenza dell'apposita tacca. Rimuovere con cautela il vetrino dall'involucro evitando di toccare le aree su cui è presente l'antigene. Il vetrino è pronto per l'uso.
2. Versare una goccia di siero diluito (da 20 a 30 µl) e i controlli sui pozzetti dell'antigene.
3. Posizionare il vetrino con il siero del paziente e i controlli in una camera umida a temperatura ambiente (circa 24°C) per 20 minuti.
4. Rimuovere il vetrino dalla camera umida e per fare gocciare il siero su un pezzo di carta assorbente. Risciacquare delicatamente il siero rimanente sul vetrino con spruzzetta per lavaggio facendo attenzione a non dirigere il getto direttamente sul pozzetto.
5. Lavare in tampone fosfato per cinque minuti. Ripetere la procedura utilizzando tampone fosfato fresco.
6. Posizionare un tampone di carta assorbente sul tavolo del laboratorio con il lato assorbente rivolto verso l'alto. Rimuovere il vetrino dal tampone fosfato e capovolgerlo in modo che il lato su cui è applicato il campione di tessuto sia a contatto con il lato assorbente del tampone. Allineare i pozzetti con i fori del tampone. Posizionare il vetrino sulla parte superiore del tampone. Non lasciare asciugare il tessuto. Pulire la parte posteriore del vetrino con carta assorbente asciutta che non lasci residui. Assorbire il tampone fosfato con la carta esercitando una leggera pressione ed accertarsi che il vetrino sia asciutto.
7. Versare 1 goccia (20-30 µl) di coniugato in ciascun pozzetto di antigene. Ripetere le fasi da 3 a 6.
8. Versare 4-5 gocce di soluzione di montaggio sul vetrino.
9. Applicare un vetrino coprioggetto da 22 x 70 mm. Esaminare il vetrino al microscopio a fluorescenza.
Nota: per mantenere la fluorescenza, conservare il vetrino montato in una camera umida all'interno di un refrigeratore al buio.

Controllo di qualità:
1. I controlli di siero positivo e negativo devono essere inclusi in tutti i test del giorno per confermare la riproducibilità, la sensibilità e la specificità della procedura.
2. Il controllo di siero negativo deve visualizzare una fluorescenza minima (+) o nulla. Una eventuale fluorescenza evidente di questo controllo indica un problema a livello di controllo, di antigene, di coniugato o di procedura tecnica.
3. Il controllo di siero positivo deve visualizzare una fluorescenza evidente da 3+ a 4+. Una eventuale fluorescenza minima o nulla di questo controllo indica un problema a livello di controllo, di antigene, di coniugato o di procedura tecnica.
4. In aggiunta ai controlli di siero positivi e negativi, eseguire un controllo con tampone fosfato per stabilire se il coniugato è libero da colorazioni non specifiche del substrato dell'antigene. Se l'antigene mostra una fluorescenza evidente nel controllo con tampone fosfato, ripetere la procedura utilizzando coniugato fresco. La presenza continua di fluorescenza indica un problema a livello del coniugato o dell'antigene stesso.

Interpretazione del titolo:
Il titolo è la diluizione più alta del siero del paziente che mostra una fluorescenza debole (1+) in presenza di una colorazione granulare del citoplasma che si estende fino al bordo del substrato di granulociti umani e in assenza di colorazione nucleare.
Inferiore a 1:20 Normale, negativo
Maggiore di 1:20 Positivo

La generale sensibilità/specificità/corrispondenza clinica tra i risultati del test IFA ANCA ALPHADIA e la diagnosi clinica di glomerulonefrite crescente idiopatica (ICGN), sindrome polmonarerenale (PRS) e poliarterite microscopica (MPA) in questo studio è riassunta nella seguente tabella:

