

 YALE-NEW HAVEN HOSPITAL	TITLE: Tosoh A1A 900		DEPT OF LAB MEDICINE Immunology, Flow Cytometry, and Molecular Diagnostics Laboratories
			DOCUMENT NO: IMM200
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I. Introduction

The **AIA-900** (Loader Model) is a fully automated continuous random access analyzer which employs the Fluorescent Enzyme Immunoassay (FEIA) method. The AIA-900 has a throughput of up to 90 assays per hour. After the start of the assays, the first result is output in about 18 minutes.

II. Overview

The AIA-900 analyzer consists of printer, touch screen operation panel and keys, external connection ports, sample loader, substrate bottle mounting position, and reagent and tip cover. The instrument sits on a wheeled base that contains a cabinet that has receptacles for dry and liquid waste along with Diluent and Wash tanks.

The AIA-900 performs two assays currently: $\beta 2$ microglobulin (BMG) and CA 27.29. These are one-step sandwich assays that utilize two sites on the antigen. One-step sandwich assays are designed for high molecular weight analytes. There are two types of antibody used in AIA-PACK test cups: monoclonal antibody immobilized on magnetic beads and enzyme-labeled monoclonal or polyclonal antibody. The latter uses antibody conjugated to bovine alkaline phosphatase with sodium azide as a preservative. Both types of antibody used bind the same antigen at different sites, forming an antigen-antibody “sandwich”. Excess antibody is bound to the bead so that formation of the antigen-antibody complexes is not limited by the concentration of the analyte. The amount of enzyme activity (fluorescence) following addition of substrate is directly proportional to the concentration of the anal yte.

III. Daily Maintenance/System Start Up

Consumables needed for daily use:

Waste Bag for AIA-900	Cat # 0022936	40 bags/pack
Probe Tip	Cat # 0020107	6 tips/bag

AIA-Pack Detector Standardization Test Cups (STD cup)	Cat # 0020970	200 cups/box
Sample Cup	Cat # 0018581	1000 cups/bag
Thermal Paper	Cat # 00890001	one each
Sample Tips	Cat # 0019215	1000 tips/bag
AIA-Pack BMG Test Cup	Cat # 0025259	100 cups/box
AIA-Pack 27.29 Test Cup	Cat # 0025202	100 cups/box

Common reagents needed for daily operation:

Substrate (stored at 2-8°C)	Cat # 0020968	2 sets/box
Diluent concentrate (stored at 2-8°C)	Cat # 0020956	100mL bottle, 4 bottles/box
Wash concentrate (stored at 2-8°C)	Cat # 0020955	100mL bottle, 4 bottles/box
AIA-Pack Sample Treatment Cup (STC cup)	Cat # 0020971	200 cups/box

1. Turn ON instrument using power switch at left. AIA-900 will request to “Wait for a while.” Wait at least 30 minutes to allow internal temperatures to stabilize.
2. Next, the LOGON screen will appear. Press OPERATOR button to find your initials.

When using the MODIFY button to change/add/delete operators, a keypad will appear, using the keypad enter the operators name, when done press the OK button.

3. Replace the 70% isopropyl alcohol bottle held in the substrate bottle mounting position with Substrate. Be sure to wipe the outside of the plastic tubing with a kimwipe to remove any residual alcohol before inserting the tube into Substrate bottle. Tube should reach the bottom of the Substrate bottle.

When installing the Substrate bottle, be sure that the label is facing you. The amount of substrate remaining in the bottle is monitored by an optical type sensor, so if label is facing the rear, a malfunction may occur.

To prepare Substrate, allow reagents to come to 18-25°C. Then, add the entire 100mL of substrate reconstituent fluid to the lyophilized substrate reagent. Mix and allow solid material to fully dissolve before using. Substrate is stable for 7 days at 2-8°C. Before reconstituting, substrate ingredients are stable until expiration date at 2-8°C.

4. From HOME screen, press the DAILY CHECK button. The following items will appear in daily check screen:

- Check Reagents
- Empty Waste

- Check Paper
- Set STD Cup to Position-2
- ✓ In cabinet, confirm that there is adequate Diluent and Wash solutions.

To prepare Wash, take the Wash tank out and remove cap. After removing the suction tube, place the end into a beaker to prevent shock or electrical leakage. Combine two 100mL bottles of wash concentrate with 4800mL of CLRW in tank. Mix thoroughly by inversion 6-8 times. Reconnect the tank back to its tubing. This Wash solution is stable for 30 days at room temperature.

