theranos	Standard Operating Procedure	Document Number: CL SOP-06029 Revision: B
	CLIA Laboratory	Effective Date: 12/2/2014

Use and Maintenance of the Idaho Technology (BioFire) FilmArray® System for the Identification of Microorganisms by Multiplex PCR

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The Laboratory Director or designee will review this procedure at least annually including revisions.

Reviewed By:	Date:	Comments:

Contents

1. Purpose and Principle

1.1 Intended Use

The FilmArray instrument is an automated in vitro diagnostic (IVD) device designed to work with specific pouches to detect multiple nucleic acid targets contained in clinical specimens. The instrument interacts with the reagent pouch to both purify nucleic acids and amplify targeted nucleic acid sequences using nested multiplex PCR in a closed system. The resulting PCR products are evaluated using DNA melting analysis. The FilmArray software then automatically interprets the results.

This standard operating procedure (SOP) outlines in detail how to set up, use and maintain the Idaho Technology, Inc. FilmArray® system for testing clinical samples using multiplex PCR detection of different microorganisms within a panel. This SOP applies to the use and maintenance of the FilmArray® System for identifying the presence of organism(s) specific to the panel for a given commercial pouch type.

1.2 The FilmArray®

The FilmArray® reagent pouch stores all the necessary reagents for sample preparation, reverse transcription-PCR, PCR, and detection in a freeze-dried format. Prior to a run, the user injects hydration solution and unprocessed sample into the pouch. The FilmArray® instrument processes the sample, performs nested multiplex PCR, and the software interprets the results.

First, the FilmArray® extracts and purifies all nucleic acids from the unprocessed sample. Next, the FilmArray® performs a nested multiplex PCR. During the first-stage PCR, the FilmArray® performs a single, large volume, massively multiplexed reaction. Last, individual singleplex second-stage PCR reactions detect the products from the first stage PCR. Using endpoint melting curve data, the FilmArray® software automatically generates a result for each target.

1.3 Respiratory Panel

The targets included in the Respiratory Panel assay are:

- Adenovirus
- Coronavirus HKU1
- Coronavirus NL63
- Coronavirus 229E
- Coronavirus OC43
- Human Metapneumovirus
- Human Rhinovirus/Enterovirus
- Influenza A
- Influenza A/H1
- Influenza A/H1-2009

- Influenza A/H3
- Influenza B
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory Syncytial Virus

2. Definitions and Abbreviations

2.1 LA: Laboratory Assistant

2.2 CLRW: Clinical Laboratory Reagent water

2.3 CLS: Clinical Laboratory Scientist (e.g., state-licensed Testing Personnel)

2.4 PPE: Personal Protective Equipment (goggles, gloves, lab coat etc.)

2.5 Pre-Amp: Pre-Amplification (low amplicon) environment

3. Responsibilities

It is the responsibility of all employees to first read the manufacturer's User Guide before using the FilmArray® and handling the reagents. It is also the responsibility of all employees to follow this SOP when setting up the FilmArray® within the CLIA Laboratory at Theranos Inc. This is a CLIA moderate complexity test. Because there are no manual measurements external controls or calibrators, trained Laboratory Assistants/Laboratory Scientists are permitted to operate the instrument under supervision of Testing Personnel (e.g., Clinical Laboratory Scientists).

4. Materials

4.1 Reagents

4.1.1 Idaho Technology, Inc. (Salt Lake City, UT) FilmArray® Panel Pouch kit (IVD) of the microorganism panel type ordered stored at 18-30ºC up until expiry date (e.g., Respiratory Panel (RP) kit), containing:

- FilmArray® Pouch

- Sample Solution Vial, 0.5 mL (Red Capped)
- Hydration Solution Vial, 1.5 mL (Blue Capped)
- Red Capped 1.0 mL Sample Loading Syringe
- Blue Capped 3.0 mL Pouch Hydration Syringe

4.1.2 Transfer medium appropriate for the sample and kit type

4.1.3 Water, Molecular Biology Grade (RNase free, sterile)

4.1.4 10% Bleach

4.1.5 DNAZap™ (store at 2 – 8 ºC until expiry) or equivalent nucleic acid degradation solution

4.2 Equipment

4.2.1 Personal protective equipment (PPE) appropriate for the specimen type.

4.2.2 Biological safety cabinet or hood

4.2.3 Idaho Technology, Inc. (BioFire) FilmArray® System (Model FLM1-ASY-0001, S/N FA1310) which consists of a specialized multiplex PCR thermocycler, and a notebook PC computer (Dell Computers, Latitude ES420) with proprietary controller and analysis software.

4.2.4 Barcode Scanner (Symbol Technologies, Inc., # LS2208-SR2000 7R-UR)

4.2.5 FilmArray® Pouch Loading Station

4.2.6 Eppendorf or equivalent pipette and LTS filtered pipette tips P1000 (100 uL-1000 uL)

5. Specimen Type and handling

5.1 Respiratory Panel

Nasopharyngeal Swab (NPS) specimens should be collected according to standard technique, and immediately placed in viral transport media. 330uL of NPS specimen is required for testing. Specimens should be processed and tested as soon as possible. If storage is required, specimens can be held:

- At room temp for up to 4 hours
- At 2-8°C for up 3 days
- At <-15°C for up to 30 days

6. Procedure

One of the most important rules when performing PCR is to avoid contamination. To maintain a contamination-free environment perform sample collection and pouch preparation, and operate the FilmArray instrument in separate locations – pouch preparation will take place in the Biosafety Cabinet, and operation of the FilmArray will take place on the Benchtop.

6.1 Preparation of Loading Station

1. Open the vacuum sealed pouch and remove the metal canister containing the FilmArray® pouch.
   Note: Once the pouch has been opened, it should be rehydrated within 30 minutes.
2. Label pouch with the sample ID in the space provided.
3. Slide the FilmArray® Pouch into the FilmArray® Pouch Loading Station with the larger film portion entering first with the barcode facing out. Make sure that the Red and Blue labels on the FilmArray® Pouch align with the Red and Blue arrows present on the Film Array Pouch Loading Station.
4. Place the blue-capped Hydration Solution Vial into the blue well on the FilmArray® Pouch Loading Station
5. Place the red-capped Sample Solution Vial into the red well on the FilmArray® Pouch Loading Station.

6.2 Hydrating the Pouch

1. Remove the blue labeled Pouch Hydration Syringe and make sure the tip is firmly attached.
2. Uncap the Hydration Solution Vial.
3. Draw approximately 1 mL of Hydration Solution into the syringe. Try to avoid bubbles; if you notice bubbles at the base of the syringe, dislodge them by gently tapping the side of the syringe body with your finger while leaving the tip of the cannula in the vial.
4. Holding the body of the syringe insert the syringe tip into the syringe inlet port directly below the blue arrow of the FilmArray® Pouch Loading Station. Holding the barrel of the syringe, forcefully push down to puncture the port seal until you feel a soft pop. Let go of the syringe and allow the liquid to be drawn into the FilmArray® Pouch.
   DO NOT push the syringe plunger or the pouch may overfill. The appropriate volume will be pulled into the pouch automatically.
   NOTE: If the Hydration Solution is not automatically drawn into the pouch, discard the current pouch and start from the beginning with a new pouch. (See Pouch Troubleshooting section in Chapter 8 of the Instruction Manual for additional details.)
5. Leave the syringe to drain for approximately 30-40 seconds or until the syringe is no longer draining and is at a stable level.
6. Remove the syringe from the port and discard in a sharps container.

6.3 Adding Sample and Sample Buffer to the Pouch

1. Uncap the Sample Buffer Vial
2. Using the P1000 pipette, add 300uL of sample into the Sample Buffer Vial. Alternatively, us the provided disposable pipettes, and fill to the third line on the pipette, adding the sample into the Sample Buffer Vial.
3. Gently pipette up and down to mix.

4. Using the red-labeled Sample Loading Syringe (making sure the tip is firmly attached) draw approximately 800 uL of Sample/ Buffer mixture.
5. Holding the body of the syringe, insert the tip into the inlet port directly below the red arrow of the Pouch Loading Station. Holding the barrel of the syringe, forcefully push down to puncture the seal until you feel a soft pop.
   DO NOT push down on the syringe plunger or the pouch will overfill. The correct volume will be drawn into the pouch automatically due to the pressure gradient.
   NOTE: If the Buffer solution is not automatically drawn into the pouch, discard the pouch and start at the beginning with a new pouch (Refer to Operator's Manual Chapter 8- Pouch Troubleshooting for more details).
6. Leave the syringe to drain for approximately 30-40 seconds or until the syringe is no longer draining and is at a stable level.
7. Remove syringe from the port and discard in a sharps container.
8. NOTE: Once rehydrated, use the pouch within 30 minutes.

6.4 Loading the FilmArray® Pouch AND SYSTEM OPERATION

The FilmArray® Software includes a step by step Wizard that:

- Walks the operator through loading the FilmArray® Pouch into the FilmArray® Instrument
- Demonstrates how to perform a run
- Analyzes Results
- Prints Reports

1. Open the FilmArray® Software by double clicking the FilmArray® Instrument desktop icon.
2. Wait for the bar at the top of the screen to read "Connected". This happens within 5 seconds.
3. Press the blue flashing button on the instrument to open the lid of the FilmArray® and follow the directions on the bottom panel of the screen.
4. Lining up the red side of the pouch with the red arrow and the blue side of the pouch with the blue arrow, slide the FilmArray® pouch between the two plates inside the machine. Make sure the barcode is facing out. If inserted correctly, the barcode is visible and the label is readable on the top of the FilmArray® pouch. There will be a soft click once the pouch is locked in place.
5. Scan the FilmArray® Pouch barcode only after inserting the pouch. If you scan the barcode prior to inserting the pouch into the instrument a "CANNOT SCAN NOW" message is displayed. Once scanned the first three fields of the FilmArray Pouch section of the screen will be filled in with the lot, serial number and pouch type. NOTE: To reduce data entry errors, it is recommended that the pouch information be entered using the barcode scanner. However if the scanner is not working it is possible to manually enter the information into the appropriate fields.
   TIP: Keep the lens of the barcode reader free of dust and scratches. See Chapter 8 "Barcode Reader Window Cleaning"
6. Enter the Sample ID of the samples within the pouch

7. Enter the operator's name and password. Be sure to select "Add Operator" if it is the first time for an operator to run the FilmArray®. The username and password will appear red until it is recognized by the software. See Chapter 5 "FilmArray Software", for more information on entering user name, passwords and adding or deleting operators.
8. Select the correct protocol from the protocol drop down list. The available protocols are dependent on the FilmArray® Pouch type. Protocols are typically selected based on the sample type being tested. Refer to the package insert for the specific pouch for further information about selecting the appropriate protocol. NOTE: If only one protocol is available, it will automatically selected.
9. Verify that the correct pouch type is selected and whether it is IVD or RUO.
10. Close the FilmArray® lid.
11. Click the Start Run button. In the NEXT STEP section of the screen a checklist of steps is displayed. As the instrument proceeds through each step, a checkmark appears by the step. The approximate time left in the run is displayed in the Run Box. The run takes about 1hr and 3 minutes to run.
12. If you need to stop the run before it is complete, select Abort Run. When you abort a run, any data that has been generated for that run will not be available for that analysis.
13. Once the run finishes, the blue button will flash to indicate completion.
14. Discard the used pouch in the appropriate biohazard waste.
15. Wipe down surfaces with DNA*Zap*, then a wipe moistened in 10% Bleach.
16. Wipe surfaces with a wipe moistened in Molecular Grade Water.
17. Allow for surfaces to air dry.

7. Procedural notes

1. When loading the syringes for the Sample Buffer and the Hydration Buffer be sure to not push the syringe down into the pouch.
2. Once the pouch is hydrated it must be run within 30 minutes.
3. When mixing the sample into the Sample Buffer ensure that as few bubbles as possible form.
4. When drawing both the Sample/Buffer and the Hydrating Buffer into their respective syringes that as few bubbles as possible form.

8. Interpretation of Results

8.1 When run is complete, the possible results for the unknown samples include:

- **Detected:** A "Detected" result means that an analyte was definitely identified in that sample.
- **Not Detected:** A "Not Detected" result means that an analyte was not identified in that sample.

- **Equivocal:** An "Equivocal" result means that the software was unable to interpret a test as being either positive or negative. **The sample should be retested.**
- **Invalid:** An "Invalid" result means that there has been an error with the software, hardware or the control has failed. **The sample should be retested.**

8.2 The report contains the following information:

a. *Run Summary*

i. *Sample ID:* Describes the sample identification entered by the operator.

ii. *Detected:* Lists all analytes that were detected. If there are no analytes detected this section displays "None". If the internal controls fail, then this section will display "Invalid".

iii. *Equivocal:* Lists all the equivocal interpretations. If there are no equivocal interpretations this section displays "None". If the controls fail, this section will display "Invalid".

iv. *Run Date:* Displays the date and time the run was performed.

v. *Controls:* Indicates whether the test Passed, Failed, or was Invalid.

b. *Result Summary*

i. *Result Column:* Lists all the analytes and whether they were "Detected" or "Not Detected". Detected targets will also have a check mark next to them for emphasis.

ii. *Interpretation Column:* Lists all of the analyte in the order they are found in the pouch definition.

iii. *Call Column:* Found only on the detailed report. Lists the type of call, "Positive" or "Negative" for that assay.

iv. *Assay Column:* Found only on detailed report. Displays all of the assays that are associated with the interpretations.

c. *Run Details*

i. *Pouch:* Displays the panel type being performed, for example Respiratory Panel.

ii. *Run Status:* Indicated if the run was Completed Successfully or Aborted.

iii. *Serial Number:* The serial number is a unique identifier for the pouch that was used in the run.

iv. *Lot Number:* The lot number is an identifier for the pouch used in the run.

v. *Protocol:* Identifies the instrument instructions used to perform the test.

vi. *Operator:* Describes the name of the operator that started the run.

vii. *Instrument:* Displays the instrument ID.

9. Reporting results

Viewing and Printing the FilmArray® System Results

9.1 Within the FilmArray® Software

a. When the Run is complete the results will be displayed in the View Report section of the screen
   i. Save the report as an .xps file, print a copy and file with the test requisition form.
b. When the Run is complete, select the Browse Runs tab at the top of the screen
   i. Select Quick Search button. The last 100 runs will be displayed with the most current run at the top of the list.
   ii. Select the desired run.
   iii. Click the View Report tab on the bottom right hand side of the screen once the desired run is highlighted.
   iv. Results may now be Printed.
   v. Results may now be Exported
      1. Select desired Run.
      2. Select Export Runs to File.
      3. A window will open that will show a list of directories. Find the desired location.
      4. Name the file and click Save.

9.2 Within the PCR Evaluator Software

a. The following information is available within this software
   i. *FilmArray® Menu:* This menu is used to import pouches, get information about the software, and exit the application.
   ii. *Browse Runs Tab:* Displays information for the runs based on search criteria.
   iii. *Information:* Displays information about the current run that is in view in the Instrument Control section. This screen summarizes information about the pouch.
   iv. *Summary Tab:* Displays results for the pouch and control, and interpretations for the current run in view. This shows the pouch status, organisms that tested positive in the run and whether or not the controls passed or failed. If no results are displayed then the controls failed or the run failed. Double clicking in the text will display the curves.
   v. *Control Tab:* Displays melting curve data for the controls within the specific panel for the current run in view.
      i. *Control Box:* Select the control name from the list on controls found here.

ii. *Assay Box:* The selected control's curve will display in the graph and information regarding the control will appear here.

iii. *Additional Information:* When the cursor is placed over the curve more information is displayed.

vi. *Interpretation Tab:* Displays melting curve data for all of the organisms tested for the current run in view. Organisms that tested positive will be highlighted in Red. To view information and curves for each interpretation, click on the individual organism.

i. *Interpretation Box:* Select the organism interpretation from the list found here.

ii. *Assays Box:* The selected curves will display in the graph and information regarding the assay used for the interpretation will appear here.

iii. *Additional Information:* When the cursor is placed over the curve more information is displayed.

b. To open a run

i. Highlight desired run in the Browse Runs tab.

ii. Double click the desired run.

10. Quality control

10.1 Internal Quality Control

Automated internal quality control is performed. If the quality control fails, the results are invalid and must be repeated.

10.2 Additional Control Samples

10.2.1 Negative Control

As a measure of contamination, a negative control must be performed after each contamination incident to ensure proper decontamination (11.2).

In addition, monthly negative control testing should be performed to ensure that the instrument is free of contamination.

300uL of viral transport medium, or PCR grade water should be used in place of the sample, then the test performed as normal. If any results are positive, then the instrument should be decontaminated, and the negative control repeated.

10.2.2 Positive Control

Positive control samples should be performed weekly, using previously positive samples.

These samples can be previous positives that have been tested within the laboratory and found to be positive, or purchased positives from an external source, such as Discovery Life Sciences.

A bacterial positive control of a suspension of *Bordetella pertussis* can also be used.

11. Decontamination and Cleaning Procedures

To be performed on an as needed basis.

11.1.1 Materials

- 10% bleach solution (prepare fresh daily per CL SOP-02008) in a squeeze or spray bottle
- CLRW in a squeeze or spray bottle
- DNA*Zap*™ or equivalent DNA degrading system
- Paper towels
- Bleach Wipes

11.1.2 Pouch Loading Station Decontamination

1. Fill a sink or bin with water and add bleach to make a 10% bleach solution.
2. Submerge the Pouch Loading Station until completely covered with bleach solution. Soak for 15 minutes.
3. Remove Pouch Loading Station from sink or bin. Replace bleach solution with CLRW.
4. Rinse the Pouch Loading Station by completely submerging in water two additional times.

11.1.3 Pouch Loading Chamber Decontamination

1. Remove pouch from instrument and discard in biohazard waste container.
2. Wet a paper towel with 10% bleach and wipe the inner sample chamber and under the lid.
3. Repeat step 2 twice with fresh paper towels for a total of three bleach wipes.
4. Wet a paper towel with CLRW and wipe sample chamber.
5. Repeat step 4 with a fresh paper towel.

11.1.4 FilmArray® Instrument Decontamination

1. Put on PPE.
2. Wet a paper towel with 10% bleach solution and wipe all exterior of the instrument, including the bottom and the bench top where the instrument had contact.
3. Change gloves.
4. Repeat step 2 twice with fresh paper towels and clean gloves for a total of three bleach wipes.

5. Change gloves and then wet a new paper towel with CLRW and wipe the surfaces of the inner chamber, including under the lid, and the entire exterior of the instrument, including the bottom and the bench top where the instrument had contact.
6. Repeat step 5 once more.

11.1.5 Decontamination of Bench Tops and Other Areas

1. Put on clean PPE.
2. Spray the surface with 10% bleach solution and let stand 5 min. Wipe with a clean paper towel or wipe. Change gloves.
3. Repeat step 2 twice with fresh paper towels and clean gloves for a total of three bleach wipes.
5. Change gloves and then spray the surface with CLRW.
6. Wipe the area dry with a new paper towel or wipe. Change gloves.
7. Spray the area with DNA*Zap*™ or an equivalent nucleic acid degradation solution according to the product's instructions. Change gloves.
8. Rinse the area by spraying it with CLRW and wiping it dry.

11.1.6 In case of Pouch Leakage

1. Put on clean PPE.
2. Ensure no one else uses potentially contaminated areas or equipment.
3. Decontaminate and dispose of the pouch as follows:
a. Dispose of potentially contaminated gloves and lab coat and put on clean ones.
b. Clean up the leaked pouch and dispose of in biohazard waste.
c. Change gloves again.
6. Follow decontamination procedure above.
7. Run a pouch without sample as in section 13.2 below.

WARNING: If the pouch contained potentially infectious material, the risk of biohazard contamination exists in addition to sample contamination.

11.2 FUNCTION CHECK OF THE INSTRUMENT

11.2.1 Test for contamination by preparing a pouch following the procedure outlined below using molecular grade water in the place of a sample.

11.2.2 If the run is successful and the result is negative, continue using the instrument as normal.

11.2.3 If unexpected positive results are obtained or the run fails, please contact ITI Technical Support (800-735-6544) for further instructions.

11.3 GENERAL MAINTENANCE

11.3.1 As needed, wipe down the surface of the FilmArray® instrument, including the sample chamber, with a cloth or paper towel and 10% bleach solution (one part household chlorine bleach and nine parts water). See also Chapter 6, Safety Precautions, for detailed instructions.

11.3.2 Periodically, use a lens cloth and lens cleaner to clean the lens of the barcode reader.

11.3.3 The pouch loading station should be wiped down between runs or otherwise decontaminated daily and whenever a pouch leak occurs.

11.3.4 After each decontamination step discard soiled cloth or paper towel in a biohazardous waste container.

11.3.5 Do not use decontamination or cleaning agents that could cause a hazard as a result of a reaction with parts of the equipment or with material contained in it.

11.3.6 If you are unsure whether a cleaning agent will react negatively with the parts of the equipment or with the material contained in it contact Idaho Technology's Technical Support (800-735-6544).

11.3.7 In the event that an instrument is taken out of service and disposed of for any reason, follow applicable state and local regulations. Contact Idaho Technology if there are questions.

12. Limitations

12.1 The assay is validated only for use with the specified sample type(s), for example, the Respiratory Panel is validated for use by the FDA with Nasopharyngeal swabs in viral transport media. If a different sample type is received, the sample should be rejected and a report issued to state inappropriate sample received. Store the sample in the freezer for 30 days.

13. Safety

1. When handling the Sample Buffer ensure that it does not touch the skin as there are some agents that may cause irritation.
2. Never touch switches or power cords with wet hands.
3. Follow all safety instructions printed on or attached to the FilmArray® instrument.

4. Handle all samples and waste materials as if they were capable of transmitting infectious agents.
5. It is EXTREMELY important that contamination from a leaking and/or punctured pouch must be contained and cleaned with DNA*Zap*™ or equivalent IMMEDIATELY. Pouches that break after PCR can contain large quantities of contaminants. This material, although non-infectious, is easily spread by normal human activity. Consequently, very small (molecular) quantities can be amplified by PCR in future runs, which can then be identified as positive by the FilmArray® instrument.
6. Do NOT return to a previous laboratory area without first completing decontamination procedures (i.e., washing and changing PPE).

14. Records

Records will be retained for at least 3 years.

15. Attachments

CL-FRM-06029 FilmArray® System Maintenance Logsheet.

16. References

FilmArray® Operator's Manual IVD, Idaho Technology, Inc., Salt Lake City, UT, 84108, # FLM1-PRT-0001-01, 2007-2011

17. Revision History

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**ECO Number**
A	05/01/2012	Arnold Gelb	ECO-00047
B	12/2/2014	Lindsay Marsh	CL DCO-00068

Use and Maintenance of the Idaho Technology (BioFire) FilmArray® System for the Identification of Microorganisms by Multiplex PCR

Section Number	Description and Justification of Changes
All	Initial Release
All	Logo changed, revision of format, and updating test procedure

theranos	Standard Operating Procedure	Document Number: **CL SOP-06033** Revision: B
	CLIA Laboratory	Effective Date: 12/5/2014

Operation and Maintenance of the DiaSorin ETI-Max 3000 System

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The Laboratory Director or designee will review this procedure at least annually including revisions.

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Contents

1. PURPOSE AND PRINCIPLE

1.1 This SOP is to assist the laboratory user with the utilization of DiaSorin ETI-Max3000 system.

1.2 The ETI-Max 3000 system, consisting of the ETI-Max 3000 instrument and the ETI-Max 3000 software, is a fully automated micro-plate analyzer performing the complete sample processing (sample dilutions, sample and reagent dispensing, incubations, wash processes, plate transports) as well as the photometric measurement and evaluation.

1.3 For in Vitro Diagnostic use only.

1.4 The ETI-Max 3000 system has generally been designed and validated for the determination of infectious diseases by ELISA methods and evaluation by colorimetric and point determination.

2. SCOPE

2.1. This procedure applies to all authorized CLIA Laboratory personnel using the DiaSorin ETI-Max 3000 system.

3. DEFINITIONS AND ABBREVIATIONS

3.1. ELISA – Enzyme-linked Immunosorbent Assay

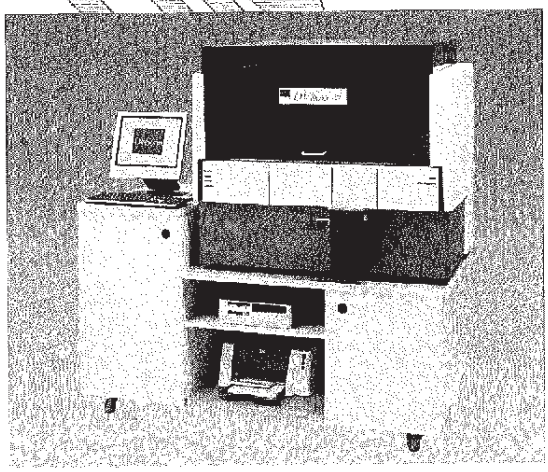
3.2. LIS – Laboratory Information System

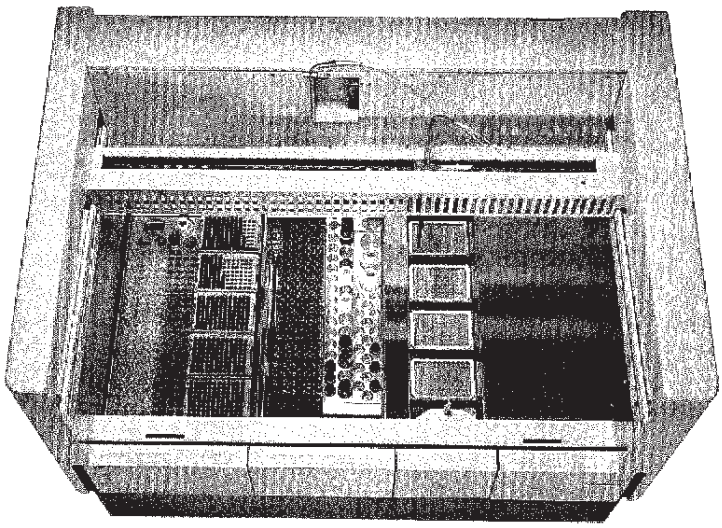
4. RESPONSIBILITIES

4.1. It is the responsibility of supervisors to ensure that the personnel using the system are aware of all safety precautions.

