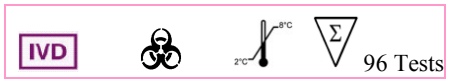
Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

CE

AccuDiag™ Measles IgM ELISA Kit

REF 1409-1



Test	Measles IgM ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	ELISA - Indirect; Antigen Coated Plate
Detection Range	Qualitative Positive; Negative control
Sample	10µL serum
Total Time	~ 75 min
Shelf Life	12 Months from the manufacturing date
Specificity	93%
Sensitivity	93%

INTENDED USE

The Diagnostic Automation, Inc. (DAI) Measles IgM ELISA is intended for the qualitative detection of Measles IgM antibodies in human serum of patients suspected of measles (rubeola) infection. This assay is intended for use as an aid in the diagnosis of a current or recent measles (rubeola) infection in conjunction with other clinical information and laboratory findings.

Performance characteristics have not been evaluated in immunocompromised or Immunesuppressed individuals. This test is not intended for use in neonatal screening or for use at a point of care. This test is not intended for use in screening blood and plasma donors. **High Complexity test.**

SUMMARY AND EXPLANATION

Since the introduction of a measles virus vaccine, many countries have mounted an effective immunization program which has essentially eliminated measles as a major childhood disease in those countries. However, as a result of vaccine failure or the failure to be vaccinated, a recent and persistent shift in the susceptible population towards young adults has 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 the immune status of the young adult population entering college or the military. In addition, the linkage between measles infection and premature delivery or spontaneous abortion supports screening pregnant mothers 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 had an incubation period of two weeks and was contagious but that life-long immunity followed primary infection (4). Over 100 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.

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). Ironically, because of the lower incidence of measles, younger physicians often diagnose the illness late in infection after the patient has exposed others. This has resulted in small isolated mini-epidemics among the susceptible population.

Several diseases in addition to measles 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 titer 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.

The immune response to infection (or vaccination) with measles virus is rapid and characteristic: Measles specific IgM and IgG begin to appear in the circulation simultaneously. The IgM response is relatively short- lived (1-3 months), while the IgG response is sustained, resulting in life-long immunity (9). Therefore, the identification of circulating measles specific IgM antibodies is useful in defining a primary infection. The Diagnostic Automation Inc. Measles IgM ELISA kit provides all the necessary reagents for the rapid determination of measles virus IgM antibody in human serum.

The sensitivity, specificity, and reproducibility of Enzyme-Linked Immunosorbent Assays are comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radioimmunoassays (14,15,16).

TEST PRINCIPLE

Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (e.g., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen- antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgM globulin conjugated with horseradish peroxidase which will bind to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H_2SO_4, The contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader (10, 11, 12, 13).

MATERIALS AND COMPONENTS

Materials provided with the test kits

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. **Purified Measles antigen coated microassay plate**: 96 wells, configured in twelve 1x8 strips stored in a foil pouch with desiccant. (96T: one plate)
2. **Calibrator:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day- to-day fluctuations in temperature and other testing conditions.(96T:one vial, 0.4mL) *
3. **Positive Control:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Positive Control is utilized to control the positive range of the assay. (96T: one vial, 0.4 mL) *

4. **Negative Control:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay (96T: one vial, 0.4 mL) *
5. **Horseradish-peroxidase (HRP) Conjugate:** Ready to use. Goat anti- human IgM, containing proclin(0.1%) and gentamicin as preservatives. (96T: one bottle, 16 mL)
6. **Serum Diluent Plus:** Ready to use, Contains goat/sheep anti-human IgG for serum absorption to remove competing IgG, with protein stabilizers and proclin (0.1%) as a preservative. (96T: two bottles, 45 mL each)
7. **Wash Buffer Type I (20X concentrate):** dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and proclin (0.1%) as a preservative. (96T: one bottle, 50 mL)
8. **Chromogen/Substrate Solution Type I:** Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 mL)
9. **Stop Solution:** Ready to use, contains a 1N H_2SO_4 solution. (96T: one bottle, 15 mL)

***Note: serum vials may contain excess volume.**

The following components are not kit lot # dependent and may be used interchangeably within the Diagnostic Automation Inc ELISA IgM assays: Chromogen/Substrate Solution Type I, Wash Buffer Type I, and Stop Solution. Please check that the appropriate Diagnostic Automation Inc. Reagent Type (Type I, Type II, etc.) is used for the assay.