To prepare the Diluent, take the Diluent tank out and remove cap. After removing the suction tube, place the end into a beaker to prevent shock or electrical leakage. Combine one 100mL bottle of diluent concentrate with 4900mL of CLRW in tank. Mix thoroughly by inversion 6-8 times. Reconnect the tank back to its tubing. This Diluent solution is stable for 30 days at room temperature.

NOTE: The tanks for the Wash and Diluent are of the same shape. Be sure to add the appropriate reagent to each tank. Each tank has an appropriate ID label on it. Do NOT mix up the tanks, as this will lead to incorrect assay results.

- ✓ If dry waste container is full, remove plastic bag and dispose properly into a biohazard waste container. Replace with a new plastic bag. If liquid waste reservoir should be emptied, disconnect the tubing and dispose of waste down an appropriate drain. Flush the drain with plenty of water. Reconnect the waste reservoir to its tubing and slide the waste reservoir back into the stand.
- ✓ Replace the printer paper if needed
- ✓ Place one STD cup in a cup holder, and set the cup holder in the No.2 position of a sample rack (per instructions on screen). Press the OK button. The daily check takes about 5 minutes. The following will appear on the screen during the daily check: Initialization, Prime Diluent, Prime Wash, Prime Substrate, and Substrate BG Measurement.

5. If the daily check ends normally after the assay of the substrate background, the word "Complete!" will appear on the screen. Press the PRINT button and save printout; record results of daily check in maintenance binder. The following check items have been performed:

a) Substrate Replacement - Indicates whether or not liquid replacement by the Substrate is adequate.

If adequate: OK

If not adequate: ERR→Check out the level of the substrate in the bottle and repeat daily check

b) 4MU Background - Measures the degree of fluorescence (background intensity) of the Substrate, and indicates whether or not there is a problem.

< 1500 nM: OK

>=1500 nM: BH→Replace the Substrate with fresh Substrate and repeat daily check

c) Lamp Intensity Level - Indicates the status of the lamp intensity of the detector lamp.

>= 0.5 (light intensity is adequate): OK

< 0.5 (light intensity is insufficient): LL→Contact Tosoh service

NOTE: An error occurs when one of the daily check items deviates from the normal range. The message “Incomplete!” will appear on the screen. Press OK button and take appropriate actions, repeating the daily check afterwards. If an error occurs during Substrate background assay, check to see if 70% isopropyl alcohol was replaced and confirm adequate Substrate volume.

6. Press the RT.OPEN key on the right side of the display. The RT.OPEN LED at the bottom of the screen flashes green for a while. When the flashing changes to a steady green, the cover is unlocked. Open the reagent and tip cover.

7. For CA 27.29 reagent, uncap and place in L1 position. For B2M reagent, remove stopper and place in L2 position.

The message “If reagent is changed, please register on INVENTORY screen” appears.

To update a new lot of reagent:

- a. Unless the reagent tip cover is open, you cannot enter reagent registration.
- b. Set the reagent rack in line with the positioning pin.
- c. After setting reagent bottles, press the INVENTORY button from HOME screen. The message “INVENTORY Wait for a while...” will appear. Next, the INVENTORY screen will appear.
- d. Set the cursor at the position where the reagent (sample diluting solution) has been set.
- e. Enter the reagent stock information in the numbered position. If you enter the reagent information in the wrong position, inspections may be interrupted or abnormal assay data may occur.
- f. Press the MODIFY button. Press the DILUTING SOLUTION button. The reagent stock screen will appear. Select the desired analyte. That analyte’s reagent will now be registered in the system.
- g. Next, the cursor will move to the lot position. Enter the lot number and press the OK button. Registration of the reagent is now completed.
- h. Press the Exit button. The display returns to HOME screen.
- i. After registering the reagent from the reagent stock screen, close the reagent tip cover. The RT.OPEN LED flashes red for a while. Next, the LED goes out and the reagent tip cover is locked.

To prepare sample tips:

- a. Press the RT.OPEN key on the right side of the display. The RT.OPEN LED at the bottom of the screen flashes green for a while. When the flashing changes to a steady green, the cover is unlocked. Open the reagent and tip cover.
- b. Place the sample tips into the light green tip rack holder. Set the tip rack in line with the positioning pin. One rack holds 96 sample tips. A maximum of two racks is held in the tip drawer.
- c. After the tips have been loaded, close the reagent and tip cover. After the cover has been closed, the number of tips is automatically counted. The RT.OPEN LED flashes red and then goes out, the compartment is now locked.