4.2. It is the responsibility of all testing personnel to follow Universal/ Standard Precautions, this SOP and any related SOPs referenced below.

5. MATERIALS AND EQUIPMENT





5.1. DiaSorin ETI-Max 3000 system Components

5.1.1. Analyzer

5.1.1.1. Tray for tip racks and dilution tubes

5.1.1.2. Patient sample and reagent racks unit with bar code scanner

5.1.1.3. Tip eject station, pipettor wash station, pipetting station

5.1.1.4. Test plate compartment, plate transport unit

5.1.1.5. Drawer with wash unit and photometer

a. Spectral range: 400 – 700nm

b. Dynamic Range: -0.100 to 3.000 O.D

5.1.1.6. Heated and ambient incubators

5.1.1.7. Solid waste container

5.1.1.8. Guide rail for pipettor

5.1.1.9. Wash buffer containers

5.1.1.10. Liquid waste container

5.1.2. Computer

5.1.3. Keyboard

5.1.4. Mice

5.1.5. Monitor

5.1.6. Printer

5.1.7. Power Supply

5.2. Consumables:

Item	Vendor	Part Number
1100 uL Tips	DiaSorin	X0002
300 uL Tips	DiaSorin	X0003
2 mL Screw Cap Vial	DiaSorin	14022R
7 mL Brown Vial w/ Cap	DiaSorin	X0004
20 mL Brown Vial w/ Cap	DiaSorin	P001662
25 mL Bottle w/ Cap	DiaSorin	X0013
60 mL Bottle w/ Cap	DiaSorin	P001664
Eti-Max Metal Plate Carrier	DiaSorin	76303202
Eti-Max Nunc Flat Microplates	DiaSorin	41051
Reagent/Control Racks	DiaSorin	A0016-18
Eti-Max Sample Rack 20 pos	DiaSorin	76308260
Eti-Max Sample Rack Holder	DiaSorin	A0021
Eti-Max Tip Waste Container	DiaSorin	A0006
Liquinox , 1 Quart	DiaSorin	X0005
12x75mm Dilution Tubes	Fisher Scientific	14959AA

6. LABORATORY PRECONDITIONS

6.1. Dimensions and weight

6.1.1. Width – 114cm, Depth – 156cm, Height – 100cm

6.1.2. Weight – 130 kg

6.1.3. Accessories: 84 kg

6.2. Electrical Requirements

6.2.1. Power: 100-260 VAC, 47-63 Hz

6.3. Operation Requirements

6.3.1. 15 - 25 °C

6.4. Consumable Preparations

6.4.1. Refer to assay package inserts.

7. MAINTENANCE AND OPERATIONS

7.1. System Basics:

7.1.1. LED display: located on the front panel next to the left and right door.

7.1.1.1. PWR – Power: Green when turned on, otherwise off.

7.1.1.2. RDY – Ready: Green when ready for a run, otherwise off.

7.1.1.3. ERR – Error: Yellow when instrument error has occurred, otherwise off.

7.1.1.4. DWR – Drawer: Green when left drawer has been cleared for loading and unloading of pre-dilution tubes and tips, otherwise off.

7.1.1.5. LD – Load: Green when plate compartment can be opened, otherwise off.

7.1.1.6. INSTR: Instrument: Green when instrument drawer can be opened, otherwise off.

7.2. Starting the system:

7.2.1. Start the ETI-Max 3000 instrument.

7.2.2. Start the computer

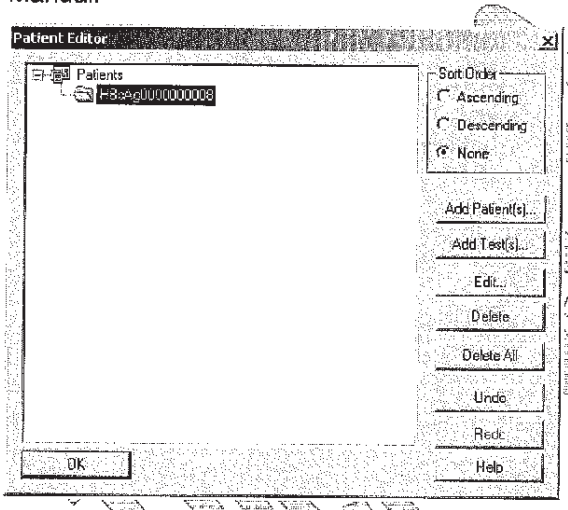
7.2.3. Start the ETI-Max 3000 software by click on the ETI-Max 3000 icon located on the desktop.

7.2.4. Click on the OK button.

7.2.5. After the system self-test and system checks, the results are displayed on the screen. Do not proceed until systems checks have passed.

7.3. Patient Data Entry:

7.3.1. Manual:



7.3.1.1. Select Utilities → Patient Details or click on the Patient Editor icon located on the tool bar.

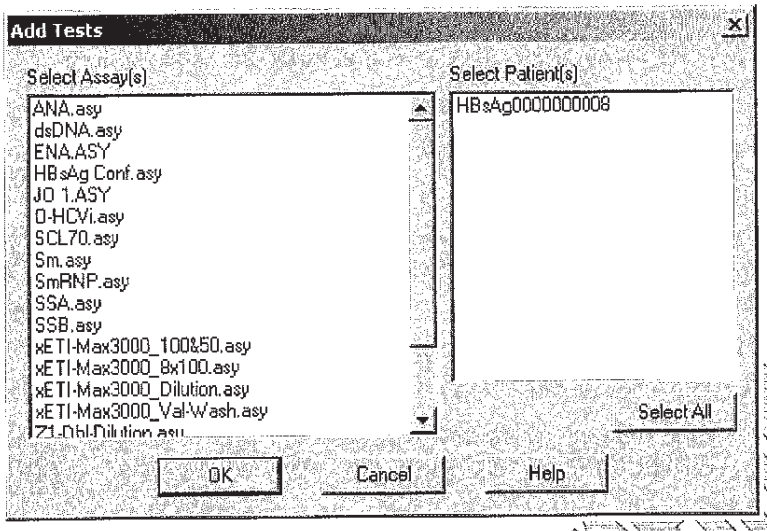
7.3.1.2. Click on Add Patient(s) to add patients.

7.3.1.3. Enter the first sample name in the First Patient ID. To automatically add consecutive sample IDs, enter the number of patients in the Number of Patients text box.

a. Only numbers and characters are accepted for patient ID with a max of 20 characters.

7.3.1.4. Click on the OK button to accept.

7.3.1.5. Once back to the Patient Editor screen, click on the Add Test(s) to add test to the sample IDs.



7.3.1.6. Under the Add Tests window, highlight the patients and then the assay(s).

7.3.1.7. Click on the OK button to accept.

7.3.2. Using the Barcode Scanner:

7.3.2.1. With the worklist closed, insert samples racks with the barcode facing to the right. Insert samples from the highest lane number to the lowest (from right to left).

a. If barcode fails to scan, remove rack and rescan the rack or manually enter the sample ID for that missing patient.

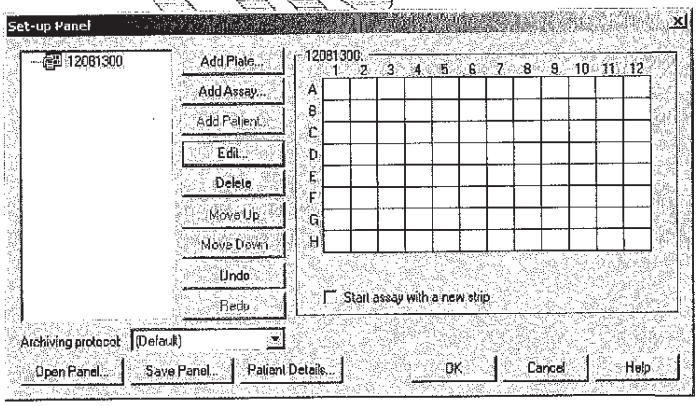
7.3.2.2. Select the assay from the Assay Column Header and assigning the test to each sample ID by checking the appropriate box under the assay column.

7.3.2.3. To accept, click on the close button.

7.3.2.4. If an LIS is in place, the assays will be automatically assigned to each sample ID. Select OK to confirm with the host.

7.4. Creat a New Worklist:

7.4.1. Click on the New → Worklist button or select File → New → Worklist.



7.4.2. The Set-up Panel appears.

7.4.2.1. For manually entered samples, the dialogue box is empty.

a. Click on the Add Plate button. Plate 1 is entered in the Explorer tree.

b. To rename the plate, click on the Edit button.

c. Select on the Plate and click on the Add Assay button.

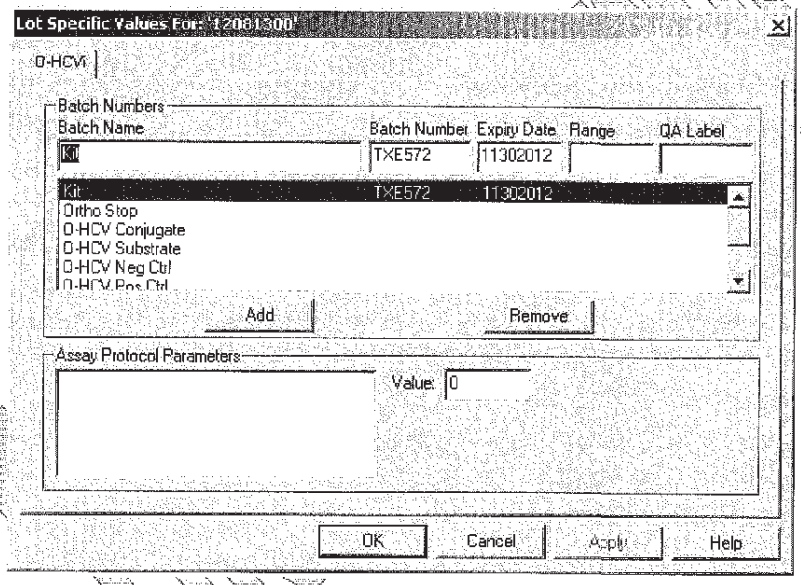
d. Select the assay to be added and then click on the Open button.

e. Click on the Add patient button to assign the patient to the selected assay. To select all samples that are loaded, click on the Select Loaded or to select all samples by clicking on the Select All button.

f. For multiple plates, repeat steps **a.** thru **d.** from above.

g. Once complete, click on the OK to accept.

7.4.2.2. For barcoded samples, assay and patient is displayed in the Explorer tree. Click on the OK button to accept or Edit to modify the plate. Alternatively, the worklist can be deleted and redefined.



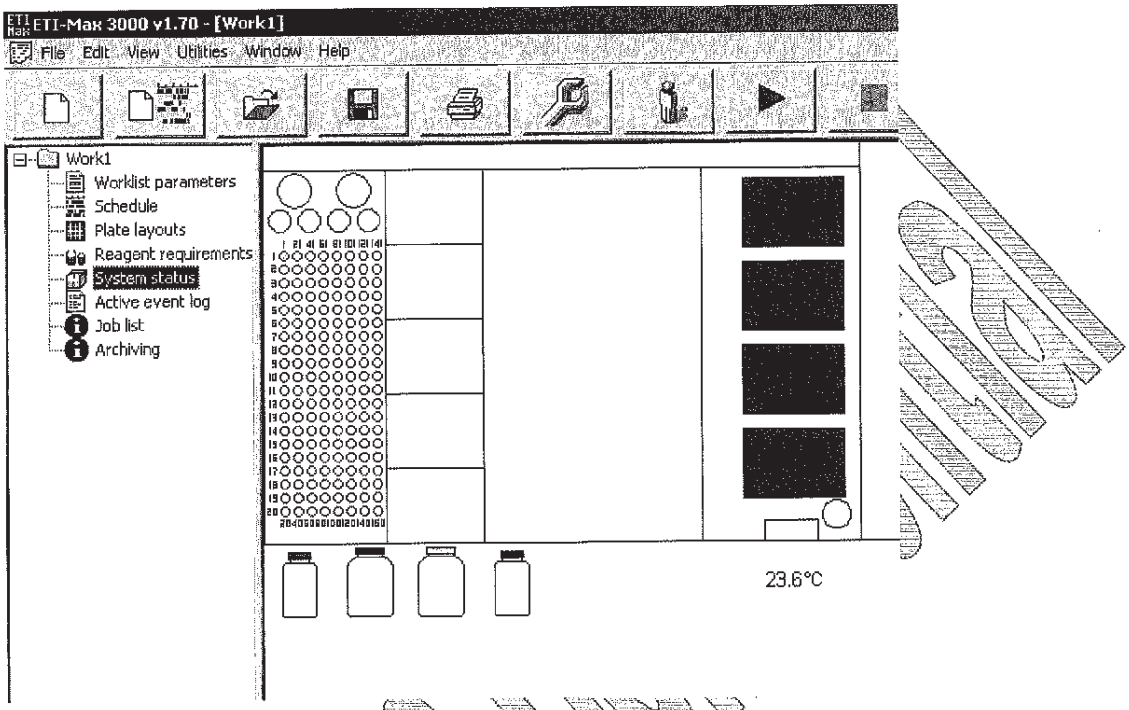
7.4.3. Input reagent data.

7.4.3.1. Enter the lot specific values for each assay tab.

7.4.3.2. Once complete select Okay to accept data.

7.4.4. Starting the Worklist

7.4.4.1. Once complete entering all the data, the worklist window appears displaying all the worklist requirement and parameters. If editing is required, select Edit from the menu item.



7.4.4.2. The worklist window contains the following information:

a. Schedule: Shows the process timeline diagram for the worklist.

b. Plate Layout: Shows the plate diagram location for standards, controls, and samples.

c. Reagent Requirements: Shows the required total reagent and wash volume for the run.

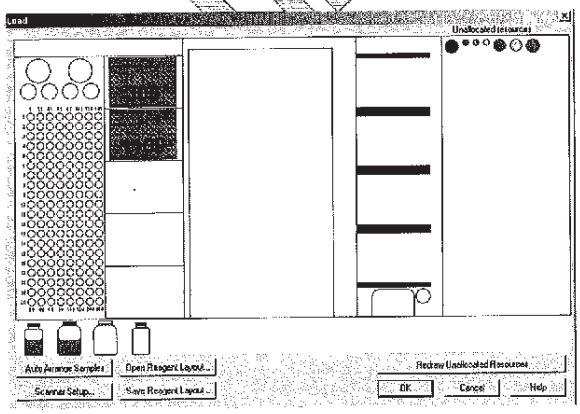
d. System Status: Shows the working area including status messages.

e. Active Event Log: Shows the communication protocol, listing all steps of ETI-Max 3000 which includes warning and error messages and user actions.

f. Job List: Shows allocation of patient IDs and tests to be performed in a list.

7.4.4.3. Once complete preparing all necessary reagents, select the Start button to start the run.

7.5. Loading consumables, reagents, controls, and calibrators:



7.5.1. Place reagents onto reagent racks with barcode facing to the right (if applicable). For reagents without barcodes, manually drag the reagent icon (located under the Unallocated resources) to the appropriate rack location.

7.5.2. Load dilution tubes according to the dilution tube diagram.

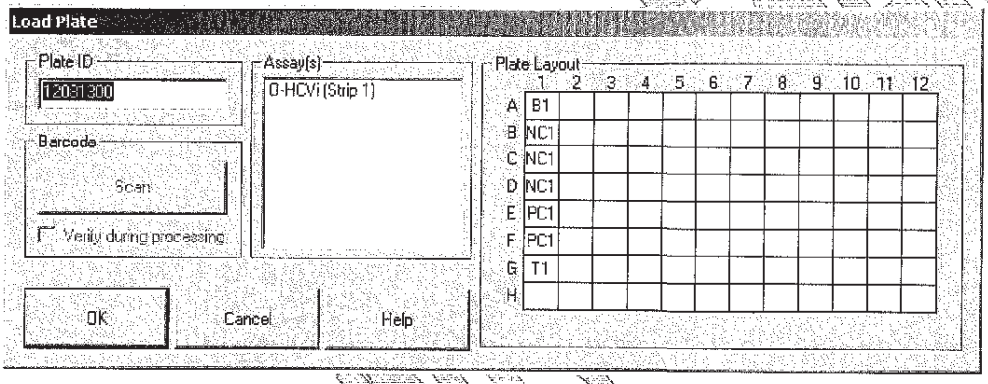
7.5.3. Load/Check disposable tips (both 300 uL and 1100 uL).

7.5.4. Load/Check washes and DI water.

7.5.5. Empty liquid and solid waste (if necessary).

7.5.6. Once complete, click on OK button. The system will perform a volume check of all reagents. A system error dialog will appear if there is insufficient volume. Add additional reagents and click on the Refill bottle.

7.6. Loading the Plate:



7.6.1. Once all reagents are loaded, the plate compartment LED lights green.

7.6.2. Open the plate loader door and load the appropriate plate with plate loader onto the instrument. A1 should be on the upper left corner.

7.6.3. Close the loader door.

7.6.4. Enter the Plate ID.

7.6.5. Click OK to accept and start the run.

7.7. Pipetting Errors:

7.7.1. Clot Errors:

7.7.1.1. To retry, click on the Continue button.

a. Users can either Log and Continue or Manually pipette once the system completes pipetting.

7.7.1.2. To skip, click on the Skip Sample button.

7.7.2. No Liquid Detected:

7.7.2.1. To retry, click on the Retry button.

7.7.2.2. To skip, click on the Abort button (not to be confused with the Abort Plate button).

7.7.2.3. The Ignore button will move the pipettor to the lowest position and aspirate.

7.8. Loading Additional Plates

7.8.1. During the incubation steps, the instrument will notify the operator that additional plates can be loaded.

7.9. Maintenance:

7.9.1. Daily Maintenance:

7.9.1.1. Check the system liquid container level.

7.9.1.2. Empty and clean the waste container for tips.

7.9.1.3. Check the waste container liquid level, empty and clean if necessary.

7.9.1.4. Check the level and fill the clean fluid bottle (red bottle).

7.9.1.5. Check the needles of the washing head and clean using the cleaning tools if necessary.

7.9.1.6. Clean the instrument surface if necessary.

7.9.1.7. Start the system first and then the computer, and then the software.

7.9.1.8. Perform the self-test. Ensure that all test passes.

7.9.1.9. Once assays are complete:

a. Close the finished worklist.

b. Remove/discard reagent, patient samples, washes, and dilution tubes.

7.9.2. Weekly Maintenance:

7.9.2.1. Startup the instrument.

7.9.2.2. Perform self-test. Weekly maintenance will execute after daily maintenance is performed.

7.9.2.3. Prepare 500 mL of 1% solution of Liqui-Nox per reagent bottle, which means 2.0 Liter is required in total. 500 mL is sufficient for two Weekly Maintenance cycles.

7.9.2.4. Follow the instructions on the screen.

a. Replace all four reagent bottles with 1% Liqui-Nox, and allow it to prime.

b. Wait for 15 minutes when prompted.

c. Replace all four reagent bottles with DI water.

d. Empty waste bottles 1 & 2 when prompted.

7.9.2.5. Clean the following areas:

a. Dilution area

b. Reagent and patient rack area

c. Pipetting station

d. Pipettor wash station

- Open drawer
- Using the supplied cleaning needle, clean the 8 dispense and the 8 aspirate needles of the wash head.

e. Tip eject station

7.9.2.6. Change the System Liquid Fluid.

7.9.2.7. Clean and disinfect the waste container.

7.9.3. Monthly Maintenance

7.9.3.1. Clean the reagent and patient racks

7.9.3.2. Clean all four washer buffer bottles and system liquid container with DI water

7.9.3.3. Check the internal waste bottle '2' and empty it if necessary.

7.9.3.4. Clean and disinfect the external waste container.

7.9.3.5. Clean the plate carriers.

7.9.4. As needed Maintenance:

7.9.4.1. Replacing the Photometer Bulb:

a. Switch off the system.
b. Disconnect the main power from the system.
c. Open the instrument drawer up to the first stop.
d. Unlock the safety catch with an Allan screw driver.
e. Pull the drawer out to the second stop.
f. Release the two retaining screws on the back of the photometer.
g. Pull out the photometer bulb and filter drawer.
h. Remove the bulb connector. Caution, the bulb will be hot.
i. Lift the bulb retaining clip and remove the lamp.
j. Reverse the process after inserting the new bulb.
k. Lock the drawer safety catch after insertion of the instrument drawer again.

7.9.4.2. Replacing the Main Fuses:

a. Switch off the system.
b. Disconnect the main power from the system.
c. Pull out the fuse carrier with a screw driver.
d. Change the faulty fuse(s): 4AT, 250V
e. Insert the fuse carrier.
f. Connect the main power.
g. Switch on the system.

7.9.4.3. Backing up the Results Files:

a. Open the Windows explorer.
b. Select the ETI-Max 3000 folder.
c. Select the Results folder
d. Select the results file (.res) to save.
e. Copy them on the desired data drive.

8. TROUBLESHOOTING

8.1. Refer to the Operator Manual.

8.2. Contact Technical Support.

9. PROCEDURAL NOTES

9.1. Not applicable

10. LIMITATIONS

10.1. Not applicable

11. SAFETY

11.1. Make sure that the working area is clean and kept clear.

11.2. Promptly clean any fluid spills.

11.3. Decontaminate with 5% sodium hypochlorite (1 part bleach and 19 parts water) solution, if needed.

11.4. Follow local guidelines for disposal of waste material according to federal, state and local laws.

11.5. If any part of the system breaks down, contact Siemens Technical Support.

12. RECORDS

12.1. Records will be kept for a minimum period of three years.

13. ATTACHMENTS

13.1. CL SOP-06033-F1 DiaSorin ETI-Max 3000 System Maintenance Form

14. REFERENCES

14.1. DiaSorin ETI-Max 3000 User's Guide Rev H

14.2. DiaSorin ETI-Max Maintenance Book

14.3. Burtis CA, Ashwood ER, Bruns DE (editors). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed., Philadelphia, WB Saunders, 2012.

15. TECHNICAL ASSISTANCE

15.1. DiaSorin Technical Support: 1-800-328-1482 ext 2100

15.2. Serial Number: 9163501010

16. REVISION HISTORY

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**ECO Number**
A	08/14/2012	A. Gelb	CL ECO-066
B	12/5/2014	L. Gee	CL DCO-00067
Section Number	**Description and Justification of Changes**		
All	Initial Release		
All	Annual Review		

theranos	Standard Operating Procedure	Document Number: **CL SOP-06051** Revision: A
	CLIA Laboratory	Effective Date: 12/5/2014
SOP Advia 2120i Operation and Maintenance		

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Reviewer(s):

Signature:	Date: 12/3/14
Name: Hoda Alamdar	Title: General Supervisor

Approver(s):

Signature: DocuSigned by: Lynette Sawyer 566AB114EEBD4B1...	Date: 5/5/2015
Name: Adam Rosendorff, MD	Title: Laboratory Director

Lynette Sawyer

SOP Advia 2120i Operation and Maintenance

1 PURPOSE/ PRINCIPLE

The ADVIA 2120 Hematology System by Siemens is a bench-top analyzer that utilizes flow cytometry for light scatter, differential white blood cell (WBC) lysis, and myeloperoxidase and oxazine 750 staining to provide a complete blood cell count, a WBC differential, and a reticulocyte count. A cyanide-free method is used to measure hemoglobin colorimetrically. The system is automation ready; in addition to its capability for analyzing peripheral blood specimens, the analyzer is also equipped to smear and stain slides for microscopic analysis.

2 SCOPE

This procedure applies to all authorized CLIA Laboratory personnel using the ADVIA 2120I analyzer.

3 DEFINITIONS AND ABBREVIATIONS

CBC Results: WBC, RBC, HB, HCT, MCV, MCH, MCHC, CHCM, RDW, HDW, CH, CHDW, PLT
Differential Results: (Absolute and %) NEUT, LYMPH, MONO, EOS, BASO, LUC (Large Unstained Cells)
Platelet Results: PLT, MPV, PDW, PCT
Reticulocyte Results: Absolute and % RETIC, MCVr, CHCMr, RDWr, HDWr ,CHr, CHDWr

4 RESPONSIBILITIES

4.1 It is the responsibility of all testing personnel to follow Universal/ Standard Precautions.

4.2 It is the responsibility of all hematology laboratory personnel to perform maintenance on the appropriate manufacturer-recommended schedules and document accordingly.

4.3 The hematology laboratory personnel will troubleshoot analyzer errors with the Siemens Customer Service if necessary.

4.4 The Hematology CLS shall properly QC the instrument each shift the instrument is in use

4.5 All calibrations and gain adjustments will be performed by the Hematology CLS, in accordance with CLIA regulation.

4.6 The Laboratory Quality Assurance staff shall be responsible for reviewing the QC data on a monthly basis

4.7 A Pathologist will give interpretations of results that require a pathologist expert opinion before the results can be reported.

5 EQUIPMENT AND REAGENTS

Advia 2120i Analyzer
Barcode Scanner
Laser Printer
17" Touchscreen Color LCD Monitor
Full-sized Keyboard
Desktop/Tower

Advia 2120i Reagents:

T03-3682-54	Siemens Healthcare Diagnostics	OptiPOINT Calibrator
T03-3685-52	Siemens Healthcare Diagnostics	SETpoint Calibrator
T03-3688-54	Siemens Healthcare Diagnostics	TESTpoint Control - High
T03-3686-54	Siemens Healthcare Diagnostics	TESTpoint Control - Low
T03-3687-54	Siemens Healthcare Diagnostics	TESTpoint Control - Normal
T01-3621-52	Siemens Healthcare Diagnostics	ADVIA DIFF Timepac
T01-3623-01	Siemens Healthcare Diagnostics	ADVIA Sheath Rinse 20L
4871500	Siemens Healthcare Diagnostics	ADVIA EZ WASH
664-0829-01	Siemens Healthcare Diagnostics	Filter, Perox Sheath
518-3148-06	Siemens Healthcare Diagnostics	Filter, RBC Baso Sheath
T01-3625-54	Siemens Healthcare Diagnostics	ADVIA Defoamer
T01-3626-52	Siemens Healthcare Diagnostics	ADVIA CBC CN-Free Timepac
CL010	R&D Systems	CBC-Line (Linearity)
PLT12	R&D Systems	PLT-Line (Linearity)
RCL002	R&D Systems	RET-Line (Linearity)

6 SPECIMEN

Collect samples according to the blood and sampling procedures. Both K2 and K3 Citrated EDTA specimens collected in 2ml, 4.4 ml or 7ml tubes are acceptable. 175uL of sample is required for Manual Open Mode analysis, and at least 300uL of sample for Closed Mode Sampling (samples 175uL, rest is to allot for dead space).