Materials Required But Not Provided

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µLvolumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water (dH20), CAP (College of American Pathology) Type 1 or equivalent (20, 21).
9. Timer capable of measuring to an accuracy of +/- 1 second (0 – 60minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950mL dH20).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Note: Use only clean, dry glassware.

SPECIMEN COLLECTION AND PREPARATION

Specimen Collection

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. Optimal performance of the kit depends upon the use of fresh serum samples (clear, non-hemolyzed, non-lipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture (18). Early separation from the clot prevents hemolysis of serum.
3. Store serum between 2° and 8° C if testing will take place within two days. If specimens are to be kept for longer periods, store at -20° C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. The CLSI provides recommendations for storing blood specimens (Approved Standard Procedures for the Handling and Processing of Blood Specimens, H18-A4. 2010).(18)

Preparation

1. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21° to 25° C). Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer Type I to 1 L with distilled and/or deionized H_20 Mix well.

Serum Treatment

Solid phase immunoassays for the detection of virus-specific IgM are known to be sensitive to interfering factors. The goat/sheep anti-human IgG in the Serum Diluent Plus diminishes competing virus-specific IgG, which would be responsible for false negative reactions. False positives are similarly minimized by removing the IgG, thus neutralizing the bound rheumatoid factor in the samples.

PRECAUTIONS

1. For *in vitro* diagnostic use.
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that laboratory specimens are pathogen-free, specimens should be handled at Biosafety Level 2, as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, Feb. 200717, and CLSI Approved Guideline M29-A3, Protection of Laboratory Workers from Occupationally Acquired Infections. 27
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.17
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type I. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21° to 25° C) before running assay. Remove only the volume of reagents that is needed. Do not pour reagents back into vials as reagent contamination may occur.
7. Before opening Control and Calibrator vials, tap firmly on the bench top to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Wash hands before and after handling reagents. Cross-contamination of reagents and/or samples could cause false results.
11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
12. Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for theConjugate addition so that sodium azide is not carried over from other reagents.
13. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
14. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing ProClin®, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.
15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not

expose to work area during actual test procedure because of potential interference with enzyme activity.

16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with water.
17. Caution: Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
18. The concentrations of anti-Measles in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

The safety data sheet is available upon request.

WARNING
Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300®, a biocidal preservative
that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic
reaction in certain sensitive individuals.
H317: May cause an allergic skin reaction.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P302 + **P352**: IF ON SKIN: Wash with plenty of soap and water.
P333 + **P313**: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.
WARNING
Serum Diluent and Controls contain < 0.1% sodium azide.
H302: Harmful if swallowed
P264: Wash thoroughly with plenty of soap and water after handling
P270: Do not eat, drink or smoke when using this product
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
P330: If swallowed, rinse mouth
P501: Dispose of contents/container to in accordance to local, regional, national and international
regulations.

ASSAY PROCEDURES

1. Place the desired number of strips into a microwell frame. Allow four (4) Control/ Calibrator determinations (one Negative Control, two Calibrators And one Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configurations. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Patient # 4
1B	NC	2B	Patient # 5
1C	Cal	2C	Patient # 6
1D	Cal	2D	Patient # 7
1E	PC	2E	Patient # 8
1F	Patient # 1	2F	Patient # 9
1G	Patient # 2	2G	Patient # 10
1H	Patient # 3	2H	Patient # 11

RB = Reagent Blank-well without serum addition run with all reagents. Used to blank reader.
NC = Negative Control
Cal = Calibrator
PC = Positive Control

2. Dilute test sera, Calibrator and Control sera 1:81 (e.g., 10 µL + 800 µL) in Serum Diluent Plus. For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum. Mix well (Vortexing recommended).
3. To individual wells add 100 µL of diluted patient sera, Calibrator and Control sera. Add 100 µL of Serum Diluent Plus to the reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21° to 25° C) for 30 minutes +/- 2 minutes.
5. Aspirate or shake out liquid from all wells. Using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out to remove all liquid. Repeat the wash procedure two times (for a total of three washes) for semi-automated equipment or four times (for a total of five washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

****IMPORTANT NOTE**: Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semiautomated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of five (5) washes may be necessary with automated equipment. **Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.**

6. Add 100 µL Conjugate to each well, including the reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21° to 25° C) for 30 minutes +/- 2 minutes.
8. Repeat wash as described in Step 5**.
9. Add 100 µL Chromogen/Substrate solution (TMB) solution to each well, including reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21° to 25° C) for 15 minutes +/- 2 minutes.
11. Stop reaction by addition of 100 µL of Stop Solution (1N H_2SO_4) following the same order of Chromogen/Substrate addition, including reagent blank well. Tap the plate gently along the outsides to mix contents of the wells. The plate may be held up to one (1) hour after addition of the Stop Solution before reading.
12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is > 0.150, the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

RESULTS

Calculations

1. Mean Calibrator O.D. (Optical Density) - Calculate the mean O.D. value for the Calibrator from the two Calibrator determinations.
2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by Diagnostic Automation Inc,. for each lot of kits. The Correction Factor is printed on the Calibrator vial.
3. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1.

4. ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. Value by the Cutoff Calibrator Value determined in Step 3.

EXAMPLE

O.D's obtained for Calibrator	=0.38,0.42
Mean O.D. for Calibrator	=0.40
Correction factor	=0.50
Cutoff Calibrator Value	=0.50 x 0.40 = 0.20
O.D. obtained for patient sera	=0.60
ISR Value	=0.60/0.20 = 3.00

INTERPRETATION

The patients' ISR (Immune Status Ratio) values are interpreted as follows:

ISR Value	Results	Interpretation
≤ 0.90	Negative	No detectable IgM antibody to Measles. The results should be interpreted in the context of the clinical information and other laboratory findings
0.91-1.09	Equivocal	Samples should be retested in singlicate by the same method. Samples that remain equivocal after repeat testing should be tested on an alternate method e.g. for measles IgG testing and by another measles IgM test. Depending on the time of the collection of the first sample from the onset of clinical symptoms and the IgG results collecting a second sample within 7-10 days for repeat IgM testing
≥ 1.10	Positive	Presence of detectable IgM antibody to Measles. The results should be interpreted in the context of clinical and other laboratory findings (false positive results may occur).

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met:

1. Calibrator and Controls must be run with each test run.
2. Reagent blank (when read against air blank) must be <0.150 Absorbance (A) at 450 nm.
3. Negative Control must be ≤ 0.250 A at 450 nm (when read against reagent blank).
4. Each Calibrator must be ≥ 0.300 A at 450 nm (when read against reagent blank).
5. Positive Control must be ≥ 0.250 A at 450 nm (when read against reagent blank).
6. The ISR (Immune Status Ratio) Values for the Positive and Negative Controls should be in their respective ranges printed on the vials. If the Control values are not within their respective ranges, the test should be considered invalid and the test should be repeated.
7. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
8. Refer to CLSI C24-A3 for guidance on appropriate Quality Control practices.(19)
9. If above criteria are not met on repeat testing, contact Diagnostic Automation, Inc. Technical Services.

PERFORMANCE CHARACTERISTICS

Three (3) studies were conducted to evaluate the performance of the Diagnostic Automation, Inc. Measles IgM ELISA test kit.

Studies 1 and 2

188 samples were collected from individuals in whom a measles IgM test was ordered (suspected of measles infection in outbreak settings by the Texas Department of Health, Austin, Texas (66 samples) and the State Laboratory of Public Health in Raleigh, North Carolina (122 samples).
All serum samples were tested on the DAI Measles IgM ELISA test kit. All but one (1) sample was tested by a comparator testing algorithm used at each institution to determine the presence of current or recent measles infection, referred to as "comparator algorithm" in the performance tables. The algorithms used consisted of the results of other laboratory confirmation methods including comparator Measles IgM ELISA test kit, a comparator Measles IgM IFA test kit, Complement Fixation (CF), Hemagglutionation Inhibition (HI) and the Diagnostic Automation, Inc. Measles ELISA IgG test kit. See Table below for results.

		+	Comparator Algorithm Indeterminate	-
DAI Measles IgM	+	88	1	6
	Equivocal	3	3	10
	-	5	1	70
	# Agree	Total	Result	95% CI
% Positive Agreement	88	96	91.67%	84.2-96.3%
% Negative Agreement	70	86	81.40%	71.6-89.0%

Percent of Measles IgM Results that are Equivocal = 8.6% (16 of 187)

Performance with the indeterminate results from the comparator algorithm calculated against the performance of Measles IgM:

		+	Comparator Algorithm Indeterminate	-
DAI Measles IgM	+	88	1	6
	Equivocal	3	3	10
	-	5	1	70
	# Agree	Total	Result	95% CI
% Positive Agreement	88	97	90.7%	83.3-95.0%
% Negative Agreement	70	87	80.5%	70.9-87.4%