NOTE: When the Reagent and Sample Tip compartment is closed, the presence of tips is automatically detected by the front sensor. If there is a tip in the front row of the rack, the instrument will assume the entire column (12 tips) contains tips. As a result, fill each column with tips so that there are no blank spaces.

IV. Loading of Sample, Calibrators and Controls (Loader Model)

- Primary tube - Load a 75 x 13mm primary tube in position No.1 of the rack. After the primary tube is loaded, two cup holders must follow every sample for every test ordered. The first holds the Sample Treatment Cup (STC Cup) and is followed by a test cup in the second holder. Every test cup must be preceded by a STC Cup. *Samples are loaded into rack from right to left. An Endmarker must be set or an empty sample rack used at the end, otherwise, sample loader will continue to circulate. Each rack is placed onto sample loader, which is located at left on instrument, with the slits facing toward you. All bar-coded tubes must be loaded with the barcode showing through the slit. The racks move through the instrument in a clockwise motion. **The dead volume of a primary tube for testing is 500 μ L. Do not use a tube with a tapered bottom (i.e. conical bottom-shaped tube.)***
- Dedicated sample cup for Calibrators/Controls/Low volume - Place a Tosoh sample cup into a clear sample cup adaptor. Pipette the appropriate sample in the sample cup. Next, place the sample cup adaptor in position No. 1 of a sample rack. Then, follow the sample cup adaptor with two cup holders. The first holds the STC cup and the second holds the test cup. *Load the rack as described previously for primary tube. **The dead volume for a sample cup is 100 μ L.***

NOTE: Be sure that the sample rack is set in line with the groove on the left side of the sample rack when placed on the machine. Unless the rack is set correctly, the rack can topple over and the sample rack will not be fed through the machine correctly.

V. Calibration Set Up (6-point calibration curve)

1. Press the ORDER(NON-BAR) button on the HOME screen.
2. Press the CALIBRATION button. This enables you to enter the calibration name, the lot number of the calibrator set and the concentration of the calibrators.
3. Select the test to be calibrated. If the test name does not appear on the screen, press the up and down arrow keys to select the desired test on the screen.
Simultaneous calibrations for different analytes can be assayed at the same time.
4. Confirm that the lot number and assigned values on the calibrator set agree with the lot number and concentration values displayed.
 - If correct, press the OK button. The calibration program for the requested analyte will appear on the ORDER(NON-BARCODE) screen.
 - If incorrect, they must be changed. Press the ORDER(NON-BAR) button to display the ORDER(NON-BARCODE) screen. Press the CALIB button and use up or down arrows next to select test. Display the NUMERIC KEYPAD screen to enter the lot number. Press the CL button to delete the current lot number and enter new lot number. Press OK. Next, the cursor will move to CAL 1 position and the numeric keypad will appear. Use CL button to delete, and then enter the correct concentration value and press OK. The cursor will then move on to the next position for CAL 2. Follow the same procedure for each of the next five calibrators.

NOTE: The concentrations can also be set by using the barcode scanner to read the concentration labels on the calibration sheet. The same can be done for lot number.

5. Print the calibration program as a work list by pressing the FUNCTION button. Then, select the WORK LIST button. Press OK. This work list gives you all of the positions where the sample cups and test cups should be placed in the sample racks. **No STC cups are used during Calibration!**
6. Load prepared calibrators and test cups onto sample racks following the sample program created and printed as a work list. Then, load the sample racks onto the instrument. **Don't forget to use an Endmarker or set an empty sample rack!**
7. Press the ASSAY START(NON) button on the HOME screen. Be sure there are sufficient volumes of Diluent, Wash, and Substrate. If insufficient, normal results will not be obtained and calibration must be repeated.
8. Confirm the value displayed (6). Press START.
9. From HOME screen, the MONITOR button will allow the progress of the current assay to be checked. The following colors appear on the screen to indicate the status of the assay:

Black	Before sampling
Blue	Sampled
Green	Measurement completed
Red	Re-Assay

10. Once calibration is complete, press the SUB MENU button on HOME screen to display the SUB MENU screen.

- a. Press the CALIBRATION button to display the CALIBRATION REVIEW (PENDING) screen.
- b. The calibration results will appear on the screen. Determine acceptability based on criteria from test procedures.
- a. Press CALCULATE button. The CALIBRATION CURVE GRAPH screen will appear.
- d. Press the ACCEPT button to finalize the calibration curve. Once finalized, you will no longer be able to change or recalculate the curve assay data.
- e. Press the PRINT button to keep curve data.

NOTE: If you set the order of concentrations of the calibrators in a wrong order, you can change over the data to ascending or descending order by pressing the (REPOSIT) button.

