

Laboratory Procedure Manual

Analyte: **Endomysial Antibody Assay (EMA)**

Matrix: **Serum**

Method: **Indirect Immunofluorescence**

Method No: **Test #9360**

Revised:

as performed by:

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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.

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Public Release Data Set Information

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

Data File Name	Variable Name	SAS Label
TGEMA_G	LBXEMA	Endomysial Antibody Assay (EMA)

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1. Summary of Test Principle and Clinical Relevance

Testing is performed by Indirect Immunofluorescence using cryostat prepared sections of rhesus monkey esophagus as substrate. (The endomysium surrounding the smooth muscle bundles of the muscularis mucosa of distal end of Rhesus monkey esophagus serve as the antigen source.) Sections are incubated first with dilutions of patient sera, followed by incubation using fluorescein conjugated anti-IgA. Slide preparation is automated using the Binding Site ASP 1200 automated slide stainer. Slides are screened and read using a fluorescent microscope.

IgA-EMA is useful for diagnosing DH and celiac disease and monitoring adherence to a gluten free diet. There is a gradual decrease in titer or absence of circulating endomysial antibodies within months after instituting a gluten free diet. Because of the high specificity of EMA for celiac disease, the test may obviate the need for multiple small bowel biopsies to verify the diagnosis. This may be particularly advantageous in the pediatric population, including evaluation of children with failure to thrive.

2. Safety Precautions

Consider all plasma specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats, and safety glasses during all steps of this method. Discard any residual sample material by autoclaving after analysis is completed. Place disposable plastic, glass, and paper (pipet tips, autosampler vials, gloves, etc.) that contact plasma in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab. If needed, MSDS for other chemicals can be viewed at <http://www.ilpi.com/msds/index.html> or at <http://intranet.cdc.gov/ohs>.

3. Computerization; Data System Management

For NHANES, data is transmitted electronically weekly to Westat's ISIS computer system and transferred from there to NCHS. Abnormal values are confirmed by the analyst, and codes for missing data are entered by the analyst and are transmitted as part of the data file to the Westat ISIS computer, and are eventually forwarded to NCHS. Westat also prepares the abnormal report notifications for the NCHS Survey Physician.

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4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

There are no requirements for fasting or avoidance of medication. If the patient has been following a gluten-free diet, this should be noted on the request form

Collect 5 ml of blood in plain red top or serum separator tube (SST).

Centrifuge and aliquot serum into 13 x 75 mm aliquot tube or screw cap plastic vial. For internal delivery or same day laboratory delivery, ambient temperature is acceptable.

For external delivery or next day sample delivery, refrigerated or frozen samples are acceptable.

Preferred sample: 1 mL serum.

Minimum acceptable 0.50 mL serum.

Unacceptable samples include plasma and hemolyzed samples are rejected.

After to delivery to laboratory and prior to analysis, serum samples should be stored in refrigerator (approximately 4° C) until assays are complete.

Samples are stored in the lab frozen for two weeks after testing.

Slides are stored refrigerated and discarded two weeks after resulting.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Inadequately prepared slides are rejected.

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

a. Reagent Preparation

Veronal buffer is stored at room temperature until the expiration date of the reagent is reached.

Tween is stored at room temperature until the expiration date of the reagent is reached.

p-Phenylenediamine is stored at room temperature until the expiration date of the reagent is reached.

Glycerol is stored at room temperature until the expiration date of the reagent is reached.

PBS (Working Buffer) is stored at room temperature until the expiration date of the reagent is reached. In 1L bottle: add 250 ml Veronal Buffer (line marked on bottle), fill bottle to rim with DI water, and add 5 ml Tween; OR In 2L bottle: add 500 ml Veronal Buffer (line marked on bottle), fill bottle to rim with DI water, and add 10 ml Tween.

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Mountant is stored refrigerated until the expiration date of the reagent is reached. Using a brown colored plastic bottle, add 2.0 mL Veronal Buffer, 8.0 mL distilled water, 100 mg p-Phenylenediamine, and swirl gently until dissolved. Add 90 mL Glycerin, cap, and shake gently to mix.

Anti-sera Conjugate Concentrate (FITC Conjugate Fab'2GXH I_{gA} 1.0 mL) IgA is stored refrigerated at 4-8 degree C until the expiration date of the reagent is reached. 1 mL anti-IgA aliquots are stored in -70 freezers in screw top conical tubes until needed for working solution.

Conjugate working Solution: combine 1 ml conjugate concentrate to 49 ml DI water; fill conjugate instrument bottle with working solution to rim as needed. Expires in 48 hrs.