Percent of Measles IgM Results that are Equivocal = 8.6% (16 of 187)

Study 3

Eight (8) samples were submitted for testing at the Kansas Department of Health and Environment Laboratory (KDHE), Topeka, Kansas. All samples were tested on the Measles IgM and Measles IgG ELISA test kits as well as compared to the method used by the testing institution to determine the presence of current or recent measles infection, referred to as "comparator algorithm" in the performance table. The comparator algorithm included RNA polymerase chain reaction (PCR) on urine, nasopharyngeal swab (NPS) and/or throat swab (TS) samples. The final % agreement is presented in the table below:

		+	Comparator Algorithm Indeterminate	-
DAI Measles IgM	+	3	0	0
	Equivocal	0	0	0
	-	0	1*	4
	# Agree	Total	Result	95% CI
% Positive Agreement	3	3	100.00%	29.2-100.0%
% Negative Agreement	4	4	100.00%	39.8-100.0%

* This sample tested NPS negative at two sites, urine positive at one site and urine indeterminate at one site (all testing done by PCR).

Performance with the indeterminate results from the comparator algorithm calculated against the performance of the Measles IgM:

		+	Comparator Algorithm Indeterminate	-
DAI Measles IgM	+	3	0	0
	Equivocal	0	0	0
	-	0	1*	4
	# Agree	Total	Result	95% CI
% Positive Agreement	3	4	75%	30.1-95.4%
% Negative Agreement	4	4	100.00%	39.8-100.0%

PRECISION

The DAI Measles IgM ELISA Test Kit was evaluated for inter-assay precision.

This study consisted of six (6) blinded proficiency panel members, varying in reactivity: Four (4) low to moderate Positive samples and two (2) Negative samples run in triplicate at three (3) separate facilities. Testing at each site was done over 10 days by two (2) operators at each site resulting in a total number of 180 data points. The results are summarized below:

Precision Between All Sites

Sample Id	ISR Site1 N=60	IRS Site2 N=60	ISR Site 3 N=60	Mean ISR	STD DEV	% CV
1	1.30	1.34	1.23	1.29	0.06	4.3%
2	0.57	0.68	0.67	0.64	0.06	9.5%
3	1.16	1.26	1.07	1.16	0.11	8.2%
4	1.92	1.84	1.76	1.84	0.08	4.3%
5	0.67	0.82	0.84	0.78	0.09	12.0%
6	1.28	1.18	1.01	1.16	0.14	11.8%

The DAI Measles IgM ELISA Test Kit was evaluated for lot to lot precision.
This study consisted of six (6) blinded proficiency panel members, varying in reactivity: four (4) low to Moderate Positive samples and two (2) Negative samples run in triplicate on three (3) separately manufactured kit lots. The results are summarized below:

Inter-lot Reproducibility

Sample Id	ISR's Lot 1	IRS's Lot 2	ISR's Lot 3	Mean ISR	STD DEV	% CV
1	1.22	1.43	1.36	1.34	0.12	9.02%
2	0.57	0.42	0.46	0.48	0.11	22.55%
3	1.19	1.31	1.24	1.25	0.10	8.27%
4	2.13	1.96	1.56	1.88	0.28	14.83%
5	0.79	0.75	0.56	0.70	0.11	15.72%
6	1.27	1.32	1.30	1.29	0.04	3.19%

A separate precision/reproducibility study was performed internally (Diagnostic Automation, Inc) using one (1) lot of the DAI Measles IgM ELISA Test Kit. The study compared the consistency between two (2) Operators. This study consisted of six (6) blinded proficiency panel members, varying in reactivity: Four (4) Low to moderate Positive samples, and two (2) Negative samples run by each of the Operators over 10 days. The results are summarized below:

Intra-run Precision/Reproducibility Between users:
Total Precision at Site 1 with Two Operators Over Ten Days

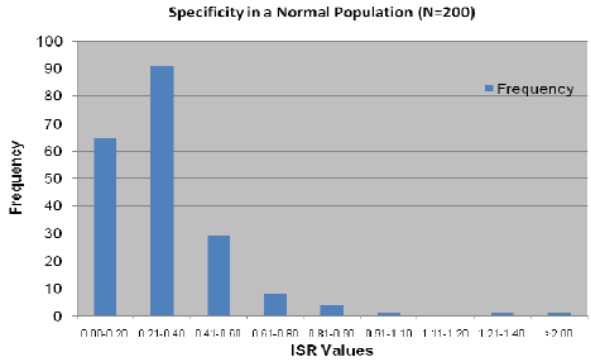
Sample ID	ISR's Operator 1 N=30	IRS's Operator 2 N=30	Mean ISR	STD DEV	% CV
1	1.26	1.33	1.30	0.05	3.80%
2	0.58	0.55	0.57	0.02	3.80%
3	1.14	1.17	1.16	0.02	1.80%
4	1.92	1.91	1.92	0.01	0.40%
5	0.69	0.65	0.67	0.03	4.20%
6	1.32	1.23	1.28	0.06	5.00%

