

### INTENDED USE

The ZEUS IFA Measles IgG Test System is designed for the qualitative and quantitative detection of Measles antibody in human serum by the indirect fluorescent antibody (IFA) technique. The assay can determine or confirm recent infection or immune status and is for *In Vitro* diagnostic use.

### SIGNIFICANCE AND BACKGROUND

Measles (Rubeola) is a highly contagious viral disease resulting from infection with a paramyxovirus (genus *Morbillivirus*). Eight to twelve days following infection, a prodromal phase of measles begins, which is marked by fever, cough, coryza, and conjunctivitis. In many cases, the onset of the prodromal symptoms is followed (2 - 3 days) by the appearance of a specific enanthem (Koplik's spots), and a generalized maculopapular eruption (3 - 4 days after onset) (1). In uncomplicated measles, the appearance of the rash is followed by a peak in temperature one to two days later, and a rapid defervescence on the third or fourth day of the rash.

Under normal circumstances, the appearance of the prodromal symptoms, especially the highly specific and pathognomic Koplik's spots, is sufficient for clinical diagnosis. However, since the introduction of the measles vaccine in 1963, the incidence of measles has dramatically decreased (2). As a result, medical professionals have had less experience in the clinical diagnosis of the disease and may require laboratory assistance for confirmation.

Diagnosis of measles can be further complicated by the appearance of an atypical form of measles in persons who were immunized with an inactivated measles vaccine between 1963 and 1967, and were subsequently reinfected with wild-type virus (3). The atypical form of measles may be severe and clinically confused with Rocky Mountain Spotted Fever. In addition, acute measles may be complicated by secondary bacterial infections of the respiratory tract and middle ear. Additional complications may include a post-infectious encephalitis and a rare, but often time fatal disease, subacute sclerosing panencephalitis (SSPE) (1).

Antibodies to measles virus begin to appear with the development of the rash. A transient IgM antibody response (3 - 6 weeks) may appear first or in conjunction with IgG. IgG antibodies peak in 2 - 6 weeks, decline gradually over 6 months, and remain relatively stable thereafter. Following administration of live, attenuated measles vaccine, antibody can be detected 11 - 14 days after inoculation (1). Subclinical reinfections can occur in persons with either vaccine-induced or natural immunity resulting in a boost in measles-specific IgG titer (1).

In spite of the wide spread vaccination program, many individuals remain susceptible to measles as a result of primary vaccine failure or non-immunization. Serology is a useful tool for ascertaining the immune status of previously vaccinated individuals, and detection of seroconversion in recently vaccinated individuals. In addition, measles serology can be a valuable tool in the diagnosis of subacute sclerosing panencephalitis which may occur years after the original measles infection (3).

### PRINCIPLE OF THE ASSAY

The ZEUS IFA Measles IgG Test System is designed to detect circulating IgG antibodies to measles virus in human serum. The assay employs measles virus-infected HEp-2 substrate cells and fluorescein isothiocyanate (FITC)-labeled anti-human IgG adjusted for optimum reactivity and free of nonspecific background staining. The reaction occurs in two steps:

- Step one is the interaction of measles-specific antibodies in patient sera with the measles-infected substrate cells.
- Step two is the interaction of FITC-labeled anti-human IgG with the measles IgG antibodies attached to the measles antigen(s) localized in the nucleus and/or cytoplasm of the infected substrate cells.

### TEST SYSTEM COMPONENTS

#### Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. SAVe Diluent® contains Sodium Azide (<0.1% w/v) as a preservative.**

- |           |   |
|-----------|---|
| • • •     | 1. Measles Antigen Substrate Slides: Ten, 10-well Slides containing measles ( <i>Edmonston</i> strain) infected human cells, and 70 - 90% uninfected (control) cells in each well. Also includes absorbent blotter and desiccant pouch. |
| CONJ      | 2. Conjugate: Goat anti-human IgG (γ chain specific) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, amber-capped, bottle. Ready to use.                               |
| CONTROL + | 3. Positive Control (Human Serum): Will produce positive apple-green fluorescence in the nucleus and/or cytoplasm of the infected cells. One, 0.5mL, red-capped, vial. Ready to use.  |
| CONTROL - | 4. Negative Control (Human Serum): Will produce no detectable staining of the infected cells. One, 0.5mL, green-capped, vial. Ready to use.   |
| DIL SPE   | 5. SAVe Diluent®: One, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. <b>NOTE: The SAVe Diluent® will change color when combined with serum.</b>  |
| BUF PBS   | 6. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.      |
| MNTMED    | 7. Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dripper tipped vials.  |

#### NOTES:

- The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems, as long as the product numbers are identical: SAVe Diluent® (Product #: FA005CC), Mounting Media (Product #: FA0009S), and PBS (Product #: 0008S).
- Test System also contains a Component Label containing lot specific information inside the Test System box.