7 CALIBRATION AND QUALITY CONTROL

7.1 Calibration of all parameters except retics shall be performed if the control values or moving averages shift significantly, after checking gains. This may occur after a major part change (i.e. laser). Calibration is performed using Advia Setpoint Calibrator.

7.2 QC is required at each shift the analyzer is in use, reagent lot number change, major maintenance procedure (i.e. part replacement). All parameters are tested with fixed Testpoint control blood samples.

7.3 The 3 levels include:
- 7.3.1 Abnormal Low
- 7.3.2 Normal
- 7.3.3 Abnormal High

7.4 The Testpoint 3-in-1 controls are used to measure WBC w/ differential, RBC w/ indices Retic and Platelets, and have an open expiration of 14 days. All controls in use shall be marked with an open date, an expiration date and tech initials who first put the material into use.

7.5 QC will be processed each shift the analyzer is in use in the Closed Automatic Tube Mode. **Once per week**, QC will also be run in both the Closed Manual Tube Mode as well as the Open Manual Tube Mode.

7.6 While the Advia 2120 does technically utilize the same pathways to process specimens, all three modes operate with different tubings.

7.7 QC material, as well as all patient specimens, must be allowed to come to room temperature prior to running (10 minutes).

7.8 QC material processed through the Closed Automatic Mode does not require mixing prior to placing in the sample rack loading area. However, for QC testing of the other two modes, QC should be mixed according to Advia Testpoint manufacturer's recommendations prior to running.

7.9 Control values must be within the target range specified in the Advia 2120 software. If the data points for all three levels do not adhere to Westgard Rules for QC Analysis, measurement of the outlying QC data point must be repeated.

7.10 All control data are managed using the Advia 2120 software that provides graphical reports. To view QC results, go to Data Manager Tab → Sample Control Panel. Highlight the first control, then click on Review/Edit. Controls within 2SD will be green squares, between 2><3 SD will be yellow squares and >3SD will be red squares. To validate the QC results, scroll down to view all tests and click on the hand on the right hand side of the screen.

8 PROCEDURE/ USE

Maintenance:

8.1 Daily and Weekly maintenance shall be performed and documented prior to running specimens.

8.2 The Advia 2120 Analyzer Quick Guide as well as the Advia 2120 Autoslide Quick Guides offer step-by-step directions for both completing maintenance procedures and reagent changes.

8.3 All maintenance procedures shall be documented on the on-board maintenance schedule. In addition, daily startup of the Advia 2120 shall be documented on the QA manager's paper maintenance form found in the Advia 2120 Maintenance binder.

8.4 Daily Startup is set to automatically commence prior to morning shift. Staff shall verify that Daily Startup completed. Go to Operations →Startup to confirm background is within limits, or repeat background by clicking on the refresh button on the Startup screen.

8.5 To Install reagents, go to Logs→Reagent Log→ Click on Reagent Installation. Scan in the reagent you are replacing, then click on Import Barcode, then OK. The system will ask you if you want to do a prime, click yes, then increment the cycles you want (1 is usually acceptable), and click Start.

8.6 The Advia 2120 is set up for 5 automated 6-minute wash cycles per day. The wash cycles are scheduled, but the scheduled times can be adjusted. Three of the cycles are scheduled to run back-to-back after the largest specimen run of the day. If you need to perform a system wash for troubleshooting outside of the auto washes, go to the Utilities Tab → Hydraulic Functions, and click on System Wash. Increment to the number of cycles desired, and click Start.

8.7 To empty waste, disconnect the waste sensor, then turn dial to the empty position. Make sure the system is NOT in standby. When container is empty, flip dial back to the normal position and reconnect waste sensor. System will build the vacuum in the container and will come back to the Ready to Run state.

To run QC or patient samples:

8.8 Analyzer must not be in Stand-by mode. If in Stand-by mode (yellow light on Touchpad), press Stand-by button. Bringing the analyzer out of Standby does not perform a background, but it does prime the reagents.

8.9 Samples must be brought to room temperature. Samples to be analyzed using the Automated Closed Mode do not require mixing.

8.10 For Autosampler: Place samples in the Advia 2120i sample rack with barcode facing out, and place rack in the Autosampler input queue on the right side of the analyzer.

8.11 Press "Start" to begin processing. Sample racks will move through the instrument and exit the Autosampler Output Queue.

8.12 For Manual Open Tube Sampling: Scan barcode with ADVIA 2120i barcode scanner. Place sample probe into blood tube and press the blue aspirate bar. Remove sample when green light above aspirate bar stops flashing (do not leave on sample tube, as it will continue to aspirate even after the green light stops flashing.

8.13 For Manual Closed Tube Sampling: Scan barcode with ADVIA 2120i barcode scanner. Place sample into MCTS needle chamber and press down on tube to activate needle. Add resistance by holding sample onto needle until the needle retracts and the light stops flashing, indicating sample has been aspirated.

9 RESULTS REPORTING

The following parameters are reported from the Advia 2120i:

9.1 CBC Results: WBC, RBC, HB, HCT, MCV, MCH, MCHC, CHCM, RDW, HDW, CH, CHDW, PLT
Differential Results: (Absolute and %) NEUT, LYMPH, MONO, EOS, BASO, LUC (Large Unstained Cells)
Platelet Results: PLT, MPV, PDW, PCT
Reticulocyte Results: Absolute and % RETIC, MCVr, CHCMr, RDWr, HDWr ,CHr, CHDWr

9.2 The Advia 2120i will have patient resulting reported from the Theranos LIS.

9.3 Theranos will follow the Advia 2120i reference ranges set by the manufacturer with regard to mean RBC, Hgb, Hct, Platelet and Retic Populations.

9.4 Flagging parameters for grading of indices and reference ranges of some WBC populations have been adjusted to Clinical Director specifications

Linearity of the Advia 2120i is as follows:

WBC	0.02 to 400 x 10^3/µL
RBC	0.0 to 7.0 x 10^6/µL
PLT	5.0 to 3500 x 10^3/µL
HB	0.0 to 22.5 g/dL
Retic	0.2 to 24.5%

10 CALCULATIONS

There are no calculations associated with the operation of ADVIA 2120i.

11 PROCEDURAL NOTES

Troubleshooting procedures as well as advanced maintenance procedures are available in both the Quick Reference Guide as well as the Online Operator's Manual.

12 LIMITATIONS

The ADVIA 2120i has been validated for its intended use. However, error can occur due to potential operator errors and ADVIA 2120i technology limitations. Results obtained MUST be used with other clinical data, for example, symptoms, other test results, patient history, clinical impressions, information available from clinical evaluation, and other diagnostic procedures. All data MUST be considered for patient care management. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

Interfering Substances which may impact the Advia 2120i:
WBC: incomplete RBC lysis (peroxidase WBC only; Baso WBC not affected)
RBC: cold agglutinins, extreme sickle cell
Hgb: EXTREME lipemia, High WBC, EXTREMELY high Bilirubin (interference with colorimetric Hgb only—none with cellular Hgb)
Differential: incomplete RBC lysis, complet Myeloperoxidase Deficiency.

13 SAFETY

13.1 Make sure that the instrument working area is clean and kept clear.

13.2 Wear PPE. Do not pipette by mouth.

13.3 Do not eat, drink, smoke, apply cosmetics, or handle contact lenses.

13.4 Promptly clean any fluid spills.

13.5 Decontaminate with 5% sodium hypochlorite (1 part bleach to 19 parts water) solution, if needed.

13.6 Consult MSDS in case of chemical spill.

13.7 Follow local guidelines for disposal of waste material according to federal, state and local laws.

13.8 If any part of the system breaks down, contact Siemens Technical Support

13.9 Refer to Operations Manual for additional safety information.

14 RECORDS

14.1 Maintenance and QC records will be kept electronically for a minimum period of three years.

15 REFERENCES

15.1 Advia 2120i Operator's Manual (Hematology 2013)
15.2 Advia 2120i Quick Reference Guide

16 REVISION HISTORY

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**DCO Number**
A	12/5/2014	T. Alton	CL DCO-00067
Section Number	**Description and Justification of Changes**		
ALL	New Document/New Analyzer		

Abbott i-STAT Operation and Maintenance

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Signature: DocuSigned by: Lynette Sawyer 566A6114FEBD481...	Date: 5/5/2015
Name: Adam Rosendorff, MD	Title: Laboratory Director

Lynette Sawyer

The Laboratory Director or designee will review this procedure at least annually including revisions.

Reviewed By:	***Date:***	***Comments:***

CONTENTS

1 PURPOSE

1.1 The Abbott i-STAT 1 Analyzer is intended for use with i-STAT cartridges for the *in vitro* quantification of various analytes in whole blood.

2 SCOPE

2.1 This procedure applies to all CLIA Laboratory testing personnel using the Abbott i-STAT 1 Analyzer.

3 DEFINITIONS AND ABBREVIATIONS

Not applicable

4 RESPONSIBILITIES

4.1 It is the responsibility of the supervisors to ensure that all their testing personnel are aware of all safety precautions.

4.2 It is the responsibility of all testing personnel to follow Universal/ Standard Precautions, this SOP and any related SOPs reference below.

5 MATERIALS AND EQUIPMENT

5.1 Abbott i-STAT®1 Analyzer

5.2 Appropriate cartridges for testing

6 LABORATORY PRECONDITIONS

CARTRIDGES:

6.1 Store at temperatures between 2 and 8°C (35-46°F). Do not use after expiration date on cartridge pouch and box.

6.2 Equilibrate a single cartridge for 5 minutes or a box of cartridges for 1 hour at room temperature before opening pouches.

6.3 Store cartridges at room temperature for the timeframe indicated on the cartridge box. Mark the cartridge box or cartridge pouch with the room temperature expiration dates. Do not expose the temperature above 30°C (86°F). Do not return cartridges to the refrigerator after room temperature equilibration.

6.4 Use cartridge immediately after opening pouch. If the pouch has been punctured, the cartridge should not be used.

ANALYZER:

6.5 Storage/Transport temperature: -10 to 46°C (14-115°F).

6.6 The handheld's opening temperature range is 16 to 30°C (61-86°F).

6.7 Store handhelds near the testing location or in an area close to the temperature of the testing area. Do no store handhelds near equipment that gives off heat or in direct sunlight.

7 QUALITY CONTROL AND QUALITY ASSURANCE

7.1 Quality Control

N/A

7.2 Quality Assurance

Perform an electronic check on each handheld in use once a day with either the internal or external Electronic Simulator or as needed for regulatory compliance. The internal simulator check is initiated, every 24 hours or according to a customized schedule, when a cartridge is inserted into the cartridge port. If the internal simulator result is PASS, the cartridge test proceeds and the simulator results are stored. If FAIL is displayed for the internal simulator, reinsert the cartridge or use an external simulator. The external simulator check is performed as follows:

7.2.1 Turn the handheld on.
7.2.2 Press "MENU" to access the Administration Menu.
7.2.3 Press "3" for Quality Tests.
7.2.4 Press "4" for Simulator.
7.2.5 Scan or enter Operator ID.
7.2.6 Enter the Simulator ID.
7.2.7 Insert the simulator into the cartridge port.
7.2.8 View results on the handheld's screen.
7.2.9 If PASS is displayed, continue to use the handheld.
7.2.10 If FAIL is displayed for the external simulator, reinsert the simulator.
If FAIL is displayed a second time, do not use the handheld and contact your Support Service representative at 800-366-8020. Serial Number: 369208.

8 PROCEDURE

8.1 Patient Test Procedures (Cartridge Test Procedure)

- The i-STAT 1 handheld must be customized through the Central Data Station (CDS) or through the handheld's Customization menu for the following option(s): Cartridge Information First Required AND Cartridge Lot Number Required.
- DO NOT insert cartridge to start test.
- DO NOT open cartridge pouch before scanning the barcode.

8.1.1 Press "1" to turn on handheld
8.1.2 Press "2" i-STAT Cartridge
8.1.3 Follow handheld prompts
8.1.4 Scan the lot number on the cartridge pouch
- Position barcode 3-9 inches from scanner window on the handheld
- Press and hold "SCAN" to activate the scanner
- Align the red laser light so it convers entire barcode
- The handheld will beep when it reads the barcode successfully

8.1.5 Continue normal procedures for preparing the sample, filling and sealing cartridge

8.1.6 Push the sealed cartridge into the handheld port until it clicks into place. Wait for the test to complete.

Note: For ACT, PT/INR, Hct and immunoassay testing, the handheld must remain on a level surface with the display facing up during testing. A level surface includes running the handheld in the Downloader/Recharger.

8.1.7 Review results

8.2 Printing Results

8.2.1 Turn printer on if green power light is not on.
8.2.2 Align IR windows of handheld and printer
8.2.3 Display results
8.2.4 Press "PRT"
8.2.5 Do not move handheld or printer until printing is complete
8.2.6 If printer is not powered from a wall outlet, turn printer off.

8.3 Replacing Batteries

8.3.1 Slide the battery compartment door off.
8.3.2 Tilt the handheld slightly to slide out the battery carrier.
8.3.3 Remove the old batteries from the carrier and replace with 2 new 9V lithium batteries.
8.3.4 Insert the carrier back into the compartment – label facing up and electrical contacts first.
8.3.5 Slide the battery compartment door into place.

8.4 Charging the Rechargeable Battery

8.4.1 Placing a handheld in a Downloader/Recharger will automatically initiate recharging of the rechargeable battery. The indicator light on top of the Downloader/Recharger will be green (trickle charge), red (fast charge), or blinking red (fast charge pending) when a handheld with a rechargeable battery is placed in the Downloader/Recharger.
8.4.2 No damage will be caused if a handheld with disposable batteries installed is placed in the Downloader/Recharger.

8.5 Replacing Paper in the i-STAT Printer

8.5.1 Open the paper compartment lid by pulling up on the release lever and remove any remaining paper.
8.5.2 Reel off a few centimeters of paper from the new paper roll, with the leading edge of the paper feeding forward from the bottom of the roll.
8.5.3 Sit the new paper roll in the compartment such that the leading edge is resting outside the compartment on the printer casing.
8.5.4 Close the lid until it snaps into place.

9 MAINTENANCE

9.1 Clean the display and case with a gauze pad moistened with a mild non-abrasive cleaner, detergent, soap and water, alcohol or 10% bleach solutions. Rinse with another pad moistened with water and dry.

10 TROUBLESHOOTING

10.1 Refer to Abbott i-STAT User Guide located at:

J:\CLIA\Instrument Manuals\Point of Care

11 LIMITATIONS

11.1 Interfering substances in the patient's sample may cause an increase or decrease in a result. Refer to the Cartridge and Test Information Sheets and Technical Bulletins for substances and/or conditions that may interfere with cartridge tests.

12 RECORDS

12.1 Records will be kept for a minimum period of three years.

13 REFERENCES

13.1 Refer to current Reference Ranges in the User Guide
13.2 Refer to Quality Check Messages and Codes in the User Guide

14 REVISION HISTORY

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**DCO Number**
A	12/5/2014	L. Gee	CL DCO-00067
Section Number	**Description and Justification of Changes**		
All	Initial release		

theranos	Standard Operating Procedure	Document Number: **CL SOP-09100** Revision: C
	CLIA Laboratory	Effective Date: 12/2/2014

HEMOGLOBIN A1C (HBA1C) AUTOMATED PRETREATMENT METHOD (A1C) IN WHOLE BLOOD ON THE ADVIA 1800 AND 2400 CHEMISTRY SYSTEMS

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Approver(s):

Signature: DocuSigned by: Lynette Sawyer 566A6114FEBD481...	Date: 5/5/2015
Name: Adam Rosendorff, MD	Title: Laboratory Director

Lynette Sawyer

The Laboratory Director or designee will review this procedure at least annually including revisions.

Reviewed By:	***Date:***	***Comments:***

CONTENTS

1. Principle of the Test

HbA1c is formed by the non-enzymatic glycation of the N-terminus of the ß-chain of hemoglobin A. The level of HbA1c is proportional to the level of glucose in the blood and is widely accepted as an indicator of the mean daily blood glucose concentration over the preceding 2 months. The concentration of HbA1c and the concentration of total hemoglobin are measured and their ratio is reported.

The automated pretreatment assays (ADVIA Chemistry A1c_3 and ADVIA Chemistry tHb_3) use 3 ADVIA Chemistry reagents:

- A1c_3 Agglutinator/Total Hemoglobin Reagent 1 (A1c_3 R1)
- A1c_3 Antibody Reagent 2 (A1c_3 R2)
- A1c_3 Denaturant Reagent (A1c_3 DENAT)

In an automated pretreatment step, the whole-blood sample is mixed with the A1c_3 Denaturant Reagent. The red blood cells are lysed and the hemoglobin chain is hydrolyzed by the protease present in the reagent.

For the measurement of total hemoglobin, the A1c_3 Agglutinator Reagent (A1c_3 R1) is used. The assay is based on the determination of released heme in the Soret region at 410 nm.

A latex agglutination inhibition assay is used for the measurement of specific HbA1c. A second protease in the R1 reagent further hydrolyzes the HbA1c sample to a glycated pentapeptide, which competes with the agglutinator (synthetic polymer containing multiple copies of the immunoreactive portion of HbA1c) for the anti-HbA1c antibody, thereby reducing the rate of agglutination. A concentration curve is obtained by monitoring the change in scattered light at 694 nm as a change of absorbance. The actual change in absorbance is inversely proportional to the concentration of HbA1c in the sample.

2. Clinical Application and Usefulness

For *in vitro* diagnostic use in the quantitative determination of Hemoglobin A1c, a diabetes marker, in whole blood on the ADVIA Chemistry systems. Such measurements are used for monitoring the long-term glycemic control of persons with diabetes. The A1c and total hemoglobin (tHb) values generated as part of the HbA1cN and HbA1cI results are intended for use in the calculation of the A1c/total hemoglobin ratio, and must not be used individually for diagnostic purposes.

3. PREANALYTIC PROCESS

3.1. Specimen Collection and Handling

3.1.1. Specimen Collection

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

- Use human or capillary whole blood samples for this assay.
- Validated anticoagulants are lithium heparin and potassium ethylenediaminetetracetate (K2-EDTA).
- Samples should be thoroughly mixed and immediately placed on the ADVIA Chemistry system or as a STAT sample on the laboratory automation system to ensure that the sample is properly aspirated. Results will be achieved if the sample is tested within 10 minutes of mixing.

  If the sample sits for too long, the red blood cells will settle and only plasma will be aspirated. If this occurs, the system flags the result as having an unacceptably low tHb result. In this case, remix and retest the sample.

  For most samples, you can extend the 10-minute limitation by transferring 200 µL of the sample to a sample cup for testing. This approach extends the time between mixing and aspiration to at least 45 minutes.

3.1.2. Specimen Storage and Stability

3.1.3. Keep tubes stoppered and upright at all times.

3.1.4. Whole blood preserved with K2-EDTA or lithium heparin may be stored at room temperature for 48° hours, at 2–8°C for 7 days, or at -70°C for 6 months.

3.1.5. Thaw frozen samples at room temperature and mix thoroughly prior to use. Do not refreeze thawed samples.

3.1.6. Specimen Rejection Criteria

- Specimen clotted
- See also CL SOP-05002 Specimen Collection, Transport, Receipt, Storage and Rejection Criteria.

3.1.7. Specimen Referral Criteria

- See CL QOP-00012 Referral Testing.

3.2. Reagents

3.2.1. Storage and Stability

- Store the reagents at 2–8°C.
- Unopened reagents are stable until the expiration date on the pack label.
- Reagents are stable on board the system as follows:

System	Reagent On Board Stability With Reagent Container Inserts	Reagent On Board Stability Without Reagent Container Inserts
ADVIA 1200 Systems	30 days	7 days
ADVIA 1650/1800 Systems	30 days	7 days
ADVIA 2400 Systems	30 days	7 days

- For all systems, A1c_3 Denaturant Reagent that has been opened and stored at 2° to 8°C is stable for 30 days.

NOTE: Reagent container inserts are not recommended for the A1c_3 Denaturant Reagent (A1c_3 DENAT).

CAUTION:

- Do not freeze reagents.
- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

3.2.2. Ingredients

Reagent ingredients for the ADVIA Chemistry systems HbA1c assay are as follows:

Reagent	REF	Amount	Ingredients
Agglutinator/ Total Hemoglobin Reagent 1	10379673 10485591	2 x 8.0 mL 4 x 17.6 mL	HbA1c hapten covalently attached polymer (1 µg/mL), Bovine serum albumin (1.5%), Buffer, Preservative (sodium azide < 0.1%), Surfactant
Antibody Reagent 2	10379673 10485591	2 x 6.0 mL 4 x 11.6 mL	Anti-HbA1c antibody coupled with latex (mouse) (1.8 mg/mL), Bovine serum albumin (0.2%), Buffer, Preservative (contains 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one), Surfactant
A1c_3 Denaturant Reagent	10379673 10485591	1 x 31.0 mL 4 x 36.5 mL	Porcine pepsin (0.3%), Buffer, Preservative (contains 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one)

CAUTION: This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

IRRITANT: Contains 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one. Potential cancer hazard. May cause sensitization by skin contact. Avoid contact with skin. Wear suitable gloves.

NOTE: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

Reagents Special Preparation

Reagents are ready to use. Before use, gently swirl the reagent to disrupt bubbles and assure homogeneity. If bubbles still exist or foam is present, using a clean transfer pipette, aspirate them from the reagent container prior to use.

When running the A1c and tHb_2 on the ADVIA 1650, ADVIA 1800, and ADVIA 2400 Chemistry systems, the A1c Denaturant Reagent (A1c DENAT) must be placed in a 10-mL glass test tube on the CTT tray in position 59. When running these methods on the ADVIA 1200 Chemistry system, the A1c Denaturant Reagent (A1c DENAT) must be placed in an empty 40-mL reagent container on the RTT-1 tray in position 13. For all systems, the container type must be defined using the same process used for other positions on the control or reagent trays.

3.3. Calibration

Use Siemens A1c Calibrator (REF 06854752) for calibration of the A1c and tHb_2 assays. A reagent blank (RBL) is measured whenever a calibration is performed.

The ADVIA HbA1cN method is traceable to the NGSP reference method (Tosoh G7) via patient sample correlation. Assigned values of Siemens A1c Calibrators are traceable to this standardization. The ADVIA HbA1cI method is traceable to the IFCC reference method via the NGSP reference method and the NGSP/IFCC Master Equation.

For setup and use instructions, refer to the *Calibration Overview* section of the system-specific Operator's Guide.

3.3.1. Calibration Frequency

Perform a calibration and RBL when this method is implemented on the system, and then perform a calibration and RBL every 14 days if using reagent container inserts or every 30 days if reagent container inserts are not used.

Additionally, recalibrate when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

Siemens recommends calibrating a new reagent pack if the previous reagent pack of the same lot number was calibrated at any time during its on-board use rather than as a fresh pack.

3.3.2. RBL Frequency

The reagent blank (RBL) is measured at the time of assay calibration. If, on the previous reagent pack, RBLs were run other than as a fresh pack RBL, run an RBL on the new reagent pack.

3.3.3. Setting Up Multi-Point Calibrations

Multi-point calibrations are used for the A1c assay.

1. Locate the applicable method in the **Proc. Test No.** list area of the Calibration Setup window.

NOTE: Multipoint calibration methods have a **Setting** button in the **MSTD** column of the **Proc. Test No.** list.

2. Select the **Setting** button to display the Multi Standard setup window.
3. In the **TT No.** area, enter the sample tray number (i.e., STT-98 or STT-99) on which the calibrators and blank solution will be loaded.

   NOTE: When performing a multi-point calibration, the blank solution and calibrators are loaded in the outer section (STT) of the sample tray.
4. In the **Posi.** box of the blank row, enter the cup position of the blank solution on the sample tray (STT).
5. For each of the calibrators that will be loaded in the outer section (STT) of the sample tray, enter the lot number, lot name, expiration date, cup position, and assigned value:
   a. In the **Lot No.**, **Lot Name**, and **Exp. Date** boxes, enter the lot number, lot name, expiration date.
   b. In the **Posi.** box, enter the cup position on the sample tray (STT).
   c. In the **Coeff (FV)** box for the method, enter the assigned value. This information can be obtained from the package insert for the lot number of calibrator that is being used.

      NOTE: Do not enter the factor values in the View Calibration Curve window at this time, as these may not be saved.
6. Select **Return**.
7. At the Calibration Setup window, select **Save**.
8. Select **Ctrl/Cal Setup**.
9. Locate the cup positions occupied by the blank solution and the calibrators and perform the following for each:
   a. At **Container Type**, select the type of tube or cup that is used.
   b. In the **Meas. Times** box, enter the number of aspirations you want taken.
   c. In the **Comment** box, enter the applicable text that describes the blank or calibrator.
   d. Close the window.
10. At the window button menu, select **Save**, then select **Yes** to confirm.
11. At the Calibration Setup window, select **Save**.

3.3.4. Scheduling Multi-Point Calibrations

Use the Calibration Setup window to request an automatic calibration after a specific time interval and/or after a new reagent container is loaded. If the calibration interval expires while the system is not running samples, calibration is automatically performed at the beginning of the next run.

1. Select **Auto calib. set**.
2. Enter the following information:
   a. Enter the test number in the **Test** box.
   b. Select both **Blank** and **Standard** in the *Sample Select* area.

c. In the **Control Select** box, enter the letter that identifies each control you want to run after the calibration.

d. In the *Enforcement* area, do the following:

(1) Select the **Time check** box to recalibrate the test when the Interval time expires.

(2) Select the **Bottle check** box to recalibrate the test whenever the system switches to another reagent bottle of the same or a different lot for the assay item.

e. In the **Interval time** box, enter the time interval in days between each calibration. You can enter a value between **1** and **9999** (or **0**).