NOTE: You can finalize no more than two lots of the calibrators per analyte. You cannot finalize the calibration curve of the same lot. If you do so, the calibration curve finalized lately will overwrite the current curve of the same lot number.

VI. ASSAY/CONTROL Requests

A. AUTO mode

1. Place the primary sample tube in the sample rack followed by the appropriate STC and test cup (from Section IV.) The AIA-900 is equipped with a camera that reads the analyte name and lot number printed on the test cup. A built-in barcode reader will read specimen ID from primary tube.

Up to five assays can be performed on one specimen.

2. Press the ASSAY START (BAR) button on HOME screen, then START.

B. Manual Entry - For programming short samples

1. Press the ORDER(NON-BAR) button on HOME screen

2. Press the SAMP. ID button

3. Press SP.1. This is specimen type for serum. Enter specimen ID using order input screen. Press OK.

4. Select an analyte from the ANALYTE SELECTION screen. Press OK.

5. Print a work list by pressing FUNCTION button in ORDER(NON-BARCODE) screen, then press PRINT. Load the sample cup with short sample into sample rack with STC cup and appropriate test cup by following the work list.
6. Press ASSAY START (NON). Input rack ID in START RACK ID window using keypad.
7. Press START

C. Requesting Controls

1. Press ORDER(NON-BAR)
2. Press CONTROL. Controls are pre-registered and available for selection.
3. Request desired controls individually by pressing the control ID, followed by OK.
4. Print a work list by pressing FUNCTION, then WORK LIST.
5. Press EXIT. Load controls according to work list.
6. Press ASSAY START (NON), then START.

VII. Dilution Requests

Refer to individual assay procedures for instructions on how to request dilutions beyond initial factory dilution.

VIII. End of Day Shutdown

1. Replace the substrate with 70% isopropyl alcohol solution.
2. From the HOME screen, press the SUBMENU button.
3. Next press the SHUTDOWN button. Follow the prompts under the shutdown menu that appear on the screen. Once the OK button is selected, a message will appear on the screen, it will ask for "SHUTDOWN Are you sure?" Press OK.
4. Next a message will appear "If backup parameters to USB stick, set it and push OK". Be sure the back up USB memory stick is in place and select OK. This will take a few minutes to back up the data.
5. After all the data has been backed up, the following message will appear: "Set substrate solution Are you ready?" Press OK.
6. Then, the instrument will start replacing the substrate with 70% isopropyl alcohol. When this is finished, the following message will appear: "Shutdown complete!" You may now press OK and turn off the power.

IX. Weekly Maintenance

A. Washing the Substrate Line

1. Under SUBMENU: Press the MAINTENANCE button. Next be sure the 70% isopropyl alcohol solution is on the instrument.

2. Select step #7, REPLACE SUBSTRATE. Then press the execute button. The substrate replacement operation will start. Do this step 5 times. Press exit to return to the submenu.
3. Be sure to check the button on the Daily Maintenance screen: "Substrate Lines(1W). This will then reset the date due for the next maintenance.

B. Cleaning of the BF Probes

1. Be sure that AIA-900 is not running. Next, press the BF Open Key. The lock on the BF probe cover becomes unlocked.
2. Turn off the power to the instrument.
3. There are two BF probes on this instrument. Loosen the set screw on the probe nearest to you and place the probe in an upward direction. Clean the probe tip using 70% isopropyl alcohol wetted kimwipe. Blot the probe tip dry with a dry kimwipe. Re-install the probe tip by aligning it with the center of the stainless steel pipe. Repeat this step with the other BF probe. Power up the instrument again and close the BF probe cover.
4. Under the Daily Check screen be sure to click Wash Probe Button(1W). This will reset the date due for the next Wash probes maintenance. All maintenance requirements that are due will appear flashing on the daily maintenance screen under the header "Periodic Maintenance." Once the maintenance has been performed, pressing the button that the maintenance has been done will set a new due date.

X. Monthly Maintenance

A. Replacement of BF probe tips

1. Open the BF probe cover by selecting the BF open cover key. Next, turn off the power.
2. Unscrew the setscrew and remove the BF probe from its station. Next, pull the old plastic probe tip off and discard it.
3. Replace the probe tip with a new one and fit it securely over the pipe (probe shaft). If the probe tip has been correctly installed, the stainless steel pipe will protrude slightly beyond the end of the tip.
4. Repeat the above steps for the other BF probe.

