Simplexa Group A Strep PCR Assay Procedure

PURPOSE

- This procedure provides instructions for preparing samples, setting up the PCR reaction and running the *Simplexa* Group A Strep assay for the detection of *Streptococcus pyogenes* from throat swabs

POLICY STATEMENT

- PCR testing is performed daily, 0700 –1530

ABBREVIATIONS

- ABC: Analyzer Before Computer
- BSC: BioSafety Cabinet
- BSL: BioSafety level
- CBA: Computer Before Analyzer
- CFU: colony forming unit
- Ct: crossing threshold
- F/T: freeze/thaw
- GAS: group A strep
- IC: internal control
- MM: master mix
- NA: Nucleic Acid
- NEGC: negative control
- NFW: nuclease free water
- PCR: polymerase chain reaction
- PCTL: process control
- POSC: positive control
- pp: primer – probe
- PPE: personal protective equipment
- SEAC: Simplexa extraction and amplification control
- TE buffer: Tris – EDTA buffer
- Area/Room 1: Clean room
- Area/Room 2: Processing room
- Area/Room 3: Amplification room

DOCUMENTATION/RECORDS

- Simplexa run-specific Segment Report
- LIS Incomplete and Completed worksheets
- Daily Maintenance Log

SAFETY CONSIDERATIONS

- Standard precautions for infectious agents. Refer to MB 2.02, Biohazard containment
- Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination
- General Safety: MB 2.01 Safe Work Practices
- *Caution:* PPE including protective eyewear must be worn when working with concentrated Extran

MATERIALS REQUIRED

Equipment	Reagents	Supplies
Room 1: Clean room ▪ Laminar-flow hood, Clean rm 1 ▪ Freezer, -10 to -30° C ▪ Refrigerator, 2 to 8° C ▪ Microcentrifuge ▪ Nalgene cooling block ▪ Vortex ▪ Eppendorf Repeater pipette ▪ Dedicated set of pipettes: 2 µl, 10 µl, 20 µl, 100 µl, 200 µl, and 1000 µl pipettes Room 2: Processing ▪ BSC, Process rm 2 ▪ Refrigerator, 2 to 8° C ▪ Freezer, ≥ - 70°C ▪ Nalgene cooling block ▪ Vortex	TE buffer	Micro tube racks
	Nuclease Free Water (NFW)	2 ml cryovials
	SEAC ▪ Internal control pp ▪ Internal control DNA	Sterile filtered pipette tips for 10 µl, 20 µl, 100 µl, 200 µl, 1000 µl pipettes
	GAS pp	Micro tubes 1.5 ml, RNase/DNase free
	GAS positive control (POSC)	Universal disc
	GAS process control (PCTL)	Universal disc sealer
	TA MasterMix	Nitrile gloves (powder-free)
	Sani-Cloth Bleach wipes	Sharps disposal container
	70% alcohol	Gripper rack, rm 2
	5% Extran	Orange barrier wipes

Equipment	Reagents	Supplies
▪ Micro-centrifuge ▪ Dedicated set of pipettes: 2 µl, 10 µl, 20 µl, 100 µl, 200 µl, and 1000 µl pipettes ▪ Gilson Concept pipette, 100 µl Room 3: Amplification and detection ▪ Focus Simplexa Integrated Cycler Room: Microbiology McFarland densitometer (micro)		Culturette swabs

QUALITY CONTROL

A. Assay Controls
   1. A PCTL, POSC and NEGC must be included in each assay run.
   2. An IC is incorporated into each reaction mixture.

B. QC Monitors:

Control	Control Monitor
Positive Control (POSC)	Reagent failure and primer-probe integrity
Negative Control (NEGC)	Reagent and/or environmental contamination, cumulative effect
Process Control (PCTL)	Elution and/or lysis failure; cross contamination; reagent failure
Internal Control (IC)	PCR inhibition in specimen, reagent failure or process error

C. Before reporting patient results, all controls must yield valid results. Refer to GAS 005, Procedures F and G, Evaluating and Interpreting Results.

