Laboratory Procedure Manual

Analyte: **Measles Antibody**

Matrix: **Serum**

Method: **Measles IgG ELISA II**

as performed by: National Center for Infectious Diseases
Division of Adolescent and School Health
Group 81 MS C22
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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label
MMRV_F	LBXMEA	Measles antibody

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Wampole Measles IgG ELISA II test system is designed to detect IgG antibodies to Measles in human sera. Wells of plastic microwell strips are sensitized by passive absorption with Measles antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components
2. Peroxidase Conjugated goat anti-human IgG (y chain specific) is added to the wells and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

Measles (Rubeola) is a highly contagious viral disease resulting from infection with a paramyxovirus. Eight to twelve days following infection, a prodromal phase of measles begins which is marked by fever, cough, coryza, and conjunctivitis. 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. Subclinical reinfections can occur in persons with wither vaccine-induced or natural immunity resulting in a boost in measles-specific IgG titer (1,2,3).

2. SAFETY PRECAUTIONS

Consider all serum specimens for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions; wear protective gloves, eye wear, and lab coat during all steps of this method because of infectious contamination hazards. Place all plastic and glassware contaminated with serum in a plastic autoclave bag for disposal. Keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution when work is finished. Biosafety level 2 containment and practice as described in CDC/NIH publication #88-8395 are recommended for handling test specimens and kit reagents(4).

Material Safety Data Sheets (MSDS) for horseradish peroxidase, tetramethylbenzidine (TMB), DMSO, H_2SO_4, HCl, and Tween-20 are available through the National Center for Infectious Diseases (NCID) computer network. Risk

is minimal because the chemicals are required in small quantities, are well-packaged, and require limited handling by the operators.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Currently, raw data are transferred electronically from instrument readout files into a computerized spreadsheet. This spreadsheet is designed for the management of CDC National Laboratory test results. It functions within the Excel (Microsoft Corporation, Redmund, WA) software program. For IgG ELISA test results, adjusted mean test optical density readings are recorded (average optical density from two normal tissue control wells, is subtracted from the average optical density from two test antigen wells). Positive test cut-offs were established empirically by analyzing operator and test variation of results from 16 serum specimens (8 positive, 8 negative) tested twice a day (two different operators) on three different days. Internal standards (strong positive, weak positive, negative) are run on each test plate. Reporting of test results includes both the adjusted mean test optical density reading and the practical outcome (positive/negative) is done directly from the spreadsheet file in printed form or by electronic transfer.

b. A physical, written log is generated for all specimens received. The log includes batch receipt dates and updated details concerning the handling, manipulation, and analysis of specimens, as well as any problems that occur.

c. Finished data sheets are reviewed by the supervisor. All data files are printed out and stored in hard copy form and are archived to CD-ROM format and on hard drive.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a. No special instructions, such as fasting or special diets, are required.

b. Serum is the preferred sample, although specimens of plasma and blood collected and dried onto filter paper blots for reconstitution in the laboratory are also acceptable.

c. Minimum sample volume is 0.2 mL. For blood spots, two completely filled circles of blood (reconstituted to 0.5 mL) are required.

d. The appropriate amount of serum/plasma is dispensed into a Nunc cryovial or other plastic screw-capped freezing vial labeled with the participant's ID. Blood spots are collected through finger or heal prick onto the underside of prepared filter blot pads (provided by the lab) as instructed.

e. Serum and plasma specimens collected in the field should be frozen, and then shipped on dry ice by overnight mail. Once received, specimens should be stored at –20^{o}C until analyzed. Residual specimens are refrozen at -20 ^{o}C. Blood spot specimens must be permitted to completely dry before placing them into

individual zip-lock bags. They should be shipped at ambient temperature within one week of collection.

f. Specimens may be stored at 4-8 ^{o}C for up to 7 days, or stored frozen at -20 ^{o}C.

g. Subsequent freezing and thawing must be avoided as it may lead to loss of activity.

