

CLSI DISCLAIMER:

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For Individual Laboratory to Complete:

**MAGO[®] Plus or
Aptus[®] Automated
EIA Processor**

Laboratory Name		
Adopted		
Reviewed		
Reviewed		
Revised		
Supercedes		

METHOD:

Diamedix Corporation's MAGO[®] Plus and Aptus[®] Automated EIA Processors are designed to perform all steps of a range of enzyme immunoassay procedures in an automated platform. Steps include dilution of samples and/or controls, transfer of dilutions into wells, incubations, reagent additions, washes, spectrophotometric readings, calculations and printouts of results.

PRINCIPLE OF THE PROCEDURE:

In general, diluted samples are incubated with reactant (e.g. antigen) bound to the solid surface of a microtiter well. If antibodies (e.g. IgG, IgM, or IgA) against the antigen are present in the samples, they will bind to the antigen forming antigen-antibody complexes. Residual sample is eliminated by aspiration and washing. Conjugate (e.g. horseradish peroxidase enzyme-labeled goat anti-human IgG, IgM or IgA) is added and will bind to these complexes. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at e.g. 450 nm (reference 600-630). Color development above a certain level denotes the presence of antibody.

Unlike the above assays, in the Capture assays the microwells are coated with antibody (anti-human IgM). Patient sample IgM binds to the wells during the first incubation step. Residual sample is eliminated by aspiration and washing. This is then incubated with a 'tracer' comprised of a complex of highly purified antigen with specific monoclonal antibody which has been conjugated to horseradish peroxidase enzyme. If patient sample contains IgM antibodies to the specific antigen, the antigen in the tracer complex will bind to them. Unbound conjugate is then removed by aspiration and washing. Substrate is then converted in the presence of bound enzyme to a colored end-product, the absorbance of which can be determined spectrophotometrically.

REAGENTS:

Diamedix Test Kits have been FDA cleared for use on the instrument.

Individual test parameters for these have been pre-programmed into the system software. In addition, 20 Custom Mode channels may be programmed and validated for other tests by the user.

OTHER MATERIALS REQUIRED

1. One liter measuring containers/cylinders
2. Deionized or distilled water
3. Pre-dilution cups (available separately cat. # 250-050)

PREPARATION PROCEDURE

1. (1X) Wash Buffer: Dilute the Wash Concentrate to 1 L with distilled or deionized water to yield a 1 X concentration, unless run size is large enough to require more than 1 L (instrument screen prompt will indicate). Mix thoroughly to dissolve any crystals that may be present. Before use check the solution visually to ensure it is clear.
2. ProbeClean® Solution: To 1 L of distilled or deionized water, add 1 ml 1000 X ProbeClean® concentrate to yield a 1 X working solution. Before use check the solution visually to ensure it is clear.
3. Sample Diluent, Substrate, and Stop Solutions are ready to use. Conjugates may be ready-to-use or may need pre-dilution. Refer to package insert for instructions.
4. All components must be brought to room temperature prior to start of assay. Remove reagents from the insulated box during the warming process. After components are equilibrated, gently mix or swirl each component to ensure homogeneity. Failure to do this may adversely affect performance/results.
5. Wipe the instrument probe with an alcohol swab with gentle downward strokes. The probe is NOT Hollow. Do Not Try to Insert any object into the Bottom of the Probe.
6. Once per day, clean the manifold pins with the stylus provided to remove any clogs or debris. Wipe each pin with an alcohol swab with gentle downward strokes.
7. The Wash and Waste bottles should be washed and thoroughly rinsed and dried once per week.

Warnings:

1. Handle samples, Calibrator, controls and the materials that contact them as potential biohazards. Each donor unit in the standards and controls has been found negative for Hepatitis B surface antigen and HIV-I antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1, Hepatitis B virus, 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.

2. Never pipette by mouth.
3. Avoid contact with open skin and mucous membranes.
4. Certain of the test components contain sodium azide as a preservative. Azides are reported to react with lead and copper in plumbing to form compounds that may become explosive. When disposing of solutions containing sodium azide, flush with copious amounts of water to minimize the build up of metal azide compounds.

GENERAL ASSAY SETUP PROCEDURE

Refer to the MAGO[®]Plus or APTUS[®] Operating Manual for detailed instructions on instrument set-up and assay procedure. The following SUMMARY may serve as a guide for SETUP.

