Subject/Title: **ASI MEASLES IgG EIA TEST**		Doc#: 850096AG
Effective Date: 10/24/25	Supersedes Revision/Date: 10/26/17 (**2**)	Revision: 10/24/25 (**3**)
Prepared by:	QA Approval by:	Copy/Dept.:

FOR IN VITRO DIAGNOSTIC USE

1.0 INTENDED USE: For the qualitative and semiquantitative detection of human IgG antibodies to measles virus in human serum by enzyme immunoassay. This assay has not been cleared / approved by the FDA for blood / plasma donor screening.

2.0 SUMMARY AND EXPLANATION:

Measles is a highly contagious, acute, exanthematous disease. It is generally self-limiting and without serious consequences, although complications such as bronchopneumonia and otitis media do occur. The most serious consequence, encephalomyelitis, is fortunately rare (about 1 in 10,000 cases). Natural infection with measles virus confers permanent immunity.

Prior to the advent of vaccines, measles was an almost universally acquired disease of childhood. With the widespread introduction of vaccines however, the incidence of measles has been dramatically reduced[1], and physicians have become increasingly less familiar with this disease. Populations vaccinated in childhood with attenuated measles vaccines have presented atypical forms of measles[2]; and children vaccinated before 15 months of age may be susceptible to measles infection despite being vaccinated[3]. Finally, measles infection poses a serious threat to immunosuppressed, or immunocompromised patients[4]. For these reasons, the laboratory diagnosis of measles has become increasingly important, notwithstanding the reduction in the incidence due to the introduction of vaccines.

The usual means of laboratory diagnosis of acute measles is serologic, either by the demonstration of a four-fold or greater rise in virus-specific IgG antibody in acute / convalescent serum pairs, or by the detection of virus-specific IgM antibody in a single, early, serum specimen. The traditional serologic test, hemagglutination-inhibition, has been replaced by enzyme-linked immunosorbent assays (ELISA), for practical reasons[5].

The Measles IgG EIA test is an ELISA test which utilizes a microwell format. Test results are obtained after one and one-half hours incubation time. They are objective and normalized as Index values, permitting uniformity of reporting

3.0 PRINCIPLE OF THE PROCEDURE:

Diluted samples are incubated in antigen-coated wells. Measles antibodies (if present) are immobilized in the wells. Residual sample is eliminated by washing and draining, and conjugate (enzyme-labeled antibodies to human IgG) is added and incubated. If IgG antibodies to Measles are present, the conjugate will be immobilized in the wells. Residual conjugate is eliminated by washing and draining, and the substrate is added and incubated. In the presence of the enzyme, the substrate is converted to a yellow end product which is read photometrically.

4.0 REAGENTS

Coated Wells	Coated with Measles antigen (Edmonston strain). 12 eight-well strips.
Well Support	One.
Diluent*	25 ml (pink color). Phosphate-buffered saline with a protein stabilizer.
Calibrator 1*	0.3 ml. Human serum. Strongly reactive for Measles IgG antibodies. Index value shown on vial label.
Calibrator 2*	0.3 ml. Human serum. Moderately reactive for Measles antibodies. Index value shown on vial label.
Positive Control*	0.3 ml. Human serum. Reactive for Measles antibodies. Index value range shown on vial label.
Negative Control*	0.3 ml. Human serum. Nonreactive for Measles antibodies.
Conjugate	12 ml (green color). Goat anti-human IgG labeled with alkaline phosphatase (calf).
Substrate	12 ml. p-nitrophenyl phosphate. ***Note**: The substrate may develop a slight yellow color during storage. One hundred microliters of substrate should yield an absorbance value less than 0.35, when read in a microwell against air or water.*
Wash Concentrate*	30 ml. Tris-buffered saline with Tween 20, pH 8.0. Prepare Wash Solution by adding the contents of the Wash Concentrate bottle to 1 liter of distilled or deionized water.
Stop Reagent	12 ml. Trisodium Phosphate 0.5 M.

* Contains 0.1% sodium azide.

Store these reagents according to the instructions on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water.

5.0 WARNINGS AND PRECAUTIONS

5.1 For *in vitro* diagnostic use.

5.2 Test samples, Calibrator(s), Controls and the materials that contact them, should be handled as potential biohazards. The calibrators and controls have been found to be negative for HIV, hepatitis B surface antigen and HCV antibodies by licensed tests. However, because no method can offer complete assurance that HIV, hepatitis B virus, HCV or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories", 1993, or latest edition.