Monkey Esophagus Slides (10 well or single well slides imbedded with tissue cut from distal end of rhesus monkey esophagus): Remove enough slides from refrigerator for the day's workload; remove slides from foil pouch as needed; unopened slides may be returned to the refrigerator; opened and unused slides must be discarded at end of day.

b. Supplies

Coverglass (24 × 60)
Graduated cylinder
Measuring pipettes (Fisher Scientific)
Disposable transfer pipettes
Plastic test tubes (12 × 75mm polystyrene) with caps
Sharpie marker pen
Test tube racks
Gauze – sponges 12 × 12 × 4
Pipette tips
Zebra printer labels

c. Preparation of Quality Control Materials

Buffer blank
NHS (Normal Human Serum/Negative)
EMA UP (Positive High Titer Control) and EMA LOW (Positive Low Titer Control)

1. Pool together approximately 15-20 previously tested patient samples
 - a. EMA UP pool samples with titer results 1:320 and higher
 - b. EMA LOW pool samples with titer results between 1:10 and 1:160
2. Mix pool well and allow to sit 20 minutes
3. Remove any fibrin clots and mix well again
4. Record control pool in Quality Control notebook
 - a. PC### (use next available control #)
 - b. Pool date and tech initials
 - c. Expiration date one year
 - d. Date placed into use
5. Aliquot 0.1 mL control material into plastic tubes
6. Add 2.0 mL buffer
7. Label tubes

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- a. Control name
- b. Lot#
- c. Exp date

Place labeled control material tubes in labeled white box and place in -80° freezer.

d. Instrumentation

Binding Site ASP 1200 automated slide processor
Zeiss Axioplan or Leica fluorescence and bright field microscope
Hand-held pipette
Pipette mp-50, mp-500
Zebra printer

7. Calibration and Calibration Verification Procedures

The reagents are stable until the expiration date on the reagent bottle is reached.

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Pre-Analytical

Build Load

- 1. Complete Endomysial Antibodies (IgA), Serum (Test #9360) Control Sheet with load number.
Staple control sheet to load worksheet.
Place worksheets in "EMA Working Drafts" file

Aliquot Samples

- 1. Print labels
- 2. Label 12 x 13 aliquot tube
- 3. Pour sample into aliquot tube
- 4. Cap
- 5. Label rack with load list number
- 6. Place rack in refrigerator until day of testing

Start Up

Create Worklist

- 1. Double click <Protocols> icon
- 2. Select <Worklist Menu>
- 3. Select <Create Worklist>
- 4. Select <Mayo 10 Well EMA Screen> protocol
- 5. Confirm by selecting <Open> and a blank worklist will appear
- 6. Enter assay control information in the Patient/Control Name column
- 7. Enter c1, c2 or c3.....test column after each control
- 8. Enter patient sample information in the Patient/control Name by scanning the barcode off the working draft for all 48 patient samples

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9. Enter "Y" into the Test column and the titer column after each patient sample
10. Select <**Worklist Menu**>
11. Select <**Save Worklist**>
12. A window appears allowing the worklist to be named
13. Name the worklist EMA followed by the worklist number
eg: EMA110901
14. Select <**Save**>
15. Select <**Exit Program**>

Load Instrument

1. Remove all reagents, slides, controls and patient samples from storage and allow to come to room temperature
2. Turn on power to ASP 1200
3. Fill system fluid bottle with PBS (document)
4. Empty waste containers verifying waste bottle cap is securely tightened (document)
5. Place dilution tube rack in position, verifying rack is positioned correctly.
6. Place working conjugate in far left position of conjugate rack
7. Place all 4 controls in labeled positions of control rack
8. Remove caps from patient samples and place tubes in sample rack,
 - a. verifying tube order matches order on working draft
 - b. load from top left to the right
9. Remove 10 slides from foil packages
10. For a full run, place 10 10-well slides on the slide carrier
11. Using a sharpie marker, label slides with run number and slide number as shown below
Load serial dilution micro titer plate.

B. Analytical

Process Work List

1. After instrument is completely loaded, close safety lid
2. Select <**Protocols**>
3. Select <**IFA Protocols**> to open listing of saved work lists
4. Select correct worklist
5. Select <**Open**>
6. The loading instructions are displayed with prompts.
7. Select <**OK**> after each step verifying instrument is loaded correctly
8. Select <**Start**>
9. During the assay, the screen will show green colored dots wherever probe aspirates liquid successfully
10. Red dots appear when there is insufficient liquid in a container
 - a. When a reagent container is low, processing will stop and the operator will be prompted to refill the container
 - b. When a sample is insufficient, an error will be flagged, the sample is skipped and processing continues until all the remaining samples are completed

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- c. A pipetting warning message is displayed for 5 minutes to allow operator to prepare dilution manually in the correlating empty space of the dilution rack
11. The assay is complete when the "Processing Completed" window is displayed.
12. Select <No> at the two print prompts.
13. Select <Continue> to perform an end of run rinse.

Remove samples from sample rack, cap and place in sample rack until slides are screened

Cover Slip Slides

1. Remove slides from slide carrier, blotting away excess wash solution by holding slide on edge of gauze.
2. Add a thin stream of mounting material to coverslip
3. Place slide on coverslip, pressing firmly to remove bubbles and spaces
4. Wipe away excess mounting material from slide edges
5. Place mounted slides in a slide box labeled with run number.