PROCEDURE A: Follow the steps in the table below to organize and label samples

Numbering and Labeling

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td rowspan="2">Sample order
Room 2</td><td>1</td><td>Call worksheet GASD; use this worksheet for sample identification throughout testing.</td><td>MB 1.01 Specimen Management</td></tr>
<tr><td>2</td><td>Process patient samples plus one PCTL per run. POSC and NEGC do not go through specimen processing. Position samples and controls in disc as follows:
<table>
<tr><th>Sample</th><th>Position</th></tr>
<tr><td>Patient samples</td><td>1 – nn</td></tr>
<tr><td>PCTL</td><td>3rd to last position</td></tr>
<tr><td>POSC</td><td>2nd to last position</td></tr>
<tr><td>NEGC</td><td>Last position</td></tr>
</table>
</td><td>MB 3.01 Engineering Controls

MB 2.01 Safe Work Practices</td></tr>
<tr><td>Organizing run

Room 2</td><td>3</td><td>Using the GASD worksheet as a layout, organize patient samples and labels
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Color code worksheets and labels per run</td></tr>
<tr><td>b</td><td>Number patients on worksheet in consecutive order</td></tr>
<tr><td>c</td><td>Number corresponding patient labels according to worksheet, color coded by run</td></tr>
<tr><td>d</td><td>Number each primary patient specimen according to worksheet</td></tr>
</table>
</td><td></td></tr>
</table>

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td rowspan="3">Organizing run Cont.</td><td>4</td><td>Number and label one 250 µl TE buffer tube per patient sample and a PCTL per run<table><tr><th>Step</th><th>Action</th></tr><tr><td>a</td><td>Place required number of Sample buffer tubes in gripper rack</td></tr><tr><td>b</td><td>Number each cap consecutively</td></tr><tr><td>c</td><td>Place corresponding label on each tube according to worksheet</td></tr></table></td><td rowspan="3"></td></tr>
<tr><td>5</td><td>Number each patient swab according to GASD worksheet</td></tr>
<tr><td>6</td><td>Place numbered swabs in a rack in consecutive order</td></tr>
<tr><td rowspan="5">Processing

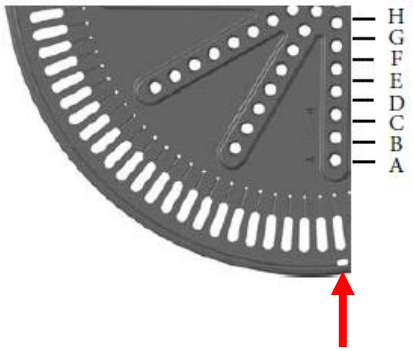
Room 2</td><td>7</td><td>Loosen caps on each sample buffer tube, allowing the cap to sit lightly on tube
Only one tube can be open at a time</td><td rowspan="5"></td></tr>
<tr><td>8</td><td>Remove numbered swab from the Culturette container</td></tr>
<tr><td>9</td><td>Lift cap on corresponding sample buffer tube</td></tr>
<tr><td>10</td><td>Place swab into tube</td></tr>
<tr><td>11</td><td>Break swab as follows:<table><tr><th>Step</th><th>Action</th></tr><tr><td>a</td><td>Using an orange barrier protector, hold the swab near the rim of the tube</td></tr><tr><td>b</td><td>Lift the swab up 1 – 1.5 cm from the bottom of the tube</td></tr><tr><td>c</td><td>Bend the swab against the edge of the tube to break (final height: between the top and bottom of gripper threads)</td></tr><tr><td>d</td><td>Return swab shaft to original transport tube</td></tr><tr><td>e</td><td>Discard barrier protector</td></tr><tr><td>f</td><td>Screw cap tightly</td></tr></table></td></tr>
<tr><td>Change gloves</td><td>12</td><td>Change gloves when possible contamination is suspected or every 16 samples</td><td></td></tr>
</table>