5.3 The concentrations of anti-Measles IgG in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

5.4 Avoid contact with open skin.

5.5 Never pipet by mouth.

5.6 Certain of the test reagents contain sodium azide. Azides are reported to react with lead and copper in plumbing to

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form compounds that may detonate on percussion. When disposing of solutions containing sodium azide, flush drains with large volumes of water to minimize the build-up of metal-azide compounds.

5.7 R 21/22: Harmful in contact with skin and if swallowed.

5.8 S24/25 - 36/37/39: Avoid contact with skin and eyes. Wear suitable protective clothing, gloves and eye/face protection. For further information, refer to product SDS.

5.9 Do not interchange reagents from different reagent lots, except for Wash Concentrate, Diluent, Substrate and Stop Reagent.

5.10 Do not use reagents beyond their stated expiration date.

5.11 Incubation times recommended in the Test Procedure section should be adhered to.

5.12 Unused Coated Wells should be kept in their resealable bag with dessicant, and stored in the refrigerator.

5.13 Do not smoke, eat, drink, or apply cosmetics in areas where plasma/serum samples are handled.

6.0 HANDLING AND PROCEDURAL NOTES

6.1 In order to obtain reliable and consistent results, the instructions in the package insert must be strictly followed. Do not modify the handling and storage conditions for reagents or samples.

6.2 Do not use past the expiration date indicated on the kit.

6.3 Do not interchange components of one kit with those of another kit.

7.0 STORAGE INSTRUCTIONS

Store all reagents at 2 to 8° C in an upright position when not in use. Do not freeze reagents.

8.0 INDICATIONS OF DETERIORATION

8.1 Turbidity or precipitation in controls is indicative of deterioration and the component should not be used.

8.2 Bacterial contamination of reagents or specimens may cause false positive results.

9.0 SPECIMEN COLLECTION AND STORAGE

9.1 Sera should be separated from clotted blood.

9.2 If specimens are not tested within 8 hours, they should be stored at 2 to 8° C for up to 48 hours. Beyond 48 hours specimens should be stored at -20° C or below.

9.3 Multiple freeze-thaw cycles should be avoided.

9.4 Samples containing visible particulate matter should be clarified by centrifugation; hemolyzed, icteric, or grossly contaminated samples should not be used.

9.5 Samples should not be heat-inactivated before testing.

10.0 PERFORMANCE OF TEST

Materials Provided:

96 Tests			
Coated Wells	12 eight well strips	Negative Control	0.3 ml
Well Support	1	Conjugate	12 ml
Diluent	25 ml	Substrate	12 ml
Calibrator 1	0.3 ml	Wash Concentrate	30 ml
Calibrator 2	0.3 ml	Stop Reagent	12 ml
Positive Control	0.3 ml		

Additional Materials Required

1. Microplate washer
2. Pipettors for dispensing 4, 100 and 200 µl
3. Timer
4. 1 or 2 liter container for Wash Solution
5. Distilled or deionized water
6. Dilution tubes or microwells
7. Microwell reader capable of reading absorbance at 405 nm. Dual wavelength readers are recommended. Dual or single wavelength readers may be used. Data on file.

11.0 TEST PROCEDURE

Preparation for the Assay

11.1 Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use. The test procedure follows:

11.2 Prepare 1:51 dilutions of test samples, Calibrator(s), Positive and Negative Controls, in the test set Diluent. For example: add 4 µl of sample to 200 µl of Diluent in a dilution well or tube, and mix well.

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Note: *For qualitative assays, a single Calibrator (Calibrator 2) may be used; for semiquantitative assays, use Calibrator 1 and Calibrator 2.*

12.0 ASSAY PROTOCOL

12.1 Place an appropriate number of Coated Wells in the Well Support.
***Note**: For combination testing (multiple assays per plate), the strips should be assembled on a white background with good lighting. Be sure to note the placement of each strip.*

12.2 Transfer 100 µl of each diluted Calibrator, Control and patient sample to the wells.
***Note**: Include one well which contains 100 µl of Diluent only. This will serve as the reagent blank and will ultimately be used to zero the photometer before reading the test results.*

