



Diagnostic Automation/Cortez Diagnostics, Inc.

I M M U N O D I A G N O S T I C S

AccuDiag™ Measles IgG IFA Kit

REF 250611

PIC ID250611-DWW1

IVD See External Label 2°C 8°C Σ 8x12 Wells

Measles IgG IFA

Principle	Indirect Fluorescent Antibody
Detection	Qualitative
Sample	10 μl serum
Incubation Time	60 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Easy to use with minimal equipment and expertise
- High sensitivity and specificity
- Versatile tool to detect wide range of antigens and antibodies
- Visual Interpretation of results using Fluorescence microscope

INTENDED USE

The AccuDiag™ Measles IgG indirect fluorescent antibody (IFA) test is intended for the detection and titration of measles IgG antibodies in human sera.

SIGNIFICANCE AND SUMMARY

Since the introduction of a measles (rubeola) virus vaccine in 1963, the United States has mounted an effective immunization program with the result that measles has essentially been eliminated as a major childhood disease. However, as a result of vaccine failure or the failure to be vaccinated, persistent shifts in the susceptible population towards young adults have been recorded.¹ In the case of measles, severity of illness and mortality rates are highest among adults.² Thus, serology has become increasingly important as a tool for determining immune status, particularly that of the young adult

population. In addition, the linkage between measles infection and premature delivery or spontaneous abortion supports the screening of pregnant women for susceptibility.³

Although measles has been recognized as a disease for over two thousand years, a description of its epidemiology first appeared in a paper by Panum in 1849. In his study of an epidemic in the Faroe Islands, Panum observed that measles has an incubation period of two weeks and is contagious, but years later, in 1963, the first live measles vaccine was licensed in the U.S. Vaccine development was made possible by Enders and Peebles' discovery, in 1954, that the virus could be successfully grown in an in vitro tissue culture system.⁵ The success of the vaccine is evident by the precipitous drop in the annual incidence of measles infection from 500,000 to less than 3,000 reported cases per year.

Classified as a paramyxovirus, measles produces a highly contagious respiratory infection. The disease is spread during the prodromal phase through direct contact with respiratory secretions in the form of droplets.³

In addition to measles, several other diseases have been associated but not causally linked to measles virus. This list includes subacute sclerosing panencephalitis (SSPE),⁶ systemic lupus erythematosus (SLE)⁷ and multiple sclerosis (MS).⁸ Patients with SSPE, a chronic degenerative neurologic disease, have documented high titers of antibody to measles virus. However, for SLE and MS there is less pronounced but statistically significant elevation in antibody titers. The significance or role that infection by measles virus plays in these disease states is unknown at the present time.

Since the presence of circulating IgG antibody to measles virus is indicative of previous infection or vaccination, screening pregnant women, young adults and other high risk patients for seropositivity is a valuable tool for determining their immune status.

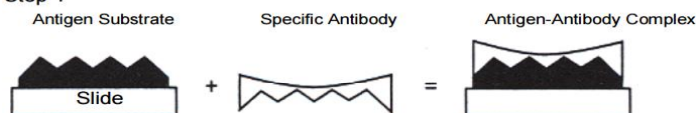
The AccuDiag™ Measles Kit manufactured by Diagnostic Automation, Inc., provides all necessary reagents for the rapid determination of measles antibody in human sera. Antigenic substrate, control sera, FITC conjugate, buffer, coverslip mounting media and an instructional insert are included in the kit.

ASSAY PRINCIPLE

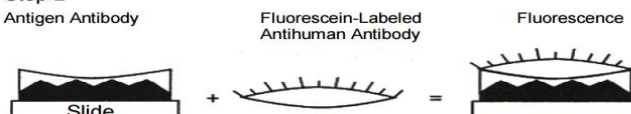
The AccuDiag™ fluorescent antibody assays utilize the indirect method of fluorescent antibody staining, first described by Weller and Coons in 1954.⁹ The procedure is carried out in two basic reaction steps. In step one, the human serum to be tested is brought in contact with the antigenic substrate. Antibody, if present in the test serum, will attach to the antigen, forming an antigen-antibody complex. If the serum being tested does not contain antibody for this particular antigen, no complex is formed and all the serum components are washed away in the rinse step. The second step involves adding a fluorescein labeled anti-human antibody to the test wells. If the specific antigen-antibody complex is formed in step one, the fluorescein labeled antibody will attach to the antibody moiety of the complex in step two. A positive reaction, bright apple-green fluorescence, can be seen with the aid of a fluorescence microscope.