B. Replacement of Tank Filters

1. Once per month, the tank filters on the Diluent tank and the Wash tank must be changed.
2. Remove the wash tank from the instrument. Unscrew the line with the tubing attached. At the end of the tubing is a white plastic filter. Pull the plastic filter off the end of the line and discard the filter. Next, place a new filter onto the end of the plastic tubing. Attach the tubing to the nipple end of the filter. Next, reinstall the cap and place the wash bottle back on to the instrument.
3. Repeat Step 2 for the Diluent tank.

C. Clean Outside of Instrument.

1. Clean the outside of the instrument with a solution of 70% isopropyl alcohol.

XI. Quarterly Maintenance

A. Decontaminating Wash and Diluent Tanks

1. Remove the Wash and Diluent tanks from the instrument.
2. Pour 500mL of tap water into each tank. Next add 5mL of bleach to each tank, put the covers back on and shake each tank vigorously.
3. Rinse each tank thoroughly with tap water and then CLRW until no smell of bleach remains.
4. Place new Wash and Diluent into each tank and return the tanks onto instrument.

B. Decontamination of Wash and Diluent Lines

1. Pour 1 liter of CLRW into a large 2 liter beaker or flask. Next, add 10mL of bleach and mix thoroughly. Next, submerge the tubing of both the Wash and Diluent lines into beaker.
2. Under the Maintenance program, select step #5 and step #6. Do each step a total of 5 times.

XII. References

1. Tosoh A1A-900 Operator's Manual, Revision C. Tosoh Bioscience, Tokyo, Japan.

XIII. Appendix

200-A	Tosoh A1A 900 Maintenance Chart
200-B	Tosoh A1A 900 Checklist
200-C	Tosoh A1A 900 System Problem Solution Log
200-D	Tosoh A1A 900 Substrate Background
200-E	Tosoh A1A 900 Common Reagent Log Substrate II
200-F	Tosoh A1A 900 Common Reagent Log Wash
200-G	Tosoh A1A 900 Common Reagent Log Diluent
200-H	Tosoh A1A 900 Calibration Chart
200-I	Tosoh A1A 900 Calibration Data
200-Q	Tosoh A1A 900 Quality Control Chart

TOSOH AIA-900 Maintenance Schedule

Doc. # IMM 200-A

Month _____ Year _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Daily																																
Empty solid waste container																																
Empty liquid waste reservoir																																
Check diluent and wash fluids, replace as needed																																
Replace ethanol with substrate																																
Check paper supply, replace if necessary																																
Refill pipet tips																																
Record results from Substrate Background																																
End of day, replace substrate with Ethanol																																
Operator Initials																																

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Weekly																															
Clean B/F Wash Probe Tip																															
Clean Substrate line with 70% Isopropyl x 5																															
Back up Data to O drive																															
Operator Initials																															

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Monthly																															
Clean Sampler area with Isopropyl																															
Replace B/F Wash Probe Tip																															
Change Tank Filters																															
Operator Initials																															

Quarterly																														
Clean instrument surfaces after any spills																														
Clean diluent/wash reservoirs with 1:100 dilution of Clorox																														
Rinse reservoirs with Reagent Grade Type I water																														
Replace worn or damaged B/F Wash Probe Tip																														
Date																														
Operator Initials																														

Supervisor Review _____ **Date** _____

Tosoh A1A 900, B2 Microglobulin, C2729 Checklist

Initial 6 Months

Instrument

Yes/No

1. Performs Daily Start Up/Shutdown _____
2. Performs Daily, Weekly, Monthly, Trimonthly(Quarterly) Maintenance _____
3. Able to calibrate and accept/not accept calibration run. _____
4. Able to process barcoded and non-barcoded samples. _____
5. Able to enter new lots of controls, calibrators, and diluent lot numbers. _____
6. Understands all reagent stability requirements. _____
7. Able to assess sample requirements for running of all assays. _____
8. Able to add tests and change dilutions. _____
9. Can perform basic troubleshooting of instrument or assay. Able to access maintenance program. _____
10. Able to independently load and run the Tosoh 900 without supervision. _____
11. Aware of inventory/par levels. _____
 - a. Entry of all new Lot #s in L Drive. _____
 - b. Pretesting of all new Lot #s received. _____

Quality Control

1. Able to ascertain QC validation by utilizing the 10X, 2-2S, and 1-3S Westgard rules. _____
2. Able to ascertain validation of standard curve. _____
3. Proficient using SOFT Total QC . _____

Test

B2Microglobulin

CA 27.29

Training Completed _____

Signature _____
Learning Technologist

Verified By _____
Teaching Technologist