12.3 Incubate the wells at room temperature (20 to 25° C.) for 30 ± 5 minutes.

12.4 Wash wells four times with at least 250 µL/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.

12.5 Place 2 drops (or 100 µl) of Conjugate into each well.

12.6 Incubate the wells at room temperature for 30 ± 5 minutes.

12.7 Wash wells four times with at least 250 µL/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.

12.8 Place 2 drops (or 100 µl) of Substrate into each well.

12.9 Incubate at room temperature for 30 ± 5 minutes.

12.10 Place 2 drops (or 100 µl) of Stop Reagent into each well.

12.11 Read and record the absorbance of the contents of each well at 405 nm against the reagent blank.
***Note**: Adjust the photometer to zero absorbance at 405 nm against the reagent blank. Readings should be made within 2 hours after the reactions have been stopped.*

13.0 QUALITY CONTROL

Quality control requirements must be performed in accordance with applicable local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control Procedures. Controls and calibrator(s) with graded reactivity must be included. If they do not yield the expected response, the assay should be considered invalid and the assay repeated. If the repeat assay does not elicit the expected results for the controls and calibrator(s), discontinue use of the kit and contact ASI Technical Support at 800-654-0146.

14.0 INTERPRETATION OF RESULTS –

Calculation of Results
Qualitative results may be calculated using a single calibrator.

Single Calibrator (Calibrator 2)
Determine the Index value for each test sample (or Control) using the following formula:

$$\frac{\text{Calibrator 2 Index}}{\text{Calibrator 2 Absorbance}} \times \text{Test Sample Absorbance} = \text{Test Sample Index}$$

If the Calibrator is run in duplicate, use the average absorbance value to calculate results.

Calibration Curve
Alternatively, test results may be calculated from a three-point curve comprised of: Calibrator 1 (high-point), Calibrator 2 (mid-point) and the reagent blank (zero / origin), using a point-to-point curve fit.

The upper range of the curve may be expanded by adding additional points. For example: the concentration of Calibrator 1 may be increased 1.5-fold, and 2-fold, by adding 6 µl and 8 µl of Calibrator 1 to 200 µl of the test set Diluent, and transferring 100 µl of each dilution to coated wells. The Index values, assigned to these points, should be 1.5 and 2 times respectively, the value shown on the Calibrator 1 label. The extent to which the upper range of the standard curve may be expanded, will be limited by the Calibrator being used.

Test Validation Criteria
1. The Calibrator(s), Positive and Negative Controls must be included in each test run.
2. The absorbance value of Calibrator 1 must be at least 0.4, when read against the reagent blank.
3. The absorbance value of the reagent blank should be less than 0.35.
4. The Negative Control must have an Index value less than 0.9. This control is used to validate the assay below the cutoff of the assay.
5. The Positive Control must have an Index value within the range printed on the labels. When performing qualitative tests, users may supply alternative positive controls if they wish.
6. The Negative and Positive Controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff. Users may wish to establish an in-house control, having a quantitative value determined by replicate testing, at or near the cutoff of the assay, to monitor the precision of the assay cutoff. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. For guidance on appropriate quality control practices, please refer to NCCLS document C24-A, *Internal Quality Control Testing: Principles and Definitions*.

Interpretation of Results

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Index Value	Interpretation
< 0.9	Negative for Measles IgG, presumed non-immune to measles infection.
≥ 0.9 to < 1.1	Equivocal
≥ 1.1	Positive for Measles IgG, presumed immune to measles infection.

When equivocal results are obtained, another specimen should be obtained ten to fourteen days later, and tested in parallel with the initial specimen.

Values obtained with different manufacturer's assay methods may not be used interchangeably. The magnitude of the reported IgG level cannot be correlated to an endpoint titer. When the assay is used qualitatively, the magnitude of results above the cut-off is not an indicator of total antibody present.

15.0 LIMITATIONS OF THE PROCEDURE

The results obtained with the Measles IgG EIA test serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.

A single positive result only indicates previous immunologic exposure.

The assay performance characteristics have not been established for matrices other than serum. The assay performance characteristics of vaccinees have not been established.

If the assay is used with cord blood as the specimen source, positive results should be interpreted with caution. The presence of IgG antibodies to Measles in cord blood may be the result of passive transfer of maternal antibody to the fetus. Performance characteristics have not been determined with neonatal or cord blood.

The performance characteristics of the Measles IgG test with automated analyzers have not been established.

The performance characteristics of the Measles IgG EIA test with specimens obtained from immunosuppressed individuals, have not been established.