Principle of Indirect Fluorescent Antibody Testing

Step 1



Step 2



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SPECIMEN COLLECTION AND HANDLING

1. Serum samples may be stored at room temperature for up to 24 hours. For longer term storage, they may be stored at 2-8°C (for up to three days), or frozen at -20°C or colder. Place at 37°C only until the samples are thawed. Remove and mix thoroughly before use. Self-defrosting freezers are not recommended. Avoid multiple free-thaw cycles.¹²
2. Optimal performance of the AccuDiag™ Measles IgG IFA depends upon the use of fresh serum samples. Specimens should be collected aseptically. Early separation from the clot prevents hemolysis of serum.¹³
3. For best results, another sample should be drawn if bacteriological contamination or lipids are present. If another sample cannot be obtained, filtering (0.45µ) or centrifugation (approximately 3000 x G for 10 minutes) is required.
4. Excess lipids in the test serum may produce a “filming” reaction. The lipids “stick” nonspecifically to the glass and are extremely difficult to remove. Experience will enable the trained technician to differentiate this “film” reaction from the specific reaction.
5. Occasionally, the specimen may contain certain proteolytic enzymes which attack and digest the substrate. This is especially true of specimens contaminated with microorganisms. Such specimens may be heated to 56°C for 30 minutes. If this fails to reduce the enzymatic activity, another sample should be obtained from the patient.

REAGENTS

96 Tests	Number of Tests per Kit
8	12-Well Slides: Measles infected (Edmonston strain) and uninfected cells.
1	Vial Positive Control: Liquid human serum with sodium azide as preservative.
1	Vial Negative Control: Liquid human serum with sodium azide as preservative.
1	Vial FITC Conjugate: Liquid inactivated goat anti-human IgG (heavy and light chains) counterstain with sodium azide as preservative.
3	*Packages Powdered Phosphate Buffer: (PBS) pH 7.4 ± 0.2
1	*Vial (2 mL) Buffered Glycerol
1	*Package (8 each) Blotters

*These components may be interchanged between different master lots. Additional supplies are available from Diagnostic Automation Inc.

Materials required but not provided

1. Test tubes and racks for making dilutions
2. Pipettes for preparing dilutions
3. Coverslips, 22 x 50 mm., No. 1 thickness
4. Humidified chamber
5. Magnetic stir plate (optional)
6. Staining dish and slide-holder rack
7. Fluorescence microscope. Refer to manufacturer's instruction manual for the filter system that gives optimum results for FITC (Maximum excitation wavelength = 490 nm. Mean emission wavelength = 520 nm.)

REAGENT STORAGE AND STABILITY

1. Store kit at 2-8°C. Powdered PBS and Buffered Glycerol can be stored at 2-30°C if desired. The test kit can be used through the expiration date on the outer box label.

2. Positive Control, Negative Control and FITC Conjugate should be stored at 2-8°C.

NOTE: Precautions were taken in the manufacture of this product to protect the reagents from contamination. Care should be exercised to protect the reagents in this kit from contamination. If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit.

REAGENT PREPARATION

Allow reagents and slides to reach room temperature 15 to 30 minutes before use.

1. **Slides and Glycerol:** Ready to use.
2. **PBS:** Dissolve contents of one package in 1 liter of distilled or deionized water. Seal container to prevent contamination or evaporation.
3. **Controls:** Ready to use in dropper vials provided at the 1:8 screening dilution.
4. **Conjugate:** Ready to use dropper vial at the appropriate working dilution.

ASSAY PROCEDURE

Specimens may contain infectious agents and should be handled accordingly.

For optimal results, DO NOT allow substrate wells to dry out while performing the test.

1. **Remove the slides and required reagents from the refrigerator** and allow them to reach room temperature (15 to 30 minutes).
2. **Remove the slides from the pouch** just before use and label.
3. **Dilute the samples** with PBS to the 1:8 screening dilution or prepare serial two-fold dilutions for quantitative determination.
4. **It is necessary to test 1 Positive Control, 1 Negative Control, and 1 PBS Buffer Control** for each batch of slides tested.
5. **Cover each well with diluted samples or controls** (~10-20 µL per well).
6. **Incubate in a humidified chamber at 37° ± 2°C for 30 minutes.**
7. **Rinse the slides briefly** in a light stream of PBS. Do not direct the stream into the wells.
8. **Rinse the slides thoroughly for 7 minutes** in a staining dish of PBS. **Change the buffer and wash for an additional 8 minutes.** Gentle agitation of the buffer is necessary for efficient slide washing.
9. **Blot the painted mask of the slide** with the blotters provided. **Do not allow the wells to dry before conjugate addition.**
10. **Cover each well by touching off 15-20 µL of FITC Conjugate from the dropper bottle tip.** Do not touch tip to substrate.
11. **Incubate in a humidified chamber at 37° ± 2°C for 30 minutes.** Protect from intense light.
12. **Repeat steps 8 and 9.** Blot the painted mask of the slide with the blotters provided. Do not allow the wells to dry before the addition of glycerol.
13. **Place a small drop of Buffered Glycerol in each well** and cover with a coverslip.
14. For best results, the **slides should be read immediately at a magnification of 200-500X.** Alternatively, the slides may be read within 24 hours. However, they should be stored at 2-8°C in the dark, and sealed to prevent the mounting fluid from drying.