Diagnosi di ICGN, MPA e PRS

		+	-
ALPHADIA P-ANCA	+	50	51
	-	16	325

Sensibilità = 75,7%
Specificità = 86,4%
Corrispondenza = 84,8%

La generale sensibilità/specificità/corrispondenza clinica tra i risultati del test IFA ANCA ALPHADIA e la diagnosi clinica di granulomatosi di Wegener in questo studio è riassunta nella seguente tabella:

Diagnosi di granulomatosi di Wegener

		+	-
ALPHADIA C-ANCA	+	38	52
	-	6	346

Sensibilità = 86,3%
Specificità = 86,9%
Corrispondenza = 86,9%

Caratteristiche di rendimento:
Confronto É stato effettuato uno studio utilizzando 244 campioni di pazienti ottenuti da un laboratorio specializzato in malattie autoimmuni e 198 campioni normali di donatori di sangue. Questi campioni sono stati testati con il kit IFA ANCA ALPHADIA confrontando i risultati del modello C-ANCA con un kit ELISA anti-PR3. I risultati del test hanno mostrato l'86,0% di sensibilità, il 97,1% di specificità e il 94,8% di corrispondenza con la procedura ELISA. I risultati sono riassunti nella seguente tabella:

EIA (PR3) di riferimento

		+	-
ALPHADIA ANCA IFA	+	80	10
	-	13	339

Sensibilità relativa = 86,0%
Specificità relativa = 97,1%
Corrispondenza relativa = 94,8%

Gli stessi campioni sono stati testati con il kit IFA ANCA ALPHADIA confrontando i risultati del modello P-ANCA con un kit ELISA anti-MPO. I risultati del test hanno mostrato il 98,7% di sensibilità, il 93,1% di specificità e il 94,1% di corrispondenza con la procedura ELISA. I risultati sono riassunti nella seguente tabella:

(MPO) di riferimento

		+	-
ALPHADIA P-ANCA	+	76	25
	-	1	340

Sensibilità relativa = 98,7%
Specificità relativa = 93,1%
Corrispondenza relativa = 94,1%

Limitazioni della procedura:
La diagnosi non deve essere mai basata sul risultato di un singolo test sierologico, in quanto è necessario prendere in considerazione diversi fattori dell'ospite.

Precauzioni:
1. Tutti i componenti umani sono stati testati mediante test radioimmunologico per (HBsAg) e HTLVIII/LAV con metodo approvato dalla FDA e sono risultati negativi (non reattivi ripetutamente). Tuttavia, questo non garantisce l'assenza di HBsAg o HTLVIII/LAV. Tutti i componenti umani devono essere manipolati con estrema cautela.
2. Sodium azide (0,095%) è compresa in controlli e coniugato.
3. Non utilizzare componenti scaduti.
4. Per garantire risultati validi, seguire le istruzioni procedurali esattamente come vengono descritte in questo inserto.
5. Per uso diagnostico in vitro.
6. Manipolare i vetrini prendendoli dai bordi in quanto la pressione diretta sui pozzetti dell'antigene può danneggiare l'antigene stesso.
7. Dopo aver iniziato la procedura, non fare asciugare l'antigene nel pozzetto. Ciò può comportare risultati falsi negativi o artefatti inutili.
8. Separate le punte della pipetta per ogni campione e dei reagenti per evitare la contaminazione incrociata.
9. I reattivi devono essere controllati per la prova di contaminazione batterica o fungina.
10. Non riutilizzare vetrini con substrate.

Componente	AD PBS1 Confezione tampone PBS AD AMM3 soluzione di montaggio	**Consiglio di prudenza** **Prevenzione:** P264 Lavare accuratamente … dopo l'uso. P280 Indossare guanti/indumenti protettivi/Proteggere gli occhi/il viso. **Risposta:** P305+P351+P338 IN CASO DI CONTATTO CON GLI OCCHI: sciacquare accuratamente per parecchi minuti. Togliere le eventuali lenti a contatto se è agevole farlo. Continuare a sciacquare. P337+P313 Se l'irritazione degli occhi persiste, consultare un medico.
Pittogramma		
AVVERTENZA	**ATTENZIONE**	
Indicazione di Pericolo	H319 Provoca grave irritazione oculare.	

