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ImmuGlo™
Anti-Keratin Antibody (AKA) Test System

PRODUCT INSERT

IVD

REF 1122 48 Determination

An indirect immunofluorescence antibody test for the detection and quantitation of anti-keratin antibodies (AKA) in human serum.

SUMMARY AND EXPLANATION

Rheumatoid arthritis (RA) is the most prevalent systemic rheumatic disease afflicting 1-2% of the population. It is characterized by mononuclear cell infiltration and proliferation of synovial cells. The disease results in membrane inflammation followed by irreversible degradation of joint cartilage and bone structure. The pathogenesis of RA is unknown, however, it is characterized by the presence of various circulating autoantibodies such as to rheumatoid factor (RF), anti-keratin antibodies (AKA), and anti-perinuclear factor (APF). RF is present in 70-90% of patients with RA and is included in the ARA* classification criteria.

Anti-keratin antibodies, initially described by Young et al[1], are found to be highly specific for RA. AKA can be detected by indirect immunofluorescence (IFA) on rat esophagus substrate, even prior to the onset of joint symptoms[2-6]. They occur in approximately 40% of patients with RA, of which approximately 14% are RF negative. The association between RF and AKA is close. Circulating immune complexes are found in significantly higher concentrations in RA patients positive for AKA. This may explain the association of AKA with severe forms of RA.

PRINCIPLES OF PROCEDURE

In the indirect immunofluorescence method used in this kit, patients' sera are incubated on rat esophagus sections to allow binding of antibodies to the tissue substrate. Any antibodies not bound are removed by rinsing. Bound antibodies of the IgG class are detected by incubation of the substrate with fluorescein-labeled, anti-human IgG. Reactions are observed under a fluorescence microscope equipped with appropriate filters. The presence of AKA demonstrated by an apple green fluorescence of the stratum corneum of the mucosal epithelium. The titers (the reciprocal of the highest dilution giving a positive reaction) are then determined by testing serial dilutions.

REAGENTS

Storage and Preparation

Store all reagents at 2°-8°C. Ready for use after equilibration to room temperature.

Precautions

All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. All human serum specimens and human derived products should be treated as potentially hazardous. Follow good laboratory practices in storing, dispensing, and disposing of these materials[7].

WARNING - Sodium azide (NaN_3) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this insert to ensure valid results. Do not interchange kit components with those from sources other than the same catalog number from IMMCO DIAGNOSTICS. Do not use beyond expiration date.

Materials Provided

ImmuGlo™ Anti-Keratin Antibody Test System REF *1122*

Kit contains sufficient reagents for 48 determinations

8x	SORB SLD 6	6-well Rat Esophagus Substrate Slides
1 x 0.5 ml	CONTROL + *	Positive Control*
1 x 0.5 ml	CONTROL - *	Negative Control*
1 x 5.0 ml	CONJ FITC *†	Goat anti-human IgG FITC Conjugate. Ready to use. **Protect from light***.
1 x 60 ml	BUF *	Buffered Diluent. Ready to use.
2 vials	BUF WASH	Phosphate Buffered Saline (PBS). Dissolve each vial to 1 liter.
1 x 5.0 ml	MOUNTING MEDIUM *	Mounting Medium. **Do not freeze**.
1 x 1.0 ml	EVANS	Counterstain
12 x	COVER SLD	Coverslips

***CAUTION - Contains <0.1% NaN_3

Materials Required but not Provided

- Fluorescence microscope
- Micropipette or Pasteur pipette
- Serological pipettes
- Staining dish (e.g. Coplin jar)
- Small test tubes (e.g. 13 x 75 mm) and test tube rack

- Distilled or deionized water
- 1 liter container
- Wash bottle
- Absorbent paper towels
- Incubation chamber

SPECIMEN COLLECTION AND PREPARATION

Only serum specimens should be used for this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of this test and should not be used. Store specimens at 2°- 8°C for no longer than one week. For longer storage, serum should be frozen at -20°C. Avoid repeated freezing and thawing of samples.

PROCEDURE

Test Method

The indirect immunofluorescence staining procedure is illustrated in the following figures:

1.Let pouch equilibrate to room temperature, then remove slide(s) from pouch. and controls.

2. Place slide(s) into moisture chamber and add samples Cover and incubate 30 minutes.

3. Rinse slide(s) by dipping into beaker with PBS. Transfer slide(s) into Coplin jar and wash 10 minutes.

4. Blot edge of slide(s) on absorbent paper. Proceed immediately with next step.

5. Apply Conjugate to each well. Cover and incubate 30 minutes.

6. Rinse slide(s) by dipping into beaker with PBS Transfer slide(s) into Coplin jar and wash 10 minutes..

7. Blot edge of slide(s) on absorbent paper. next step

. **8**. Mount cover slip and read under fluorescent immediately with microscope.

A. Screening:

Step 1. Dilute each patient serum **1:10** with the Buffered Diluent (0.1 ml serum + 0.9 ml diluent). **Do not dilute Positive or Negative Controls**. Save the undiluted sera to determine antibody titers if screening tests are found to be positive.

Step 2 Allow pouches containing substrate slides to equilibrate to room temperature for **10-15 minutes**. Carefully remove the slides without touching the substrate.

Step 3 Label the slides and place them in an incubation chamber lined with paper towels moistened with water to prevent slides from drying.