SPECIFICITY IN A NORMAL POPULATION

A total of 200 random serum samples were tested to establish the expected values in a population of individuals between the ages 18-65 with no known clinically apparent Measles infection. The table below summarizes the distribution of DAI Measles IgM assay ISR Values observed for the population.

Distribution of Diagnostic Automation, Inc. Measles IgM Assay ISR Values from a Normal Population

Measles ISR Range	Number of Specimens (Frequency)	Percent Total
0.00-0.20	65	32.5%
0.21-0.40	91	45.5%
0.41-0.60	29	14.5%
0.61-0.80	8	4.0%
0.81-0.90	4	2.0%
0.91-1.10	1	0.5%
1.11-1.20	0	0%
1.21-1.40	1	0.5%
>2.00	1	0.5%



CROSS REACTIVITY

47 samples were used for establishing the potential cross reactivity of the DAI Measles IgM ELISA Test Kit. The samples were selected as confirmed positive by DAI CMV IgM, HSV1 IgM, HSV2 IgM, Rubella IgM, VZV IgM and Mumps IgM test kits, for each of the following potentially cross-reacting agents: CMV, HSV-1, HSV-2, Rubella, VZV, Mumps and Parvo-B19. Fourteen (14) of the suspected cross reactive samples tested as measles IgM antibody tested positive or equivocal (12 positive and 2 equivocal) with the DAI Measles IgM ELISA test kit. These 14 samples were then run on a comparator Measles IgM IFA test kit and a comparator Measles IgM ELISA test kit. A sample was determined as measles IgM antibody positive if the results on two (2) measles IgM tests were positive. A sample was determined as measles IgM antibody negative if the results on two (2) measles IgM tests were negative. The results are presented in the table below:

Cross Reacting Agents	Number of Samples	DAI Measles IgM Result			Consensus Comparator Result *	
		Neg	Pos	Equiv	Neg	Pos
Mumps IgM	4	4	0	0	NT	NT
VZV IgM	4	4	0	0	NT	NT
Rubella IgM	4	4	0	0	NT	NT
CMV IgM	7	3	3	1	3	1
HSV1 IgM	16	9	7	0	5	2
HSV 2 IgM	10	7	2	1	2	1

NT=Not tested
*Tested only if DAI Measles IgM result was positive

Potential cross reactivity was observed with CMV IgM, HSV1 and HSV2 IgM. Potential crossreactivity with Parvovirus, Respiratory Syncytial Virus (RSV) and parainfluenza has not been ruled out, as either specimens were not tested or a limited number were tested.

INTERFERENTS
Interfering substance testing was conducted using three (3) characterized samples for measles IgM, spiked with recommended interfering substances according to the guidance in the CLSI document EP7-A2 (Interference Testing in Clinical Chemistry; Approved Guideline 2nd Ed). Results from the three (3) samples tested as spiked with the interfering substance and unspiked (as a control) were favorable. No adverse results or significant change in results occurred with these samples tested with the following:

Potential Interferent	Concentration
Hemoglobin	≥500 mg/dL
Bilirubin (unconjugated)	≥ 20mg/dL

Interferents with substances such as red cells, cholesterol, gamma globulin, triglycerides, beta carotene, protein, ascorbic acid and anticoagulants have not been tested.

HIGH DOSE HOOK EFFECT
When antigen is evaluated for purchase, it is titrated to ensure there is no dose hook effect (prozone) of antigen on the plate. If prozoning is apparent then the antigen is titrated out further past the prozone point.

Due to the nature of obtaining IgM positive serum in general, positive serum purchased for the DAI Measles IgM generally contains less antibody than would be present to demonstrate dose hook effect (prozone), although the positive control in each kit lot is titrated to negative at each kit QC test stage to ensure the positive control is not prozoning.