Screen Slides

1. Using fluorescent microscope, scan each slide and well for fluorescent activity.
2. Grade each well using the following criteria
 - a. Negative
 - b. +/-
 - c. 1+
 - d. 2+
 - e. 3+
3. Record control results on Endomysial Antibodies (IgA), Serum Control Sheet
4. Record patient results on EMA Load Worksheet
5. Result negative samples. See Reporting/Interpreting Results section below.

Titers

Select Samples

1. All positive samples must be removed from load and tittered.
2. Place barcode labels on ASP 1200 Titer Worksheet: EMA and/or CIFS
3. Staple Endomysial Antibodies (IgA), Serum Control Sheet to front of titer worklist
4. Place samples in refrigerator until testing
5. Place worklist in "EMA Working Draft" file

Create Worklist

1. Double click <Protocols> icon
2. Select <Worklist Menu>
3. Select <Create Worklist>
4. Select <Mayo 10 Well EMA Titer> protocol
5. Confirm by selecting <Open> and a blank worklist will appear
6. Enter assay control information in the Patient/Control Name column
7. Enter c1, c2 or c3.....test column after each control
8. Enter patient sample information in the Patient/control Name by scanning the barcode off the working draft for all 48 patient samples
9. Enter "Y" into the Test column and the titer column after each patient sample
10. Select <Worklist Menu>
11. Select <Save Worklist>
12. A window appears allowing the worklist to be named

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13. Name the worklist EMA followed by the worklist number
14. Select <Save>

Load Instrument

Follow the same steps listed above

Process Work List

Follow the same steps listed above

Cover Slip Slides

Follow the same steps listed above

Screen Slides

1. Using fluorescent microscope, scan each slide and well for fluorescent activity.
2. Grade each well using the following criteria
 - a. Negative
 - b. +/-
 - c. 1+
 - d. 2+
 - e. 3+
3. Record control results on Endomysial Antibodies (IgA), Serum (Test #9360) Control Sheet
4. Record patient results on ASP 1200 Titer Worksheet: EMA and/or CIFS
5. Place titer slide box in refrigerator and worksheet in read out room for consultant review.

Consultant Review

1. Consultant will review titer slides and results
2. Consultant results will be recorded on ASP 1200 Titer Worksheet: EMA and/or CIFS
3. Results varying more than 2 titers will be reported to Supervisor
4. After completion, technologist will report consultant read results. See Reporting/Interpreting Results section below.

C. Special Method Notes

There are no special method notes for this procedure.

D. Calculations

Using fluorescent microscope, each slide and well are scanned for fluorescent activity and graded.

E. Mayo modifications

The method is run exactly as stipulated; The laboratory has introduced no modifications.

F. Interpretation of results

Negative Screens may be answered by the laboratory technologist

1. Negative screens may be answered in a batch mode using the worklist
NOTE: Positive samples for titers must be removed first
- 2.
3. Release Load

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Positive Titers are resulted by the laboratory technologist after consultant review

1. LAB3 function 3, 1
2. Worklist **EMA**
3. Scan barcode label
4. Type one of the following comment codes
 - a. P10 (Positive titer 1:10)
 - b. P20 (Positive Titer 1:20)
 - c. P40
 - d. P80
 - e. P160
 - f. P320
 - g. P640
 - h. P1280
 - i. P2560
 - j. P5120
 - k. I (Indeterminate); will auto-populate comment "This may be seen in gluten sensitive enteropathy (GSE). Suggest ELISA tissue transglutaminase (tTG) assay for confirmation of the diagnosis GSE."
5. Release All Results: <Y>

9. Reportable Range of Results

Negative up to positive titer >1:5120.

10. Quality Control (QC) Procedures

A blank and four Bench QC specimens are inserted into each run.

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

Verify control material was placed on ASP in correct position

Verify correct slide was read

Receive 2nd opinion from co-worker

Re-stain slide

Repeat run

Prepare new QC material

Document all actions on Endomysial Antibodies (IgA), Serum (Test #9360) Control Sheet

12. Limitations of Method; Interfering Substances and Conditions

Patients with IgA deficiency may test negative or have low titers.

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13. Reference Ranges (Normal Values)

Negative in normal individuals.

Negative in patients with dermatitis herpetiformis (DH) and/ or celiac sprue adhering to gluten-free diets

14. Critical Call Results (“Panic Values”)

There are no critical results for this procedure.

15. Specimen Storage and Handling during Testing

Specimens are allowed to reach room temperature during preparation. The unused portion of the patient specimen is returned to the freezer.

16. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

If the analytical system fails, we recommend that the specimens be stored at -20°C until the analytical system is restored to functionality.

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

We recommend that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual serum from these analyses for non-NHANES studies may be discarded at the request of the principal investigator, or may be transferred to the CDC CASPIR facility for use by other investigators. Very little residual material will be available after NHANES analyses are completed, and these vials may be routinely autoclaved.

The exact procedure used to track specimens varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are kept by the supervisor.

19. Summary Statistics and QC Graphs

Since the data is qualitative there are no summary statistics or QC graphs associated with the data.

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References
None.