Step 4 Invert dropper vial and gently squeeze to apply **1 drop** (approximately 50 µl) of the **Negative Control** to well #1. Similarly apply **1 drop** of **Positive Control** to well #2. Avoid overfilling the wells.

Step 5 Using a micropipette or Pasteur pipette, apply **1 drop** of patient's diluted serum (approximately 50 µl) to the other wells. Avoid overfilling the wells.

Step 6 Place the lid on the incubation chamber and incubate slides **30 minutes** at room temperature.

Step 7 Remove a slide from the incubation chamber. Hold slide at tab end and rinse gently with approximately **10 ml** of PBS using a pipette, or rinse slide in a beaker filled with PBS. Do not use wash bottle. Transfer slide immediately into Coplin jar and wash **10 minutes**. Repeat process with all remaining slides.

Step 8 Remove slide(s) from Coplin jar. Blot the edge of the slide on a paper towel to remove excess PBS. Place the slide in the incubation chamber. Immediately invert the **Conjugate** dropper vial and gently squeeze to apply **1 drop** (approximately 50 µl) to each well. Repeat procedure for remaining slides.

Step 9 Replace the lid on the incubation chamber. Incubate **30 minutes** at room temperature.

Step 10 Remove a slide from incubator. Hold the slide at the tab end and dip the slide in a beaker containing PBS to remove excess conjugate. Place slide(s) in a staining dish filled with PBS for **10 minutes**. If desired, 2-3 drops of Evans blue counterstain may be added to the final wash. Repeat for the remaining slides. **NOTE:** Improper washing may lead to increased background fluorescence.

Step 11 Remove a slide from the staining dish. Blot the edge of the slide on a paper towel to remove excess PBS. **To prevent slide from drying, proceed immediately with Step 13 while the slide is still wet.**

Step 12 Place **3 drops** of the Mounting Medium evenly spaced on a coverslip and invert the slide onto the coverslip. To remove any air bubbles gently apply pressure along the edge of the coverslip. Avoid any lateral movement of the coverslip. Repeat procedure for remaining slides.

Step 13 Examine for specific fluorescence under a fluorescence microscope at a magnification of **200x** or greater. The presence of the antifading agent in the mounting medium allows extended viewing of a field without appreciable loss of staining intensity.

Slides may be read as soon as prepared. However, because of the presence of antifading agent in the mounting medium, no significant loss of staining intensity occurs if reading is delayed. Slides should be stored in the dark at 2°- 8°C.

B: End Point Determination (Titration)

A serum positive in the screening test may be further tested following **Steps 5 through 13** to determine the titer. Each run should include the appropriate Positive and Negative Controls. Make serial two-fold dilutions starting at **1:10**. Using one slide, a serum may be tested at dilutions ranging from 1:20 to 1:320. If positive at a 1:320 dilution, the titer is reported as greater or equal to 320. Alternatively, additional slides may be used to obtain endpoints for those sera still positive at a 1:320 dilution. The reciprocal of the highest dilution producing a positive reaction is the titer.

Preparation of Serial Dilutions

Number six tubes 1 through 6. Add 0.9 ml of buffered diluent to tube 1 and 0.2ml to tubes 2 through 6. Pipette 0.1 ml of undiluted serum to tube 1 and mix thoroughly. Transfer 0.2 ml from tube 1 to tube 2 and mix thoroughly. Continue transferring 0.2 ml from one tube to the next after mixing to yield the dilutions depicted below:

Tubes	1	2	3	4	5	6
Serum	0.1 ml					
	+					
Diluent	0.9ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Transfer	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	
Final dilution	1:10	1:20	1:40	1:80	1:160	1:320

Quality Control

Both the Positive and Negative Controls should be included with each test run. The negative control should show no specific fluorescence, whereas the positive control should have 2+ or greater specific staining.

If expected results are not obtained, the run should be repeated. If inadequate results continue to occur with the controls, these may be due to:

- Contamination as a result of improper storage or handling. If signs of contamination such as turbidity are seen, discard and use another control.

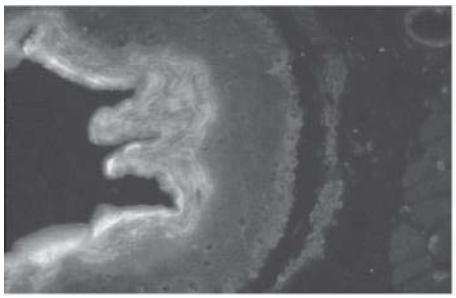
- Problems with the optical system of the fluorescence microscope. These may include: improper alignment, use of the bulb beyond the expected performance life. etc.

- Allowing the slide to dry during the procedure.

RESULTS

The results of the tests for AKA should be considered negative (< 10), positive (greater or equal to 320), or alternatively, positive with titer.

Read for staining of the stratum corneum layer of the epithelium for anti-keratin antibodies, as shown in the following figure:



LIMITATIONS OF THE PROCEDURE

In some cases, sera positive for anti-keratin antibodies may either be very weak or negative at the initial screening dilution (prozone phenomenon). In such doubtful cases the sera should be screened at higher dilutions and, if positive, antibody titers determined.
The presence of two or more antibodies in a serum which react with the same substrate may cause an interference in their detection by immunofluorescence. This interference may cause either a failure to detect AKA or a suppression of its titer if the interfering antibody has a higher titer than anti-keratin antibodies.

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