IgM/IgG COMPETITION
Specific IgG may compete with the IgM for sites and may result in a false negative. Conversely, rheumatoid factor in the presence of specific IgG may result in a false positive reaction. The Serum Diluent Plus Solution diminishes competing virus-specific IgG and minimizes rheumatoid factor interference in samples. Studies indicate that the maximum amount of IgG which can be removed by the kit Serum Diluent Plus is in excess of the expected high end of the normal range for IgG > 1380 mg/dL. The highest titer of RF+ tested (1: 1280; 500 IU/mL) did not adversely affect the performance of the assay.

SEROCONVERSION STUDY
The BIOMEX Rubeola IgG and IgM Antibody Seroconversion Panel (Cat No. SCP-MEA-001) contained 15 samples depicting the onset and decline of IgG and IgM antibodies to Rubeloa (Measles) from one individual over a period of 55 days. The 15 member panel demonstrated an IgM response consistent with an antibody response for seroconversion when tested on four (4) different lots of the DAI Measles IgM ELISA Test Kit.

The seroconversion panel consisted of one patient with 15 draws during an approximately two month period from May 31st to July 25th, 1994. Seroconversion was demonstrated at Day 20 on the DAI Measles IgM ELISA Test Kit, and remained positive until Day 22. Comparator 1 demonstrated seroconversion at Day 22 and was positive only at that bleed date. Comparator 2 sero-conversions happened earlier than the DAI kit by 2 days and remained positive for the remainder of timeframe. The results are summarized below:

	Diagnostic Automation, Inc. Measles IgM ELISA								Comparator 1		Comparator 2	
	Lot DA027		Lot DA028		Lot DA029		Lot DA030		Rubeola IgM		Rubeola IgM	
Bleed Day	ISR	Result	ISR	Result	ISR	Result	ISR	Result	S/CO	Result	S/CO	Result
Day 1	0.22	Neg	0.29	Neg	0.2	Neg	0.12	Neg	NT	NT	0.429	Neg
Day6	0.29	Neg	0.33	Neg	0.24	Neg	0.15	Neg	0.02	Neg	0.449	Neg
Day 8	0.25	Neg	0.31	Neg	0.21	Neg	0.14	Neg	-0.04	Neg	0.389	Neg
Day 13	0.26	Neg	0.34	Neg	0.27	Neg	0.21	Neg	0.28	Neg	1.101	Pos
Day 15	0.44	Neg	0.51	Neg	0.54	Neg	0.43	Neg	0.46	Neg	3.045	Pos
Day 20	1.53	Pos	1.71	Pos	1.77	Pos	1.28	Pos	0.92	Neg	7.332	Pos
Day 22	1.36	Pos	1.4	Pos	1.49	Pos	1.24	Pos	1.09	Pos	5.919	Pos
Day 27	0.84	Neg	0.92	Equi	0.96	Equi	0.71	Neg	0.5	Neg	3.785	Pos
Day 29	0.59	Neg.	0.8	Neg	0.89	Neg	0.54	Neg	0.33	Neg	3.296	Pos
Day 35	0.54	Neg	0.63	Neg	0.62	Neg	0.47	Neg	0.61	Neg	2.449	Pos
Day 37	0.44	Neg	0.53	Neg	0.53	Neg	0.79	Neg	0.24	Neg	2.113	Pos
Day 43	0.44	Neg	0.6	Neg	0.54	Neg	0.38	Neg	0.17	Neg	2.040	Pos
Day45	0.37	Neg	0.5	Neg	0.5	Neg	0.31	Neg	0.15	Neg	1.802	Pos
Day 49	0.34	Neg	0.59	Neg	0.39	Neg	0.28	Neg	0.04	Neg	1.471	Pos
Day 54	0.38	Neg	0.5	Neg	0.42	Neg	0.29	Neg	0.27	Neg	1.470	Pos