PROCEDURE B: Follow the steps in the table below for setting up the computer

Computer set-up

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related Doc</th></tr>
<tr><td>Computer Set-up Room 3</td><td>1</td><td>Set up Simplexa; take run specific patient labels into room 3
<table>
<tr><th>Step</th><th>Prompt</th><th>Action/Entry</th></tr>
<tr><td>a</td><td>------</td><td>Turn on the Simplexa Integrated Cyclers (ABC)</td></tr>
<tr><td>b</td><td>------</td><td>Turn on the Simplexa computer</td></tr>
<tr><td>c</td><td>------</td><td>Log on computer</td></tr>
<tr><td>d</td><td>User name</td><td>administrator</td></tr>
<tr><td>e</td><td>Password</td><td>focusIC#1</td></tr>
<tr><td>f</td><td>------</td><td>Double-click on Integrated Cycler icon DX icon to open program</td></tr>
<tr><td>g</td><td>User name</td><td>Enter personal user code</td></tr>
<tr><td>h</td><td>Password</td><td>Enter personal password code</td></tr>
<tr><td>i</td><td>-----</td><td>Select Setup Run from Quick pick list</td></tr>
<tr><td>j</td><td>Assay definition</td><td>Select GAS from drop down box</td></tr>
<tr><td>k</td><td>Run Name Prefix</td><td>GAS</td></tr>
<tr><td>l</td><td>Lot information</td><td>PP lot: Add/deactivate reagent lot numbers as needed</td></tr>
<tr><td>m</td><td>Add Samples</td><td>Scan barcode ID from each label consecutively</td></tr>
<tr><td>n</td><td>Controls</td><td>Assign controls according to layout</td></tr>
<tr><td>o</td><td>-----</td><td>Click Move to Disc button</td></tr>
<tr><td>p</td><td>-----</td><td>Click Save to save the run for later use or</td></tr>
<tr><td>q</td><td>-----</td><td>Click Run to save the run and open the Start Run window</td></tr>
<tr><td>r</td><td>-----</td><td>(Optional) Click the Print Preview button to generate a layout report, refer to Fig.1</td></tr>
<tr><td>s</td><td>-----</td><td>Recycle labels when run is complete; do not take back to room 2</td></tr>
</table></td><td></td></tr>
<tr><td>New user</td><td>2</td><td>To switch users: Select File: Switch Users; cannot be done while instrument is running</td><td></td></tr>
<tr><td>Delete or Edit Segment</td><td>3</td><td>To delete or edit segments, right click one of the wells in the segment
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Select action: Edit Segment or Delete Segment
▪ Delete Segment will remove all test samples from run
▪ Edit Segment will move samples from the disc back to the sample list where changes can be made</td></tr>
<tr><td>b</td><td>To move samples back to disc, click starting well location in Disc View</td></tr>
<tr><td>c</td><td>Click Move to Disc button</td></tr>
</table></td><td></td></tr>
<tr><td rowspan="2">Change PPE</td><td>4</td><td>Remove lab coat</td><td rowspan="2"></td></tr>
<tr><td>5</td><td>Change gloves; move to room 1</td></tr>
</table>

Figure 1: Spoke 1 is identified by the open slot on the outer ring of the disc. The wells are identified from the outer–edge inward A – H. Numerical assignment of the wells is in vertical order.



Well Identity Matrix - Universal Disc

Well-Spoke 1	2	3	4	5	6
1 (1A)	9 (2A)	17 (3A)	25 (4A)	33 (5A)	41 (6A)
2 (1B)	10 (2B)	18 (3B)	26 (4B)	34 (5B)	42 (6B)
3 (1C)	11 (2C)	19 (3C)	27 (4C)	35 (5C)	43 (6C)
4 (1D)	12 (2D)	20 (3D)	28 (4D)	36 (5D)	44 (6D)
5 (1E)	13 (2E)	21 (3E)	29 (4E)	37 (5E)	45 (6E)
6 (1F)	14 (2F)	22 (3F)	30 (4F)	38 (5F)	46 (6F)
7 (1G)	15 (2G)	23 (3G)	31 (4G)	39 (5G)	47 (6G)
8 (1H)	16 (2H)	24 (3H)	32 (4H)	40 (5H)	48 (6H)