NOTE: KEYS IN THE MAGO SCREENS ARE LABELED WITH LETTERS **KEYS IN THE APTUS SCREENS ARE LABELED WITH NUMBERS**

1. Select OK (to run the previous tests selected) and proceed to Step 5.
OR: select X: Modify and proceed to step 2.
2. Press (T for MAGO) or (1 for Aptus): Tests
3. Press X: to clear all tests
Press: up or down arrows to highlight preferred test or tests
Press (Spacebar) to select/deselect up to 9 tests.
Press: OK (Enter key)
4. Press: Yes at prompt
Press: Esc to exit Setup menu.
5. Press: (S for MAGO) or (2 for Aptus) (Sample Input)
Instrument instructs user to open drawer. To do this, grasp the recessed handgrip in the center of the drawer, press in slightly first, then pull.
6. Type in lot no. and expiration date or barcode lot information label.
TAB to OK.
Press Enter key.
7. Verify correct tests selected
Press: OK
8. a) Press TAB to highlight Patient Code box. Input Patient Code (bar code or accession #)
b) TAB to Name box. Input Last name, First name.
c) TAB to Birth date (optional).
d) TAB to Test selection (3 options)
 - 1) F2 (list) (Use spacebar to select/deselect)
 - 2) Select/deselect 1-2-3-4 using # keys (must know order of tests--from F2 list)
 - 3) Press (R for MAGO) or (1 for Aptus): (Repeat function to batch test--repeat test selection on certain number of patients)

- e) TAB to Department (optional)
- f) Options Available:
 - 1) Repeat --to batch test--multiple samples with same tests.
 - 2) List --to view and print patient list
 - 3) Edit Test --arrow to patient sample, edit previous test selection
 - 4) Clear List --remove list
 - 5) Clear --arrow to sample and remove that sample (will be blank)
 - 6) Next Rack --switch from rack to rack
- Press: Esc to exit
- 9. Press: (P for MAGO) or (3 for Aptus) (Plate Configuration)
- 10. Press: OK (Enter)
- 11. Press: OK (Enter)
- 12. Press: Esc after loading required wells (Full strips--Please be sure to use blank wells to complete partial strips)
- 13. a) Press: (R for MAGO) or (4 for Aptus) (Reagent Configuration)
 - b) Calculates dilution cups & denotes graphically. Place number of cups on deck as directed. Ensure deck and dilution cups are flat.
 - c) Ensure sample racks are positioned properly.
- 14. Press: Esc
- 15. a) Reagent rack
 - Place reagents according to screen prompts.
 - Press (N for MAGO) or (2 for APTUS): to advance through reagents/controls/standards
 - To view required reagent volumes continue to press (N) or (2)
 - b) Highlight C
 - c) Press: X Modify if needed
 - Enter Correction Factor or variable lot data
 - Press: Esc to exit
- 16. Press: OK (Enter)
- 17. Press: OK (Enter)
 - (Prepare by adding 1 ml. ProbeClean[®] to 1 liter DI H2O)
- 18. Press: OK (Enter)
- 19. Press: Esc to advance software to Main Menu
- 20. Press: (R for MAGO) or (2 for Aptus) (Run Menu)
- 21. Press: (S for MAGO) or (1 for Aptus) (Start Run)
- 22. Press: Yes at 'Start Run?' query
- 23. Press: Yes at 'Check Volumes?' query
- 24. When run is completed press: (V for MAGO) or (2 for Aptus) to View Results and Archive Data (this must be done in order to print results)
- 25. Press: (T for MAGO) or (3 for Aptus) to Print Results. A drop down menu will allow selection of print-out options.
 - Press: Esc to advance to Main Menu
- 26. Press: Esc
 - Press: Yes to terminate session

QUALITY CONTROL

The following items are pre-programmed into the software for each of the Diamedix tests:

1. Instructions for placement of the appropriate reagent blank, Standards, Calibrators, positive, negative controls and all reagents.
2. Criteria for determination of valid test runs according to the individual Package Insert specifications. Pass/Fail indicated as OK/NO on printout.
3. Calibration method specific to the test.
4. Calculations as required and result interpretations on the print-out for each sample.

Twenty additional tests may be programmed by the user, if desired.

CALCULATION AND INTERPRETATION OF RESULTS:

Calculations: All calculations are automatically performed by the instrument according to the calculation method prescribed in the Package Insert for each test. Units are reported as Index Value, EU/ml, IU/ml, OD Ratio, or as required by the individual test.