3. Select **Save**.

A multi-point calibration and RBL can be included in any run by requesting them in the Start Conditions window that is displayed when the run is started.

3.3.5. Setting Up Single-Point Calibrations

Single point calibrations are used for the tHb_2 assay.

Use the Calibration Setup window to enter the ADVIA Chemistry System SSV (System Specific Value) for the calibrator, to specify the number of aspirations, to specify the type of sample container that is used, and to specify the location of the reagent blank solution and calibrator on the sample tray.

NOTE: Refer to the package insert supplied with the Siemens A1c Calibrator for additional information.

1. Locate the applicable method in the **Proc. Test No.** list area of the Calibration Setup window.
2. In the **Blk posi.** box for the method, enter the position of the reagent blank solution on the calibrator/control tray (CTT).
3. In the **STD posi.** box for the method, enter the position of the calibrator cup on the calibrator/control tray (CTT).
4. In the **Coeff (FV)** box for the method, enter the ADVIA Chemistry System SSV (System Specific Value) that is provided on the package inserts for the lot number of calibrator that is being used.

   NOTE: Do not enter the factor values in the View Calibration Curve window at this time, as these may not be saved.
5. Select **Ctrl/Cal Setup**.
6. Locate the cup positions occupied by the blank solution and the calibrator and perform the following for each:

   a. At **Container Type**, select the type of tube or cup that is used.

   b. In the **Meas. Times** box, enter the number of aspirations you want taken.

   NOTE: Do not use more than three replicates for the tHb calibrator.

   c. In the **Comment** box, enter the applicable text that describes the blank or calibrator.

   d. Close the window.
7. At the window button menu, select **Save**, then select **Yes** to confirm.

3.3.6. Scheduling Single-Point Calibrations and RBLs

Use the Calibration Setup window to request an automatic calibration and RBL after a specific time interval and/or after a new reagent container is loaded. If the calibration interval expires while the system is not running samples, calibration and RBL are automatically performed at the beginning of the next run.

1. Select **Auto calib. set**.
2. Enter the following information:
   a. Enter the test number in the **Test** box.
   b. Select both **Blank** and **Standard** in the *Sample Select* area.
   c. In the **Control Select** box, enter the letter that identifies each control you want to run after the calibration.
   d. In the *Enforcement* area, do the following:
      (1) Select the **Time check** box to recalibrate the test when the Interval time expires.
      (2) Select the **Bottle check** box to recalibrate the test whenever the system switches to another reagent bottle of the same or a different lot for the assay item.
   e. In the **Interval time** box, enter the time interval in days between each calibration. You can enter a value between **1** and **9999** (or **0**).
3. Select **Save**.

A single point calibration and/or RBL can be included in any run by requesting them in the Start Conditions window that is displayed when the run is started.

3.4. Quality Control (QC)

For detailed QC procedural information, refer to the *Quality Control Overview* section of the system-specific Operator's Guide.

3.4.1. QC Materials

Bio-Rad Laboratories LiquiChek Diabetes Control Levels 1, 2, and 3.

3.4.2. QC Frequency

Analyze all levels of quality control material:

- on each day that samples are analyzed
- when the reagent lot number changes
- following the performance of any system maintenance, cleaning, or troubleshooting procedure
- after performing a new calibration

Use the QC Sample Definition window to specify cup positions for the controls and to request that controls are automatically run after a user-specified number of samples are processed. Alternately, controls can be included in a particular run by requesting them in the Start Conditions window that is displayed when the run is started.

3.4.3. Troubleshooting Out-of-Range QC Values

A QC run is acceptable when all values fall within the expected ranges.

If the A1c QC results do not fall within the defined ranges, then reevaluate all patient test results obtained in the unacceptable test run to determine if patient test results were adversely affected. Take and document appropriate corrective actions, which may include:

- Verify that the controls and reagents were prepared properly and have not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact the local technical support provider or distributor for assistance.
-

4. ANALYTIC PROCESS

4.1. Instrument Operation and System Description

The ADVIA Chemistry systems are automated clinical chemistry analyzers that can run tests on human serum, plasma, or urine in random access, batch, and STAT (interrupt) modes.

When the **Start** button is selected, the first reagent for the test is aspirated and dispensed into a cuvette on the reaction tray (RRV). Sample is then added to the cuvette and mixed with the reagent. If a second reagent is required, it is dispensed into the same reaction cuvette and the solution is mixed again. The reaction takes place for the amount of time designated in the assay. Concentration data is obtained by the spectrophotometer at timed intervals.

Refer to the *Operating the System* section of the system-specific Operator's Guide for detailed procedures that describe how to schedule samples and manage the workorders.

A. Processing Start/Stop Buttons

The Operation Panel opens after the system software is started. It includes the following buttons:

- **Start** button, to start sample processing.
- **SMP Pause** button, to temporarily stop sampling, so that samples can be added or so that the outer (STT) or inner (CTT) section of the sample tray can be replaced.
- **Stop** button, to halt startup or shutdown.

NOTE: There is also a Stop bottom on the analyzer Power Panel that can be activated in an emergency.

B. Starting the System

1. After the power is applied and the operating system is loaded, the ADVIA Chemistry system Startup window displays.
2. If an optional rack handler (ADVIA 1650/1800 and 2400 systems only) is being used, turn on the rack handler:
   * For the **rack handler**, set the control panel Standby/On switch to **I (ON)**.
   * For the **universal rack handler**, set the display panel Ready/Standby switch to **READY**.

3. At the analyzer Power Panel, set the Operate/Standby switch to **Operate**.
4. At the Startup window, logon as **user** and select **New Start** or **Re-start**, then select **OK**. After a few minutes, the Menu Panel and the Operation Panel open.

   CAUTION: Make sure that all probes and mixers are free to move without obstruction and that all analyzer covers are in place to avoid possible injury and damage to the analyzer.
5. On the Operation Panel, select **Initialize**.
6. If an optional rack handler (ADVIA 1650/1800 and ADVIA 2400 systems only) is being used, double-select the rack handler icon on the Windows desktop.
7. Log on as **supervisor** or **tech_manager**, if required.

Loading Reagents

Loading System Reagents

Visually check the system reagents. Perform a prime after replacing any system ancillary reagents.

1. At the Operation Panel, select **Prime**.
2. Select **Prime 2**, then select **Execute**.

Loading Method Reagents

Check the method reagents in the reagent trays.

1. At the Menu Panel, select **Reagent**, then select **Reagent Inventory**.
2. At the Reagent Inventory window, determine if any reagents need replenishing.
3. Replace any expired reagents:

   IMPORTANT: Do not move reagent containers on Reagent Tray 1 (RTT1) or Reagent Tray 2 (RTT2) after a barcode scan has been performed. This can cause erroneous results.

   If the operator accidentally switches barcoded reagents (i.e., if R1 reagent is loaded on RTT2 and R2 reagent is loaded on RTT1) and performs a reagent barcode scan, an error message displays to alert the operator.

   a. Place the barcoded reagent container(s) in any empty position.
   b. For multiple reagent methods, place R1 on RTT1, and place R2 on RTT2.
   c. Load multiple containers for each reagent on one tray. A maximum of eight reagents can be loaded for the same method.
4. Perform a startup wash.

C. Checking the Analyzer

1. Inspect the probes, mixing rods, cuvette washers, probe wash cups, cuvette covers, and pumps. Perform any required maintenance.
2. At the Menu Panel, select **Maint**, then select **System Monitor** and examine the system operating conditions. If there are abnormal indicators, take the appropriate corrective action.

D. Entering Workorders

Each patient sample must have a workorder that contains a sample number and at least one test request.

Workorders can be entered using several different methods. For detailed operating procedural information, refer to the *Using Workorders* section of the system-specific Operator's Guide.

Downloading Workorders from a Host Computer

1. To download workorders **automatically** from a host computer, use the *Automatic item select* feature in the *Automatic transfer* area on the Online Settings window.
2. To download workorders **manually** from a host computer, follow the procedure below:
   a. At the Menu Panel, select **Request**, then select **Order Entry**.
   b. Select **Host Request**.
   c. In the *Entry format* area, select the means for identifying the first workorder (Step e below).
   d. In the *Last no. entry format* area, select the means for identifying the last workorder (Step f below).
   e. In the Start no. box, identify the first workorder you want downloaded.
   b. In the Last no. box, identify the last workorder you want downloaded, or enter the number of workorders you want downloaded.
   g. Select **Execute**.

Creating a Single Workorder at the Analyzer

1. At the Menu Panel, select **Request**, then select **Order Entry**.
2. Select **Routine** or **Interr**.
3. In the Posi.no. boxes, enter the sample position number.
4. In the Samp.no. box, enter the sample identification number.
5. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
6. As needed, provide entries for Comment and Age.
7. Order tests by any of the following methods:
   * In the Test table, select each test or ratio you want to run.
   * In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
   * In the Profiles area, select each profile you want to run.
8. Select **Enter**. The Number of workorder box increments. If autoincrement is on, a new workorder displays with the next sample number and position number incremented.
9. You can create another workorder, or you can select **Exit** to leave.

   NOTE: If necessary, select **New** to clear the window for entry of the next workorder.

Creating Multiple Workorders at the Analyzer

1. At the Menu Panel, select **Request**, then select **Order Entry**.

2. Enter information for the first sample:

   NOTE: Enter only those items you want replicated.

   a. Select **New** located above the Enter.
   b. Select **Routine** or **Interr**.
   c. In the Posi.no. boxes, enter the starting sample position number (Tray and Cup numbers).
   d. In the Samp.no. box, enter the starting sample identification number.
   e. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
   f. As needed, provide entries for Comment and Age.
   g. Order tests by any of the following methods:
      * In the Test table, select each test or ratio you want to run.
      * In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
      * In the Profiles area, select each profile you want to run.

3. Select **Batch Entry**.
4. Select Samp.no., Posi.no., or Batch entry button, then enter corresponding information in the selected box.
5. Select **Execute**. The Posi.no. and Samp.no. fields increment by the number of workorders requested from Batch Entry.

Creating a Profile, Load List, or Work List

Refer to the *Using Workorders* section of the system-specific Operator's Guide.

F. Loading Patient Samples, Calibrators, and Control Samples

Patient samples are aspirated from the outer section (STT) of the sample tray.

An optional universal rack handler (LAS) can also be used. For instructions on loading patient samples on the universal rack handler, refer to the *Loading Patient Samples* section of the system-specific Operator's Guide.

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

Loading Patient Samples

1. Remove the sample tray evaporation cover.
2. Remove any completed samples and dispose of them in accordance with laboratory procedure.
3. Load the samples in the outer section (STT) of the sample tray.

   NOTE: You can either load samples onto the sample tray while it is in the sampler, or you can remove the tray and then load the samples.

a. When loading samples with barcode labels, make sure the labels are clean, properly positioned on the sample tube, and visible to the sample barcode reader.
b. When not using barcode labels, load each sample into the sample position number entered on the workorder.
c. Load sample cups into a plastic adapter. This adapter can hold two cup sizes. If the cup does not fit, remove the adapter and try the other end.
d. To use barcode labels with a sample cup, just insert the sample cup into a sample tube that has the correct label.
e. EZ Nest cups may also be used in primary sample tubes.

4. If the sample tray was removed for loading the samples, replace the tray and press down on the locking pins to secure it.
5. Replace the sample tray evaporation cover.

CAUTION: Seat the STT cover within the two alignment pins. The dilution probe access holes must be at the back, and the arrow labels must be aligned next to each other to avoid probe crashes.

CAUTION: Make sure all sample containers (including tube-cup combinations) are defined at the System Specification Settings window to avoid probe crashes.

Loading Calibrator and Control Samples

Calibrator samples for a multi-point calibration are loaded in the outer section (STT) of the sample tray, while calibrator samples for a single point calibration are loaded in the inner section (CTT) of the sample tray. Control samples are always loaded in the inner section (CTT) of the sample tray. Cup positions for calibrators are specified on the Calibration Setup window, while cup positions for controls are specified on the QC Sample Definition window.

Loading Urgent (Interrupt) Samples on the STT

1. Verify that an Interr. workorder exists for each sample.
2. At the Operation Panel, select **Pause**. A short delay may occur.
3. Load the new sample(s) on the STT.
4. At the Operation Panel, select **Start** to resume sampling.
5. Complete the Start Conditions window (see the following section) and start the run.

G. Starting the Run

1. In the Operation Panel, select **Start**. The Start Conditions window displays.
2. To run a manual calibration, specify this in the *Calibration* section of the Start Conditions window:
   a. Select **One-pnt.smp. Analyze** or **Multipnt.smp. Analyze**.
   b. Use **Temp.item select** to designate those tests for which a calibration is to be performed.
   c. Select **Temp.sample select**, then select the calibrator solutions.
3. To manually include controls, specify this in the *Control* section of the Start Conditions window:
   a. Select **Control smp. Analyze.**

b. Use **Temp.item select** to designate those tests for which controls are to be manually added.

c. Select **Temp.sample select**, then select the controls that are to be run.

4. If samples are run from the sample tray, start the run as outlined below:
   a. Verify that the samples are loaded.
   b. Select the upper (just below the **Tray no.** field) **General smp. Analyze** field (ADVIA 1200 systems) or **Routine smp. Analyze** field (ADVIA 1650/1800 and 2400 systems).
   c. Select either **Bar-code** or **Cup posi.** to designate how the samples are identified. Then, enter the tray number (i.e., **98** or **99**) in the **Tray no.** field and enter the tray positions (i.e., 50 and 60) in the two boxes adjacent to the **General smp. Analyze** (or **Routine smp. Analyze**) field to specify the tray number and range of tray positions that the system will scan and/or aspirate.
   d. If certain samples should be run first or if a different type of sample tube is used for the run, select **Temp.cup/tube select** and specify the priority samples and/or type of sample container that is being used.
   e. Select **Start** to begin the run.
5. If samples are run from the optional universal rack handler (ADVIA 1650/1800 and 2400 systems only), start the run as outlined below:
   a. Select the lower **Routine smp. Analyze** field (below the **Out side analyze** field).
   b. Select **Start** to begin the run.
   c. Load the sample racks on the rack handler or universal rack handler.

5. POSTANALYTIC PROCESS

5.1. Reporting Results

5.1.1. Reference Interval

Nondiabetics: 4.0 – 6.0% (20 – 42 mmol/mol)

5.1.2. Critical Values

Critical Values: >10.0%

5.1.3. Reporting Protocol for Critical Values

See CL SOP 12001 Test Result Reporting

5.1.4. Units for Reporting Results

The A1c_3 and tHb_3 assays are run on the ADVIA Chemistry systems. HbA1c results in National Glycohemoglobin Standardization Program (NGSP) equivalent units (%) are calculated from the A1c_3 and tHb_3 results using the HbA1c% ratio. HbA1c results in International Federation of Clinical Chemistry (IFCC) equivalent units (mmol/mol) are calculated using the HbA1cR ratio.

5.1.5. Acceptable Results

Patient test results are acceptable and may be reported when

- QC passes
- there are no major instrument flags
- the results are within the measurable range.

5.1.6. Corrective Action

Patient test results must be repeated and corrective action taken when:

- QC is out
- there are major instrument flags
- the results are not within the measurable range

When a test is verified by repeat testing,

- if the two results meet precision criteria (e.g., Tietz, Table 14-5), the first result is usually reported.
- if the two tests don't meet precision criteria, a third test is usually performed and reported.

5.2. Procedure Notes

5.2.1. Calculations

For detailed information about how the system calculates results, refer to the *Analysis* section of the system-specific Operator's Guide.

5.2.2. Conversion of NGSP Values to IFCC Equivalent Values

The National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry (IFCC) formed a working group to develop better primary reference methods. The relationship between HbA1c results from the NGSP network and the IFCC network was evaluated, and the following master equation developed:

IFCC = (NGSP-2.15)/0.092

Results generated on ADVIA Chemistry systems are in NGSP equivalent units. They can be converted to IFCC equivalent units using the equation shown above.

5.2.3. Disposal

Dispose of hazardous or biologically contaminated materials according to Theranos CL SOP-02007. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

5.3. Method Limitations

5.3.1. Reportable Range

The analytical ranges for the ADVIA Chemistry A1c_3 assays are listed below:

- The A1c_3 assay can be used for specific A1c concentrations from 1.0 to 8.83 µmol/L.
- The tHb_3 assay can be used for total hemoglobin concentrations from 7 to 24 g/dL.

- The HbA1c% assay is linear from 2.9 to 15.4% HbA1c.
- The HbA1cR assay is linear from 8 to 144 mmol/mol.

5.3.2. Other Limitations

The ADVIA Chemistry A1c_3 assay gives accurate results for a range of total hemoglobin varying between 7 g/dL and 24 g/dL. Most patients have hemoglobin values within this range. According to the literature, however, patients with severe anemias may have hemoglobin concentrations lower than 7 g/dL, and patients with polycythemia may have hemoglobin concentrations above 24 g/dL. Patients with hemoglobin concentrations outside of the acceptable range and who are known to have these conditions should be assayed by a test that employs a different assay principle.

Any cause of shortened red cell survival (for example, hemolytic anemia or other hemolytic diseases, pregnancy, or recent significant blood loss) will reduce exposure of red cells to glucose with a consequent decrease in HbA1c values. HbA1c results are not reliable in patients with chronic blood loss and consequent variable erythrocyte life span.

Fetal Hemoglobin (HbF) consists of 2 alpha and 2 gamma chains that are not recognized by the anti-HbA1c antibody. Samples that contain high amounts of HbF (> 10%), usually found in some people with thalassemia, in infants, and in some pregnant women, may yield a lower than expected HbA1c result with this assay. For blood samples containing HbF (> 10%), HbA1c results obtained by this assay should not be compared to published normal or abnormal results.

A panel of HbC and HbS samples were compared to an HPLC method and showed no clinically significant bias at 6% (42 mmol/mol) and 9% (74 mmol/mol) HbA1c when compared to HbA using linear regression analyses.

Samples containing HbD and HbE showed no significant interference (a percentage of ≥ 10% is considered significant interference).

Labile glycosylated hemoglobin (6% and 9% [42 and 74 mmol/mol] HbA1c) and carbamylated hemoglobin (6% and 9% [42 and 74 mmol/mol] HbA1c) showed no significant interference (a percentage effect of ≥ 10% is considered significant interference).

As with any chemical reaction, you must be alert to the possible effect on results of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

Siemens tested the following potential interferents and found the results shown below:

Interferent	*ADVIA 1200*	*ADVIA 1650/1800*	*ADVIA 2400*
bilirubin (conjugated) 60 mg/dL	NSI at 5.32%*	NSI at 5.03%*	NSI at 5.23%*
	NSI at 10.18%*	NSI at 9.78%*	NSI at 10.66%*
bilirubin (unconjugated) 60 mg/dL	NSI at 5.26%*	NSI at 5.16%*	NSI at 5.38%*

Interferent	*ADVIA 1200*	*ADVIA 1650/1800*	*ADVIA 2400*
	NSI at 10.11%*	NSI at 9.90%*	NSI at 10.52%*
lipemia (from Intralipid) 1000 mg/dL	NSI at 5.43%*	NSI at 5.16%*	NSI at 5.33%*
	NSI at 10.18%*	NSI at 9.84%*	NSI at 10.50%*
Rheumatoid Factor (RF) 936 IU/mL	NSI at 5.05%*	NSI at 5.25%*	NSI at 5.15%*
	NSI at 10.19%*	NSI at 10.24%*	NSI at 10.00%*

* NSI = No Significant Interference, where significant interference is considered a percentage effect ≥ 10%.

For additional information on performance characteristics, see the product information in the ADVIA Chemistry systems A1c_3 product insert.

5.3 Equipment and Supplies

- ADVIA A1c Reagents
- Siemens A1c Calibrators
- Quality Control material (Bio-Rad Laboratories recommended)
- Sample containers
- System solutions
- Glass test tubes (16 x 100 mm) (ADVIA 1650/1800/2400 systems)
- Reagent container adapters
- Reagent container inserts (optional)

6. References

1. Siemens Healthcare Diagnostics ADVIA Chemistry systems Hemoglobin A1c_3 (A1c) Product Insert.
2. Siemens Healthcare Diagnostics ADVIA Chemistry system-specific Operator's Guide.
3. Clinical and Laboratory Standards Institute (CLSI). Clinical Laboratory Technical Procedure Manuals; Laboratory Documents: Development and Control; Approved Guideline, GP02-A5, 2006.
4. Burtis CA, Ashwood ER, Bruns DE (ed.s). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Fourth Edition, Elsevier Saunders, 2006.

7. Technical Assistance

Siemens Healthcare Diagnostics Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

Serial Number 1800: CA1291000790079

Serial Number 2400: CA1275000100010 or CA1275000120012

8. Revision History

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**ECO Number**
A	10/20/2011	A. Gelb	CL ECO-00022
B	08/10/2012	A. Gelb	CL ECO-00065
C	12/2/2014	L. Gee	CL DCO-00067
Section Number	**Description and Justification of Changes**		
All	Initial Release		
All	Updated SOP for A1c_3 reagent		
All	Annual Review		

ALBUMIN (ALB) IN SERUM OR PLASMA ON THE ADVIA CHEMISTRY SYSTEMS

Author(s):

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Signature: DocuSigned by: Lynette Sawyer 566A8114FEBD481...	Date: 5/5/2015
Name: Adam Rosendorff, MD	Title: Laboratory Director

Lynette Sawyer

The Laboratory Director or designee will review this procedure at least annually including revisions.

Reviewed By:	***Date:***	***Comments:***

ALBUMIN (ALB) IN SERUM OR PLASMA ON THE ADVIA CHEMISTRY SYSTEMS

1. Principle of the Test

Serum or plasma albumin quantitatively binds to BCG to form an albumin-BCG complex that is measured as an endpoint reaction at 596/694 nm.

$$BCG + Albumin \xrightarrow{pH\ 4.2} BCG\ Albumin\ Complex$$

2. Clinical Application and Usefulness

For *in vitro* diagnostic use in the quantitative determination of albumin in human serum and plasma (lithium heparin) on the ADVIA Chemistry systems. Such measurements are used in the diagnosis and treatment of chronic inflammatory diseases, collagen diseases, and liver and kidney disorders.

3. PREANALYTIC PROCESS

3.1. Specimen Collection and Handling

3.1.1. Specimen Collection

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

- Serum or lithium heparinized plasma are the recommended sample types for this assay.
- Avoid hemolysis of erythrocytes during sample collection and preparation.
- For serum samples, place the collection tube in an upright position and allow the specimen to stand undisturbed for thirty minutes to allow clot to form.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells.
- Separate plasma or serum from cells within two hours of collection. If samples cannot be assayed immediately after separation, store them in stoppered containers at 2 – 8°C.
- Process samples in a manner that prevents the introduction of clots, fibrin strands, or other solid materials into the system.

3.1.2. Specimen Storage and Stability

- Serum or lithium heparin plasma may be stored at -75 – -85°C for 6 months, at -15 – -25°C for 3 months, at 2-8°C for 7 days, or at room temperature for 24 hours.

- Thaw frozen samples at room temperature and mix thoroughly prior to use. Do not refreeze thawed samples.

3.1.3. Specimen Rejection Criteria

- See CL SOP-05002 Specimen Collection, Transport, Receipt, Storage and Rejection Criteria.

3.1.4. Specimen Referral Criteria

- See CL QOP-00012 Referral Testing.

3.2. *Reagents*

3.2.1. Storage and Stability

- Store the reagents at 15 – 25°C.
- Unopened reagents are stable until the expiration date on the pack label.
- Reagents are stable on board the system as follows:

System	*Reagent On Board Stability*
ADVIA 1200 Systems	60 days
ADVIA 1650/1800 Systems	60 days
ADVIA 2400 Systems	60 days

CAUTION:

- Do not freeze reagents.
- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

3.2.2. Ingredients

Reagent ingredients for the ADVIA Chemistry systems ALB assay are as follows:

Reagent	*REF*	*Amount*	*Ingredients*
Reagent 1	07622536	4 x 68 mL	bromocresol green (0.2 mmol/L), sodium azide (0.02%)

NOTE: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

3.2.3. Reagents Special Preparation

Reagents are ready to use. Before use, gently swirl the reagent to disrupt bubbles and assure homogeneity. If bubbles still exist or foam is present, using a clean transfer pipette, aspirate them from the reagent container prior to use.

3.3. Calibration

The ADVIA ALB method is traceable to a BCG reference method, which uses reference materials from the National Institute of Standards and Technology (NIST), via patient sample correlation. Assigned values of Siemens Chemistry Calibrators are traceable to this standardization.

Use the Siemens Chemistry Calibrator (REF 09784096; T03-1291-62) to perform a single-point calibration for this method. A reagent blank (RBL) is required whenever a calibration is performed.

For setup and use instructions, refer to the *Calibration Overview* section of the system-specific Operator's Guide.

3.3.1. Calibration Frequency

Perform a calibration and RBL when this method is implemented on the system and every 60 days thereafter. Additionally, recalibrate when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

3.3.2. RBL Frequency

An RBL is run whenever a calibration is performed.

3.3.3. Setting Up Single-Point Calibrations

Use the Calibration Setup window to enter the ADVIA Chemistry System SSV (System Specific Value) for the calibrator, to specify the number of aspirations, to specify the type of sample container that is used, and to specify the location of the reagent blank solution and calibrator on the sample tray.

1. Locate the applicable method in the **Proc. Test No.** list area of the Calibration Setup window.
2. In the **Blk posi.** box for the method, enter the position of the reagent blank solution on the calibrator/control tray (CTT).
3. In the **STD posi.** box for the method, enter the position of the calibrator cup on the calibrator/control tray (CTT).

4. In the **Coeff (FV)** box for the method, enter the ADVIA Chemistry System SSV (System Specific Value) that is provided on the package inserts for the lot number of calibrator that is being used.

   NOTE: Do not enter the factor values in the View Calibration Curve window at this time, as these may not be saved.