### PRECAUTIONS

- For *In Vitro* diagnostic use.
- Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
- The wells of the Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
- The Controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Bio-safety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (20).

- Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay.** Return unused reagents to their original containers immediately and follow storage requirements.
- Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate. Do not allow the wells to dry out between incubations.
- The SAVE Diluent®, Conjugate, and Controls contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may be toxic if ingested.
- Dilution or adulteration of these reagents may generate erroneous results.
- Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
- Avoid microbial contamination of reagents. Incorrect results may occur.
- Cross contamination of reagents and/or samples could cause erroneous results.
- Reusable glassware must be washed and thoroughly rinsed free of all detergents.
- Avoid splashing or generation of aerosols.
- Do not expose reagents to strong light during storage or incubation.
- Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
- Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
- Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
- Do not apply pressure to slide envelope. This may damage the substrate.
- The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
- Unopened/opened components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
- Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
- Do not allow slides to dry during the procedure. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

### MATERIALS REQUIRED BUT NOT PROVIDED

- Small serological, Pasteur, capillary, or automatic pipettes.
- Disposable pipette tips.
- Small test tubes, 13 x 100mm or comparable.
- Test tube racks.
- Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing Slides between incubation steps.
- Cover slips, 24 x 60mm, thickness No. 1.
- Distilled or deionized water.
- Properly equipped fluorescence microscope.
- 1 Liter Graduated Cylinder.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).



The following filter systems, or their equivalent, have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies:

<b>Transmitted Light</b>			
Light Source: Mercury Vapor 200W or 50W			
Excitation Filter	Barrier Filter	Red Suppression Filter	
KP490	K510 or K530	BG38	
BG12	K510 or K530	BG38	
FITC	K520	BG38	
Light Source: Tungsten – Halogen 100W			
KP490	K510 or K530	BG38	
<b>Incident Light</b>			
Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	Dichroic Mirror	Barrier Filter	Red Suppression Filter
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

### SPECIMEN COLLECTION

- ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
- Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (4, 9). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
- Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (10).

### STORAGE CONDITIONS

	Unopened Test System.
	Mounting Media, Conjugate, SAVE Diluent®, Slides, Positive and Negative Controls. Rehydrated PBS (Stable for 30 days).
	Phosphate-buffered-saline (PBS) Packets.

## ASSAY PROCEDURE

1. Remove Slides from refrigerated storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove Slides. **Do not apply pressure to flat sides of protective envelope.**
2. Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted.** Prepare a 1:10 dilution (e.g.: 10µL of serum + 90µL of SAVE Diluent® or PBS) of each patient serum. **The SAVE Diluent® will undergo a color change confirming that the specimen has been combined with the Diluent.**  
**Dilution Options:**
  - a. As an option, users may prepare initial sample dilutions using PBS, or Zorba-NS (Zorba-NS is available separately. Order Product Number FA025 – 2, 30mL bottles).
  - b. Users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in SAVE Diluent® or PBS. When evaluated by ZEUS Scientific, an endpoint dilution is established and printed on the Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected endpoint titer for each lot of Positive Control.
  - c. When titrating patient specimens, initial dilutions should be prepared in SAVE Diluent®, PBS, or Zorba-NS and all subsequent dilutions should be prepared in SAVE Diluent® or PBS only. Titrations should not be prepared in Zorba-NS.
3. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
4. Incubate Slides at room temperature (20 - 25°C) for 30 minutes.
5. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
6. Wash slides for two, 5 minute intervals, changing PBS between washes.
7. Remove Slides from PBS one at a time. Invert Slide and key wells to holes in blotters provided. Blot Slide by wiping the reverse side with an absorbent wipe. **CAUTION: Position the blotter and Slide on a hard, flat surface. Blotting on paper towels may destroy the Slide matrix. Do not allow the Slides to dry during the test procedure.**
8. Add 20µL of Conjugate to each well.
9. Repeat steps 4 through 7.
10. Apply 3 - 5 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope.  
**NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.**

