Indirect Fluorescence Assay

for Measles Virus IgM

Antibody

Your Preferred Autoimmune and Infectious Disease Detection Company

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Package Insert

Intended Use

The SCIMEDX Indirect Fluorescence Assay (IFA) for Measles Virus IgM Antibody is intended for the qualitative and semi-quantitative detection of IgM (Immunoglobulin M) antibody to measles virus in human serum or plasma. Detection of measles IgM antibody in humans can be used as an aid in the diagnosis of primary infection with this virus.

Introduction and Summary of Test Procedures

Measles, which is an acute, extremely contagious disease, is characterized by a generalized rash and fever. Bacterial infections of the ear or respiratory tract, or encephalitis, may complicate the disease. In rare cases, the measles virus can persist and cause a late encephalitis, subacute sclerosing panencephalitis (2,5,6). Additionally, immunocompromised individuals can develop a very severe form of measles infection without a rash. Usually fatal, these infections can involve many organs, particularly lung, kidney, and brain tissue (4,8).

Since the development of a measles vaccine in 1963, the incidence of measles has dropped. However, measles remains a specified disease, especially in developing countries where it continues to be a dominant killer of young children. A recent upsurge in measles in developed countries has also added to the necessity for continued detection of the disease. Rapid laboratory confirmation of suspected measles cases has become important for control of measles outbreaks (1,7).

Among serological methods which aid in the confirmation of measles infection, the IFA has been found to be a sensitive and specific serological method for detection of measles IgM antibodies (3,9,10).

Principle of the Test

SCIMEDX fluorescent antibody assays use the indirect method of antibody detection and titer determination. Patient serum or plasma samples are applied to cultured cells containing inactivated viral antigens provided on paint delineated wells on glass microscope slides. During a 60 minute incubation, antibody specific for measles virus antigens forms an antigen/antibody complex with the measles virus antigens in the infected cells. In a brief washing step, nonspecific antibodies and other unreacted serum proteins are eliminated. Fluorescein-conjugated goat anti human IgM is then applied to the wells of the glass slide. The anti-IgM conjugate combines with human IgM, if present, during a 30 minute incubation. After a brief wash to remove unreacted conjugate, the slides are viewed by fluorescence microscopy. A positive antibody reaction is denoted by bright green fluorescence at the antigen sites.

Materials Furnished and Storage Conditions

Measles Virus Antigen Slides: Slides of fibroblast cells infected with measles virus on each glass well. The slides are ready for use after removal from protective pouch. Store at 2-8°C. Slides are stable at this storage condition until the expiration date stated on the pouch label.

Measles Virus IgM Positive Control: Each vial contains 0.5 ml measles virus IgM antibody positive human control. This component is a ready for use liquid at a 1:10 working dilution. Store at 2-8°C. Liquid positive control is stable at this storage condition until the expiration date stated on the vial label.

Measles Virus IgM Negative Control: Each vial contains 0.5 ml measles virus IgM antibody negative human control. This component is a ready for use liquid at a 1:10 working dilution. Store at 2-8°C. Liquid negative control is stable at this storage condition until the expiration date stated on the vial label.

Fluorescein Conjugate: Each vial contains 1.5 ml fluorescein conjugated goat (inactivated) antihuman IgM (μ chain specific) with Evans Blue and Rhodamine counterstains. The fluorescein conjugate is a conjugation of affinity chromatography purified anti human IgM with fluorescein isothiocyanate (FITC). Adding Evans Blue and Rhodamine counterstains to the conjugate masks nonspecific fluorescence of the tissue culture cells. This component is a ready for use liquid at its working dilution. Store at 2-8°C. Liquid conjugate is stable at this storage condition until the expiration date stated on the vial label.

Coverslip Mounting Media: Each vial contains 2.0 ml phosphate buffered glycerol with fade retardant. This component is a ready for use liquid at its working dilution. Storage temperature may range from refrigerated to room temperatures (2-30°C.). Mounting media is stable at either storage condition until the expiration date stated on the vial label.

Phosphate Buffered Saline (PBS): Each aluminized sealed packet of powdered buffer makes one liter of 1X PBS. Storage temperatures may range from refrigerated to room temperature (2-30°C.). Add the entire contents of a PBS packet to one liter of freshly prepared distilled or deionized water. Note: Addition of the salts while rapidly stirring the water will facilitate solubilization. Store PBS as a solution at 2-8°C.