Interpretations: Interpretations appear for each sample in a valid run. If any of the QC criteria is not met, the run is considered invalid and the interpretation is removed for the patient sample results.

LIMITATIONS OF THE PROCEDURE

A diagnosis should not be made on the basis of ELISA results alone. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures. The performance characteristics of this device have not been established for lipemic, hemolyzed and icteric specimens; therefore, these specimens should not be tested.

The results of this assay are not diagnostic proof of the presence or absence of disease.

MAINTENANCE

PROCEDURES FOR INSTRUMENT CALIBRATION:

A. Dilution & Dispensing Calibration

Purpose: This procedure is designed to assess the dilution and dispensing accuracy and precision of the instrument by preparing 95 separate dilutions at 1:101, and dispensing 100µl of each dilution into microwells.

Materials Needed: MAGO[®]Plus Calibration Check Kit (Cat No. 250-030)

Frequency: Every 2 to 3 months (or as CLIA/local laboratory standards require)

Calibration Check Setup Summary:

1. From the Main Menu, Press (S) to select Setup Menu.
2. Press (T) : Test Configuration Menu.
3. Press (I) : Instrument Calibration.
 Press: up or down arrows to highlight 'QC 405, 1:101' test option
 Press (Spacebar) to select/deselect this test parameter.
 Press: OK (Enter key)
4. Press: Yes at prompt (to clear previous patient list)
 Press: Esc to exit Setup menu.
5. Place Sample/Dilution Cup racks into rack positions A and B. Make sure they are seated flat inside the Sample Drawer.
6. Press (P) : Plate Configuration Screen. Place plate from Calibration Check Kit into plate A position inside the instrument.
7. Press ENTER, ENTER, and ESC to continue. (Screen messages will appear).
8. Press (R) : Reagent Configuration Screen. After dilution cups calculation, place the indicated dilution cups (60 in rack A and 35 in rack B).
 Press Esc to Reagent Rack. Place Calibration Dye tube into first tube position as indicated and Calibration Diluent into the first bottle position NOTE: The bottle of Diluent must be filled to the top by combining the contents of 2 bottles.
9. Wash bottles may contain wash solutions or deionized water. Tighten caps of all four bottles. Empty all waste bottles. Press ENTER.
10. Press Esc to Main Menu.
11. Press (R) : to Run Menu.
12. Press (S) : to Start.
13. Press Y to start run.
14. Press N for reagent volume check.
15. Run takes app 70 min.
16. Press (R) to View Results.
 Press OK to return to the Run Menu.
17. Press (T) to Print Results.

The printout will show the absorbances for 1 blank well and the 95 individual dilutions prepared and transferred to the microwells. At the lower left is the Mean absorbance and CV% calculated.

Fill out the information in the designated areas for Lot#, Expiration Date, Date performed etc.

If the CV is greater than 8%, contact the Diamedix Technical Service Department, 1-800-327-4565. Keep the printout of the QC results for your records.

B. Reader Calibration

Purpose: This procedure is designed to verify the 'Calibration', 'Repeatability', and 'Linearity' of the instrument plate reader.

Materials Needed: DRI-DYE[®] CHECK STRIPS (405/450) Cat No.790-915
 DRI-DYE[®] CHECK STRIPS (405) Cat No.790-900
 DRI-DYE[®] CHECK STRIPS (450) Cat No.790-905

Frequency: Every 6 months (or as CLIA/local laboratory standards require)

Note: Please refer to the DRI-DYE[®] package insert for detailed information if needed.

1. Carefully remove, and unwrap one of the 12-well strip. Place it in row A of a strip holder (tab end farthest away from the A marking on the holder). Reconstitute dye in each well with 200 µl DI water. Cover with film and allow to sit undisturbed for 2 hours. While holding tray on a level surface, tap the long side of the holder repeatedly with the other hand for 45 seconds to mix contents. Change hands and repeat for an additional 45 seconds.
2. With the instrument OFF, insert the DRI-DYE[®] Reader Calibration disk into the drive slot, and turn the instrument ON.
3. From the screen menu, select the desired wavelength. Arrow to highlight and press spacebar to select:
 <5> for 405 nm or <0> for 450nm
4. Enter upper and lower absorbance limits of the 50 Standard, which is listed as the Acceptable Range on the 'Calibration Record' sheet in the DRI-DYE[®] package insert (StatFax or others category).
5. Follow screen prompts requesting that the DRI-DYE[®] strip be moved into each of the eight rows (A through H).