16.0 EXPECTED VALUES

The incidence of antibodies to measles virus may vary according to patient age and geographical location. Measles is predominantly a disease of childhood which occurs in epidemics during the winter and spring, in rural areas, and is more or less endemic in urban areas. Epidemics occur in 2 to 3 year cycles in more highly developed countries, as sufficient numbers of non-immune children arise in the population. In the United States the highest incidence of measles infection is in children 5 to 7 years of age. Circulating antibodies are detected 10 to 14 days post infection, i.e. after the appearance of the rash, and they persist for life.

Serum samples obtained randomly from 73 normal adult South Florida blood donors (59 % male and 41% female) were assayed at Laboratory C (Miami, FL) using the Measles IgG EIA test. Sixty-eight samples (93.2 %) were positive for IgG antibodies to Measles, two (2.7 %) were equivocal, and three (4.1%) were negative. The positive samples yielded Index values between 1.1 and 3.8. The mean Index value was 2.3. The incidence of these values is shown in table 1.

Table 1. Results of tests of 73 Random Specimens (100% frozen), from Normal Adult South Florida Donors, Performed at Laboratory C (Miami, FL), Using the Measles IgG EIA Test.

Index Value Ranges		Specimens
< 1.1	5	6.8%
≥1.1 to < 2	13	17.8%
≥2 to < 3	15	20.5%
≥3 to ≥ 3.5	13	17.8%
>3.5	27	37.1%

17.0 PERFORMANCE CHARACTERISTICS

Comparative Testing

Measles IgG EIA test results correlate well with results of other serological tests. Sera from normal blood donors were assayed for the presence of Measles IgG antibodies, using the Measles IgG EIA test and two other commercial EIA tests, at two independent laboratories (Lab A, Miami, FL, and Lab B, W. Columbia, SC), and at Laboratory C (Miami, FL). These results are shown below in Tables 2, 3 and 4, respectively.

Table 2. Results of Tests of 150 Specimens (79.4% frozen and 20.6% fresh), from South Florida, Performed at Laboratory A (Miami, FL), Using the Measles IgG EIA Test and Another Commercial EIA Test.

Comparative	Measles IgG EIA				
Test #1	Positive	Equivocal	Negative	95%CI	
Positive	130	5	1	Relative sensitivity*	95.8 to 100**

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Equivocal	1	0	4		
Negative	0	0	9	Relative specificity	66.4 to 100**
* Excluding equivocal results				Overall Agreement*	96.1 to 100**
** Calculated by the Exact Method.					

Table 3. Results of tests of 160 Specimens (5.6% frozen and 94.4% fresh), Performed at Laboratory B (W. Columbia, SC), Using the Measles IgG EIA Test and Another Commercial EIA Test.

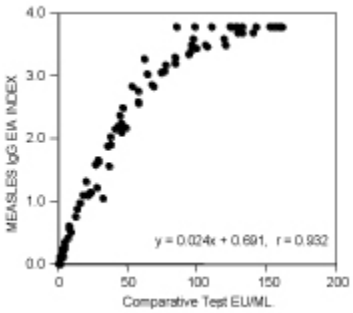
Comparative	Measles IgG EIA				
Test #2	Positive	Equivocal	Negative	95% CI	
Positive	117	2	0	Relative sensitivity*	96.9 to 100**
Equivocal	4	0	2		
Negative	11	4	11	Relative specificity*	29.1 to 70.9***
* Excluding equivocal results				Overall Agreement*	87.6 to 96.6***
** Calculated by the Exact Method.					
*** Calculated by the Normal Method.					

Table 4. Results of tests of 89 Specimens (100% frozen), from South Florida, Performed at Laboratory C (Miami, FL), Using the Measles IgG EIA Test and Another Commercial EIA Test.

Comparative	Measles IgG EIA				
Test #1	Positive	Equivocal	Negative	95%CI	
Positive	67	0	0	Relative sensitivity	94.6 to 100**
Equivocal	1	1	0		
Negative	0	1	19	Relative specificity*	82.4 to 100**
* Excluding equivocal results				Overall Agreement*	95.8 to 100**
** Calculated by the Exact Method.					

Please be advised that the term relative refers to the comparison of this assay's results to that of a similar assay. There was no attempt to correlate the assay's results with disease presence or absence. No Judgement can be made on the comparison of the assay's accuracy to predict disease.

Figure 1. Results of Tests of 89 Serum Specimens Performed at Laboratory C, Miami, FL, Using the Measles IgG EIA Test and Another Commercial EIA Test.