PROCEDURE C: Follow the steps in the table below for preparing the MM

Master Mix preparation

Activity	Step	Action	Related Doc
Thaw/warm reagents Room 1	1	Remove MM components from –20° C freezer/refrigerator; warm to room temperature (approx 15 min); use within 1 h	
Mix prior to use	2	Gently mix each MM component prior to each use; briefly centrifuge ▪ Larger volumes: Vortex 2 – 3 sec, setting 8 (IC DNA and TA MM) ▪ Lower volumes: flick tube 4 – 5 times (IC and GAS pp) ▪ Centrifuge: 1 – 2 sec	
MasterMix	3	Prepare MM in 1.5 micro-centrifuge tube according to chart volumes	MB 8.04 Refer to MM chart
Room 1	4	Gently vortex MM; briefly centrifuge ▪ Vortex setting: 8 ▪ Time: 2 sec ▪ Centrifuge: 1 – 2 sec	
	5	Return reagents to refrigerator, do not refreeze	MB 8.03 Storage and Stability
	6	Proceed to PCR set-up	
	7	Remove lab coat; move to room 2	
Room 2	8	Place MM in cooling block until use	
	9	*Keep MM protected from light. Use MM within 30 min of preparation*	

PROCEDURE D: Follow the steps in the table below for PCR set-up and amplification

PCR set-up and amplification

Activity	Step	Action	Related Doc
Vortex Room 2	1	Vortex sample buffer tubes for 1 min (vortex speed 9); use within 20 min	
	2	Remove Universal disc from package and set on disc cold block	