ALPHADIA sa/nv
DIAGNOSTIC PRODUCTS
Avenue Vésale 26
B1300 WAVRE
BELGIUM
TEL : 32 (0) 10 24 26 49
FAX : 32 (0) 10 24 55 99
contact@alphadia.be

CE

EC	REP

Emergo Europe
Prinsessegracht 20
2514 AP The Hague
The Netherlands

CE

El español

ALPHADIA S.A. / N.V.
DIAGNOSTIC PRODUCTS

IFA ANCA TEST SYSTEM

Para uso *diagnóstico in vitro*.

AD CAN60 60 Tests
AD CAN120 120 Tests

Nombre de la prueba:
Ensayo de anticuerpos ANCA IFA

Aplicación:
Ensayo de inmunofluorescencia para la detección de anticuerpos citoplásmicos antineutrófilos en suero del paciente.

Principio:
La principal reacción de la prueba consiste en la unión de anticuerpos circulantes del suero del paciente a sus antígenos homólogos. Esto sucede durante el período de incubación en el que el suero recubre la superficie de antígeno. Tras un período de lavado para eliminar los anticuerpos humanos que no se han unido se procede a una reacción secundaria. El reactivo utilizado en la reacción secundaria es un conjugado de anticuerpos contra la globulina humana marcados con fluoresceína. A continuación, la superficie de antígeno se lava a fondo para eliminar el conjugado que no se ha unido y se observa en un microscopio de fluorescencia adecuado.

Materiales suministrados:
Almacenamiento y estabilidad de los componentes
1. Portaobjetos con antígeno ANCA (almacenar a 2-8 ºC).
2. Control positivo C-ANCA (almacenar a 2-8 ºC).
3. Control negativo ANCA (almacenar a 2-8 ºC).
4. Conjugado IgG H y L de FITC ANCA con azul de Evans 2.5 ml (almacenar a 2-8 ºC) n° AD CGEA25.
5. El medio de montaje ANCA n.º AD AMM3 es estable cuando se almacena a 2-8 ºC.
6. El tampón PBS re-hidratado permanece estable durante 60 días a 2-8 ºC.

Otros materiales necesarios pero no suministrados:
Gradilla de tubos de ensayo o sistema de micro-titulación
Micropipeteador en el intervalo de 2 a 20 µL.
Pipetas de cristal graduadas.
Probeta graduada de 1 L.
Placa de tinción.
Cámara húmeda.
Contenedores limpios para tampones diluídos.
Agua destilada o desionizada.
Microscopio de fluorescencia.
Toallas de papel que no dejen pelusa.
Temporizador (intervalo de 60 minutos).

Preparación del reactivo:
1. Sobre de tampón n.º AD PBS1. Rehidratar el tampón con 1 litro de agua destilada estéril.

Toma de muestras:
Las muestras serológicas deben extraerse en condiciones asépticas. La hemólisis se evita separando rápidamente el suero del coágulo. Si se va a analizar en pocos días, el suero debe almacenarse a 2-8 ºC. Puede conservarse de 3 a 6 meses a una temperatura de -20 ºC o inferior. No conviene utilizar sueros lipémicos o fuertemente hemolíticos. Si las muestras se guardan a temperatura ambiente, es altamente recomendable añadir un conservante, como por ejemplo timerosal al 0,01% o azida sódica al 0,095%.