Specificity

The Measles IgG EIA test is specific for IgG antibodies directed against measles virus, and does not cross-react with the herpes viruses. Of five specimens which were unreactive in the Measles IgG EIA test, 5 were shown to contain moderate to high levels of IgG antibody directed against cytomegalovirus, 2 against herpes simplex virus, and 5 against Epstein-Barr virus. The IgG antibodies directed against cytomegalovirus, herpes simplex virus, and Epstein-Barr virus were detected using commercially available enzyme immunoassays.

Precision

Eight serum specimens (2 negative and 6 positive) and the Measles IgG EIA Positive and Negative Controls, were assayed in triplicate, on three separate occasions. The precision experiments were performed manually at two independent laboratories (Lab A and Lab B), and at Laboratory C. These results are shown below in tables 5 through 8, respectively.

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Table 5. Results of Intra-assay and Interassay Precision Tests Performed at Lab A. Values were calculated from the Measles IgG EIA Index values.

	INTRA-ASSAY			INTERASSAY		
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	S.D.	C.V.%
Pos. Control	1.6	0.153	9.4	1.7	0.122	7.3
Neg. Control	0.3	0.058	NA	0.3	0.000	NA
1	0.1	0.000	NA	0.1	0.044	NA
2	0.1	0.000	NA	0.1	0.053	NA
3	3.9	0.173	4.4	3.8	0.173	4.5
4	1.1	0.058	5.4	1.1	0.083	7.7
5	1.0	0.058	5.6	1.1	0.088	8.4
6	1.5	0.058	3.8	1.5	0.257	17.3
7	2.3	0.170	7.5	2.3	0.240	10.6
8	3.4	0.150	4.5	3.2	0.170	5.1

Table 6. Results of Intra-assay and Interassay Precision Tests Performed at Lab B. Values were calculated from the Measles IgG EIA Index values.

	INTRA-ASSAY			INTERASSAY		
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	S.D.	C.V.%
Pos. Control	1.7	0.056	3.2	1.6	0.091	5.5
Neg. Control	0.2	0.034	NA	0.2	0.000	NA
1	0.1	0.016	NA	0.1	0.024	NA
2	0.1	0.000	NA	0.1	0.026	NA
3	3.0	0.058	1.9	3.1	0.132	4.3
4	1.2	0.058	4.9	1.0	0.115	11.2
5	1.7	0.000	0.0	1.6	0.163	10.5
6	2.0	0.085	4.2	1.7	0.310	17.7
7	1.9	0.050	2.6	1.6	0.190	11.5
8	2.7	0.120	4.3	2.5	0.190	7.7

Table 7. Results of Intra-assay and Interassay Precision Tests Performed at Lab C. Values were calculated from the Measles IgG EIA Index values.

	INTRA-ASSAY			INTERASSAY		
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	S.D.	C.V.%
Pos. Control	1.8	0.115	6.5	1.6	0.142	8.7
Neg. Control	0.2	0.000	NA	0.2	0.000	NA
1	0.0	0.000	NA	0.0	0.000	NA
2	0.0	0.000	NA	0.0	0.000	NA
3	3.6	0.100	2.8	3.2	0.359	11.2
4	1.2	0.058	4.9	1.1	0.105	9.5
5	1.9	0.058	3.0	1.8	0.179	10.1
6	2.2	0.058	2.6	2.1	0.188	9.1
7	2.3	0.060	2.5	2.2	0.210	9.6
8	3.4	0.150	4.5	3.0	0.330	11.0

Table 8. Interlaboratory Precision. Tests Were Performed at Lab A, Lab B and Lab C. Values were calculated from the Measles IgG EIA Index values.

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	INDEXES		
SAMPLE	MEAN	S.D	C.V. %
Low Pos. Control	1.6	0.142	8.7
Neg. Control	0.2	0.000	NA
1	0.1	0.000	NA
2	0.1	0.000	NA
3	3.4	0.359	11.2
4	1.1	0.101	9.5
5	1.5	0.143	9.6
6	1.8	0.252	14.2
7	2.0	0.213	10.5
8	2.9	0.230	7.9

18.0 REFERENCES

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19.0 TECHNICAL INFORMATION: (801) 489-8911 or (800) 654-0146

	Manufactured for: Arlington Scientific, Inc. 1840 N Technology Drive, Springville, UT 84663 (USA)		JB Morphet Ltd. 34 Ashdale Road Kesgrave Suffolk IP5 2PA United
	Lot No.		Expiration Date
	96 Tests		Catalog No.
	*In vitro* diagnostic use only		Temperature Limitations