Special Blotters: Absorbent blotters have pre-cut holes for use in drying the slide mask. Storage temperatures may range from refrigerated to room temperature (2-30°C.).

General Precautions

IFA Test Kit: No US Standard of Potency. For *in vitro* diagnostic use only.

- Store all kit components at their recommended or suggested temperatures. **Do not freeze**.
- Do not use the components beyond the stated expiration date of each component.
- SCIMEDX optimizes all of the active components in each lot of its IFA kits as a unit. Do not mix components from different lots or from different sources.
- The controls and conjugate contain 0.095% sodium azide that, if allowed to accumulate, can form explosive compounds in lead and/or copper plumbing. Flush drains thoroughly if used to dispose of these materials.

Antigen slides: All IFA antigen slides have fixed cells that do not contain any viable infectious agents. However, good laboratory practices (GLP) require careful slide handling and disposal as with any other potentially biohazardous laboratory material.

- Do not remove the slides from their protective pouch until ready for use.

Human Controls: The human controls in these kits have all been tested for hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV) antibody by FDA licensed methods and found to be non-reactive. However, no test system can ensure the absence of these agents. Handle all human serum components, including those received in your laboratory for testing, as potentially biohazardous.

Specimen Collection, Storage and Limitations of Test Samples

- Separate aseptically collected serum or plasma from the red blood cells and store frozen (-10°C or lower) until ready for testing. Avoid repeated freezing and thawing.
- If desired, store fresh liquid serum or plasma samples at 2° to 8°C for up to one week without loss of antibody activity.
- Do not use excessively lipemic samples without delipidization.
- Do not use contaminated samples.
- SCIMEDX recommends screening dilutions of 1:10 and 1:40 for testing purposes unless a pretreatment step removes interfering IgG. If serum is pretreated, testing at the 1:10 dilution is sufficient. We have available a pretreatment reagent. Please call customer service for additional information.

Additional Materials Required

- Test tubes, racks, pipettes, microtiter plates and safety pipetting devices for making sample dilutions
- 37°C. incubator
- Moist chamber for incubating slides
- Slide holder rack and staining dish for washing slides
- Coverslips: 22 X 50 mm No. 1 thickness glass

Fluorescence microscope: A fluorescence microscope equipped with the following was used to calibrate the controls and conjugate:

- 10X eyepiece
- 16X or 40X objectives
- Epi-illuminator with 50W halogen lamp
- FITC-excitation filter KP490
- Yellow absorbing filter K530
- Red suppression filter BG38

The fluorescein label has an excitation peak of 490 nm and an emission peak of 520 nm. Differences in endpoint reactivities and fluorescence intensities may be due to the type and condition of the fluorescence equipment used in your laboratory.

IFA Procedure

1. For IgM antibody determination, prepare a 1:10 and 1:40 screening dilution of each test sample in PBS. Prepare all dilutions in a minimum volume of 0.10 ml with PBS as the diluent. For samples pretreated to remove IgG a 1:10 dilution is sufficient. SCIMEDX can provide a pretreatment reagent to remove IgG by the following procedure: Add one drop (approximately 45 μl) of the reagent to 5 μl of test sample and mix well. The resulting 1:10 dilution can be used immediately. Precipitation may occur but will not interfere with the IFA test results.
2. Remove slides from protective pouch and apply 1 drop (approximately 20 μl) of the diluted test sample(s) to each well. Add sufficient volume to completely cover each well, but cross-mixing of contents between wells should not occur.

Note: Each day's test run requires one well each for positive control, negative control, and PBS (conjugate control). Positive and negative controls represent 1:10 screening dilutions.

3. Incubate the slides in a moist chamber for 60 minutes at 37°C.
4. Rinse the slides in a light stream of buffer. Avoid directing the stream at the wells.
5. Wash the slides for 10 minutes in PBS, changing the PBS solution after 5 minutes. Agitate the slides by moving the rack up and down in the buffer.
6. Blot the paint mask surrounding the test wells with the special blotters provided in the kit.
7. Apply one drop of the ready to use conjugate to each test well.
8. Incubate the slides in a moist chamber for 30 minutes at 37°C.
9. Repeat steps 4 (PBS rinse), 5 (10 minute PBS wash), and 6 (blot).