5. Select **Ctrl/Cal Setup**.
6. Locate the cup positions occupied by the blank solution and the calibrator and perform the following for each:
   a. At **Container Type**, select the type of tube or cup that is used.
   b. In the **Meas. Times** box, enter the number of aspirations you want taken.
   c. In the **Comment** box, enter the applicable text that describes the blank or calibrator.
   d. Close the window.
7. At the window button menu, select **Save**, then select **Yes** to confirm.

3.3.4. Scheduling Single-Point Calibrations

Use the Calibration Setup window to request an automatic calibration and RBL after a specific time interval and/or after a new reagent container is loaded. If the calibration interval expires while the system is not running samples, calibration and RBL are automatically performed at the beginning of the next run.

1. Select **Auto calib. set.**
2. Enter the following information:
   a. Enter the test number in the **Test** box.
   b. Select both **Blank** and **Standard** in the *Sample Select* area.
   c. In the **Control Select** box, enter the letter that identifies each control you want to run after the calibration.
   d. In the *Enforcement* area, do the following:
      (1) Select the **Time check** box to recalibrate the test when the Interval time expires.
      (2) Select the **Bottle check** box to recalibrate the test whenever the system switches to another reagent bottle of the same or a different lot for the assay item.
   e. In the **Interval time** box, enter the time interval in days between each calibration. You can enter a value between **1** and **9999** (or **0**).
3. Select **Save**.

A single point calibration and RBL can be included in any run by requesting them in the Start Conditions window that is displayed when the run is started.

3.4. Quality Control (QC)

For detailed QC procedural information, refer to the *Quality Control Overview* section of the system-specific Operator's Guide.

3.4.1. QC Materials

Siemens recommends the use of commercially available quality control materials with at least 2 levels (low and high).

3.4.2. QC Frequency

Analyze all levels of quality control material:

- on each day that samples are analyzed
- when the reagent lot number changes
- following the performance of any system maintenance, cleaning, or troubleshooting procedure
- after performing a new calibration

Use the QC Sample Definition window to specify cup positions for the controls and to request that controls are automatically run after a user-specified number of samples are processed. Alternately, controls can be included in a particular run by requesting them in the Start Conditions window that is displayed when the run is started.

3.4.3. Troubleshooting Out-of-Range QC Values

A QC run is acceptable when all values fall within the expected ranges.

If the ALB QC results do not fall within the defined ranges, then reevaluate all patient test results obtained in the unacceptable test run to determine if patient test results were adversely affected. Take and document appropriate corrective actions, which may include:

- Verify that the controls and reagents were prepared properly and have not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact the local technical support provider or distributor for assistance.

4. ANALYTIC PROCESS

4.1. Instrument Operation and System Description

The ADVIA Chemistry systems are automated clinical chemistry analyzers that can run tests on human serum, plasma, or urine in random access, batch, and STAT (interrupt) modes.

When the **Start** button is selected, the first reagent for the test is aspirated and dispensed into a cuvette on the reaction tray (RRV). Sample is then added to the cuvette and mixed with the reagent. If a second reagent is required, it is dispensed into the same reaction cuvette and the solution is mixed again. The reaction takes place for the amount of time designated in the assay. Concentration data is obtained by the spectrophotometer at timed intervals.

Refer to the *Operating the System* section of the system-specific Operator's Guide for detailed procedures that describe how to schedule samples and manage the workorders.

A. Processing Start/Stop Buttons

The Operation Panel opens after the system software is started. It includes the following buttons:

- **Start** button, to start sample processing.
- **SMP Pause** button, to temporarily stop sampling, so that samples can be added or so that the outer (STT) or inner (CTT) section of the sample tray can be replaced.
- **Stop** button, to halt startup or shutdown.

  NOTE: There is also a Stop bottom on the analyzer Power Panel that can be activated in an emergency.

B. Starting the System

1. After the power is applied and the operating system is loaded, the ADVIA Chemistry system Startup window displays.
2. If an optional rack handler (ADVIA 1650/1800 and 2400 systems only) is being used, turn on the rack handler:

- For the **rack handler**, set the control panel Standby/On switch to **I (ON)**.
- For the **universal rack handler**, set the display panel Ready/Standby switch to **READY**.

3. At the analyzer Power Panel, set the Operate/Standby switch to **Operate**.
4. At the Startup window, logon as **user** and select **New Start** or **Re-start**, then select **OK**. After a few minutes, the Menu Panel and the Operation Panel open.

   CAUTION: Make sure that all probes and mixers are free to move without obstruction and that all analyzer covers are in place to avoid possible injury and damage to the analyzer.
5. On the Operation Panel, select **Initialize**.
6. If an optional rack handler (ADVIA 1650/1800 and ADVIA 2400 systems only) is being used, double-select the rack handler icon on the Windows desktop.
7. Log on as **supervisor** or **tech_manager**, if required.

Loading Reagents

Loading System Reagents

Visually check the system reagents. Perform a prime after replacing any system ancillary reagents.

1. At the Operation Panel, select **Prime**.
2. Select **Prime 2**, then select **Execute**.

Loading Method Reagents

Check the method reagents in the reagent trays.

1. At the Menu Panel, select **Reagent**, then select **Reagent Inventory**.
2. At the Reagent Inventory window, determine if any reagents need replenishing.
3. Replace any expired reagents:

   IMPORTANT: Do not move reagent containers on Reagent Tray 1 (RTT1) or Reagent Tray 2 (RTT2) after a barcode scan has been performed. This can cause erroneous results.

   If the operator accidentally switches barcoded reagents (i.e., if R1 reagent is loaded on RTT2 and R2 reagent is loaded on RTT1) and performs a reagent barcode scan, an error message displays to alert the operator.

   a. Place the barcoded reagent container(s) in any empty position.
   b. For multiple reagent methods, place R1 on RTT1, and place R2 on RTT2.
   c. Load multiple containers for each reagent on one tray. A maximum of eight reagents can be loaded for the same method.
4. Perform a startup wash.

C. Checking the Analyzer

1. Inspect the probes, mixing rods, cuvette washers, probe wash cups, cuvette covers, and pumps. Perform any required maintenance.
2. At the Menu Panel, select **Maint**, then select **System Monitor** and examine the system operating conditions. If there are abnormal indicators, take the appropriate corrective action.

D. Entering Workorders

Each patient sample must have a workorder that contains a sample number and at least one test request.

Workorders can be entered using several different methods. For detailed operating procedural information, refer to the *Using Workorders* section of the system-specific Operator's Guide.

Downloading Workorders from a Host Computer

1. To download workorders **automatically** from a host computer, use the *Automatic item select* feature in the *Automatic transfer* area on the Online Settings window.
2. To download workorders **manually** from a host computer, follow the procedure below:
   a. At the Menu Panel, select **Request**, then select **Order Entry**.
   b. Select **Host Request**.
   c. In the *Entry format* area, select the means for identifying the first workorder (Step e below).
   d. In the *Last no. entry format* area, select the means for identifying the last workorder (Step f below).
   e. In the Start no. box, identify the first workorder you want downloaded.
   b. In the Last no. box, identify the last workorder you want downloaded, or enter the number of workorders you want downloaded.
   g. Select **Execute**.

Creating a Single Workorder at the Analyzer

1. At the Menu Panel, select **Request,** then select **Order Entry**.
2. Select **Routine** or **Interr**.
3. In the Posi.no. boxes, enter the sample position number.
4. In the Samp.no. box, enter the sample identification number.
5. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
6. As needed, provide entries for Comment and Age.
7. Order tests by any of the following methods:

- In the Test table, select each test or ratio you want to run.
- In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
- In the Profiles area, select each profile you want to run.

8. Select **Enter**. The Number of workorder box increments. If autoincrement is on, a new workorder displays with the next sample number and position number incremented.
9. You can create another workorder, or you can select **Exit** to leave.

   NOTE: If necessary, select **New** to clear the window for entry of the next workorder.

Creating Multiple Workorders at the Analyzer

1. At the Menu Panel, select **Request,** then select **Order Entry**.
2. Enter information for the first sample:

   NOTE: Enter only those items you want replicated.

   a. Select **New** located above the Enter.

b. Select **Routine** or **Interr**.
c. In the Posi.no. boxes, enter the starting sample position number (Tray and Cup numbers).
d. In the Samp.no. box, enter the starting sample identification number.
e. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
f. As needed, provide entries for Comment and Age.
g. Order tests by any of the following methods:
   - In the Test table, select each test or ratio you want to run.
   - In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
   - In the Profiles area, select each profile you want to run.

3. Select **Batch Entry**.
4. Select Samp.no., Posi.no., or Batch entry button, then enter corresponding information in the selected box.
5. Select **Execute**. The Posi.no. and Samp.no. fields increment by the number of workorders requested from Batch Entry.

Creating a Profile, Load List, or Work List

Refer to the *Using Workorders* section of the system-specific Operator's Guide.

F. Loading Patient Samples, Calibrators, Control Samples and RBL Samples

Patient samples are aspirated from the outer section (STT) of the sample tray, while RBL, calibrator, and control samples are aspirated from the inner section (CTT) of the sample tray. An optional universal rack handler (LAS) can also be used. For instructions on loading patient samples on the universal rack handler, refer to the Loading Patient Samples section of the system-specific Operator's Guide.

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

Loading Patient Samples

1. Remove the sample tray evaporation cover.
2. Remove any completed samples and dispose of them in accordance with laboratory procedure.
3. Load the samples in the outer section (STT) of the sample tray.

   NOTE: You can either load samples onto the sample tray while it is in the sampler, or you can remove the tray and then load the samples.

a. When loading samples with barcode labels, make sure the labels are clean, properly positioned on the sample tube, and visible to the sample barcode reader.
b. When not using barcode labels, load each sample into the sample position number entered on the workorder.
c. Load sample cups into a plastic adapter. This adapter can hold two cup sizes. If the cup does not fit, remove the adapter and try the other end.
d. To use barcode labels with a sample cup, just insert the sample cup into a sample tube that has the correct label.
e. EZ Nest cups may also be used in primary sample tubes.

4. If the sample tray was removed for loading the samples, replace the tray and press down on the locking pins to secure it.
5. Replace the sample tray evaporation cover.

CAUTION: Seat the STT cover within the two alignment pins. The dilution probe access holes must be at the back, and the arrow labels must be aligned next to each other to avoid probe crashes.

CAUTION: Make sure all sample containers (including tube-cup combinations) are defined at the System Specification Settings window to avoid probe crashes.

Loading RBL, Calibrator and Control Samples

RBL, calibrator, and control samples are loaded in the inner section (CTT) of the sample tray. The blank solution (water) used to run the reagent blanks/rates is typically assigned to Position 1 (CTT-1) of the inner section of the sample tray. Cup positions for calibrators are specified on the Calibration Setup window, while cup positions for controls are specified on the QC Sample Definition window.

Loading Urgent (Interrupt) Samples on the STT

1. Verify that an Interr. workorder exists for each sample.
2. At the Operation Panel, select **Pause**. A short delay may occur.
3. Load the new sample(s) on the STT.
4. At the Operation Panel, select **Start** to resume sampling.
5. Complete the Start Conditions window (see the following section) and start the run.

G. Starting the Run

1. In the Operation Panel, select **Start**. The Start Conditions window displays.
2. To run a manual calibration, specify this in the *Calibration* section of the Start Conditions window:
   a. Select **One-pnt.smp. Analyze** or **Multipnt.smp. Analyze**.

b. Use **Temp.item select** to designate those tests for which a calibration is to be performed.

c. Select **Temp.sample select**, then select the calibrator solutions.

3. To manually include controls, specify this in the *Control* section of the Start Conditions window:

   a. Select **Control smp. Analyze**.

   b. Use **Temp.item select** to designate those tests for which controls are to be manually added.

   c. Select **Temp.sample select**, then select the controls that are to be run.

4. If samples are run from the sample tray, start the run as outlined below:

   a. Verify that the samples are loaded.

   b. Select the upper (just below the **Tray no.** field) **General smp. Analyze** field (ADVIA 1200 systems) or **Routine smp. Analyze** field (ADVIA 1650/1800 and 2400 systems).

   c. Select either **Bar-code** or **Cup posi.** to designate how the samples are identified. Then, enter the tray number (i.e., **98** or **99**) in the **Tray no.** field and enter the tray positions (i.e., 50 and 60) in the two boxes adjacent to the **General smp. Analyze** (or **Routine smp. Analyze**) field to specify the tray number and range of tray positions that the system will scan and/or aspirate.

   d. If certain samples should be run first or if a different type of sample tube is used for the run, select **Temp.cup/tube select** and specify the priority samples and/or type of sample container that is being used.

   e. Select **Start** to begin the run.

5. If samples are run from the optional universal rack handler (ADVIA 1650/1800 and 2400 systems only), start the run as outlined below:

   a. Select the lower **Routine smp. Analyze** field (below the **Out side analyze** field).

   b. Select **Start** to begin the run.

   c. Load the sample racks on the rack handler or universal rack handler.

5. POSTANALYTIC PROCESS

5.1. Reporting Results

5.1.1. Reference Interval

3.2 to 4.8 g/dL (32 to 48 g/L)

5.1.2. Critical Values

For children <1.7 or >6.8 g/dL (<17 or >68 g/L)

5.1.3. Reporting Protocol for Critical Values

See CL SOP-12001

5.1.4. Units for Reporting Results

The system reports serum or plasma albumin results in g/dL (common units) or g/L (SI units), depending on the units defined when setting up the assay. The conversion formula is 1 g/dL = 10 g/L. Units are user-defined in the system software.

5.1.5. Acceptable Results

Patient test results are acceptable and may be reported when

- QC passes
- there are no major instrument flags
- the results are within the measurable range.

5.1.6. Corrective Action

Patient test results must be repeated and corrective action taken when:

- QC is out
- there are major instrument flags
- the results are not within the measurable range

When a test is verified by repeat testing,

- if the two results meet precision criteria (e.g., Tietz, Table 14-5), the first result is usually reported.
- if the two tests don't meet precision criteria, a third test is usually performed and reported.

5.2. Procedure Notes

5.2.1. Calculations

For detailed information about how the system calculates results, refer to the *Analysis* section of the system-specific Operator's Guide.

5.2.2. Disposal

Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, local and Theranos laboratory requirements.

5.3. Method Limitations

5.3.1. Reportable Range

The reportable range of the ADVIA Chemistry systems ALB assay is 1 to 6 g/dL (10 to 60 g/L).

Siemens has validated an automatic rerun condition for this method that extends the reportable range for serum and plasma up to 12 g/dL (120 g/L).

5.3.2. Other Limitations

As with any chemical reaction, you must be alert to the possible effect on results of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

Siemens tested the following potential interferents and found the results shown below:

Interferent	ADVIA 1200	ADVIA 1650/1800	ADVIA 2400
bilirubin 22.5 mg/dL	--	--	+9.4% at 3.2 g/dL*
	--	--	NSI at 4.7 g/dL*
bilirubin 25 mg/dL	NSI at 3.5 g/dL*	--	--
bilirubin 30mg/dL	--	NSI at 2.6 g/dL*	+12.8% at 3.2 g/dL*
	--	NSI at 3.8 g/dL*	NSI at 4.7 g/dL*
hemolysis (hemoglobin) 250 mg/dL	--	NSI at 2.6 g/dL*	--
	--	NSI at 3.8 g/dL*	--
hemolysis (hemoglobin) 500 mg/dL	--	--	NSI at 3.3 g/dL*
hemolysis (hemoglobin) 525 mg/dL	NSI at 3.5 g/dL*	+11.5% at 2.6 g/dL*	--
	--	NSI at 3.8 g/dL*	--
lipemia (from Intralipid) 280 mg/dL **	--	NSI at 2.6 g/dL*	--
	--	NSI at 3.8 g/dL*	--
lipemia (from Intralipid) 625 mg/dL **	NSI at 3.6 g/dL*	--	NSI at 2.7 g/dL*
lipemia (from Intralipid) 650 mg/dL **	--	+11.5% at 2.6 g/dL*	--

Interferent	*ADVIA 1200*	*ADVIA 1650/1800*	*ADVIA 2400*
	--	+10.5 at 3.8 g/dL*	--

* Interference at the indicated concentration of albumin. NSI = No Significant Interference, where significant interference is considered a percentage effect ≥10%.

** as triolein

For additional information on performance characteristics, see the product information in the ADVIA Chemistry systems ALB product insert.

5.4. Equipment and Supplies

- ADVIA Chemistry Albumin Reagent
- Siemens Chemistry Calibrator
- Quality Control material
- Sample containers
- System solutions
- Reagent container adapters

6. References

1. Siemens Healthcare Diagnostics ADVIA Chemistry systems ALB Product Insert.
2. Siemens Healthcare Diagnostics ADVIA Chemistry system-specific Operator's Guide.
3. Clinical and Laboratory Standards Institute (CLSI). Clinical Laboratory Technical Procedure Manuals; Laboratory Documents: Development and Control Approved Guideline, GP02-A5, 2006.
4. Burtis CA, Ashwood ER, Bruns DE (ed.s). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Fourth Edition, Elsevier Saunders, 2006.

7. Technical Assistance

Siemens Healthcare Diagnostics Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

Serial Number 1800: CA1291000790079

Serial Number 2400: CA1275000100010 or CA1275000120012

8. Revision History

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**ECO Number**
A	10/20/2011	A. Gelb	CL ECO-00022
B	12/5/2014	L. Gee	CL DCO-00067
Section Number	**Description and Justification of Changes**		
All	Initial Release		
All	Annual Review		

theranos	Standard Operating Procedure	Document Number: **CL SOP-09102** Revision: B
	CLIA Laboratory	Effective Date: 12/5/2014

ALKALINE PHOSPHATASE (ALP) AMP IN SERUM OR PLASMA ON THE ADVIA CHEMISTRY SYSTEMS

Author(s):

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Signature:	Date: 12/4/14
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Approver(s):

Signature: DocuSigned by: Lynette Sawyer 566A6114FEBD481...	Date: 5/5/2015
Name: Adam Rosendorff, MD	Title: Laboratory Director

Lynette Sawyer

The Laboratory Director or designee will review this procedure at least annually including revisions.

Reviewed By:	***Date:***	***Comments:***

1. Principle of the Test

Alkaline phosphatase hydrolyzes ρNPP substrate to form ρ-nitrophenol. The reaction is followed by the colorimetric measurement of the rate of formation of ρ-nitrophenol at 410/478 nm, which is proportional to the alkaline phosphatase activity.

A 2-amino-2-methyl-1-propanol (AMP) buffer is used to maintain the reaction pH at 10.3 to 10.4. Magnesium and zinc ions are added to the AMP buffer to activate and stabilize the enzyme.

$$\rho NPP + AMP \xrightarrow{ALP,\ Mg^{2+},\ Zn^{2+}} \rho\text{-}Nitrophenol + P\text{-}AMP$$

2. Clinical Application and Usefulness

For *in vitro* diagnostic use in the quantitative determination of alkaline phosphatase in human serum and plasma on the ADVIA Chemistry systems. Such measurements are used in the diagnosis and treatment of hepatobiliary and bone disease.

3. PREANALYTIC PROCESS

3.1. Specimen Collection and Handling

3.1.1. Specimen Collection

BIOHAZARD

All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

- Serum or lithium heparinized plasma are the recommended sample types for this assay.
- Avoid hemolysis of erythrocytes during sample collection and preparation.
- For serum samples, place the collection tube in an upright position and allow the specimen to stand undisturbed for thirty minutes to allow clot to form.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells.
- Separate plasma or serum from cells within two hours of collection. If samples cannot be assayed immediately after separation, store them in stoppered containers at 2 – 8°C.
- Process samples in a manner that prevents the introduction of clots, fibrin strands, or other solid materials into the system.

3.1.2. Specimen Storage and Stability

- Freshly collected serum or lithium heparin plasma should be kept at room temperature and assayed as soon as feasible, preferably within 4 hours. Otherwise, in samples refrigerated at 2 – 8°C activity may increase slowly (~2%/d). Alternatively, samples may be stored at -15 – -25 °C for 3 months or at -75 – -85°C for 6 months.
- Thaw frozen samples at room temperature for 18 to 24 hours to achieve full enzyme reactivation and mix thoroughly prior to use. Do not refreeze thawed samples.

3.1.3. Specimen Rejection Criteria

- Hemolysis
- See CL SOP-05002 Specimen Collection, Transport, Receipt, Storage and Rejection Criteria.

3.1.4. Specimen Referral Criteria

- See CL QOP-00012 Referral Testing.

3.2. *Reagents*

3.2.1. Storage and Stability

- Store the reagents at 2 – 8°C.
- Unopened reagents are stable until the expiration date on the pack label.
- Reagents are stable on-board the system as follows:

System	*Reagent On Board Stability*
ADVIA 1200 Systems	7 days
ADVIA 1650/1800 Systems	10 days
ADVIA 2400 Systems	10 days

CAUTION:

- Do not freeze reagents.
- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

3.2.2. Ingredients

Reagent ingredients for the ADVIA Chemistry systems Alkaline Phosphatase AMP assay are as follows:

Reagent	*REF*	*Amount*	*Ingredients*
Reagent 1	03035814	7 x 38 mL	AMP (0.438 mol/L), sodium azide (0.09%)
Reagent 2	03035814	7 x 11.7 mL	Paranitrophenyl Phosphate (60 mmol/L), sodium azide (0.09%)

NOTE: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

3.2.3. Reagents Special Preparation

Reagents are ready to use. Before use, gently swirl the reagent to disrupt bubbles and assure homogeneity. If bubbles still exist or foam is present, using a clean transfer pipette, aspirate them from the reagent container prior to use.

3.3. Calibration

Calibration uses a fixed system Factor Value (FV), which is based on the established molar extinction coefficient of ρ-nitrophenol at 410 nm, adjusted by the patient sample correlation to the IFCC reference method. One unit is the amount of enzyme required to produce 1 µmole of ρ-nitrophenol per minute under the conditions of the method. For setup and use instructions, refer to the *Calibration Overview* section of the system-specific Operator's Guide.

The ADVIA Alkaline Phosphatase AMP method is traceable to the IFCC reference method via patient sample correlation. Refer to the Siemens Healthcare Diagnostics ADVIA Chemistry Systems ALPAMP Product Insert for correlation data.

3.3.1. Calibration Frequency

Calibration is not required.

Run a reagent blank (RBL) daily. Additionally, run an RBL when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

3.3.2. Setting up RBLs

Use the Calibration Setup window to specify the number of RBL aspirations, to specify the type of RBL sample container that is used, and to specify the location of the reagent blank solution on the sample tray.

1. Locate the applicable method in the **Proc. Test No.** list area of the Calibration Setup window.

2. In the **Blk posi.** box for the method, enter the position of the reagent blank solution on the calibrator/control tray (CTT).
3. Select **Ctrl/Cal Setup**.
4. Locate the cup position occupied by the blank solution and perform the following:
   a. At **Container Type**, select the type of tube or cup that is used.
   b. In the **Meas. Times** box, enter the number of aspirations you want taken.
   c. In the **Comment** box, enter the applicable text that describes the blank.
   d. Close the window.
5. At the window button menu, select **Save**, then select **Yes** to confirm.

3.3.3. Scheduling RBLs

Use the Calibration Setup window to request an automatic RBL after a specific time interval and/or after a new reagent container is loaded. If the calibration interval expires while the system is not running samples, an RBL test is automatically performed at the beginning of the next run.

1. Select **Auto calib. set**.
2. Enter the following information:
   a. Enter the test number in the **Test** box.
   b. Select **Blank** in the *Sample Select* area.
   c. In the **Control Select** box, enter the letter to identify each control to be run after the RBL.
   d. In the *Enforcement* area, do the following:
      (1) Select the **Time check** box to run an RBL when the Interval time expires.
      (2) Select the **Bottle check** box to run an RBL whenever the system switches to another reagent bottle of the same or a different lot for the assay item.
   e. In the **Interval time** box, enter the time interval in days between each RBL. You can enter a value between 1 and 9999 (or 0).
3. Select **Save**.

An RBL can be included in any run by requesting it in the Start Conditions window that is displayed when the run is started.

3.4. Quality Control (QC)

For detailed QC procedural information, refer to the Quality Control Overview section of the system-specific Operator's Guide.

3.4.1. QC Materials

Siemens recommends the use of quality control material from Bio-Rad Laboratories with at least 2 levels (low and high).

3.4.2. QC Frequency

Analyze all levels of quality control material:

- on each day that samples are analyzed
- when the reagent lot number changes
- following the performance of any system maintenance, cleaning, or troubleshooting procedure
- after performing a new calibration

Use the QC Sample Definition window to specify cup positions for the controls and to request that controls are automatically run after a user-specified number of samples are processed. Alternately, controls can be included in a particular run by requesting them in the Start Conditions window that is displayed when the run is started.

3.4.3. Troubleshooting Out-of-Range QC Values

A QC run is acceptable when all values fall within the expected ranges.

If the ALP QC results do not fall within the defined ranges, then reevaluate all patient test results obtained in the unacceptable test run to determine if patient test results were adversely affected. Take and document appropriate corrective actions, which may include:

- Verify that the controls and reagents were prepared properly and have not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact the local technical support provider or distributor for assistance.

4. ANALYTIC PROCESS

4.1. Instrument Operation and System Description

The ADVIA Chemistry systems are automated clinical chemistry analyzers that can run tests on human serum, plasma, or urine in random access, batch, and STAT (interrupt) modes.

When the **Start** button is selected, the first reagent for the test is aspirated and dispensed into a cuvette on the reaction tray (RRV). Sample is then added to the cuvette and mixed with the reagent. If a second reagent is required, it is dispensed into the same reaction cuvette and the solution is mixed again. The reaction takes place for the amount of time designated in the assay. Concentration data is obtained by the spectrophotometer at timed intervals.

Refer to the *Operating the System* section of the system-specific Operator's Guide for detailed procedures that describe how to schedule samples and manage the workorders.

A. Processing Start/Stop Buttons

The Operation Panel opens after the system software is started. It includes the following buttons:

- **Start** button, to start sample processing.
- **SMP Pause** button, to temporarily stop sampling, so that samples can be added or so that the outer (STT) or inner (CTT) section of the sample tray can be replaced.
- **Stop** button, to halt startup or shutdown.

NOTE: There is also a Stop bottom on the analyzer Power Panel that can be activated in an emergency.