CRITERIA FOR GRADING FLUORESCENCE INTENSITY

- 4+ Brilliant apple-green fluorescence
- 3+ Bright apple-green fluorescence
- 2+ Clear distinguishable apple-green fluorescence
- 1+ Dull apple-green fluorescence



- o No fluorescence or barely visible fluorescence

GUIDELINES FOR CHARACTERIZING FLUORESCENCE

Measles Associated Fluorescence

A positive measles antibody reaction is identified by the presence of bright fluorescence in the cytoplasm of infected cells.

As an additional control, uninfected cells are mixed with the infected cells. Each high power field should contain cells that exhibit no specific fluorescence. These uninfected "negative" cells should exhibit dull red or orange staining of the cytoplasm with a greenish-black to black nucleus.

Non-specific Fluorescence

All the cells exhibit positive apple-green fluorescence, either nuclear, cytoplasmic, or both. A reaction produced by a disease state unrelated to or in addition to a measles infection, e.g., antinuclear antibody or antimitochondrial antibody should be considered.^{14,15}

No Fluorescence

Absence of or less than 1+ fluorescence in the cytoplasm of infected cells.

NOTES:

1. In quantitative determinations, the endpoint titer is the highest dilution showing a 1+ fluorescence in the cytoplasm.

INTERPRETATION OF SAMPLE RESULTS

RESULTS	SIGNIFICANCE
Screening:	
No fluorescence or fluorescence intensity < 1+ at the 1:8 screening dilution.	No detectable Measles IgG antibodies. Such individuals are presumed to be uninfected, therefore, susceptible to a primary measles infection.
≥1+ fluorescence at 1:8 or greater on a single serum sample.	Positive by IFA for Measles IgG antibodies. Such individuals are presumed to be immune to measles infection. Not diagnostic for recent or current infection without paired serum sample.
To confirm acute infection, paired samples are required. The first sample (acute) should be taken as soon as possible after clinical signs of infection. The second (convalescent) sample should be taken within 10-14 days of the first. Both samples must be tested at the same time using an identical lot of reagents.	
Paired Sera:	
A difference of one two-fold dilution or less in endpoint titer or paired serum samples taken 10-14 days apart.	No evidence of recent or current infection.
Four-fold (or greater) dilution increase in titer of paired serum samples taken 10-14 days apart.	Diagnostic for recent or current infection.

QUALITY CONTROL

1. The control sera are representative of positive and negative reactions. At the screening dilution, the Positive Control represents a strong (3-4+)

reaction. If the fluorescence intensity of the Positive Control is less than the acceptable range, the test is invalid and should be repeated.

2. Each lot of Positive Control must be titrated to an endpoint dilution. The endpoint titer must be within ± one two-fold serial dilution of the Positive Control titer reported in the AccuDiag™ Measles IgG IFA Kit Notice. If the results obtained are out of range, the test is invalid and should be repeated.
3. At the screening dilution, the Negative Control should not display apple-green fluorescence. If apple-green fluorescence is observed, the test is invalid and should be repeated.
4. Quality Control results were obtained on a Nikon® microscope equipped for epiillumination with a 50W HBO mercury ARC lamp, B filter system for FITC and a 40X dry objective (NA 0.65). Differences in endpoint reactivity and fluorescence intensity may be affected by the type and condition of fluorescence equipment used (see Microscope Specifications at the end of the package insert).

MICROSCOPE SPECIFICATIONS

Comparable filter systems are shown below:

Transmitted Light Fluorescence:

Light Source	Exciter Filter	Barrier Filter
Mercury Vapor 200W 50W	KP490 + BG38 (4 mm.) or BG12 (4 mm.) + BG38 (4 mm.)	KP510, K530
Tungsten Halogen 50W 100W	KP490 + BG38 (4 mm.)	KP510, K515, K530

Incident Light Fluorescence:

Light Source	Exciter Filter	Dichroic Beam Splitting Mirror	Barrier Filter
Mercury Vapor 200W 100W 50W	KP500 + BG38 (4 mm.) or BG23 (4 mm.) for stronger red suppression. Edge filter 450 nm., 480 nm. For narrow band excitation, suppression of tissue auto- fluorescence	TK-510	K510 K520
Tungsten Halogen 50W 100W	KP500 + BG38 (4 mm.)	TK510	K510 K515 K530

LIMITATIONS OF THE ASSAY

1. The AccuDiag™ Measles IgG IFA Test Procedure and the Interpretation of Sample Results must be followed closely to obtain reliable test results.
2. Lack of a significant rise in antibody titer does not exclude the possibility of measles infection.
3. When measuring IgG antibody levels, positive results in neonates must be interpreted with caution since maternal antibody is transferred passively from the mother to the fetus before birth. IgM assays are



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generally more useful indicators of infection in children below the age of six months.