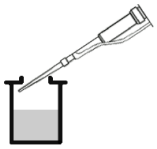
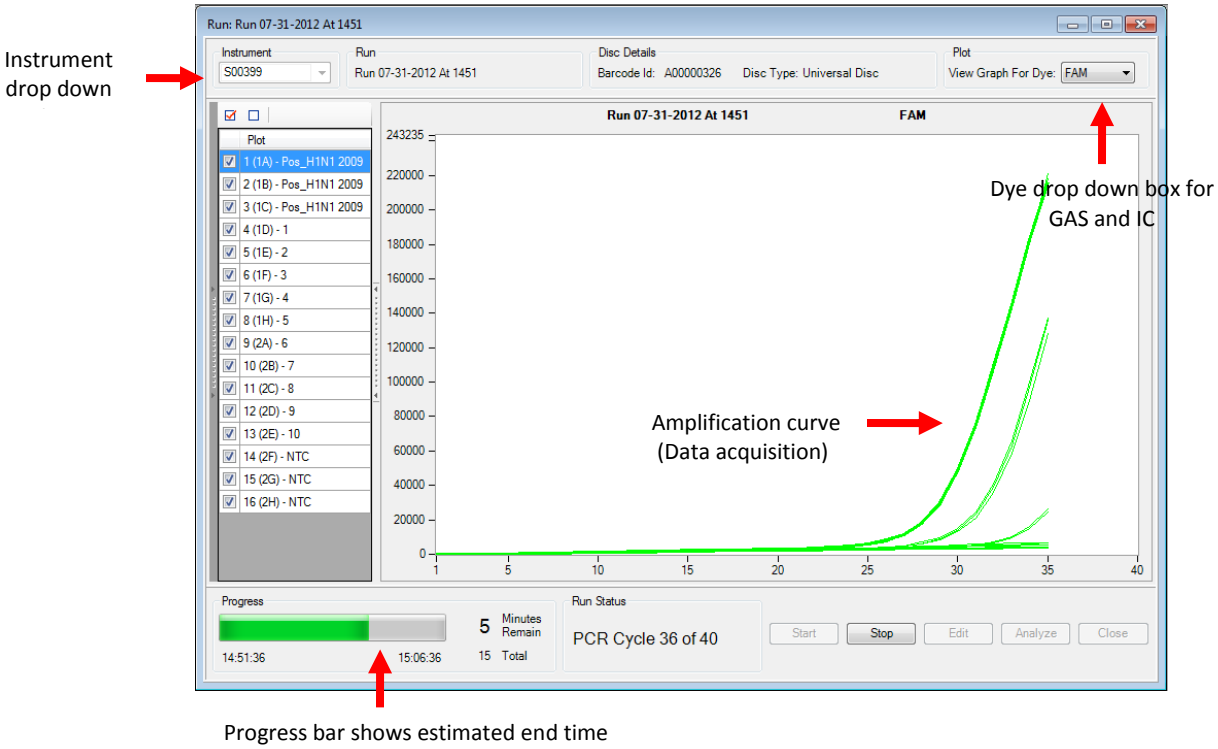
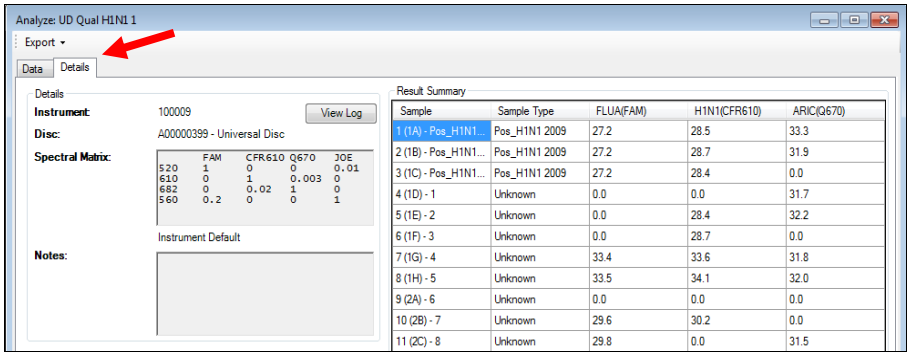
Activity	Step	Action	Related Doc
Load MM **Room 2**	3	Position spoke 1 over colored tape (refer to Fig. 1)	Simplexa Operator Manual
	4	Pipette 8 µl of MM into each well to be used *Tip* ▪ Automatic pipettor: hold at slight angle to maintain accuracy ▪ Manual pipetting: hold the pipette at a 30-degree angle inserting the tip under the roof of the well to reduce possible contamination 	
Load samples	5	Pipette 2 µl of each patient sample and each control into appropriate well ▪ PCTL: swab elution ▪ POSC: undiluted ▪ NEGC: NFW *Caution*: Do not go to second stop to avoid introduction of bubbles and producing aerosols	
Seal disc	6	Apply the cover tape on the disc in horizontal position	
	7	Use the disc applicator to seal the cover tape  	
	8	Remove cover tape tabs by gently pulling outwards	
Change gloves	9	Remove lab coat	
	10	Change gloves; move to room 3	
Room 3 **Start Run**	11	Place disc into the instrument; close lid	
	12	Click **Run** button to move to status screen	
	13	Select test instrument from drop down box	
	14	Click **Start** *Note:* Once the run is started, it cannot be canceled and then restarted using the same disc. Canceling will require a new disc.	
Change gloves	15	Remove lab coat	
	16	Change gloves before leaving room 3	
Run	17	Approximate run time: 1 h	
	18	Run progress can be viewed in the **Run Status Window**: refer to Fig. 2	
Run completion	19	Remove disc from instrument; *check well volumes for pipetting accuracy*	
	20	Place in bio-bag	
	21	Discard in red biohazard container	

Figure 2: The graph plots detection progress in Real-Time



PROCEDURE E: Follow the steps in the table below for analysis of data

Analyzing Completed Runs

Activity	Step	Action	Related doc
Analyze Results	1	Click the Analyze button at the bottom of the screen to open the Analysis Window	
Summary Room 3	2	Click on the run **Details** tab to display a summary of the run, target Ct and IC Ct values 	

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related doc</th></tr>
<tr><td>Room 3