B. Starting the System

1. After the power is applied and the operating system is loaded, the ADVIA Chemistry system Startup window displays.
2. If an optional rack handler (ADVIA 1650/1800 and 2400 systems only) is being used, turn on the rack handler:

   * For the **rack handler**, set the control panel Standby/On switch to **I (ON)**.

   * For the **universal rack handler**, set the display panel Ready/Standby switch to **READY**.
3. At the analyzer Power Panel, set the Operate/Standby switch to **Operate**.
4. At the Startup window, logon as **user** and select **New Start** or **Re-start**, then select **OK**. After a few minutes, the Menu Panel and the Operation Panel open.

   CAUTION: Make sure that all probes and mixers are free to move without obstruction and that all analyzer covers are in place to avoid possible injury and damage to the analyzer.
5. On the Operation Panel, select **Initialize**.
6. If an optional rack handler (ADVIA 1650/1800 and ADVIA 2400 systems only) is being used, double-select the rack handler icon on the Windows desktop.
7. Log on as **supervisor** or **tech_manager**, if required.

Loading Reagents

Loading System Reagents

Visually check the system reagents. Perform a prime after replacing any system ancillary reagents.

1. At the Operation Panel, select **Prime**.
2. Select **Prime 2**, then select **Execute**.

Loading Method Reagents

Check the method reagents in the reagent trays.

1. At the Menu Panel, select **Reagent**, then select **Reagent Inventory**.
2. At the Reagent Inventory window, determine if any reagents need replenishing.
3. Replace any expired reagents:

   IMPORTANT: Do not move reagent containers on Reagent Tray 1 (RTT1) or Reagent Tray 2 (RTT2) after a barcode scan has been performed. This can cause erroneous results.

   If the operator accidentally switches barcoded reagents (i.e., if R1 reagent is loaded on RTT2 and R2 reagent is loaded on RTT1) and performs a reagent barcode scan, an error message displays to alert the operator.

   a. Place the barcoded reagent container(s) in any empty position.
   b. For multiple reagent methods, place R1 on RTT1, and place R2 on RTT2.
   c. Load multiple containers for each reagent on one tray. A maximum of eight reagents can be loaded for the same method.
4. Perform a startup wash.

C. Checking the Analyzer

1. Inspect the probes, mixing rods, cuvette washers, probe wash cups, cuvette covers, and pumps. Perform any required maintenance.
2. At the Menu Panel, select **Maint**, then select **System Monitor** and examine the system operating conditions. If there are abnormal indicators, take the appropriate corrective action.

D. Entering Workorders

Each patient sample must have a workorder that contains a sample number and at least one test request.

Workorders can be entered using several different methods. For detailed operating procedural information, refer to the *Using Workorders* section of the system-specific Operator's Guide.

Downloading Workorders from a Host Computer

1. To download workorders **automatically** from a host computer, use the *Automatic item select* feature in the *Automatic transfer* area on the Online Settings window.
2. To download workorders **manually** from a host computer, follow the procedure below:
   a. At the Menu Panel, select **Request**, then select **Order Entry**.
   b. Select **Host Request**.
   c. In the *Entry format* area, select the means for identifying the first workorder (Step e below).
   d. In the *Last no. entry format* area, select the means for identifying the last workorder (Step f below).
   e. In the Start no. box, identify the first workorder you want downloaded.

b. In the Last no. box, identify the last workorder you want downloaded, or enter the number of workorders you want downloaded.

g. Select **Execute**.

Creating a Single Workorder at the Analyzer

1. At the Menu Panel, select **Request,** then select **Order Entry**.
2. Select **Routine** or **Interr**.
3. In the Posi.no. boxes, enter the sample position number.
4. In the Samp.no. box, enter the sample identification number.
5. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
6. As needed, provide entries for Comment and Age.
7. Order tests by any of the following methods:
   * In the Test table, select each test or ratio you want to run.
   * In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
   * In the Profiles area, select each profile you want to run.
8. Select **Enter**. The Number of workorder box increments. If autoincrement is on, a new workorder displays with the next sample number and position number incremented.
9. You can create another workorder, or you can select **Exit** to leave.

   NOTE: If necessary, select **New** to clear the window for entry of the next workorder.

Creating Multiple Workorders at the Analyzer

1. At the Menu Panel, select **Request,** then select **Order Entry**.
2. Enter information for the first sample:

   NOTE: Enter only those items you want replicated.

   a. Select **New** located above the Enter.
   b. Select **Routine** or **Interr**.
   c. In the Posi.no. boxes, enter the starting sample position number (Tray and Cup numbers).
   d. In the Samp.no. box, enter the starting sample identification number.
   e. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
   f. As needed, provide entries for Comment and Age.
   g. Order tests by any of the following methods:
      * In the Test table, select each test or ratio you want to run.

* In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
* In the Profiles area, select each profile you want to run.

3. Select **Batch Entry**.
4. Select Samp.no., Posi.no., or Batch entry button, then enter corresponding information in the selected box.
5. Select **Execute**. The Posi.no. and Samp.no. fields increment by the number of workorders requested from Batch Entry.

Creating a Profile, Load List, or Work List

Refer to the *Using Workorders* section of the system-specific Operator's Guide.

F. Loading Patient Samples, Control Samples and/or an RBL Sample

Patient samples are aspirated from the outer section (STT) of the sample tray, while RBL and control samples are aspirated from the inner section (CTT) of the sample tray.

An optional universal rack handler (LAS) can also be used with the ADVIA 1650/1800 and ADVIA 2400 systems. For instructions on loading patient samples on the universal rack handler, refer to the *Loading Patient Samples* section of the system-specific Operator's Guide.

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

Loading Patient Samples

1. Remove the sample tray evaporation cover.
2. Remove any completed samples and dispose of them in accordance with laboratory procedure.
3. Load the samples in the outer section (STT) of the sample tray.

   NOTE: You can either load samples onto the sample tray while it is in the sampler, or you can remove the tray and then load the samples.

   a. When loading samples with barcode labels, make sure the labels are clean, properly positioned on the sample tube, and visible to the sample barcode reader.
   b. When not using barcode labels, load each sample into the sample position number entered on the workorder.
   c. Load sample cups into a plastic adapter. This adapter can hold two cup sizes. If the cup does not fit, remove the adapter and try the other end.
   d. To use barcode labels with a sample cup, just insert the sample cup into a sample tube that has the correct label.

e. EZ Nest cups may also be used in primary sample tubes.

4. If the sample tray was removed for loading the samples, replace the tray and press down on the locking pins to secure it.
5. Replace the sample tray evaporation cover.

CAUTION: Seat the STT cover within the two alignment pins. The dilution probe access holes must be at the back, and the arrow labels must be aligned next to each other to avoid probe crashes.

CAUTION: Make sure all sample containers (including tube-cup combinations) are defined at the System Specification Settings window to avoid probe crashes.

Loading RBL and Control Samples

RBL and control samples are loaded in the inner section (CTT) of the sample tray. The blank solution (water) used to run the reagent blanks/rates is typically assigned to Position 1 (CTT-1) of the inner section of the sample tray. Cup positions for controls are specified on the QC Sample Definition window.

Loading Urgent (Interrupt) Samples on the STT

1. Verify that an Interr. workorder exists for each sample.
2. At the Operation Panel, select **Pause**. A short delay may occur.
3. Load the new sample(s) on the STT.
4. At the Operation Panel, select **Start** to resume sampling.
5. Complete the Start Conditions window (see the following section) and start the run.

G. Starting the Run

1. In the Operation Panel, select **Start**. The Start Conditions window displays.
2. To run a manual calibration, specify this in the *Calibration* section of the Start Conditions window:
   a. Select **One-pnt.smp. Analyze** or **Multipnt.smp. Analyze**.
   b. Use **Temp.item select** to designate those tests for which a calibration is to be performed.
   c. Select **Temp.sample select**, then select the calibrator solutions.
3. To manually include controls, specify this in the *Control* section of the Start Conditions window:
   a. Select **Control smp. Analyze**.
   b. Use **Temp.item select** to designate those tests for which controls are to be manually added.
   c. Select **Temp.sample select**, then select the controls that are to be run.

4. If samples are run from the sample tray, start the run as outlined below:
   a. Verify that the samples are loaded.
   b. Select the upper (just below the **Tray no.** field) **General smp. Analyze** field (ADVIA 1200 systems) or **Routine smp. Analyze** field (ADVIA 1650/1800 and 2400 systems).
   c. Select either **Bar-code** or **Cup posi.** to designate how the samples are identified. Then, enter the tray number (i.e., **98** or **99**) in the **Tray no.** field and enter the tray positions (i.e., 50 and 60) in the two boxes adjacent to the **General smp. Analyze** (or **Routine smp. Analyze**) field to specify the tray number and range of tray positions that the system will scan and/or aspirate.
   d. If certain samples should be run first or if a different type of sample tube is used for the run, select **Temp.cup/tube select** and specify the priority samples and/or type of sample container that is being used.
   e. Select **Start** to begin the run.
5. If samples are run from the optional universal rack handler (ADVIA 1650/1800 and 2400 systems only), start the run as outlined below:
   a. Select the lower **Routine smp. Analyze** field (below the **Out side analyze** field).
   b. Select **Start** to begin the run.
   c. Load the sample racks on the rack handler or universal rack handler.

5. POSTANALYTIC PROCESS

5.1. Reporting Results

5.1.1. Reference Interval

45 to 129 U/L

5.1.2. Critical Values

Not applicable

5.1.3. Reporting Protocol for Critical Values

Not applicable

5.1.4. Units for Reporting Results

The system reports serum or plasma albumin results in g/dL (common units) or g/L (SI units), depending on the units defined when setting up the assay. The conversion formula is 1 g/dL = 10 g/L. Units are user-defined in the system software.

5.1.5. Acceptable Results

Patient test results are acceptable and may be reported when

- QC passes

- there are no major instrument flags
- the results are within the measurable range.

5.1.6. Corrective Action

Patient test results must be repeated and corrective action taken when:

- QC is out
- there are major instrument flags
- the results are not within the measurable range

When a test is verified by repeat testing,

- if the two results meet precision criteria (e.g., Tietz, Table 14-5), the first result is usually reported.
- if the two tests don't meet precision criteria, a third test is usually performed and reported.

5.2. Procedure Notes

5.2.1. Calculations

For detailed information about how the system calculates results, refer to the *Analysis* section of the system-specific Operator's Guide.

5.2.2. Disposal

Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, local and Theranos laboratory requirements.

5.3. Method Limitations

5.3.1. Reportable Range

The reportable range of the ADVIA Chemistry systems Alkaline Phosphatase AMP assay is 0–1100 U/L.

Siemens has validated an automatic rerun condition for this method that extends the reportable range for serum and plasma up to 3300 U/L on the ADVIA 1200 Chemistry system, and up to 6600 U/L on the ADVIA 1650/1800 and ADVIA 2400 Chemistry systems.

5.3.2. Other Limitations

As with any chemical reaction, you must be alert to the possible effect on results of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

Siemens tested the following potential interferents and found the results shown below:

Interferent	ADVIA 1200	ADVIA 1650/1800	ADVIA 2400

Interferent	*ADVIA 1200*	*ADVIA 1650/1800*	*ADVIA 2400*
bilirubin 25 mg/dL	NSI at 56 U/L*	--	NSI at 111 U/L*
bilirubin 30 mg/dL	--	NSI at 63 U/L*	--
hemolysis (hemoglobin) 125 mg/dL	NSI at 62 U/L*	--	--
hemolysis (hemoglobin) 250 mg/dL	-19% at 62 U/L*	-11% at 63 U/L*	--
	NSI at 243 U/L*	--	--
hemolysis (hemoglobin) 375 mg/dL	-23% at 62 U/L*	--	--
	NSI at 243 U/L*	--	--
hemolysis (hemoglobin) 500 mg/dL	-35% at 62 U/L*	--	NSI at 113 U/L*
	NSI at 243 U/L*	--	--
hemolysis (hemoglobin) 525 mg/dL	--	-27% at 63 U/L*	--
	--	NSI at 293 U/L*	--
lipemia (from Intralipid) 500 mg/dL **	NSI at 54 U/L*	--	NSI at 113 U/L*
lipemia (from Intralipid) 650 mg/dL **	--	NSI at 63 U/L*	--

* Interference at the indicated level of alkaline phosphatase activity. NSI = No Significant Interference, where significant interference is considered a percentage effect ≥10%.

** as triolein

For additional information on performance characteristics, see the product information in the ADVIA Chemistry systems ALP product insert.

5.4. Equipment and Supplies

- ADVIA Chemistry Alkaline Phosphatase AMP Reagents
- Quality Control material (Bio-Rad Laboratories recommended)

- Sample containers
- System solutions
- Reagent container adapters

6. References

1. Siemens Healthcare Diagnostics ADVIA Chemistry Systems ALP AMP Product Insert.
2. Siemens Healthcare Diagnostics ADVIA Chemistry system-specific Operator's Guide.
3. Clinical and Laboratory Standards Institute (CLSI). Clinical Laboratory Technical Procedure Manuals; Laboratory Documents: Development and Control; Approved Guideline, GP02-A5, 2006.
4. Burtis CA, Ashwood ER, Bruns DE (ed.s). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Fourth Edition, Elsevier Saunders, 2006.

7. Technical Assistance

Siemens Healthcare Diagnostics Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

Serial Number 1800: CA1291000790079

Serial Number 2400: CA1275000100010 or CA1275000120012

8. Revision History

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**ECO Number**
A	10/20/2011	A. Gelb	CL ECO-00022
B	12/5/2014	L. Gee	CL DCO-00067
Section Number	**Description and Justification of Changes**		
All	Initial Release		
All	Annual Review		

theranos	Standard Operating Procedure	Document Number: **CL SOP-09103** Revision: B
	CLIA Laboratory	Effective Date: 12/5/2014

ALANINE AMINOTRANSFERASE (ALT) IN SERUM OR PLASMA ON THE ADVIA CHEMISTRY SYSTEMS

Author(s):

Signature:	Date: 12/4/14
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Reviewer(s):

Signature:	Date: 12/3/14
Name: Hoda Alamdar	Title: General Supervisor

Approver(s):

Signature: DocuSigned by: Lynette Sawyer 566A6114FEBD481...	Date: 5/5/2015
Name: Adam Rosendorff, MD	Title: Laboratory Director

Lynette Sawyer

The Laboratory Director or designee will review this procedure at least annually including revisions.

Reviewed By:	***Date:***	***Comments:***

1. Principle of the Test

The reaction is initiated by the addition of α-ketoglutarate as a second reagent. The concentration of NADH is measured by its absorbance at 340/410 nm and the rate of absorbance decrease is proportional to the alanine aminotransferase activity.

$$\textit{L-Alanine} + \alpha\textit{-Ketoglutarate} \xrightarrow{\textit{ALT}} \textit{Pyruvate} + \textit{L-Glutamate}$$

$$\textit{Pyruvate} + \textit{NADH} \xrightarrow{\textit{Lactate Dehydrogenase}} \textit{Lactate} + \textit{NAD}$$

2. Clinical Application and Usefulness

For *in vitro* diagnostic use in the quantitative determination of alanine aminotransferase activity in human serum and plasma on ADVIA Chemistry systems. Such measurements are used mainly in the diagnosis and treatment of liver disease and to monitor the course of treatment for hepatitis and active post necrotic cirrhosis.

3. PREANALYTIC PROCESS

3.1. Specimen Collection and Handling

3.1.1. Specimen Collection

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

- Serum or lithium heparinized plasma are the recommended sample types for this assay.
- Avoid hemolysis of erythrocytes during sample collection and preparation.
- For serum samples, place the collection tube in an upright position and allow the specimen to stand undisturbed for thirty minutes to allow clot to form.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells.
- Separate plasma or serum from cells within two hours of collection. If samples cannot be assayed immediately after separation, store them in stoppered containers at 2 – 8°C.
- Process samples in a manner that prevents the introduction of clots, fibrin strands, or other solid materials into the system.

3.1.2. Specimen Storage and Stability

- Serum or lithium heparin plasma kept at room temperature is preferably tested within 2 hours when stored at room temperature or the same day at 2 –8° C. Stability is at -75 – -85°C is up to 6 days.
- Thaw frozen samples at room temperature and mix thoroughly prior to use. Do not refreeze thawed samples.

3.1.3. Specimen Rejection Criteria

- Hemolysis
- See CL SOP-05002 Specimen Collection, Transport, Receipt, Storage and Rejection Criteria.

3.1.4. Specimen Referral Criteria

- See CL QOP-00012 Referral Testing.

3.2. Reagents

3.2.1. Storage and Stability

- Store the reagents at 2 – 8°C.
- Unopened reagents are stable until the expiration date on the pack label.
- Reagents are stable on board the system as follows:

System	*Reagent On Board Stability*
ADVIA 1200 Systems	60 days
ADVIA 1650/1800 Systems	60 days
ADVIA 2400 Systems	60 days

CAUTION:

- Do not freeze reagents.
- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.
- .

3.2.2. Ingredients

Reagent ingredients for the ADVIA Chemistry systems ALT assay are as follows:

Reagent	*REF*	*Amount*	*Ingredients*
Reagent 1	03036926	6 x 68 mL	L-alanine (610 mmol/L), LD (pig heart) (≥ 1.2 KU/L), sodium azide (0.09%)
	07501976	7 x 38 mL	
	02760612	4 x 68 mL	
Reagent 2	03036926	6 x 20 mL	α-ketoglutarate (93 mmol/L), NADH (1.41 mmol/L), sodium azide (0.09%)
	07501976	7 x 11.2 mL	
	02760612	2 x 39 mL	

CAUTION: This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

NOTE: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

3.2.3. Reagents Special Preparation

Reagents are ready to use. Before use, gently swirl the reagent to disrupt bubbles and assure homogeneity. If bubbles still exist or foam is present, using a clean transfer pipette, aspirate them from the reagent container prior to use..

3.3. Calibration

Calibration uses a fixed-system Factor Value (FV), which is based on the established molar extinction coefficient of NADH at 340 nm, adjusted by the patient sample correlation to the IFCC reference method. One unit is the amount of enzyme required to produce 1 µmol of NAD per minute under the conditions of the method. For setup and use instructions, refer to the *Calibration Overview* section of the system-specific Operator's Guide.

The ADVIA ALT method is traceable to the IFCC reference method, which uses IFCC-454 reference material via patient sample correlation. Refer to the Siemens Healthcare Diagnostics ADVIA Chemistry Systems ALT Product Insert for correlation data.

3.3.1. Calibration Frequency

Calibration is not required.

Run a reagent blank (RBL) daily. Additionally, run an RBL when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

3.3.2. Setting up RBLs

Use the Calibration Setup window to specify the number of RBL aspirations, to specify the type of RBL sample container that is used, and to specify the location of the reagent blank solution on the sample tray.

1. Locate the applicable method in the **Proc. Test No.** list area of the Calibration Setup window.
2. In the **Blk posi.** box for the method, enter the position of the reagent blank solution on the calibrator/control tray (CTT).
3. Select **Ctrl/Cal Setup**.
4. Locate the cup position occupied by the blank solution and perform the following:
   a. At **Container Type**, select the type of tube or cup that is used.
   b. In the **Meas. Times** box, enter the number of aspirations you want taken.
   c. In the **Comment** box, enter the applicable text that describes the blank.
   d. Close the window.
5. At the window button menu, select **Save**, then select **Yes** to confirm.

3.3.3. Scheduling RBLs

Use the Calibration Setup window to request an automatic RBL after a specific time interval and/or after a new reagent container is loaded. If the calibration interval expires while the system is not running samples, an RBL test is automatically performed at the beginning of the next run.

1. Select **Auto calib. set**.
2. Enter the following information:
   a. Enter the test number in the **Test** box.
   b. Select **Blank** in the *Sample Select* area.
   c. In the **Control Select** box, enter the letter to identify each control to be run after the RBL.
   d. In the *Enforcement* area, do the following:
      (1) Select the **Time check** box to run an RBL when the Interval time expires.
      (2) Select the **Bottle check** box to run an RBL whenever the system switches to another reagent bottle of the same or a different lot for the assay item.
   e. In the **Interval time** box, enter the time interval in days between each RBL. You can enter a value between **1** and **9999** (or **0**).
3. Select **Save**.

An RBL can be included in any run by requesting it in the Start Conditions window that is displayed when the run is started.

3.4. Quality Control (QC)

For detailed QC procedural information, refer to the Quality Control Overview section of the system-specific Operator's Guide.

3.4.1. QC Materials

Siemens recommends the use of commercially available quality control materials with at least 2 levels (low and high).

3.4.2. QC Frequency

Analyze all levels of quality control material:

- on each day that samples are analyzed
- when the reagent lot number changes
- following the performance of any system maintenance, cleaning, or troubleshooting procedure

3.4.3. Troubleshooting Out-of-Range QC Values

A QC run is acceptable when all values fall within the expected ranges.

If the ALT QC results do not fall within the defined ranges, then reevaluate all patient test results obtained in the unacceptable test run to determine if patient test results were adversely affected. Take and document appropriate corrective actions, which may include:

- Verify that the controls and reagents were prepared properly and have not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact the local technical support provider or distributor for assistance.

4. ANALYTIC PROCESS

4.1. Instrument Operation and System Description

The ADVIA Chemistry systems are automated clinical chemistry analyzers that can run tests on human serum, plasma, or urine in random access, batch, and STAT (interrupt) modes.

When the **Start** button is selected, the first reagent for the test is aspirated and dispensed into a cuvette on the reaction tray (RRV). Sample is then added to the cuvette and mixed with the reagent. If a second reagent is required, it is dispensed into the same reaction cuvette and the solution is mixed again. The reaction takes place for the amount of time designated in the assay. Concentration data is obtained by the spectrophotometer at timed intervals.

Refer to the *Operating the System* section of the system-specific Operator's Guide for detailed procedures that describe how to schedule samples and manage the workorders.

A. Processing Start/Stop Buttons

The Operation Panel opens after the system software is started. It includes the following buttons:

- **Start** button, to start sample processing.
- **SMP Pause** button, to temporarily stop sampling, so that samples can be added or so that the outer (STT) or inner (CTT) section of the sample tray can be replaced.
- **Stop** button, to halt startup or shutdown.

NOTE: There is also a Stop bottom on the analyzer Power Panel that can be activated in an emergency.

B. Starting the System

1. After the power is applied and the operating system is loaded, the ADVIA Chemistry system Startup window displays.
2. If an optional rack handler (ADVIA 1650/1800 and 2400 systems only) is being used, turn on the rack handler:
   * For the **rack handler**, set the control panel Standby/On switch to **I (ON)**.
   * For the **universal rack handler**, set the display panel Ready/Standby switch to **READY**.
3. At the analyzer Power Panel, set the Operate/Standby switch to **Operate**.
4. At the Startup window, logon as **user** and select **New Start** or **Re-start**, then select **OK**. After a few minutes, the Menu Panel and the Operation Panel open.

   CAUTION: Make sure that all probes and mixers are free to move without obstruction and that all analyzer covers are in place to avoid possible injury and damage to the analyzer.
5. On the Operation Panel, select **Initialize**.
6. If an optional rack handler (ADVIA 1650/1800 and ADVIA 2400 systems only) is being used, double-select the rack handler icon on the Windows desktop.
7. Log on as **supervisor** or **tech_manager**, if required.

Loading Reagents

Loading System Reagents

Visually check the system reagents. Perform a prime after replacing any system ancillary reagents.

1. At the Operation Panel, select **Prime**.
2. Select **Prime 2**, then select **Execute**.

Loading Method Reagents

Check the method reagents in the reagent trays.

1. At the Menu Panel, select **Reagent**, then select **Reagent Inventory**.
2. At the Reagent Inventory window, determine if any reagents need replenishing.
3. Replace any expired reagents:

   IMPORTANT: Do not move reagent containers on Reagent Tray 1 (RTT1) or Reagent Tray 2 (RTT2) after a barcode scan has been performed. This can cause erroneous results.

   If the operator accidentally switches barcoded reagents (i.e., if R1 reagent is loaded on RTT2 and R2 reagent is loaded on RTT1) and performs a reagent barcode scan, an error message displays to alert the operator.

   a. Place the barcoded reagent container(s) in any empty position.
   b. For multiple reagent methods, place R1 on RTT1, and place R2 on RTT2.
   c. Load multiple containers for each reagent on one tray. A maximum of eight reagents can be loaded for the same method.
4. Perform a startup wash.

C. Checking the Analyzer

1. Inspect the probes, mixing rods, cuvette washers, probe wash cups, cuvette covers, and pumps. Perform any required maintenance.
2. At the Menu Panel, select **Maint**, then select **System Monitor** and examine the system operating conditions. If there are abnormal indicators, take the appropriate corrective action.

D. Entering Workorders

Each patient sample must have a workorder that contains a sample number and at least one test request.

Workorders can be entered using several different methods. For detailed operating procedural information, refer to the *Using Workorders* section of the system-specific Operator's Guide.

Downloading Workorders from a Host Computer

1. To download workorders **automatically** from a host computer, use the *Automatic item select* feature in the *Automatic transfer* area on the Online Settings window.
2. To download workorders **manually** from a host computer, follow the procedure below:

a. At the Menu Panel, select **Request**, then select **Order Entry**.
b. Select **Host Request**.
c. In the *Entry format* area, select the means for identifying the first workorder (Step **e** below).
d. In the *Last no. entry format* area, select the means for identifying the last workorder (Step **f** below).
e. In the Start no. box, identify the first workorder you want downloaded.
b. In the Last no. box, identify the last workorder you want downloaded, or enter the number of workorders you want downloaded.
g. Select **Execute**.

Creating a Single Workorder at the Analyzer

1. At the Menu Panel, select **Request,** then select **Order Entry**.
2. Select **Routine** or **Interr**.
3. In the Posi.no. boxes, enter the sample position number.
4. In the Samp.no. box, enter the sample identification number.
5. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
6. As needed, provide entries for Comment and Age.
7. Order tests by any of the following methods:
   * In the Test table, select each test or ratio you want to run.
   * In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
   * In the Profiles area, select each profile you want to run.
8. Select **Enter**. The Number of workorder box increments. If autoincrement is on, a new workorder displays with the next sample number and position number incremented.
9. You can create another workorder, or you can select **Exit** to leave.