h. Slightly and moderately lipemic, hemolyzed, or icteric samples are acceptable. Samples that are grossly lipemic, icteric, or hemolyzed are to be brought to the attention of the laboratory manager or technical supervisor (reconstituted blood spots are invariably hemolyzed).

i. Specimens may be stored in glass or plastic vials, as long as the vials are tightly sealed to prevent dessication of the sample.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Instrumentation

(1) OPTImax tunable microplate reader, model 0112-0050 (Sunnyvale, CA)

(2) Automatic plate washer, model SKANwasher 400 (Sterling, VA)

b. Other Materials

Equipment/Supplies Required but Not Provided

ELISA microwell reader capable of reading at a wavelength of 450nm.
Pipettes capable of accurately delivering 10 to 200uL.
Multichannel pipette capable of accurately delivering (50-200uL)
Reagent reservoirs for multichannel pipettes.
Wash bottle or microwell washing system.
Distilled or deionized water.
One liter graduated cylinder.
Serological pipettes.
Disposable pipette tips.
Paper towels.
Laboratory timer to monitor incubation steps.

c. Reagent Preparation

Wash buffer concentrate (10X): dilute 1 part concentrate +9 parts deionized or distilled water.

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v).

1. Plate. 96 wells configured in twelve 1x8-well strips coated with inactivated Measles antigen. The strips are packaged in a strip holder and seated in an envelope with desiccant
2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG (y chain specific). Ready to use. One, 15 mL vial with a white cap.
3. Positive Control (Human Serum). One, 0.35 mL vial with a red cap.
4. Calibrator (Human Serum). One, 0.5 mL vial with a blue cap.
5. Negative Control (Human Serum). One, 0.35 mL vial with a green cap.
6. SAVe Diluent™ (Sample Diluent). One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 72 ± 0.2). Ready to use. Note: Shake Well Before Use. (Product # 4500CC). (NOTE: This reagent may be used with any Wampole ELISA test system utilizing Product # 4500CC). NOTE: The SAVe Diluent ™ will change color in the presence of serum.
7. TMB: One 15 mL amber bottle (amber cap) containing 3,3'.55'-tetramethylbenzidine(TMB). Ready to use. Contains DMS0 < 15% (W)
8. Stop solution: One 15 mL bottle (red cap) containing1M H2SO4, 0.7M HCI. Ready to use.
9. Wash buffer concentrate (10X): dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). NOTE: 1X solution will have a pH of 7.2 ± 0.2.

The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.

Note: Kit also contains:

1. Component list containing lot specific information is inside the kit box.
2. Packages insert providing instructions for use.

d. Standards Preparation

Identify individuals who have been previously tested and determined to be high positive, low positive, or negative for measles, and obtain serum from each. Use individual serum samples (not pooled). Add 10 µL of a 10 g/dL sodium azide solution per mL of serum (final dilution of sodium azide is 1:1000), aliquot sera into 5.0 mL lots and freeze at –20°C. These QC materials are stable indefinitely, unless contamination occurs. These sera are screened for HBs antigen and antibody to HIV before accepting for use.

Controls included on each plate: High positive, low positive, negative.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibration Curve

No calibration curve is generated by the user as part of these assay methods. The calibration is either automatic or performed periodically by contracted service personnel.

b. Verification Procedure

The instrument used to read assay results (Section 6.a.1.) is equipped to analyze the positive and negative controls for each test series. If, within a testing series, positive or negative controls do not conform to specifications as defined in the product literature, the entire series is invalidated by the instrument.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

(1) Serum or plasma: Unpack samples immediately after arrival, and check for damage and leakage during transport. Check the vial specimen numbers against the packing list and box vials. If specimens arrive frozen, work rapidly to avoid thawing and store at –20°C. Thawed specimens are stored at 4°C if they are to be tested within one week.

