

**Fluorescein conjugated monoclonal antibody
for the detection of Chlamydia
FA TEST REAGENT SET
FOR CHLAMYDIA "SEIKEN"**

In the past, isolation and cultivation of Chlamydia using embryonated eggs and cell culture techniques were necessary to confirm infection, but, in recent years, with the use of highly specific monoclonal antibodies, it has become possible to detect Chlamydia antigens directly, thus speeding up diagnosis.

In this reagent, genus-specific monoclonal antibodies (mouse clone A-2 established by Veterinary Surgeon School, Hokkaido University) directed against Chlamydia psittaci and C. trachomatis are labeled with fluoresceins and are used to detect Chlamydia directly in patient's specimens.

CHARACTERISTICS

Chlamydia antigen can be detected directly from specimens using FITC-conjugated monoclonal antibody.

CONTENTS

1. **FITC-Conjugated Chlamydia Monoclonal Antibody (Lyophilized)**
1 ml equivalent 1 vial
FITC-conjugated monoclonal antibody against Chlamydia is lyophilized. The reagent contains 0.5 w/v% bovine serum albumin as a stabilizer and Evans Blue as a comparative stain.
2. **Reconstitution water** 1 ml 1 vial
Distilled water containing 0.1 w/v% sodium azide.
3. **Control slide** 2 pcs.
Chlamydia-infected cells and Chlamydia-uninfected cells are coated and fixed on a glass slide on the right and left hand side, respectively.

INTENDED USE

To detect Chlamydia psittaci and Chlamydia trachomatis antigens in patient specimens.

PRINCIPLE

The occurrence of a reaction between FITC-conjugated Anti-Chlamydia monoclonal antibody and Chlamydia is judged by observing whether or not fluorescence is present under a fluorescent microscope.

PROCEDURES

1. Materials and reagents

1) Materials required

- (1) Swabs for collecting specimens (Vinylon or polyester)
- (2) Non-fluorescent glass slide with marking
- (3) Fluorescent microscope (vertically-lit type) No transpiring-lit type can be used.

2) Reagents

- (1) FA TEST REAGENT SET FOR CHLAMYDIA "SEIKEN"
- (2) Acetone
- (3) Mounting medium (Non-fluorescent Glycerin-PBS)

Nine parts of non-fluorescent glycerin (commercially available) is mixed with 1 part of M/100 PBS (pH 7.0–7.4).

2. Reagent preparation

Add 1 ml of diluent (supplied with the kit) to the vial FITC-labeled monoclonal antibody against Chlamydia to reconstitute the reagent and leave undisturbed at 2 – 10°C for 30 minutes. The reagent should be stored at the same temperature and used within 3 months after preparation.

3. Procedures

- 1) Remove the control slide or specimens from the refrigerator and bring to room temperature at least 5 minutes before the test.
- 2) Place 25 μ l FITC-conjugated anti-Chlamydia monoclonal antibody on the smeared specimen with a micropipette and place the glass slide in a moisture box, incubating at room temperature for 15 minutes. Avoid allowing the mixture to dry, as this may cause non-specific reactions.
- 3) After incubation, wash the slide with distilled water in a wash bottle to remove excess reagent. When washing, take care not to allow the water stream to hit the sample part directly.
- 4) Allow the plate to air-dry and carry out microscopic observation, immersing the glass slide in mounting medium. If a quick observation is needed, store the slides at 2–10°C and observe results within 24 hours.

NOTE

1. Sample collection

As it is difficult to collect specimens from exudates, it is recommended that specimens be collected by abrasion of mucosal epithelial cells from the eyes, cavity of the nasopharynx, and/or genitourinary tracts.

- 1) From urinary tract:

Insert the swab into the urinary tract to a depth of 2–4 cm while rotating the swab, and take the mucosal epithelial cells by rotating it in the tract, abrasing the cells by the movement.

- 2) Since Chlamydia trachomatis proliferates only in columnar epithelial cells and not in squamous cells, exercise care when obtaining the sample specimen as follows: take columnar epithelial cells from just behind the opening of the uterus by first wiping the mucous secretions at the opening of the uterus with a cotton swab; then, inserting a fresh one to where these cells are located, collect them onto the swab with a rotating motion for 5–10 seconds. Finally, remove for smearing.

2. Smearing the specimen on the glass slide

- 1) After collecting the cells, spread them uniformly onto a non-fluorescent glass slide appropriately marked and allow it to air-dry.
- 2) Fix the cells with cold acetone for 5–10 minutes. Always use fresh acetone and never acetone that contains water. If the test is not to be carried out right away, the sample should be stored at –20°C after air-drying by sealing tightly. In this condition, the sample can be stored for several months.

INTERPRETATION

Microscopic observation is done at 400–1000 X magnification and evaluation is done while referring to the control slide. Because the reagent contains Evans Blue, the cells will be stained red, while Chlamydia antigens will appear apple-green with fluorescence.

When only a few stained particles are found throughout the smeared area, the sample should be retested. If no red-stained cells are found in the field of observation, the sample should be discarded and a new one collected.

PERFORMANCE CHARACTERISTICS

The monoclonal antibody contained in this kit is genus specific and will therefore react with both *Chlamydia trachomatis* and *Chlamydia psittaci*.

CORRELATION

Using 600 samples from male and female patients of Gonorrhoea and non-Gonococcal urethritis, the results of this test and those of MicroTrak* (Syva) were compared. A 100% correlation was found for positive and negative results.

* MicroTrak Chlamydia Direct Test (Syva)

PRECAUTIONS

1. Do not expose FITC-conjugated anti-Chlamydia monoclonal antibody to strong light.
2. Change the tips of micropipettes for each specimen.
3. Do not mouth pipette.
4. If the reagent comes in contact with hands, etc., wash the affected area as quickly as possible.
5. All equipment used in this test should be sterilized by soaking in 0.5% sodium hypochlorite (available chlorine approx. 5000 ppm) for 1 hour or longer or by autoclaving at 121°C for 20 minutes or longer.
6. This reagent contains 0.1 w/v% sodium azide as a stabilizer. As sodium azide may react with lead and copper piping to form highly explosive metal azides, dispose of by flushing with a large volume of water.

STORAGE AND SHELF LIFE

Storage : Below 10 °C

Expiration : Up to expiration date on the label

PACKAGE

FA TEST REAGENT SET FOR CHLAMYDIA "SEIKEN"

1 box (Product code 421601)