   NOTE: If necessary, select **New** to clear the window for entry of the next workorder.

Creating Multiple Workorders at the Analyzer

1. At the Menu Panel, select **Request,** then select **Order Entry**.
2. Enter information for the first sample:

   NOTE: Enter only those items you want replicated.

   a. Select **New** located above the Enter.
   b. Select **Routine** or **Interr**.
   c. In the Posi.no. boxes, enter the starting sample position number (Tray and Cup numbers).

d. In the Samp.no. box, enter the starting sample identification number.
e. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
f. As needed, provide entries for Comment and Age.
g. Order tests by any of the following methods:
   * In the Test table, select each test or ratio you want to run.
   * In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
   * In the Profiles area, select each profile you want to run.

3. Select **Batch Entry**.
4. Select Samp.no., Posi.no., or Batch entry button, then enter corresponding information in the selected box.
5. Select **Execute**. The Posi.no. and Samp.no. fields increment by the number of workorders requested from Batch Entry.

Creating a Profile, Load List, or Work List

Refer to the *Using Workorders* section of the system-specific Operator's Guide.

F. Loading Patient Samples, Control Samples and/or an RBL Sample

Patient samples are aspirated from the outer section (STT) of the sample tray, while RBL and control samples are aspirated from the inner section (CTT) of the sample tray.

An optional universal rack handler (LAS) can also be used with the ADVIA 1650/1800 and ADVIA 2400 systems. For instructions on loading patient samples on the universal rack handler, refer to the *Loading Patient Samples* section of the system-specific Operator's Guide.

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

Loading Patient Samples

1. Remove the sample tray evaporation cover.
2. Remove any completed samples and dispose of them in accordance with laboratory procedure.
3. Load the samples in the outer section (STT) of the sample tray.

   NOTE: You can either load samples onto the sample tray while it is in the sampler, or you can remove the tray and then load the samples.

   a. When loading samples with barcode labels, make sure the labels are clean, properly positioned on the sample tube, and visible to the sample barcode reader.

b. When not using barcode labels, load each sample into the sample position number entered on the workorder.

c. Load sample cups into a plastic adapter. This adapter can hold two cup sizes. If the cup does not fit, remove the adapter and try the other end.

d. To use barcode labels with a sample cup, just insert the sample cup into a sample tube that has the correct label.

e. EZ Nest cups may also be used in primary sample tubes.

4. If the sample tray was removed for loading the samples, replace the tray and press down on the locking pins to secure it.

5. Replace the sample tray evaporation cover.

CAUTION: Seat the STT cover within the two alignment pins. The dilution probe access holes must be at the back, and the arrow labels must be aligned next to each other to avoid probe crashes.

CAUTION: Make sure all sample containers (including tube-cup combinations) are defined at the System Specification Settings window to avoid probe crashes.

Loading RBL and Control Samples

RBL and control samples are loaded in the inner section (CTT) of the sample tray. The blank solution (water) used to run the reagent blanks/rates is typically assigned to Position 1 (CTT-1) of the inner section of the sample tray. Cup positions for controls are specified on the QC Sample Definition window.

Loading Urgent (Interrupt) Samples on the STT

1. Verify that an Interr. workorder exists for each sample.
2. At the Operation Panel, select **Pause**. A short delay may occur.
3. Load the new sample(s) on the STT.
4. At the Operation Panel, select **Start** to resume sampling.
5. Complete the Start Conditions window (see the following section) and start the run.

G. Starting the Run

1. In the Operation Panel, select **Start**. The Start Conditions window displays.
2. To run a manual calibration, specify this in the *Calibration* section of the Start Conditions window:
   a. Select **One-pnt.smp. Analyze** or **Multipnt.smp. Analyze**.
   b. Use **Temp.item select** to designate those tests for which a calibration is to be performed.
   c. Select **Temp.sample select**, then select the calibrator solutions.

3. To manually include controls, specify this in the *Control* section of the Start Conditions window:
   a. Select **Control smp. Analyze**.
   b. Use **Temp.item select** to designate those tests for which controls are to be manually added.
   c. Select **Temp.sample select**, then select the controls that are to be run.
4. If samples are run from the sample tray, start the run as outlined below:
   a. Verify that the samples are loaded.
   b. Select the upper (just below the **Tray no.** field) **General smp. Analyze** field (ADVIA 1200 systems) or **Routine smp. Analyze** field (ADVIA 1650/1800 and 2400 systems).
   c. Select either **Bar-code** or **Cup posi.** to designate how the samples are identified. Then, enter the tray number (i.e., **98** or **99**) in the **Tray no.** field and enter the tray positions (i.e., 50 and 60) in the two boxes adjacent to the **General smp. Analyze** (or **Routine smp. Analyze**) field to specify the tray number and range of tray positions that the system will scan and/or aspirate.
   d. If certain samples should be run first or if a different type of sample tube is used for the run, select **Temp.cup/tube select** and specify the priority samples and/or type of sample container that is being used.
   e. Select **Start** to begin the run.
5. If samples are run from the optional universal rack handler (ADVIA 1650/1800 and 2400 systems only), start the run as outlined below:
   a. Select the lower **Routine smp. Analyze** field (below the **Out side analyze** field).
   b. Select **Start** to begin the run.
   c. Load the sample racks on the rack handler or universal rack handler.

5. POSTANALYTIC PROCESS

5.1. Reporting Results

5.1.1. Reference Interval

For adults 10 to 49 U/L

5.1.2. Critical Values

Not applicable

5.1.3. Reporting Protocol for Critical Values

Not applicable

5.1.4. Units for Reporting Results

The system reports ALT results in U/L.

5.1.5. Acceptable Results

Patient test results are acceptable and may be reported when

- QC passes
- there are no major instrument flags
- the results are within the measurable range.

5.1.6. Corrective Action

Patient test results must be repeated and corrective action taken when:

- QC is out
- there are major instrument flags
- the results are not within the measurable range

When a test is verified by repeat testing,

- if the two results meet precision criteria (e.g., Tietz, Table 14-5), the first result is usually reported.
- if the two tests don't meet precision criteria, a third test is usually performed and reported.

5.2. Procedure Notes

5.2.1. Calculations

For detailed information about how the system calculates results, refer to the *Analysis* section of the system-specific Operator's Guide.

5.2.2. Disposal

Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, local and Theranos laboratory requirements.

5.3. Method Limitations

5.3.1. Reportable Range

The reportable range of the ADVIA Chemistry systems ALT assay is 0–1100 U/L.

Siemens has validated an automatic rerun condition for this method that extends the reportable range for serum and plasma up to 3300 U/L on the ADVIA 1200 Chemistry system, and up to 6600 U/L on the ADVIA 1650/1800 and ADVIA 2400 Chemistry systems.

5.3.2. Other Limitations

As with any chemical reaction, you must be alert to the possible effect on results of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

High levels of hemoglobin may cause elevation of ALT, especially in normal samples. Siemens tested the following potential interferents and found the results shown below:

Interferent	*ADVIA 1200*	*ADVIA 1650/1800*	*ADVIA 2400*
bilirubin (conjugated/unconjugated) 12.5 mg/dL	NSI at 25 U/L*	--	--
bilirubin (conjugated/unconjugated) 25 mg/dL	-18% at 25 U/L*	--	NSI at 49 U/L*
	NSI at 70 U/L*	--	--
bilirubin (conjugated) 30 mg/dL	--	NSI at 54 U/L*	--
bilirubin (unconjugated) 30 mg/dL	--	NSI at 29 U/L*	--
hemolysis (hemoglobin) 500 mg/dL	NSI at 29 U/L*	--	NSI at 49 U/L*
hemolysis (hemoglobin) 520 mg/dL	--	+16% at 29 U/L*	--
	--	NSI at 107 U/L*	--
lipemia (from Intralipid) 488 mg/dL **	--	NSI at 55 U/L*	--
lipemia (from Intralipid) 500 mg/dL **	NSI at 25 U/L*	--	NSI at 50 U/L*
lipemia (from Intralipid) 650 mg/dL **	--	-16% at 55 U/L*	--
	--	-16% at 156 U/L*	--

* Interference at the indicated level of alanine aminotransferase activity. NSI = No Significant Interference, where significant interference is considered a percentage effect ≥10%.

** as triolein

For additional information on performance characteristics, see the product information in the ADVIA Chemistry systems ALT product insert.

5.4. Equipment and Supplies

- ADVIA Chemistry Alanine Aminotransferase Reagents
- Quality Control material
- Sample containers
- System solutions

- Reagent container adapters

6. References

1. Siemens Healthcare Diagnostics ADVIA Chemistry Systems ALT Product Insert.
2. Siemens Healthcare Diagnostics ADVIA Chemistry System-specific Operator's Guide.
3. Clinical and Laboratory Standards Institute (CLSI). Clinical Laboratory Technical Procedure Manuals; Laboratory Documents: Development and Control; Approved Guideline, GP02-A5, 2006.
4. Burtis CA, Ashwood ER, Bruns DE (ed.s). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Fourth Edition, Elsevier Saunders, 2006.
5. Todoroff SS. Analysis of the stability of alanine aminotransferase (ALT) levels in donor samples. Immunohematology. 1989;5(1):18-21.

7. Technical Assistance

Siemens Healthcare Diagnostics Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

Serial Number 1800: CA1291000790079

Serial Number 2400: CA1275000100010 or CA1275000120012

8. Revision History

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**ECO Number**
A	10/20/2011	A. Gelb	CL ECO-00022
B	12/5/2014	L. Gee	CL DCO-00067
Section Number	**Description and Justification of Changes**		
All	Initial Release		
All	Annual Review		

theranos	Standard Operating Procedure	Document Number: **CL SOP-09104** Revision: B
	CLIA Laboratory	Effective Date: 12/5/2014

AMYLASE IN SERUM, PLASMA OR URINE ON THE ADVIA CHEMISTRY SYSTEMS

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Name: Adam Rosendorff, MD	Title: Laboratory Director

Lynette Sawyer

The Laboratory Director or designee will review this procedure at least annually including revisions.

Reviewed By:	***Date:***	***Comments:***

1. Principle of the Test

The amylase method uses ethylidene blocked ρ-nitrophenyl-maltoheptaoside as substrate. The indicator enzyme α-glucosidase, used to release the ρ-nitrophenol, is also employed in the method. The terminal glucose of the substrate is chemically blocked preventing cleavage by the indicator enzymes. The released ρ-nitrophenol is measured at 410/694 nm.

$$\text{Ed-G7PNP} \xrightarrow{\alpha\text{-Amylase}} \text{Ed-G}_n + \text{G}_n\text{-PNP}$$

$$\text{G}_n\text{-PNP} \xrightarrow{\alpha\text{-Glucosidase}} \text{PNP} + \text{Glucose}$$

2. Clinical Application and Usefulness

For *in vitro* diagnostic use in the quantitative determination of amylase activity in human serum, plasma, and urine on ADVIA Chemistry systems. Such measurements are used primarily in the diagnosis and monitoring of acute pancreatitis (inflammation of the pancreas).

3. PREANALYTIC PROCESS

3.1. Specimen Collection and Handling

3.1.1. Specimen Collection

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

- Serum, lithium heparinized plasma, and urine are the recommended sample types for this assay.
- Avoid hemolysis of erythrocytes during sample collection and preparation.
- For serum samples, place the collection tube in an upright position and allow the specimen to stand undisturbed for thirty minutes to allow clot to form.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells.
- Separate plasma or serum from cells within two hours of collection. If samples cannot be assayed immediately after separation, store them in stoppered containers at 2 – 8°C.
- Process samples in a manner that prevents the introduction of clots, fibrin strands, or other solid materials into the system.

3.1.2. Specimen Storage and Stability

- Serum or lithium heparin plasma may be stored at -80°C for 5 years, at 2-8°C for 14 days, or at room temperature for 4 days. Urine may be stored for up to 24 hours at 2-8°C.
- Thaw frozen samples at room temperature and mix thoroughly prior to use. Do not refreeze thawed samples.

3.1.3. Specimen Rejection Criteria

- See CL SOP-05002 Specimen Collection, Transport, Receipt, Storage and Rejection Criteria.

3.1.4. Specimen Referral Criteria

- See CL QOP-00012 Referral Testing.

3.2. Reagents

3.2.1. Storage and Stability

- Store the reagents at 2 – 8°C.
- Unopened reagents are stable until the expiration date on the pack label.
- Reagents are stable on board the system as follows:

System	*Reagent On Board Stability*
ADVIA 1200 Systems	30 days
ADVIA 1650/1800 Systems	50 days
ADVIA 2400 Systems	50 days

CAUTION:

- Do not freeze reagents.
- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

3.2.2. Ingredients

Reagent ingredients for the ADVIA Chemistry systems AMY assay are as follows:

Reagent	*REF*	*Amount*	*Ingredients*
Reagent 1	01149316	4 x 42 mL	α-glucosidase (≥ 4 KU/L), sodium azide (0.04%)
	03031177	7 x 38 mL	

	07498401	7 x 18 mL	
Reagent 2	01149316	3 x 17 mL	ethylidene-4-NP-G7 (22 mmol/L), sodium azide (0.04%)
	03031177	7 x 11.7 mL	
	07498401	7 x 6.2 mL	

NOTE: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

3.2.3. Reagents Special Preparation

Reagents are ready to use. Before use, gently swirl the reagent to disrupt bubbles and assure homogeneity. If bubbles still exist or foam is present, using a clean transfer pipette, aspirate them from the reagent container prior to use.

3.3. Calibration

Calibration uses a fixed system Factor Value (FV), which is based on the established molar extinction coefficient of ρ-nitrophenol at 410 nm, adjusted by patient sample correlation to the IFCC reference method. One unit of amylase activity is defined as that amount of enzyme that catalyzes the production of 1 µmol of ρ-nitrophenol per minute under the conditions of the method. For setup and use instructions, refer to the *Calibration Overview* section of the system-specific Operator's Guide.

The ADVIA amylase method is traceable to the IFCC reference method via patient sample correlation. Refer to the Siemens Healthcare Diagnostics ADVIA Chemistry Systems AMYLAS Product Insert for correlation data.

3.3.1. Calibration Frequency

Calibration is not required.

Run a reagent blank (RBL) daily. Additionally, run an RBL when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

3.3.2. Setting up RBLs

Use the Calibration Setup window to specify the number of RBL aspirations, to specify the type of RBL sample container that is used, and to specify the location of the reagent blank solution on the sample tray.

1. Locate the applicable method in the **Proc. Test No.** list area of the Calibration Setup window.

2. In the **Blk posi.** box for the method, enter the position of the reagent blank solution on the calibrator/control tray (CTT).
3. Select **Ctrl/Cal Setup**.
4. Locate the cup position occupied by the blank solution and perform the following:
   a. At **Container Type**, select the type of tube or cup that is used.
   b. In the **Meas. Times** box, enter the number of aspirations you want taken.
   c. In the **Comment** box, enter the applicable text that describes the blank.
   d. Close the window.
5. At the window button menu, select **Save**, then select **Yes** to confirm.

3.3.3. Scheduling RBLs

Use the Calibration Setup window to request an automatic RBL after a specific time interval and/or after a new reagent container is loaded. If the calibration interval expires while the system is not running samples, an RBL test is automatically performed at the beginning of the next run.

1. Select **Auto calib. set**.
2. Enter the following information:
   a. Enter the test number in the **Test** box.
   b. Select **Blank** in the *Sample Select* area.
   c. In the **Control Select** box, enter the letter to identify each control to be run after the RBL.
   d. In the *Enforcement* area, do the following:
      (1) Select the **Time check** box to run an RBL when the Interval time expires.
      (2) Select the **Bottle check** box to run an RBL whenever the system switches to another reagent bottle of the same or a different lot for the assay item.
   e. In the **Interval time** box, enter the time interval in days between each RBL. You can enter a value between **1** and **9999** (or **0**).
3. Select **Save**.

An RBL can be included in any run by requesting it in the Start Conditions window that is displayed when the run is started.

3.4. Quality Control (QC)

For detailed QC procedural information, refer to the Quality Control Overview section of the system-specific Operator's Guide.

3.4.1. QC Materials

Siemens recommends the use of commercially available quality control materials with at least 2 levels (low and high).

3.4.2. QC Frequency

Analyze all levels of quality control material:

- on each day that samples are analyzed
- when the reagent lot number changes
- following the performance of any system maintenance, cleaning, or troubleshooting procedure

3.4.3. Troubleshooting Out-of-Range QC Values

A QC run is acceptable when all values fall within the expected ranges.

If the AMY QC results do not fall within the defined ranges, then reevaluate all patient test results obtained in the unacceptable test run to determine if patient test results were adversely affected. Take and document appropriate corrective actions, which may include:

- Verify that the controls and reagents were prepared properly and have not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact the local technical support provider or distributor for assistance.

4. ANALYTIC PROCESS

4.1. Instrument Operation and System Description

The ADVIA Chemistry systems are automated clinical chemistry analyzers that can run tests on human serum, plasma, or urine in random access, batch, and STAT (interrupt) modes.

When the **Start** button is selected, the first reagent for the test is aspirated and dispensed into a cuvette on the reaction tray (RRV). Sample is then added to the cuvette and mixed with the reagent. If a second reagent is required, it is dispensed into the same reaction cuvette and the solution is mixed again. The reaction takes place for the amount of time designated in the assay. Concentration data is obtained by the spectrophotometer at timed intervals.

Refer to the *Operating the System* section of the system-specific Operator's Guide for detailed procedures that describe how to schedule samples and manage the workorders.

A. Processing Start/Stop Buttons

The Operation Panel opens after the system software is started. It includes the following buttons:

- **Start** button, to start sample processing.

- **SMP Pause** button, to temporarily stop sampling, so that samples can be added or so that the outer (STT) or inner (CTT) section of the sample tray can be replaced.
- **Stop** button, to halt startup or shutdown.

NOTE: There is also a Stop bottom on the analyzer Power Panel that can be activated in an emergency.

B. Starting the System

1. After the power is applied and the operating system is loaded, the ADVIA Chemistry system Startup window displays.
2. If an optional rack handler (ADVIA 1650/1800 and 2400 systems only) is being used, turn on the rack handler:

   * For the **rack handler**, set the control panel Standby/On switch to **I (ON)**.

   * For the **universal rack handler**, set the display panel Ready/Standby switch to **READY**.
3. At the analyzer Power Panel, set the Operate/Standby switch to **Operate**.
4. At the Startup window, logon as **user** and select **New Start** or **Re-start**, then select **OK**. After a few minutes, the Menu Panel and the Operation Panel open.

   CAUTION: Make sure that all probes and mixers are free to move without obstruction and that all analyzer covers are in place to avoid possible injury and damage to the analyzer.
5. On the Operation Panel, select **Initialize**.
6. If an optional rack handler (ADVIA 1650/1800 and ADVIA 2400 systems only) is being used, double-select the rack handler icon on the Windows desktop.
7. Log on as **supervisor** or **tech_manager**, if required.

Loading Reagents

Loading System Reagents

Visually check the system reagents. Perform a prime after replacing any system ancillary reagents.

1. At the Operation Panel, select **Prime**.
2. Select **Prime 2**, then select **Execute**.

Loading Method Reagents

Check the method reagents in the reagent trays.

1. At the Menu Panel, select **Reagent**, then select **Reagent Inventory**.
2. At the Reagent Inventory window, determine if any reagents need replenishing.
3. Replace any expired reagents:

   IMPORTANT: Do not move reagent containers on Reagent Tray 1 (RTT1) or Reagent Tray 2 (RTT2) after a barcode scan has been performed. This can cause erroneous results.

If the operator accidentally switches barcoded reagents (i.e., if R1 reagent is loaded on RTT2 and R2 reagent is loaded on RTT1) and performs a reagent barcode scan, an error message displays to alert the operator.

a. Place the barcoded reagent container(s) in any empty position.
b. For multiple reagent methods, place R1 on RTT1, and place R2 on RTT2.
c. Load multiple containers for each reagent on one tray. A maximum of eight reagents can be loaded for the same method.

4. Perform a startup wash.

C. Checking the Analyzer

1. Inspect the probes, mixing rods, cuvette washers, probe wash cups, cuvette covers, and pumps. Perform any required maintenance.
2. At the Menu Panel, select **Maint**, then select **System Monitor** and examine the system operating conditions. If there are abnormal indicators, take the appropriate corrective action.

D. Entering Workorders

Each patient sample must have a workorder that contains a sample number and at least one test request.

Workorders can be entered using several different methods. For detailed operating procedural information, refer to the *Using Workorders* section of the system-specific Operator's Guide.

Downloading Workorders from a Host Computer

1. To download workorders **automatically** from a host computer, use the *Automatic item select* feature in the *Automatic transfer* area on the Online Settings window.
2. To download workorders **manually** from a host computer, follow the procedure below:
   a. At the Menu Panel, select **Request**, then select **Order Entry**.
   b. Select **Host Request**.
   c. In the *Entry format* area, select the means for identifying the first workorder (Step **e** below).
   d. In the *Last no. entry format* area, select the means for identifying the last workorder (Step **f** below).
   e. In the Start no. box, identify the first workorder you want downloaded.
   b. In the Last no. box, identify the last workorder you want downloaded, or enter the number of workorders you want downloaded.
   g. Select **Execute**.

Creating a Single Workorder at the Analyzer

1. At the Menu Panel, select **Request,** then select **Order Entry.**

2. Select **Routine** or **Interr**.
3. In the Posi.no. boxes, enter the sample position number.
4. In the Samp.no. box, enter the sample identification number.
5. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
6. As needed, provide entries for Comment and Age.
7. Order tests by any of the following methods:
   * In the Test table, select each test or ratio you want to run.
   * In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
   * In the Profiles area, select each profile you want to run.
8. Select **Enter**. The Number of workorder box increments. If autoincrement is on, a new workorder displays with the next sample number and position number incremented.
9. You can create another workorder, or you can select **Exit** to leave.

   NOTE: If necessary, select **New** to clear the window for entry of the next workorder.

Creating Multiple Workorders at the Analyzer

1. At the Menu Panel, select **Request**, then select **Order Entry**.
2. Enter information for the first sample:

   NOTE: Enter only those items you want replicated.

   a. Select **New** located above the Enter.
   b. Select **Routine** or **Interr**.
   c. In the Posi.no. boxes, enter the starting sample position number (Tray and Cup numbers).
   d. In the Samp.no. box, enter the starting sample identification number.
   e. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
   f. As needed, provide entries for Comment and Age.
   g. Order tests by any of the following methods:
      * In the Test table, select each test or ratio you want to run.
      * In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
      * In the Profiles area, select each profile you want to run.
3. Select **Batch Entry**.
4. Select Samp.no., Posi.no., or Batch entry button, then enter corresponding information in the selected box.

5. Select **Execute**. The Posi.no. and Samp.no. fields increment by the number of workorders requested from Batch Entry.

Creating a Profile, Load List, or Work List

Refer to the *Using Workorders* section of the system-specific Operator's Guide.

F. Loading Patient Samples, Control Samples and/or an RBL Sample

Patient samples are aspirated from the outer section (STT) of the sample tray, while RBL and control samples are aspirated from the inner section (CTT) of the sample tray.

An optional universal rack handler (LAS) can also be used with the ADVIA 1650/1800 and ADVIA 2400 systems. For instructions on loading patient samples on the universal rack handler, refer to the *Loading Patient Samples* section of the system-specific Operator's Guide.

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

Loading Patient Samples

1. Remove the sample tray evaporation cover.
2. Remove any completed samples and dispose of them in accordance with laboratory procedure.
3. Load the samples in the outer section (STT) of the sample tray.

   NOTE: You can either load samples onto the sample tray while it is in the sampler, or you can remove the tray and then load the samples.

   a. When loading samples with barcode labels, make sure the labels are clean, properly positioned on the sample tube, and visible to the sample barcode reader.
   b. When not using barcode labels, load each sample into the sample position number entered on the workorder.
   c. Load sample cups into a plastic adapter. This adapter can hold two cup sizes. If the cup does not fit, remove the adapter and try the other end.
   d. To use barcode labels with a sample cup, just insert the sample cup into a sample tube that has the correct label.
   e. EZ Nest cups may also be used in primary sample tubes.
4. If the sample tray was removed for loading the samples, replace the tray and press down on the locking pins to secure it.
5. Replace the sample tray evaporation cover.

CAUTION: Seat the STT cover within the two alignment pins. The dilution probe access holes must be at the back, and the arrow labels must be aligned next to each other to avoid probe crashes.

CAUTION: Make sure all sample containers (including tube-cup combinations) are defined at the System Specification Settings window to avoid probe crashes.

Loading RBL and Control Samples

RBL and control samples are loaded in the inner section (CTT) of the sample tray. The blank solution (water) used to run the reagent blanks/rates is typically assigned to Position 1 (CTT-1) of the inner section of the sample tray. Cup positions for controls are specified on the QC Sample Definition window.

Loading Urgent (Interrupt) Samples on the STT

1. Verify that an Interr. workorder exists for each sample.
2. At the Operation Panel, select **Pause**. A short delay may occur.
3. Load the new sample(s) on the STT.
4. At the Operation Panel, select **Start** to resume sampling.
5. Complete the Start Conditions window (see the following section) and start the run.

G. Starting the Run

1. In the Operation Panel, select **Start**. The Start Conditions window displays.
2. To run a manual calibration, specify this in the *Calibration* section of the Start Conditions window:
   a. Select **One-pnt.smp. Analyze** or **Multipnt.smp. Analyze**.
   b. Use **Temp.item select** to designate those tests for which a calibration is to be performed.
   c. Select **Temp.sample select**, then select the calibrator solutions.
3. To manually include controls, specify this in the *Control* section of the Start Conditions window:
   a. Select **Control smp. Analyze**.
   b. Use **Temp.item select** to designate those tests for which controls are to be manually added.
   c. Select **Temp.sample select**, then select the controls that are to be run.
4. If samples are run from the sample tray, start the run as outlined below:
   a. Verify that the samples are loaded.
   b. Select the upper (just below the **Tray no.** field) **General smp. Analyze** field (ADVIA 1200 systems) or **Routine smp. Analyze** field (ADVIA 1650/1800 and 2400 systems).

c. Select either **Bar-code** or **Cup posi.** to designate how the samples are identified. Then, enter the tray number (i.e., **98** or **99**) in the **Tray no.** field and enter the tray positions (i.e., 50 and 60) in the two boxes adjacent to the **General smp. Analyze** (or **Routine smp. Analyze**) field to specify the tray number and range of tray positions that the system will scan and/or aspirate.

d. If certain samples should be run first or if a different type of sample tube is used for the run, select **Temp.cup/tube select** and specify the priority samples and/or type of sample container that is being used.

e. Select **Start** to begin the run.