(2) Blood filter spots: Unpack specimens after arrival and check the filter pad specimen identifiers against the packing list. Dried blood specimen spots are removed from the pad (2 spots per pad) using a paper punch, and are placed together in 0.5 ml of PBS. Specimen disks are soaked for 30 minutes at ambient temperature. Pads are removed and any remaining

liquid is squeezed from them using duck-bill pliers and added to the soaking liquid. Specimens processed from dried blood are considered to have a practical starting dilution of approximately 1:10.

b. Sample Preparation

(1) Serum/plasma specimens:

(i) Dilute 20.0 µl of specimen in 380 µl of blotto supplemented with 1% normal HLF cell lysate and 1% fetal calf serum (final dilution of 1:20).

(ii) Vortex all tubes.

(2) Blood filter spot eluate:

(i) Dilute 200 µl of eluate with 200 µl of blotto supplemented with 1% normal HLF cell lysate and 1% fetal calf serum (final dilution 1:2)

(ii) Vortex all tubes.

c. Instrument Set-up

(1) Operation of the SkanWASHER 400

(i) Switch on the main power.

(ii) Verify that the waste inlet reservoir tubing is connected.

(iii) Verify that inlet reservoir is filled and pressurized.

(iv) Verify that plate carriage is in position for flat bottom plates.

(v) Run prime program before washing plates.

(2) Operation of the OPTImax tunable microplate reader

(i) Switch on main power to OPTImax reader.

(ii) Switch on Compac Elite 4/75 CXL laptop computer.

(iii) Run Softmax Pro application.

(iv) Verify communication between OPTImax reader and computer.

d. Operation of Assay Procedure

(1) Remove the individual components from storage and allow them to

warm to room temperature (20-25°C).

(2) Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8°C.

(3) Prepare a 1:21 dilution (e.g.: 10uL of serum + 200uL of SAVe Diluent™. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. The SAVe Diluent™ will undergo a color change confirming that the specimen has been combined with the diluent.

(4) To individual wells, add 100uL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.

(5) Add 100uL of SAVe Diluent™ to well Al as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.

(6) Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.

(7) Wash the microwell strips 5X.

Automated Wash Procedure:

If using an automated microwell wash system, set the dispensing volume to 300-350uL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

(1) Add 100uL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.

(2) Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes

(3) Wash the microwells by following the procedure as described in step 7.

(4) Add 100uL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.

(5) Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.

(6)Stop the reaction by adding 50uL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.

(7)Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

e. Recording of Data

Templates have been created for the OPTImax reader that automatically correct the optical density readings against the blank, calculate mean test O.D. (T.O.D.) and mean normal tissue O.D. (N.O.D.) for each specimen, and adjust the final O.D. value by subtracting N.O.D. FROM T.O.D. All raw and calculated data are automatically saved to an Excel spreadsheet for each run.

(1) Quality Control Data

Stong positive, weak positive and negative sera with established performance data with this assay are used to determine whether or not individual test runs are valid. These data (both raw O.D. readings and adjusted O.D. readings are recorded together with specimen test data for each test run).

(2) Analytical Results

Both raw data and adjusted O.D. readings are automatically recorded after each test run.

(3) Data Maintenance

All test results are maintained both as electronic Excel spreadsheets and in hard copy.

f. Replacement and Periodic Maintenance of Key Components

(1) Instruments are on service contract and, except for basic daily routine maintenance are serviced by a representative from Molecular Devices Corporation.

(2) Incubator temperatures, ScanWASH fluids, and room temperature are monitored and documented weekly; Freezers and refrigerators are under continual remote monitoring, and are monitored manually and documented weekly.

(3) All micropipettors used in testing clinical specimens are checked for calibration every 6 months. Pipettors that do not conform to specifications are sent out for recalibration in accordance with the manufacturer's recommendations. Calibration records are kept for each pipettor by serial number.

g. Calculations

Correction Factor
A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box.