LIMITATION OF PROCEDURE

1. This assay is not, in and of itself, diagnostic and should be considered in conjunction with the patient's clinical presentation/history and other laboratory test results.
2. The magnitude of the measured result is not indicative of the amount of antibody present.
3. The absence of detectable IgM antibody does not rule out the possibility of recent or current infection.
4. Specific IgG may compete with the IgM for sites and may result in a false negative. Conversely, rheumatoid factor in the presence of specific IgG may result in a false positive reaction.
5. CMV IgM, HSV1 IgM and HSV2 IgM antibodies cross react with the test results and may lead to false positive results
6. Some antinuclear antibodies have been found to cause a false positive reaction on some ELISA tests.
7. Interferents with substances such as red cells, cholesterol, gamma globulin, triglycerides, beta carotene, protein, ascorbic acid and anticoagulants have not been tested.
8. Potential cross reactivity with Respiratory Syncytial Virus (RSV) and parainfluenza has not been performed and couldn't be ruled out.
9. The sensitivity of the test in detecting IgM antibodies against recent wild type virus strains was not evaluated.
10. All positive results require careful interpretation by the physician in combination with other serologic and clinical observations.
11. This test is not intended for the determination of immune status. It is intended for the presumptive determination of a person's antibody response to indicate active infection to measles virus and not as an indication of immunity.
12. Samples collected early in the course of the infection may not have detectable levels of specific IgM. A nonreactive IgM result may be due to delayed seroconversion and does not rule out current infection. If clinical exposure to measles virus is suspected despite a negative finding, a second sample should be collected and tested.
13. The longevity of the detection of IgM antibodies post infection was not determined for this device.
14. The performance was not evaluated in immunocompromised patients

15. In immunocompromised patients, the ability to produce an IgM response may be impaired and specific IgM may be falsely negative during an active infection.
16. The IgM results are not intended as a stand alone or to replace virus isolation, molecular testing or other current means of laboratory confirmation of measles
17. The predictive value of reactive or nonreactive results depends on the population's prevalence and the pretest likelihood of the test.
18. This kit must not be used after the expiration date printed on the package label.
19. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
20. Bacterial contamination or heat inactivation of the specimens may affect the test results.

EXPECTED VALUES

IgM antibodies appear in the first week of infection and usually peak within a month. Although uncommon, low levels of IgM may persist for 1 year or longer. The annual incidence rates are reported to vary in different geographical areas.
Recent reports (2004-2011) from the Centers for Disease Control and Prevention (CDC) indicate that annual measles incidence is <1 reported case per 1 million population. A large proportion of cases (40%) are international imported or associated with importation. Unknown source cases are not linked to any endemic chain (12%). Most imported cases do not lead to spread in the United States. This is attributed to high levels of vaccination coverage. (26)

STORAGE AND STABILITY

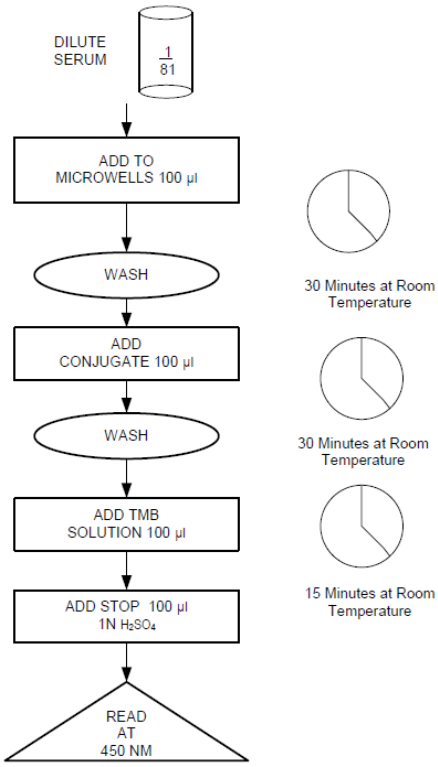
1. Store unopened kit between 2° and 8° C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2° and 8° C. Unused strips must be immediately resealed in a sealable bag with desiccant and returned to storage between 2° and 8° C.
3. Store HRP Conjugate between 2° and 8° C.
4. Store the Calibrator, Positive and Negative Controls between 2° and 8° C.
5. Store Serum Diluent Plus and 20X Wash Buffer Type I between 2° and 8° C.
6. Store the Chromogen/Substrate Solution Type I between 2° and 8° C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1X (diluted) Wash Buffer Type I at room temperature (21° to 25° C) for up to 5 days, or up to 1 week between 2° and 8° C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination. Do not use if evidence of microbial contamination or precipitation is present.