10. Apply the glycerol mounting media and 22 X 50 mm glass coverslip to cover the wells of the slide.
11. Observe the reactivity under fluorescence microscopy using 200-500X magnification. For best results, examine slides immediately after completion of the test. To obtain equivalent results, seal slides or keep humidified to minimize dehydration of mounting medium, store in dark at 2-8°C, and read within three days. Positive reactivity may range in fluorescence intensity from brilliant to weak. Grade the fluorescence reaction according to the following intensity scale: 4^+ (brilliant), 3^+ (bright), 2^+ (moderate), 1^+ (weak).

Interpretation of Results

- Bright green fluorescent staining of the infected cells denotes a measles virus IgM antibody positive reaction. To provide an internal control, each well on the microscope slide contains both measles virus infected and uninfected cells. Preparation of the slide in this manner is intentional. Uninfected cells, stained red by the counterstain, provide a contrasting background. Infectivity of the cells ranges from 20 % - 60 %. Titration of positive measles virus IgM sera is not necessary except to minimize interfering antibodies or to provide quantitative information. In a titration series, the highest serum dilution demonstrating a 1+ reaction is interpreted as the endpoint.
- Absence of specific fluorescent staining of the infected cells denotes a measles virus IgM antibody negative reaction.

Significance of Interpretation

1. No discernible fluorescence of the infected cells found at the screening dilution.	1. Test sample is measles IgM antibody negative.
2. Specific positive fluorescence of the infected cells found at any of the screening dilutions.	2. Test sample is measles IgM antibody positive, indicating current infection.
3. Fluorescence found in both infected and uninfected cells.	3. Test sample is exhibiting a non-specific reaction.

Quality Control

1. To ensure the test is working properly use the positive and negative controls at least once for each day's testing.
2. The type and age of the fluorescence microscope and hours of UV bulb usage can affect fluorescence intensity and titration endpoints to some degree. The measles antibody positive control furnished with this kit is vialed at a working dilution that demonstrates a 2^+ to 4^+ intensity reaction. The vial has a listed titer to use as an additional check for the test system (see 1^+ Dilution Notice). Use this as the calibrator for a 2^+ to 4^+ intensity reaction on your microscope.
3. Use the measles antibody negative control furnished with this kit as the calibrator for a negative reaction on your equipment.
4. Each day's test should include one PBS well in place of a test sample. This is a conjugate control to ensure the conjugate is not reacting with the cell substrate.

1^+ Dilution Notice

The positive control in this kit is vialed at a screening dilution to provide a 2^+ to 4^+ fluorescent intensity when tested. To obtain a 1^+ fluorescent intensity make two-fold dilutions to the titer indicated on the vial included in this kit. Titer the positive control with the initial use of the kit.

The titer you obtain in testing may differ from the listed endpoint titer due to a number of technical reasons. It is best to test the titer indicated on the vial, as well as the two-fold dilution immediately preceding and following the listed titer. It is normal for results obtained for an endpoint (1^+) titer to differ between laboratories due to factors affecting the intensity of fluorescence. These factors include:

1. the power rating of the UV light source in the microscope
2. the kind of light source
3. the age of the lamp
4. the length of the optical path of the microscope and the types of optical filters used
5. the accuracy of dilution techniques and the dilution equipment

Limitations of the Procedure

1. A serological test such as the IFA serves as an aid to detect viral infection, but its use should not be the sole criteria. The test results should be used in conjunction with information available from the patient, clinical evaluation and other available diagnostic procedures.
2. Nonspecific positive reactions such as antinuclear antibody and/or anticytoplasmic antibody reactions can occur in samples from patients with certain autoimmune diseases. Both infected and uninfected cells will fluoresce, and this may obscure a positive measles reaction. Therefore, observation of an autoimmune reaction cannot eliminate the possibility of measles infection.
3. SCIMEDX recommends pretreatment of test samples to remove IgG antibody. This additional step helps eliminate false negative and false positive results. When IgG antibody competes with IgM antibody for specific binding sites, IgG antibody can cause a false negative result. When IgG antibody forms immune complexes with the antigenic substrate that may then bind rheumatoid factor (IgM class), IgG antibody can cause a false positive result.

Expected Values

The pattern of antibody response is characteristic, with IgM antibodies appearing when the rash develops and peaking about 10 days later. IgM antibodies are undetectable within 30-90 days.

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