Instrucciones del ensayo:
Criba (screening): diluya los sueros de prueba en proporción 1:20 con PBS. Prepare diluciones (10 µL + 0,20 ml) en tubos de ensayo.
Titulación: prepare diluciones de suero seriadas en proporción 2x a partir de 1:20 (es decir, 1:20, 1:40, 1:80, 1:160, 1:320, etc.). Mezcle todas las diluciones agitando a baja velocidad.
1. Una vez que el portaobjetos alcance la temperatura ambiente, rasgue el envoltorio tirando de la pestaña. Saque cuidadosamente el portaobjetos, evitando tocar las zonas de antígeno. El portaobjetos está listo para usar.
2. Ponga una gota del suero diluido (de 20 a 30 µl) y de los controles en los pocillos de antígeno.
3. Coloque el portaobjetos con el suero del paciente y los controles en una cámara húmeda a temperatura ambiente (aproximadamente 24 ºC) durante 20 minutos.
4. Retire el portaobjetos de la cámara húmeda, sujételo de canto sobre una toalla de papel y dele unos golpecitos para vaciar el suero. Utilice un frasco lavador para aclarar suavemente los restos de suero del portaobjetos procurando no dirigir el chorro directamente hacia el pocillo.
5. Lávelo en PBS durante cinco minutos. Repita la operación con PBS nuevo.
6. Coloque un secante sobre la poyata de laboratorio con la cara absorbente hacia arriba. Retire el portaobjetos del PBS, dele la vuelta de modo que la cara de tejido quede orientada hacia la cara absorbente del secante. Alinee los pocillos con los orificios del secante. Coloque el portaobjetos sobre el secante. No deje que el tejido se seque. Seque el dorso del portaobjetos con una toalla de papel que no deje pelusa. Al secarlo, aplique al portaobjetos la presión necesaria para absorber el tampón.
7. Ponga 1 gota (20-30 µl) de conjugado en cada pocillo de antígeno. Repita los pasos 3 a 6.
8. Ponga 4 ó 5 gotas de medio de montaje en el portaobjetos.
9. Coloque un cubreobjetos de 22 x 70 mm. Examine el portaobjetos con un microscopio de fluorescencia.
Nota: para mantener la fluorescencia, guarde el portaobjetos montado en la nevera dentro una cámara húmeda y a oscu ras.

Control de calidad:
1. Para confirmar la reproducibilidad, sensibilidad y especificidad del ensayo es necesario incluir controles de suero positivo y negativo en los análisis todos los días.
2. El resultado del control de suero negativo debe ser poca (+) o ninguna fluorescencia. Si este control muestra una fluorescencia brillante, el problema puede estar en el control, el antígeno, el conjugado o en la técnica.
3. El resultado del control de suero positivo debe ser una fluorescencia brillante del orden de 3+ a 4+. Si este control muestra poca o ninguna fluorescencia, el problema puede estar en el control, el antígeno, el conjugado o en la técnica.
4. Además de los controles de suero positivo y negativo, debe analizarse un control de PBS para confirmar que el conjugado no tiñe el substrato de antígeno de manera inespecífica. Si el antígeno presenta una fluorescencia brillante en el control de PBS, repita el análisis usando conjugado nuevo. Si el antígeno sigue presentando fluorescencia, el problema puede estar en el conjugado o en el antígeno.

Interpretación del título:
El título es la dilución más alta de suero del paciente que muestra una fluorescencia débil (1+) cuando hay tinción granular positiva del citoplasma que se extiende hasta el borde el substrato de granulocitos humanos, y hay ausencia de tinción nuclear.
Menor de 1:20 Normal, negativo
Mayor de 1:20 Positivo

La sensibilidad/especificidad/coincidencia general entre los resultados de ALPHADIA ANCA IFA y el diagnóstico clínico de la Glomerulonefritis rápidamente progresiva idiopática, el Síndrome renal pulmonar y la Poliarteritis microscópica en este estudio son las siguientes:

Diagnóstico de GRPI. PAM y SRP

		+	-
ALPHADIA	+	50	51
P-ANCA	-	16	325

Sensibilidad = 75,7%
Especificidad = 86,4%
Coincidencia = 84,8%

La sensibilidad/especificidad/coincidencia general entre los resultados de ALPHADIA ANCA IFA y el diagnóstico clínico de la Granulomatosis de Wegener en este estudio son las siguientes:

Diagnóstico de Wegener

		+	-
ALPHADIA	+	38	52
C-ANCA	-	6	346

Sensibilidad = 86,3%
Especificidad = 86,9%
Coincidencia = 86,9%

Caracteristicas de rendimiento:
Comparación
Se realizó un estudio con 244 muestras de pacientes obtenidas de un laboratorio especializado en enfermedades autoinmunitarias, y 198 muestras de sangre de donantes normales. Estas muestras se probaron con el kit ALPHADIA ANCA IFA, y los resultados de los patrones C-ANCA se compararon con el kit ELISA anti-PR3. Los resultados fueron de un 86,0% de sensibilidad, un 97,1% de especificidad y un 94,8% de coincidencia con el procedimiento ELISA. A continuación se muestra el resumen de resultados:

Referencia EIA (PR3)

		+	-
ALPHADIA	+	80	10
ANCA IFA	-	13	339

Sensibilidad relativa = 86,0%
Especificidad relativa = 97,1%
Coincidencia relativa = 94,8%

Estas mismas muestras se probaron con el kit ALPHADIA ANCA IFA, y los resultados de la interpretación de los patrones P-ANCA se compararon con un kit ELISA anti-MPO. Los resultados fueron de un 98,7% de sensibilidad, un 93,1% de especificidad y un 94,1% de coincidencia con el procedimiento ELISA. A continuación se muestra el resumen de resultados:

Referencia EIA (MPO)

		+	-
ALPHADIA	+	76	25
P-ANCA	-	1	340

Sensibilidad relativa = 98,7%
Especificidad relativa = 93,1%
Coincidencia relativa = 94,1%

Limitaciones del procedimiento:
Ningún diagnóstico debe basarse en el resultado de una sola prueba serológica, ya que hay que tener en cuenta diversos factores del huésped.

Precauciones:
1. Todos los componentes humanos han sido probados mediante radioinmunoensayo para (HBsAg) y HTLVIII/LAV con un método aprobado por la FDA, y han dado resultados negativos (no repetidamente reactivos). No obstante, esto no garantiza la ausencia de HBsAg o HTLVIII/LAV. Todos los componentes humanos deben manipularse con las debidas precauciones.
2. Los controles y el conjugado contienen Sodium azide (0,095%).
3. No utilice ningún componente que haya sobrepasado la fecha de caducidad.
4. Para garantizar la validez de los resultados, siga las instrucciones del procedimiento exactamente como aparecen aquí.
5. Para uso diagnóstico in vitro.
6. Sujete los portaobjetos por los bordes, ya que la presión directa sobre los pocillos puede estropear el antígeno.
7. Una vez iniciado el procedimiento, no deje que el antígeno de los pocillos se seque. Esto podría dar falsos negativos o producir artefactos innecesarios.
8. Use distintas puntas de pipeta para cada una de las muestras y reactivos para evitar la contaminación cruzada.
9. Los reactivos deben inspeccionarse en busca de evidencias de contaminación bacteriana o micótica.
10. No reutilizar portaobjetos.

Componente	AD PBS1 PBS Sobre de tampón AD AMM3 medio de montaje	**Consejos de prudencia** **Prevención:** P264 Lavarse … concienzudamente tras la manipulación. P280 Llevar guantes/prendas/gafas/máscara de protección. **Respuesta:** P305+P351+P338 EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando. P337+P313 Si persiste la irritación ocular: Consultar a un médico.
Pictograma		
Palabra Clave	**ATENCIÓN**	
Indicación de Peligro	H319 Provoca irritación ocular grave.	