Cutoff OD Value
To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.
1) (CF x mean OD of Calibrator = cutoff OD value)

Index Values or OD Ratios
Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

Example

Mean OD of Calibrator	= 0.793
Correction Factor (CF)	=0.25
Cut off OD	=0.793 x 0.25 = 0.198
Unknown specimen OD	=0.432
Specimen Index Value or OD Ratio	=0.432/0.198 = 2.18

9. REPORTABLE RANGE OF RESULTS

INTERPRETATION OF RESULTS

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥ 1.10

An OD ratio greater than or equal to 1.10 is interpreted as positive for IgG Measles. An OD ratio of less than or equal to 0.90 is interpreted as negative for IgG Measles. Specimens with ratio values in the equivocal range are considered borderline for IgG to measles. These specimens should be retested. Specimens which are repeatedly equivocal should be tested using an alternative method such as the Wampole Laboratories Measles IFA test system.

Results are recorded both as mean adjusted O.D. reading and by their objective rating as positive, negative or equivocal.

10. QUALITY CONTROL (QC) PROCEDURES

a. Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay.
b. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
c. The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

	OD Range
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥0.500

d. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤ 0.9.
e. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥ 1.25.
f. If the above conditions are not met the test should be considered invalid and should be repeated.

g. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.

h. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

i. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

j. Prepare a log of control results for each run including the date tested; the run number; the mean absorbance for each control; and a notation when an individual control or run is unsatisfactory.

k. Calculate the mean and standard deviation for each control on the basis of the first 40 acceptable runs with a lot of antigen.

l. Select at random one serum specimen from every other run for repeat testing (2.5%).

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

a. Repeat the entire EIA run using new serum dilutions.

b. If controls continue to fail, consult the supervisor for other appropriate actions.

c. Do not report results from runs in which the controls did not meet acceptable limits.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. A diagnosis should not be made on the basis of anti-Measles ELISA results alone. Test results for anti-Measles should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
b. The antibody titer of a single serum specimen cannot be used to determine a recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to demonstrate seroconversion.
c. Samples collected too early in the course of an infection may not have detectable levels of IgG. In such cases, a second sample may be collected after 2-7 weeks and tested concurrently with the original sample to look for seroconversion.

NOTE: For complete details and performance of this product, refer to the package insert provided with the test kit.

13. REFERENCE RANGES (NORMAL VALUES)

There are no established normal ranges for this assay.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens reach and maintain 20-25^{o}C during testing. After analysis, the specimens are stored at –20^{o}C or lower.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

An FDA licensed latex bead agglutination test performed equivalently with this IgG EIA in a validation study conducted in this laboratory. This method, while not preferred for handling large numbers of specimens, can be substituted for the IgG EIA, or may be used as an adjunct to clarify equivocal results obtained by EIA. If the analytical system fails, the specimens should be refrigerated at 4-8^{o}C until the analytical system is restored. If long-term interruption is anticipated, specimens are refrozen at –20^{o}C or lower.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Standard record-keeping means (e.g., electronic, data files, laboratory notebook) are used to track specimens. Records are maintained indefinitely. Specimens are retained at the laboratory for at least one year, and then may be placed in archival storage at the CDC facility in Lawrenceville, GA. Only numerical identifiers are used. All personal identifiers are kept masked and available only to the project coordinator in order to safeguard confidentiality.

19. SUMMARY STATISTICS AND QC GRAPHS

Qualitative assays are assays with a positive, negative or indeterminate result. Since the controls do not generate quantitative values, plots are not generated for quality control purposes.

REFERENCES:

Norrby E, and Oxman MN: Measles Virus. In: Virology, Fields BN and Knope DM, (eds). 2nd Edition, Raven Press, Ltd., New York, 1013-1044, 1990.

Gershon AA, and Krugman S: Measles Virus. In: diagnostic Procedures for Viral, rickettsia', and Chlamydial Infections. Lennette EH and Schmidt NJ (eds), 5th Edition. American Public Health Association, Inc. 655-693, 1979.

Norrby E: Measles Virus. In: Manual of Clinical Microbiology. Lennette EH, Balows A, Hausler WJ, and Shadomy HJ (eds), 4th Edition. American Society for Microbiology, Washington, DC. 769-773, 1985.

Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.

Procedures for the collection and diagnostic blood specimens by venipuncture. 2nd edition. Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodbome Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.