NOTE: Wait at least 5 seconds after closing the drawer and before pressing ENTER (to start the reading). This wait is needed to minimize any light scatter that would adversely affect the readings.

6. After readings are completed, a screen menu will appear. Select (P) to print results.

RESULTS

Repeatability Record

Absorbance differences between two readings are calculated for each well in each of the eight row locations. If any well is outside the 'Maximum Allowed Difference' (appears below the absorbance chart), it will be marked with an asterisk and a footnote will appear 'Repeatability out of range'.

Calibration Record

The absorbance readings of well # 2 (the Standard, assigned value = 50) in each of the eight row locations are used to determine 'Calibration Within or Out of Range' for the Acceptable Range entered (Step 4 above).

Linearity Plot

Absorbances of the 25, 50, 100 and 200 dye solution wells are used to determine concentrations relative to the 50 Standard in Well #2. Each well (as a mean of the duplicates or triplicates) is analyzed for linearity of response for these dye concentrations. "Linearity Within or Out of Range" appears on the printout.

Report Summary

On the last page each parameter is summarized as:

CALIBRATION	:	PASSED	or	FAILED
LINEARITY	:	PASSED	or	FAILED
REPEATABILITY	:	PASSED	or	FAILED

If any of your results are not within the established limits, your instrument may require service. It is recommended that another check strip be run to confirm the problem before requesting service. Keep the printed results from both runs, since this information may be needed by the service technician.

ADDITIONAL FUNCTION CHECKS

1. The Temperature/Probe Indicator Panel (located in the front left section of the sample drawer):
 - a) provides the status of plate temperature controls for plates A-D
 - b) indicates when the probe detects liquid
 - c) prevents the probe from damage (Safety Crash Sensor) in case a cap is inadvertently left on a container or a dilution cup is not positioned properly.
2. Automatic Maintenance Steps- After each run and prior to startup the instrument automatically flushes the lines, cleans the probe and wash manifold and primes the tubing. No additional washing or priming by the operator is typically necessary.
3. ProbeClean[®] Solution is prepared by the user in one of the 2-Liter bottles during the assay setup. This solution reduces the adhesion of substances to the internal surfaces of the hydraulic system and is used to wash the probe, wash manifold and internal lines between all steps of the assay.
4. Assay Temperature Control-each heater is maintained at the desired temperature for the duration of the assay. The temperature of each plate is reported at 2 minute intervals to the internal computer. The software can then identify and indicate on the assay printout if an out-of-range temperature was experienced for more than 2 minutes.
5. Reagent and Specimen Volume Sensing-the instrument uses a probe composed of two insulated sleeves which is affected when the two sleeves are short-circuited by the presence of liquid on the tip of the probe. The height at which liquid is sensed allows an estimate of volume present. The instrument checks the volumes of components prior to the run (user must respond [Y] to 'Volume check?' query) to ensure that sufficient reagents/specimens are provided to complete the run. If insufficient volume is detected, an audible alarm will sound, an error light will appear, and a screen prompt will allow intervention before continuing.

6. Wash Bottle Liquid Sensing-the Wash/ProbeClean bottles are equipped with immersion sensors to warn if any solutions are insufficient for the run. An audible alarm sounds and allows intervention.
7. Spectrophotometer Filters Check-each time the instrument is powered up, there will be an automatic check of the reader filters. It is best to have the drawer and door closed at this time.
8. Start of Run Function Checks- the following parameters will be checked before the run begins; a) internal vacuum pressure, b) volume of reagents, c) volumes of Wash/ProbeClean[®] solutions, d) empty Waste bottle, e) closed drawer, f) plate holders in position.

INSTRUMENT DECONTAMINATION

This procedure may be followed prior to transport and repair of the instrument.

- (1) Empty both waste bottles.
- (2) Fill ProbeClean[®]/Distilled Water bottle with 500 ml Instrument Wash Solution (70% isopropyl alcohol).
- (3) Fill Wash 1 and Wash 2 Bottles with 500 ml each of 5% Bleach Solution (25 ml Household Bleach + 475 ml distilled water).
- (4) Insert System Cleaning disk into disk drive and re-boot the instrument.
- (5) After cleaning the system, the instrument will display the message, "CLEANING COMPLETED."
- (6) Remove the disk and turn off the instrument.
- (7) Empty the waste bottles.
- (8) Rinse all bottles thoroughly with distilled water.

This document was written February 27, 2003.

Diamedix Corporation assumes no responsibility for procedural updates following this date.