Review amplification curves</td><td>3</td><td>Review IC Ct results and amplification curves for exponential growth and possible inhibition or low target amplification, refer to Figures 3 and 4
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Select Data tab</td></tr>
<tr><td>b</td><td>Click on Print Preview</td></tr>
<tr><td>c</td><td>Check Include Graphs</td></tr>
<tr><td>d</td><td>Scroll through the report , reviewing comments, failures and amplification curves</td></tr>
<tr><td>e</td><td>A valid curve shows a smooth, exponential increase</td></tr>
<tr><td>f</td><td>Invalid curve may be linear or a curve with data “spikes” where the curve crosses the threshold</td></tr>
<tr><td>g</td><td>If curve is valid, the Ct values may be used to interpret the results</td></tr>
<tr><td>h</td><td>Confirm results by a second reviewer before releasing</td></tr>
<tr><td>I</td><td>Positive results: Confirm name and accession number on primary sample/TE buffer before releasing</td></tr>
<tr><td>j</td><td>Select or deselect results to be released</td></tr>
</table></td><td></td></tr>
<tr><td>Print Report</td><td>3</td><td>Print report after review (optional)
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Click Print Preview button for multi-page analysis report</td></tr>
<tr><td>b</td><td>Checkbox: Include Graphs</td></tr>
<tr><td>c</td><td>Print</td></tr>
</table></td><td></td></tr>
</table>

Figure 3: Analysis Window

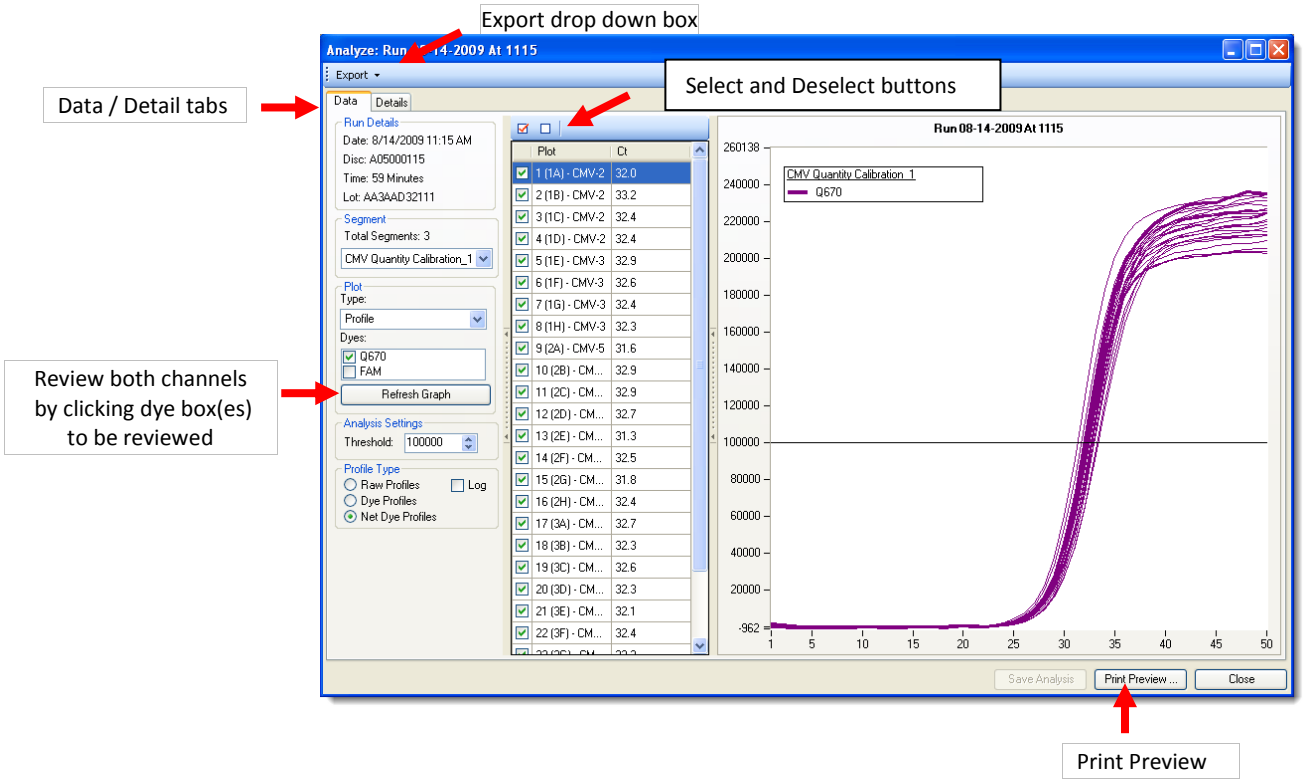
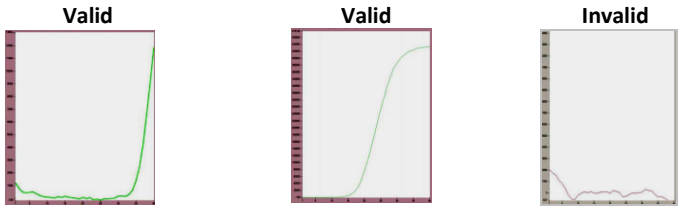