5. If samples are run from the optional universal rack handler (ADVIA 1650/1800 and 2400 systems only), start the run as outlined below:

a. Select the lower **Routine smp. Analyze** field (below the **Out side analyze** field).

b. Select **Start** to begin the run.

c. Load the sample racks on the rack handler or universal rack handler.

5. POSTANALYTIC PROCESS

5.1. Reporting Results

5.1.1. Reference Interval

For serum and plasma samples: 30 to 118 U/L

For urine samples: ≤ 650 U/L

5.1.2. Critical Values

Not applicable

5.1.3. Reporting Protocol for Critical Values

Not applicable

5.1.4. Units for Reporting Results

The system reports AMY results in U/L.

5.1.5. Acceptable Results

Patient test results are acceptable and may be reported when

- QC passes
- there are no major instrument flags
- the results are within the measurable range.

5.1.6. Corrective Action

Patient test results must be repeated and corrective action taken when:

- QC is out

- there are major instrument flags
- the results are not within the measurable range

When a test is verified by repeat testing,

- if the two results meet precision criteria (e.g., Tietz, Table 14-5), the first result is usually reported.
- if the two tests don't meet precision criteria, a third test is usually performed and reported.

5.2. Procedure Notes

5.2.1. Calculations

For detailed information about how the system calculates results, refer to the *Analysis* section of the system-specific Operator's Guide.

5.2.2. Disposal

Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, local and Theranos laboratory requirements.

5.3. Method Limitations

5.3.1. Reportable Range

The reportable range of the ADVIA Chemistry systems AMY assay is 0–1500 U/L for serum, plasma and urine.

Siemens has validated an automatic rerun condition for this method that extends the reportable range for serum and plasma up to 4500 U/L.

5.3.2. Other Limitations

As with any chemical reaction, you must be alert to the possible effect on results of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

Siemens tested the following potential interferents and found the results shown below:

Interferent	ADVIA 1200	ADVIA 1650/1800	ADVIA 2400
bilirubin 25 mg/dL	NSI at 71 U/L*	--	NSI at 149 U/L*
bilirubin 30 mg/dL	--	NSI at 55 U/L*	--
hemolysis (hemoglobin) 500 mg/dL	NSI at 72 U/L*	--	NSI at 144 U/L*
hemolysis (hemoglobin) 525 mg/dL	--	NSI at 55 U/L*	--

Interferent	*ADVIA 1200*	*ADVIA 1650/1800*	*ADVIA 2400*
lipemia (from Intralipid) 500 mg/dL **	NSI at 69 U/L*	--	NSI at 148 U/L*
lipemia (from Intralipid) 650 mg/dL **	--	NSI at 55 U/L*	--

* Interference at the indicated level of amylase activity. NSI = No Significant Interference, where significant interference is considered a percentage effect ≥10%.

** as triolein

For additional information on performance characteristics, see the product information in the ADVIA Chemistry systems AMYLAS product insert.

5.4. Equipment and Supplies

- ADVIA Chemistry Amylase Reagents
- Quality Control material
- Sample containers
- System solutions
- Reagent container adapters

6. References

1. Siemens Healthcare Diagnostics ADVIA Chemistry Systems AMYLAS Product Insert.
2. Siemens Healthcare Diagnostics ADVIA Chemistry System-specific Operator's Guide.
3. Clinical and Laboratory Standards Institute (CLSI). Clinical Laboratory Technical Procedure Manuals; Laboratory Documents: Development and Control; Approved Guideline, GP02-A5, 2006.
4. Burtis CA, Ashwood ER, Bruns DE (ed.s). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Fourth Edition, Elsevier Saunders, 2006.

7. Technical Assistance

Siemens Healthcare Diagnostics Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

Serial Number 1800: CA1291000790079

Serial Number 2400: CA1275000100010 or CA1275000120012

8. Revision History

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**ECO Number**
A	10/20/2011	A. Gelb	CL ECO-00022
B	12/5/2014	L. Gee	CL DCO-00067
Section Number	**Description and Justification of Changes**		
All	Initial Release		
All	Annual Review		

ASPARTATE AMINOTRANSFERASE (AST) IN SERUM OR PLASMA ON THE ADVIA CHEMISTRY SYSTEMS

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Approver(s):

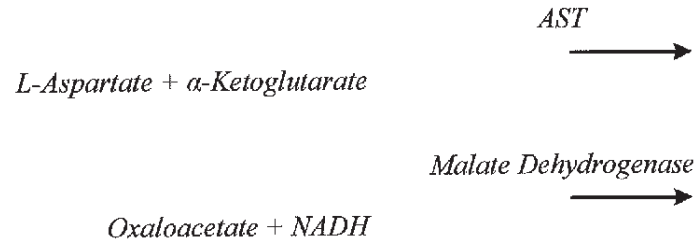
Signature: DocuSigned by: Lynette Sawyer 566A6114FEBD481...	Date: 5/5/2015
Name: Adam Rosendorff, MD Lynette Sawyer	Title: Laboratory Director

The Laboratory Director or designee will review this procedure at least annually including revisions.

Reviewed By:	*Date:*	*Comments:*

1. Principle of the Test

The reaction is initiated by the addition of α-ketoglutarate as a second reagent. The concentration of NADH is measured by its absorbance at 340/410 nm and the rate of absorbance decrease is proportional to the aspartate aminotransferase activity.



2. Clinical Application and Usefulness

For *in vitro* diagnostic use in the quantitative determination of aspartate aminotransferase activity in human serum and plasma on the ADVIA Chemistry systems. Such measurements are used mainly to determine the progress and prognosis of patients with myocardial infarction and the diagnosis and monitoring of liver disease.

3. PREANALYTIC PROCESS

3.1. Specimen Collection and Handling

3.1.1. Specimen Collection

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

- Serum or lithium heparinized plasma are the recommended sample types for this assay.
- Avoid hemolysis of erythrocytes during sample collection and preparation.
- For serum samples, place the collection tube in an upright position and allow the specimen to stand undisturbed for thirty minutes to allow clot to form.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells.
- Separate plasma or serum from cells within two hours of collection. If samples cannot be assayed immediately after separation, store them in stoppered containers at 2 – 8°C.
- Process samples in a manner that prevents the introduction of clots, fibrin strands, or other solid materials into the system.

3.1.2. Specimen Storage and Stability

- Serum or lithium heparin plasma kept at room temperature is preferably tested within 4 hours at room temperature, 48 hours at 2 – 8°C, 3 months at -15 – -25°C, or 6 months at -75 – -85°C.
- Thaw frozen samples at room temperature and mix thoroughly prior to use. Do not refreeze thawed samples.

3.1.3. Specimen Rejection Criteria

- **CAUTION:** Avoid hemolyzed samples due to high AST levels found in red blood cells.
- See CL SOP-05002 Specimen Collection, Transport, Receipt, Storage and Rejection Criteria.

3.1.4. Specimen Referral Criteria

- See CL QOP-00012 Referral Testing.

3.2. Reagents

3.2.1. Storage and Stability

- Store the reagents at 2 – 8°C.
- Unopened reagents are stable until the expiration date on the pack label.
- Reagents are stable on board the system as follows:

System	Reagent On Board Stability
ADVIA 1200 Systems	60 days
ADVIA 1650/1800 Systems	60 days
ADVIA 2400 Systems	60 days

CAUTION:

- Do not freeze reagents.
- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

3.2.2. Ingredients

Reagent ingredients for the ADVIA Chemistry systems AST assay are as follows:

Reagent	REF	Amount	Ingredients
Reagent 1	07499718	7 x 38 mL	L-aspartic acid (290 mmol/L), MDH (≥ 0.42 KU/L), LD (pig heart) (≥ 0.60 KU/L), sodium azide (0.09%)
	03039631	6 x 68 mL	

	00952166	4 x 68 mL	
Reagent 2	07499718	7 x 11.2 mL	α-ketoglutarate (74.4 mmol/L), NADH (1.41 mmol/L), sodium azide (0.09%)
	03039631	6 x 20 mL	
	00952166	2 x 39 mL	

CAUTION: This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

NOTE: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

3.2.3. Reagents Special Preparation

Reagents are ready to use. Before use, gently swirl the reagent to disrupt bubbles and assure homogeneity. If bubbles still exist or foam is present, using a clean transfer pipette, aspirate them from the reagent container prior to use.

3.3. *Calibration*

Calibration uses a fixed-system Factor Value (FV), which is based on the established molar extinction coefficient of NADH at 340 nm, adjusted by the patient sample correlation to the IFCC reference method. One unit is the amount of enzyme required to produce 1 µmol of NAD per minute under the conditions of the method. For setup and use instructions, refer to the *Calibration Overview* section of the system-specific Operator's Guide.

The ADVIA AST method is traceable to the IFCC reference method via patient sample correlation. Refer to the Siemens Healthcare Diagnostics ADVIA Chemistry Systems AST Product Insert for correlation data.

3.3.1. Calibration Frequency

Calibration is not required.

Run a reagent blank (RBL) daily. Additionally, run an RBL when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

3.3.2. Setting up RBLs

Use the Calibration Setup window to specify the number of RBL aspirations, to specify the type of RBL sample container that is used, and to specify the location of the reagent blank solution on the sample tray.

1. Locate the applicable method in the **Proc. Test No.** list area of the Calibration Setup window.
2. In the **Blk posi.** box for the method, enter the position of the reagent blank solution on the calibrator/control tray (CTT).
3. Select **Ctrl/Cal Setup**.
4. Locate the cup position occupied by the blank solution and perform the following:
   a. At **Container Type**, select the type of tube or cup that is used.
   b. In the **Meas. Times** box, enter the number of aspirations you want taken.
   c. In the **Comment** box, enter the applicable text that describes the blank.
   d. Close the window.
5. At the window button menu, select **Save**, then select **Yes** to confirm.

3.3.3. Scheduling RBLs

Use the Calibration Setup window to request an automatic RBL after a specific time interval and/or after a new reagent container is loaded. If the calibration interval expires while the system is not running samples, an RBL test is automatically performed at the beginning of the next run.

1. Select **Auto calib. set**.
2. Enter the following information:
   a. Enter the test number in the **Test** box.
   b. Select **Blank** in the *Sample Select* area.
   c. In the **Control Select** box, enter the letter to identify each control to be run after the RBL.
   d. In the *Enforcement* area, do the following:
      (1) Select the **Time check** box to run an RBL when the Interval time expires.
      (2) Select the **Bottle check** box to run an RBL whenever the system switches to another reagent bottle of the same or a different lot for the assay item.
   e. In the **Interval time** box, enter the time interval in days between each RBL. You can enter a value between **1** and **9999** (or **0**).
3. Select **Save**.

An RBL can be included in any run by requesting it in the Start Conditions window that is displayed when the run is started.

3.4. Quality Control (QC)

For detailed QC procedural information, refer to the Quality Control Overview section of the system-specific Operator's Guide.

3.4.1. QC Materials

Siemens recommends the use of commercially available quality control materials with at least 2 levels (low and high).

3.4.2. QC Frequency

Analyze all levels of quality control material:

- on each day that samples are analyzed
- when the reagent lot number changes
- following the performance of any system maintenance, cleaning, or troubleshooting procedure

Use the QC Sample Definition window to specify cup positions for the controls and to request that controls are automatically run after a user-specified number of samples are processed.

Alternately, controls can be included in a particular run by requesting them in the Start Conditions window that is displayed when the run is started.

3.4.3. Troubleshooting Out-of-Range QC Values

A QC run is acceptable when all values fall within the expected ranges.

If the AST QC results do not fall within the defined ranges, then reevaluate all patient test results obtained in the unacceptable test run to determine if patient test results were adversely affected. Take and document appropriate corrective actions, which may include:

- Verify that the controls and reagents were prepared properly and have not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact the local technical support provider or distributor for assistance.

4. ANALYTIC PROCESS

4.1. Instrument Operation and System Description

The ADVIA Chemistry systems are automated clinical chemistry analyzers that can run tests on human serum, plasma, or urine in random access, batch, and STAT (interrupt) modes.

When the **Start** button is selected, the first reagent for the test is aspirated and dispensed into a cuvette on the reaction tray (RRV). Sample is then added to the cuvette and mixed with the reagent. If a second reagent is required, it is dispensed into the same reaction cuvette and the solution is mixed again. The reaction takes place for the amount of time designated in the assay. Concentration data is obtained by the spectrophotometer at timed intervals.

Refer to the *Operating the System* section of the system-specific Operator's Guide for detailed procedures that describe how to schedule samples and manage the workorders.

A. Processing Start/Stop Buttons

The Operation Panel opens after the system software is started. It includes the following buttons:

- **Start** button, to start sample processing.
- **SMP Pause** button, to temporarily stop sampling, so that samples can be added or so that the outer (STT) or inner (CTT) section of the sample tray can be replaced.
- **Stop** button, to halt startup or shutdown.

NOTE: There is also a Stop bottom on the analyzer Power Panel that can be activated in an emergency.

B. Starting the System

1. After the power is applied and the operating system is loaded, the ADVIA Chemistry system Startup window displays.
2. If an optional rack handler (ADVIA 1650/1800 and 2400 systems only) is being used, turn on the rack handler:
   - For the **rack handler**, set the control panel Standby/On switch to **I (ON)**.
   - For the **universal rack handler**, set the display panel Ready/Standby switch to **READY**.
3. At the analyzer Power Panel, set the Operate/Standby switch to **Operate**.
4. At the Startup window, logon as **user** and select **New Start** or **Re-start**, then select **OK**. After a few minutes, the Menu Panel and the Operation Panel open.

   CAUTION: Make sure that all probes and mixers are free to move without obstruction and that all analyzer covers are in place to avoid possible injury and damage to the analyzer.
5. On the Operation Panel, select **Initialize**.
6. If an optional rack handler (ADVIA 1650/1800 and ADVIA 2400 systems only) is being used, double-select the rack handler icon on the Windows desktop.
7. Log on as **supervisor** or **tech_manager**, if required.

Loading Reagents

Loading System Reagents

Visually check the system reagents. Perform a prime after replacing any system ancillary reagents.

1. At the Operation Panel, select **Prime**.
2. Select **Prime 2**, then select **Execute**.

Loading Method Reagents

Check the method reagents in the reagent trays.

1. At the Menu Panel, select **Reagent**, then select **Reagent Inventory**.
2. At the Reagent Inventory window, determine if any reagents need replenishing.
3. Replace any expired reagents:

   IMPORTANT: Do not move reagent containers on Reagent Tray 1 (RTT1) or Reagent Tray 2 (RTT2) after a barcode scan has been performed. This can cause erroneous results.

   If the operator accidentally switches barcoded reagents (i.e., if R1 reagent is loaded on RTT2 and R2 reagent is loaded on RTT1) and performs a reagent barcode scan, an error message displays to alert the operator.

   a. Place the barcoded reagent container(s) in any empty position.
   b. For multiple reagent methods, place R1 on RTT1, and place R2 on RTT2.
   c. Load multiple containers for each reagent on one tray. A maximum of eight reagents can be loaded for the same method.
4. Perform a startup wash.

C. Checking the Analyzer

1. Inspect the probes, mixing rods, cuvette washers, probe wash cups, cuvette covers, and pumps. Perform any required maintenance.
2. At the Menu Panel, select **Maint**, then select **System Monitor** and examine the system operating conditions. If there are abnormal indicators, take the appropriate corrective action.

D. Entering Workorders

Each patient sample must have a workorder that contains a sample number and at least one test request.

Workorders can be entered using several different methods. For detailed operating procedural information, refer to the *Using Workorders* section of the system-specific Operator's Guide.

Downloading Workorders from a Host Computer

1. To download workorders **automatically** from a host computer, use the *Automatic item select* feature in the *Automatic transfer* area on the Online Settings window.
2. To download workorders **manually** from a host computer, follow the procedure below:
   a. At the Menu Panel, select **Request**, then select **Order Entry**.
   b. Select **Host Request**.
   c. In the *Entry format* area, select the means for identifying the first workorder (Step e below).
   d. In the *Last no. entry format* area, select the means for identifying the last workorder (Step f below).
   e. In the Start no. box, identify the first workorder you want downloaded.
   b. In the Last no. box, identify the last workorder you want downloaded, or enter the number of workorders you want downloaded.
   g. Select **Execute**.

Creating a Single Workorder at the Analyzer

1. At the Menu Panel, select **Request**, then select **Order Entry**.
2. Select **Routine** or **Interr**.
3. In the Posi.no. boxes, enter the sample position number.
4. In the Samp.no. box, enter the sample identification number.
5. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
6. As needed, provide entries for Comment and Age.
7. Order tests by any of the following methods:
   - In the Test table, select each test or ratio you want to run.
   - In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
   - In the Profiles area, select each profile you want to run.
8. Select **Enter**. The Number of workorder box increments. If autoincrement is on, a new workorder displays with the next sample number and position number incremented.
9. You can create another workorder, or you can select **Exit** to leave.

   NOTE: If necessary, select **New** to clear the window for entry of the next workorder.

Creating Multiple Workorders at the Analyzer

1. At the Menu Panel, select **Request**, then select **Order Entry**.
2. Enter information for the first sample:

   NOTE: Enter only those items you want replicated.

a. Select **New** located above the Enter.
b. Select **Routine** or **Interr**.
c. In the Posi.no. boxes, enter the starting sample position number (Tray and Cup numbers).
d. In the Samp.no. box, enter the starting sample identification number.
e. Verify that the System Dilution Mode, Container Type, Sample Type, Dil. factor, Sex, and Blood collection date entries are correct.
f. As needed, provide entries for Comment and Age.
g. Order tests by any of the following methods:
   - In the Test table, select each test or ratio you want to run.
   - In the Test-tbl no. box, enter the number of the test you want, then press the period (.) key.
   - In the Profiles area, select each profile you want to run.

3. Select **Batch Entry**.
4. Select Samp.no., Posi.no., or Batch entry button, then enter corresponding information in the selected box.
5. Select **Execute**. The Posi.no. and Samp.no. fields increment by the number of workorders requested from Batch Entry.

Creating a Profile, Load List, or Work List

Refer to the *Using Workorders* section of the system-specific Operator's Guide.

F. Loading Patient Samples, Control Samples and/or an RBL Sample

Patient samples are aspirated from the outer section (STT) of the sample tray, while RBL and control samples are aspirated from the inner section (CTT) of the sample tray.

An optional universal rack handler (LAS) can also be used with the ADVIA 1650/1800 and ADVIA 2400 systems. For instructions on loading patient samples on the universal rack handler, refer to the *Loading Patient Samples* section of the system-specific Operator's Guide.

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

Loading Patient Samples

1. Remove the sample tray evaporation cover.
2. Remove any completed samples and dispose of them in accordance with laboratory procedure.
3. Load the samples in the outer section (STT) of the sample tray.

NOTE: You can either load samples onto the sample tray while it is in the sampler, or you can remove the tray and then load the samples.

a. When loading samples with barcode labels, make sure the labels are clean, properly positioned on the sample tube, and visible to the sample barcode reader.
b. When not using barcode labels, load each sample into the sample position number entered on the workorder.
c. Load sample cups into a plastic adapter. This adapter can hold two cup sizes. If the cup does not fit, remove the adapter and try the other end.
d. To use barcode labels with a sample cup, just insert the sample cup into a sample tube that has the correct label.
e. EZ Nest cups may also be used in primary sample tubes.

4. If the sample tray was removed for loading the samples, replace the tray and press down on the locking pins to secure it.
5. Replace the sample tray evaporation cover.

CAUTION: Seat the STT cover within the two alignment pins. The dilution probe access holes must be at the back, and the arrow labels must be aligned next to each other to avoid probe crashes.

CAUTION: Make sure all sample containers (including tube-cup combinations) are defined at the System Specification Settings window to avoid probe crashes.

Loading RBL and Control Samples

RBL and control samples are loaded in the inner section (CTT) of the sample tray. The blank solution (water) used to run the reagent blanks/rates is typically assigned to Position 1 (CTT-1) of the inner section of the sample tray. Cup positions for controls are specified on the QC Sample Definition window.

Loading Urgent (Interrupt) Samples on the STT

1. Verify that an Interr. workorder exists for each sample.
2. At the Operation Panel, select **Pause**. A short delay may occur.
3. Load the new sample(s) on the STT.
4. At the Operation Panel, select **Start** to resume sampling.
5. Complete the Start Conditions window (see the following section) and start the run.

G. Starting the Run

1. In the Operation Panel, select **Start**. The Start Conditions window displays.
2. To run a manual calibration, specify this in the *Calibration* section of the Start Conditions window:
   a. Select **One-pnt.smp. Analyze** or **Multipnt.smp. Analyze**.

b. Use **Temp.item select** to designate those tests for which a calibration is to be performed.
c. Select **Temp.sample select**, then select the calibrator solutions.

3. To manually include controls, specify this in the *Control* section of the Start Conditions window:
   a. Select **Control smp. Analyze**.
   b. Use **Temp.item select** to designate those tests for which controls are to be manually added.
   c. Select **Temp.sample select**, then select the controls that are to be run.
4. If samples are run from the sample tray, start the run as outlined below:
   a. Verify that the samples are loaded.
   b. Select the upper (just below the **Tray no.** field) **General smp. Analyze** field (ADVIA 1200 systems) or **Routine smp. Analyze** field (ADVIA 1650/1800 and 2400 systems).
   c. Select either **Bar-code** or **Cup posi.** to designate how the samples are identified. Then, enter the tray number (i.e., **98** or **99**) in the **Tray no.** field and enter the tray positions (i.e., 50 and 60) in the two boxes adjacent to the **General smp. Analyze** (or **Routine smp. Analyze**) field to specify the tray number and range of tray positions that the system will scan and/or aspirate.
   d. If certain samples should be run first or if a different type of sample tube is used for the run, select **Temp.cup/tube select** and specify the priority samples and/or type of sample container that is being used.
   e. Select **Start** to begin the run.
5. If samples are run from the optional universal rack handler (ADVIA 1650/1800 and 2400 systems only), start the run as outlined below:
   a. Select the lower **Routine smp. Analyze** field (below the **Out side analyze** field).
   b. Select **Start** to begin the run.
   c. Load the sample racks on the rack handler or universal rack handler.

5. POSTANALYTIC PROCESS

5.1. Reporting Results

5.1.1. Reference Interval

For adults less than 60 years of age: < 34 U/L

5.1.2. Critical Values

Not applicable

5.1.3. Reporting Protocol for Critical Values

Not applicable

5.1.4. Units for Reporting Results

The system reports AST results in U/L.

5.1.5. Acceptable Results

Patient test results are acceptable and may be reported when

- QC passes
- there are no major instrument flags
- the results are within the measurable range.

5.1.6. Corrective Action

Patient test results must be repeated and corrective action taken when:

- QC is out
- there are major instrument flags
- the results are not within the measurable range

When a test is verified by repeat testing,

- if the two results meet precision criteria (e.g., Tietz, Table 14-5), the first result is usually reported.
- if the two tests don't meet precision criteria, a third test is usually performed and reported.

5.2. Procedure Notes

5.2.1. Calculations

For detailed information about how the system calculates results, refer to the *Analysis* section of the system-specific Operator's Guide.

5.2.2. Disposal

Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, local and Theranos laboratory requirements.

5.3. Method Limitations

5.3.1. Reportable Range

The reportable range of the ADVIA Chemistry systems AST assay is 0–1000 U/L.

Siemens has validated an automatic rerun condition for this method that extends the reportable range for serum and plasma up to 3000 U/L on the ADVIA 1200 Chemistry system, and up to 6000 U/L on the ADVIA 1650/1800 and ADVIA 2400 Chemistry systems.

5.3.2. Other Limitations

As with any chemical reaction, you must be alert to the possible effect on results of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

Avoid hemolyzed samples due to high AST levels found in red blood cells. Siemens tested the following potential interferents and found the results shown below:

Interferent	ADVIA 1200	ADVIA 1650/1800	ADVIA 2400
bilirubin 25 mg/dL	NSI at 29 U/L*	--	NSI at 39 U/L*
bilirubin (conjugated/unconjugated) 30 mg/dL	--	NSI at 55 U/L*	--
lipemia (from Intralipid) 488 mg/dL **	--	NSI at 55 U/L*	--
lipemia (from Intralipid) 500 mg/dL **	NSI at 30 U/L*	--	NSI at 39 U/L*

* Interference at the indicated level of aspartate aminotransferase activity. NSI = No Significant Interference, where significant interference is considered a percentage effect ≥10%.

** as triolein

For additional information on performance characteristics, see the product information in the ADVIA Chemistry systems ALT product insert.For additional information on performance characteristics, see the product information in the ADVIA Chemistry systems AST product insert.

5.4. Equipment and Supplies

- ADVIA Chemistry Aspartate Aminotransferase Reagents
- Quality Control material
- Sample containers
- System solutions
- Reagent container adapters

6. References

1. Siemens Healthcare Diagnostics ADVIA Chemistry Systems AST Product Insert.
2. Siemens Healthcare Diagnostics ADVIA Chemistry System-specific Operator's Guide.
3. Clinical and Laboratory Standards Institute (CLSI). Clinical Laboratory Technical Procedure Manuals; Laboratory Documents: Development and Control; Approved Guideline, GP02-A5, 2006.

4. Burtis CA, Ashwood ER, Bruns DE (ed.s). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Fourth Edition, Elsevier Saunders, 2006.

7. Technical Assistance

Siemens Healthcare Diagnostics Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

Serial Number 1800: CA1291000790079

Serial Number 2400: CA1275000100010 or CA1275000120012

8. Revision History

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**ECO Number**
A	10/20/2011	A. Gelb	CL ECO-00022
B	12/5/2014	L. Gee	CL DCO-00067
Section Number	**Description and Justification of Changes**		
All	Initial Release		
All	Annual Review		