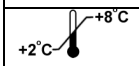
ALPHADIA sa/nv
DIAGNOSTIC PRODUCTS
Avenue Vésale 26
B1300 WAVRE
BELGIUM
TEL : 32 (0) 10 24 26 49
FAX : 32 (0) 10 24 55 99
contact@alphadia.be

CE

EC	REP

Emergo Europe
Prinsessegracht 20
2514 AP The Hague
The Netherlands

CE

	Manufactured by Prodotto da Fabricado por Fabriqué par hergestellt von
REF	Catalog number Numero di catalogo Número de Catálogo Numéro de catalogue Katalognummer
LOT	Lot Lotto Lote Lot Charge
EC REP	EC Authorized Representative Rappresentante Autorizzato CE Representante Autorizado CE CE Représentant autorisé EG authorisierter Bevollmächtigter
	EC Declaration of Conformity Dichiarazione di Conformità CE Declaración de Conformidad CE CE Déclaration de Conformité EG Konformitätserklärung
	Number of tests Numero di test Número de determinaciones Nombre de tests Anzahl der Teste
	See instructions for use Vedere le istruzioni per l'uso Consultar la instrucciones de uso Voir instructions Gebrauchsanweisung beachten
	Expiration date Data di scadenza Caducidad Date d'Expiration Haltbarkeitsdatum
	Store at 2-8 C / 35-46 F Conservare a 2-8°C/35-46 F Almacenar a 2-8ºC / 35-46ºF Conserver à 2-8°C Bei 2-8°C / 35-46°F lagern
	Caution Attenzione Precaución Précautions Achtung
	Potential biological risk Potenziale rischio biologico Riesgo potencial biológico Biohazard Risque Biologique Potentiel Potentielle biologische Gefährdung
RFU	Ready for use Pronto all'uso Listo para su uso Prêt à l'emploi Gebrauchsfertig
IVD	For in vitro diagnostic use Per uso diagnostico *in vitro* Para uso solo in vitro Usage in vitro Für in-vitro diagnostische Verwendung
RUO	For research use only Solo per ricerca Para uso solo en investigación Pour recherche Nur für Forschungszwecke
IUO	For investigational use only Solo per uso investigativo Para uso solo en investigación Pour investigation Nur für Forschungszwecke
IFA/DFA PBS	Phosphate Buffered Saline Tampone salino fosfato Fosfato Salino Tamponado Tampon phosphate salin PBS
SOR	Sorbent Assorbent Sorbente Absorbant Sorbens

SLIDE	Tissue Substrate Slide Vetrini con substrato di tessuto Porto objetos de Sustrato de Tejido Lame portant le substrat tissulaire Gewebesubstrat-Objektträger
MM	Mounting Medium Mezzo di montaggio Medio de Montaje Liquide de montage Eindeckmedium
10x	Concentration Concentrazione Concentración Concentration Konzentration
ENS	Enhancement solution Soluzione di rinforzo Solución de realce Solution d'amplification Verstärkungslösung
WASHB	Wash Buffer Tampone di lavaggio Tampón de lavado Tampon de lavage Waschpuffer
MPS 12x8	Microplate Strips Strip per Micropiastra Tiras de micro placa Microplaque Mikrotiterplattenstreifen
CONJ	Conjugate Coniugato Conjugado Conjugué Konjugat
SUB	Substrate Substrato Sustrato Substrat Substrat
STOP	Stop Solution Soluzione bloccante Solución de Parada Solution d'arrêt Stopplösung
CAL X	Calibrator(s) Calibratore (i) Calibrador (s) Calibrateur(s) Kalibrator(en)
CONTROL -	Negative Control Controllo Negativo Control Negativo Contrôle Négatif Negative Kontrolle
CONTROL +	Positive Control Controllo Positivo Control Positivo Contrôle Positif Positive Kontrolle
CONJ CNS	Counterstain Colorante di contrasto Contraste Contre colorant Gegenfärbung
CS	Coverslip Coprioggetto Cubre portaobjetos Lamelles couvre-objet Deckgläshen
CONJ +	Positive Conjugate Conjugato Positivo Conjugado Positivo Conjugué Positif Positivekonjugat
CONJ -	Negative Conjugate Conjugato Negativo Conjugado Negativo Conjugué Négatif Negativkonjugat
DIL	Sample Diluent Diluente del campione Diluyente de muestra Tampon de dilution Probenverdünnungslösung