

Anti-Submaxillary Antibody Test System

QUALITY CONTROL

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.
2. The negative serum 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.
3. The positive serum controls should result in bright 3+ to 4+ fluorescence. If these controls show little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.
4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from non-specific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

RESULTS

A positive result is observed as a bright 3-4+ staining.

TEST LIMITATIONS

1. No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.

LITERATURE REFERENCES

1. Harmon, C.E. et al. The importance of tissue substrate in the SS-A/Ro antigen antibody system. Arthritis Rheum 1984; 27: 166-73.
2. Alspaugh, M.A., Tala, D. and Tan, E.M. Differentiation and characterisation of autoantibodies and their antigens in Sjögren's syndrome. Arthritis Rheum 1976; 216.
3. Alspaugh, M.A. and Tan, E.M. 1975. Antibodies to cellular antigens in Sjögren's syndrome. J Clin Invest. 55:1067.

INSTRUCTIONS FOR USE

Anti-Submaxillary Antibody Test System

K4820 - 48 Tests

K9620 - 96 Tests

Also for: **Monkey submaxillary slides** **S4208** - 4 well **S8208** - 8 well
S5208 - 5 well **S0208** - 10 well

Parotid gland antibody Positive control

C050

INTENDED USE

The Bio-Diagnostics Anti-Submaxillary Antibody Test kit is an immunofluorescent antibody (IFA) test to detect the presence of submaxillary antibodies in human serum.

SUMMARY AND EXPLANATION

Fluorescence of the salivary duct is associated with Sjögren's syndrome. Approximately 70-75% of Sjögren's patients exhibit anti-salivary duct and epithelium cell antibodies. This antibody is seen in 10-20% of cases of Sjögren's disease with the sicca complex alone and 25% of rheumatoid arthritis without evidence of Sjögren's syndrome.

PRINCIPLE OF THE TEST

The primary reaction involves circulating antibodies present in the patient's serum. The binding of the antibody to its homologous antigen site occurs during the first incubation period whilst the serum covers the antigen surface. A secondary reaction then follows a rinsing period that removes the unbound human antibody. The reagent used in the secondary reaction is a fluorescein labelled antihuman globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope.

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WARNINGS AND PRECAUTIONS

- All human components have been tested by radioimmunoassay for (HBsAg) and HTLVIII/LAV by an FDA approved method and found to be negative (not repeatedly reactive). However, this does not assure the absence of (HBsAg) or HTLVIII/LAV. All human components should be handled with appropriate care.
- The controls included in the kit contain 0.1% sodium azide or 0.01% thiomersal as preservatives. Although these are at low concentrations, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
- Do not use components beyond their expiration date.
- Follow the procedural instructions exactly as they appear in this insert to ensure valid results.
- For in vitro diagnostic use.
- Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
- 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 unnecessary artefacts.

KIT CONTENTS

SLIDE	Monkey submaxillary substrate antigen slides (S4208, S5208, S8208 or S0208)
CONJ IgG	FITC Conjugate (for use with Primate substrates) with Evans Blue Counterstain: J502. This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.
CONTROL +	Parotid Positive Control no: C050
CONTROL -	Universal Negative Control no: C000N/C000N-0.5
IFA/DFA PBS	Buffer Pack no: R002
MM	Mounting Medium no: R005

Note: All kit components are available separately.
Please see the Bio-Diagnostics Ltd catalogue for more details.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes and rack or microtitre system	Disposable pipettes
Staining Dish and Slide Forceps	Moisture Chamber
Volumetric Flask (500 ml)	Distilled Water
Fluorescence Microscope	Paper Towels – lint free

REAGENT PREPARATION

- Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

	Manufacturer		Contains sufficient for <n> tests		Ready for use
	Temperature limitation		In vitro diagnostic medical device		

Anti-Submaxillary Antibody Test System

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2°C to +8°C. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents. Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

TEST INSTRUCTIONS

Screening: dilute test serums 1/4 (1 part patient sample to 3 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.

Titration: set up doubling dilutions of serum starting at 1/4, (i.e. 1/4, 1/8, 1/16, 1/32 etc.).

- Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
- Place a drop of diluted serum (20 to 30µl) and controls over the antigen wells.
- Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 18-24°C).
- Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly onto the well.
- Wash in PBS for 5 minutes. Repeat using fresh PBS.
- Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up the wells to blotter holes. Place slide on top of the blotter. Wipe the back of the slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer. **Do not allow tissue to dry.**
- Deliver 1 drop (20-30µl) of conjugate per antigen well. Repeat steps 3-6.
- Place 4-5 drops of mounting medium on slide.
- Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope.

Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.