## QUALITY CONTROL

1. Every time the assay is run, a Positive Control, a Negative Control and a Buffer Control must be included.
  2. It is recommended that one read the Positive and Negative Controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If Controls do not appear as described, results are invalid.
    - a. Negative Control - characterized by the absence of fluorescence.
    - b. Positive Control - characterized by a 3+ to 4+ apple-green fluorescence in the nucleus and/or cytoplasm of the infected cells. Between 10 - 30% of the cells in any one field are infected. The remaining cells in the same field (70 - 90%) are uninfected and serve as a "within-field" Negative Control.
  3. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
- NOTE: The intensity of the observed fluorescence may vary with the microscope and filter system used.**

## INTERPRETATION OF RESULTS

1. Negative Patients: The absence of characteristic staining in the infected cells represents a negative reaction. This reaction should compare favorably with the reaction observed in the negative control.
2. Positive Patients: The number of infected cells observed with positive patient sera should closely approximate the number of infected cells seen in the positive control well. The reactivity, depending on patient antibody concentration, may vary from 1+ to 4+ apple-green granular fluorescence in the nucleus and/or cytoplasm of the infected cells. The 1+ staining reaction appears as the first definitely discernible characteristic fluorescent staining within the measles infected cells in the field.

**NOTE: All positive test sera should be titered to end-point.** Serial two-fold dilutions should be prepared in PBS starting with a 1:20 (repeating the 1:10 dilution is optional) in volumes of at least 100µL. The end-point is the last dilution that produces apple-green staining (1+) in the infected cells. If, for any reason, the sample does not titer to end-point, test results should be reported as indeterminate. Non-Specific Reactions: If **all** the cells (infected and uninfected) in a test field exhibit apple-green fluorescence in the nucleus and/or cytoplasm, an autoimmune or other antibody reaction (e.g. anti-actin (5)), should be considered. **NOTE: It is recommended that samples exhibiting non-specific reactivity be titered to end-point to aid in the interpretation.** Test results should be reported as indeterminate if characteristic measles fluorescence cannot be resolved from the non-specific fluorescence.

Analysis of Titers	
Serum Titer	Significance
Less than 1:10:	No measles specific fluorescence in the substrate wells. *No detectable antibody to measles by the IFA test (see Limitations of the Assay, 1 - 2).
Greater than or equal to 1:10: (≥1+ fluorescence)	Measles specific fluorescence in infected cells only (see Limitations of the Assay, 1 & 3). *Indicates current infection *Indicates previous exposure (natural or vaccine) and patient is presumed to be immune *Indicates passively acquired antibody
Four-fold or greater rise in titer:	A four-fold rise in titer (seroconversion) between acute and convalescent specimens is highly suggestive of a current infection with measles virus (see Limitations of the Assay, 1, 2, 4, & 5).

## LIMITATIONS OF THE ASSAY

1. A single, serological IgG test result should not be the only criterion used to establish a diagnosis. The ZEUS IFA Measles IgG Test System results should be reviewed in conjunction with information available from the clinical evaluation, as well as other diagnostic procedures, by a competent medical authority.
2. A negative Measles IgG test result on a single sample generally indicates no previous exposure to measles virus and susceptibility to this agent. A negative result may also indicate that the sample was obtained during the early phase of a primary infection, prior to the development of a detectable humoral immune response. If a measles infection is suspected, a second specimen should be obtained 7 - 14 days after the first, and the two specimens should be tested in parallel to determine if seroconversion has occurred.
3. A positive measles IgG test result on a single sample generally indicates a previous exposure to the virus (natural or vaccine), and the patient is presumed to be immune. A positive test result may also indicate a current infection or passively acquired measles antibody resulting from the recent receipt of blood products containing plasma.
4. A four-fold (or greater) rise in antibody titer between acute and convalescent samples is generally indicative of an active or recently active measles infection. An increase in titer of less than four-fold when both the acute and convalescent samples are positive is generally not indicative of a current infection unless:

- a. The interval between the acute and convalescent samples was not sufficient for the development of a four-fold rise in titer.
- b. Or, the acute specimen was obtained late after onset and the antibody response has plateaued.
5. In the case of the former (a.), a third sample should be obtained 7 - 14 days after the second, and all three samples should be assayed simultaneously.
6. When evaluating acute and convalescent samples, and the acute sample is negative, and the convalescent sample is positive; seroconversion is presumed to have occurred and an active, primary infection should be suspected. If both the acute and convalescent samples are negative, an active measles infection is not likely unless the second specimen was taken too soon after the first. If the clinical situation suggests an active infection, a third sample may be obtained 7 - 14 days after the second, and all three samples should be analyzed simultaneously to determine if seroconversion has occurred.
7. End-point reactions may vary due to the type of microscope employed, the light source, age of bulb, filter assembly, and filter thickness.
8. Antibody titers against measles virus may be elevated as a result of other conditions (3), which have not, as yet, been correlated with persistent measles virus infection. For example, increased measles antibody titers, as well as antibody titers against other viruses (Rubella) have been associated with active chronic hepatitis, systemic lupus erythematosus, and infectious mononucleosis (6). In addition, elevated measles (and other virus) titers have been found in patients with multiple sclerosis (7). The significance of this finding is unknown.

### EXPECTED RESULTS

Infection with measles virus is related to many factors; including age, socioeconomic factors, and the usage of measles vaccine (1). In the absence of widespread measles vaccination, the majority of children (>95%) will become infected before the age of 15 years. In developing countries, the age of incidence of measles shifts to earlier age groups. More than 50% of children may be infected before the age of 2 years, and nearly 100% by the age of 5 years (1). With the introduction of the measles vaccine, the age-specific incidence of measles infection has shifted upwards as the overall incidence of measles has declined (2).

### PERFORMANCE CHARACTERISTICS

#### 1. Comparative Studies:

- a. The ZEUS IFA Measles IgG Test System was compared to a commercially available Measles IFA test system using 74 samples. The results of this evaluation are depicted in Table 1 below:

**Table 1: Measles Antibody Activity**

	Reactive	Non-Reactive
ZEUS IFA Measles IgG Test System	60	14
IFA (Other)	61	13

- b. A second evaluation was performed to compare the ZEUS IFA Measles IgG Test System to a commercially available Measles ELISA test system. This evaluation utilized a total of 94 specimens and the results are depicted in Table 2 below:

**Table 2: Measles Antibody Activity**

	Reactive	Equivocal	Non-Reactive
ZEUS IFA Measles IgG Test System	72	N/A	22
ELISA	71	8	15

- c. A modified endpoint titer comparisons was performed to assess the distribution of measles antibody titers within the specimen population depicted in Table 1 and compared to a commercially available Measles IFA test system. These results are depicted in Table 3 below.

**Table 3: Endpoint Titer Comparison**

ZEUS IFA Measles IgG Test System	< 1:10	1:10 to 1:40	> 1:40
	14/74 (18.9%)	34/74 (46.0%)	26/74 (35.1%)
Other IFA	< 1:8	1:8 to 1:32	> 1:32
	13/74 (17.6%)	29/74 (39.2%)	32/74 (43.2%)

#### 2. Reproducibility

Sample ID	Intra-run			
	Inter-run			
	1a	1b	2	3
0068	1:10	1:10	1:10	1:10
0111	1:10	1:10	1:10	1:10
0019	1:20	1:20	1:40	1:40
0018	1:160	1:320	1:320	1:160
0026	1:320	1:640	1:320	1:640
0512	1:640	1:640	1:640	1:640
0013	1:640	1:640	1:1280	1:640
0012	1:1280	1:1280	1:1280	1:1280

- a. **Intra-run:** Eight samples with titers ranging from 1:10 to 1:1280 were titered in duplicate on the same slide lot (1a, 1b). Six of eight samples exhibited identical endpoint titers. Two of eight samples were within one, two-fold dilution.
- b. **Inter-run:** Using the same eight samples noted above for the intra-run testing, the samples were titered in singlicate on two different manufactured slide lots on two different days. Five of eight samples exhibited identical endpoints; the other three samples were within one, two-fold dilution.

When the results from the intra-run assay are included in the inter-run analysis (1a, 1b, 2, and 3), four of eight samples had identical titers and four of eight samples were within one, two-fold dilution.

### REFERENCES

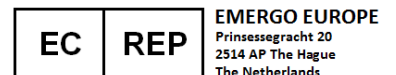
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