REFERENCES

1. Frank, J.A. et al. 1985. Major Impediments to Measles Elimination: The Modern Epidemiology of an Ancient Disease. American Journal of Disease of Children (AJDC)139:881-888.
2. Measles Surveillance Report No. 11. 1977-1981. Atlanta.
3. Kempe, C.H. and V.A. Fulginite. 1965. The Pathogenesis of Measles Virus Infection. Arch.Gesamte Virusforsch. 16:103-128.
4. Panum, P. 1989. Observations Made During the Epidemic of Measles on the Faroe Islands in the Year 1846. Med. Classics. 3:829-886.
5. Enders, J.R. and T.C. Peebles. 1954. Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles. Proc. Soc. Exp. Biol. Med. 86:277-286.
6. Connolly, J.H. et. al. 1967. Measles-virus Antibody and Antigen in Subacute Sclerosing Panencephalitis. Lancet 1:542-546.
7. 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.
8. Adams, J.M. and D.T. Imagawa. 1962. Measles Antibodies in Multiple Sclerosis. Proc. Exp. Biol. Med. 111:562-566.
9. Norrby, E. 1985. Measles Virus. Lennette, E., et al. In: Manual of DAI Technical Microbiology, 4th Edition, American Society for Microbiology Washington, D.C. pp 769-773.
10. Engvall, E., K. Jonsson, and P. Perlmann. 1971. Enzyme-Linked Immunosorbent Assay,(ELISA) Quantitative Assay of Immunoglobulin G. Immunochemistry. 8:871-874.
11. Engvall, E. and P. Perlmann. 1971. Enzyme-Linked Immunosorbent Assay, ELISA. In: Protides of the Biological Fluids. H. Peeters, ed. Proceedings of the Nineteenth Colloquium, Brugge Oxford. Pergamon Press pp. 553-556.
12. Engvall, E., K. Jonsson, and P. Perlmann. 1971. Enzyme-Linked Immunosorbent Assay.II. Quantitative Assay of Protein Antigen, Immunoglobulin-G, By Means of Enzyme- Labeled Antigen and Antibody-Coated tubes. Biochem. Biophys. Acta. 251: 427-434.
13. Van Weeman, B. K. and A.H.W.M. Schuurs. 1971. Immunoassay Using Antigen-Enzyme Conjugates. FEBS Letter. 15:232-235.
14. Bakerman, S. 1980. Enzyme Immunoassays. Lab. Mgmt. August. pp 21-29.
15. Voller, A. and D. E. Bidwell. 1975. Brit. J. Exp. Pathology. 56: 338-339.
16. Engvall, E. and P. Perlmann. 1972. Enzyme-Linked Immunosorbent Assay, ELISA. III. Quantitation of Specific Antibodies by Enzyme-Labeled Anti-Immunoglobulins in Antigen-Coated Tubes. J. Immunol. 109: 129-135.
17. CDC/NIH Interagency Working Group. 1993. Biosafety in Microbiological and Biomedical Laboratories. 5th Edition. U.S. Dept. of Health and Human Services, Public Health Service.
18. CLSI. 2010. Clinical & Laboratory Standards Institute.. Procedures for Handling & Processing of Blood Specimens. CLSI Publication H18-A4.
19. CLSI. Clinical & Laboratory Standards Institute. Statistical Quality Control for Quantitative Measurement Procedures. Principles and Definitions. CLSI Publication C24- A3.
20. http://www.cap.org/html/ftpdirectory/checklistftp.html. 1998. Laboratory General – CAP (College of American Pathology) Checklist (April 1998). pp 28-32.
21. CLSI. 2012. National Committee for Clinical Laboratory Standard. Preparation and Testing of Reagent Water in the Clinical Laboratory. CLSI Publication C3-A4-AMD.
22. CLSI. 2005.Clinical & Laboratory Standards Institute. Interference in Clinical Chemistry;CLSI Publication EP7-A2.
23. CLSI. 2004. Clinical & Laboratory Standards Institute. Evaluation of Precision performance of Quantitative Measurement Methods. CLSI Publication EP05-A2.
24. CLSI. 2008. Clinical & Laboratory Standards Institute. User Protocol for Evaluation of Qualitative Tests. CLSI Publication EP12-A2.
25. CLSI. 2010. Clinical & Laboratory Standards Institute. Method Comparison and Bias Estimation Using Patient Samples. CLSI Publication EP 9-A2.
26. CDC Report: Documentation and Verification of Measles, Rubella and Congenital RubellaSyndrome Elimination in the Region of the Americas, United States National Report, CDCMarch 28, 2012.
27. CLSI. Protection of Laboratory Workers from Occupationally Acquired Infections. CLSIPublication M 29-A3.

SUMMARY OF ASSAY PROCEDURE



ISO 13485
ISO 9001

Diagnostic Automation/
Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	**2017-04-27**
REF **1409-1**	**AccuDiag™- Measles IgM ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands.** www.cepartner4u.eu
Revision Date: 2015-03	