- Results of this test should be interpreted in the light of other clinical findings and diagnostic procedures.

PRECAUTIONS

- HANDLE ALL ASSAY SPECIMENS, SLIDES, POSITIVE AND NEGATIVE CONTROLS AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS. All human blood components of the kit have been tested by approved FDA methods and found to be negative for both hepatitis B surface antigen (HbsAg) and for antibodies to human immunodeficiency virus type 1. Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, specimens and kit reagents should be handled at the Biosafety Level 2, as recommended for any potentially infectious human serum or blood specimen.^{11,12}
- The antigenic substrates are fixed in acetone.
- Do not pipette by mouth.
- Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
- All materials used in this assay, including reagents, samples and wiping materials should be disposed of in a manner that will inactivate infectious agents.

HANDLING PRECAUTIONS

- For In Vitro Diagnostic Use.**
- Do not use the kit or individual reagents beyond their labeled expiration dates.
- The components of this kit have been tested as a unit. **Do not** interchange components from other sources or from different master lots, except as noted.
- Protect the conjugate from prolonged exposure to light.

REFERENCES

- Frank, JA et al. 1985. Major Impediments to Measles Elimination: The Modern Epidemiology of an Ancient Disease. AJDC (American Journal of Disease of Children), 139:881-888.
- Measles Surveillance Report No. 11. 1977-1981. Atlanta.
- Kempe, CH and VA Fulginit. 1965. The Pathogenesis of Measles Virus Infection. Arch Gesamte Virusforsch, 16:103-128.
- Panum, P. 1989. Observations Made During the Epidemic of Measles on the Faroe Islands in the Year 1846. Med. Classics, 3:829-886.
- Enders, JR and TC Peebles. 1954. Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles. Proc. Soc. Exp. Biol. Med., 86:277-286.
- Connolly, JH et al. 1967. Measles-virus Antibody and Antigen in Subacute Sclerosing Panencephalitis. Lancet, 1:542-546.
- Tannenbaum, M et al. Electron Microscopic Virus-like Material in Systematic Lupus Erythematosus: With Preliminary Immunologic Observations on Presence of Measles Antigen. J. Urol., 105:615-619.
- Adams, JM and DT Imagawa. 1962. Measles Antibodies in Multiple Sclerosis. Proc. Exp. Biol. Med., 111:562-566.
- Weller, TH and AH Coons. 1954. Fluorescent antibody studies with agent of varicella and herpes zoster propagated in vitro. Proc. Soc. Exp. Biol. Med., 86:789-794.

- NCCLS Doc M29-P. 1988. Protection of laboratory workers from infectious disease transmitted by blood and tissue, Proposed guideline. National Committee for Clinical Laboratory Standards. Villanova, PA.
- CDC/NIH Manual. 1988. Biosafety in Microbiological and Biomedical Laboratories, 2nd Ed. Pp 12-16.
- NCCLS Doc H18-T. 1984. Procedures for the Handling and Processing of Blood Specimens. National Committee for Clinical Laboratory Standards. Villanova, PA.
- NCCLS Doc H3-A2. 1984. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture, 2nd Edition: Approved Standard. National Committee for Clinical Laboratory Standards. Villanova, PA.
- Holborow, EJ, DM Weir and GD Johnson. 1957. A Serum factor in lupus erythematosus with affinity for tissue nuclei. Br. Med. J., 11: 732-734.
- Berg PA, I Roitt, D Doniach and HM Cooper. 1969. Mitochondrial antibodies in primary biliary cirrhosis. IV. Significance of membrane structure for the complement fixing antigen. Immunol., 17:281-293.

SUMMARY OF THE ASSAY

IMPORTANT: It is recommended that one be familiar with the detailed procedure in the package insert before using this summary.

Cover wells with the 1:8 screening dilution of sample or control. For quantitative determination, prepare serial two-fold dilutions.



Incubate slides in a humidified chamber at 37° ± 2°C for 30 minutes.



Rinse slides briefly with PBS. Wash for 15 minutes, changing buffer 1X. Blot slides.



Cover each well with FITC Conjugate.



Incubate slides in a humidified chamber at 37° ± 2°C for 30 minutes.



Repeat wash step described above.



Place a small drop of Buffered Glycerol on each well and cover with a coverslip.



Read slides immediately at 200-500X magnification on fluorescence microscope.

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016



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Date Adopted 2026-09-10

Brand Name AccuDiag™

REF 250611 AccuDiag™ - Measles IgG IFA

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Revision Date: 2020-10

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