Figure 4: Valid and invalid amplification curves



PROCEDURE F: Follow the activities below for evaluating QC acceptability

Evaluating and Interpreting QC Results

<table>
<tr><th>Activity</th><th>Step</th><th>Action</th><th>Related doc</th></tr>
<tr><td></td><td>1</td><td colspan="2">Check QC for acceptability before reporting patient results</td><td rowspan="2">Simplexa Operator Manual Appendix B: Troubleshooting</td></tr>
<tr><td></td><td>2</td><td colspan="2">Failure indications: Review highlighted yellow results, QC notes and Ct values
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>a</td><td>Click the Print Preview button to review the “Data Quality message” on the Segment report under QC Notes</td></tr>
<tr><td>b</td><td>Review associated amplification curves and Ct values</td></tr>
<tr><td>c</td><td>Click the Print button to generate a report for the QC and Equipment Failure Log documentation</td></tr>
<tr><td>d</td><td>Record corrective action on QC log</td></tr>
<tr><td>e</td><td>Record number of failed samples on Failed Run log</td></tr>
</table></td></tr>
<tr><td>QC / Valid assay</td><td>3</td><td colspan="2">For a valid run, the following QC conditions must be met: ranges are subject to change based on periodic re-calculated values
<table>
<tr><th>Control</th><th>GAS Ct</th><th>IC Ct</th><th>Assay Result</th></tr>
<tr><td>POSC</td><td>29 – 35</td><td>NA</td><td>Positive</td></tr>
<tr><td>PCTL</td><td>26 – 34</td><td>NA</td><td>Positive</td></tr>
<tr><td>NEGC</td><td>0</td><td>26 – 35</td><td>Negative</td></tr>
</table></td><td>3SD ranges periodically determined in EP Evaluator and programmed into the Simplexa</td></tr>
<tr><td rowspan="8">QC conditions not met

Invalid assay</td><td rowspan="8">4</td><td>If</td><td>Then</td><td rowspan="8">Refer to MB 8.05, Proc. I for repeat testing</td></tr>
<tr><td>Valid assay: Controls as expected</td><td>▪ Report patient results</td></tr>
<tr><td>Invalid assay conditions:
PCTL/POSC/ NEGC fail</td><td>▪ Do not report patient results
▪ Failure caused by inhibition, reagent or system failure
▪ Repeat patient testing</td></tr>
<tr><td>PCTL negative or out of range</td><td>▪ Review the specimen handling/ preparation technique
▪ Repeat patient testing</td></tr>
<tr><td>POSC negative or out of range</td><td>▪ Review the specimen handling/ preparation technique
▪ Repeat patient testing</td></tr>
<tr><td>NEGC positive</td><td>▪ Possible contamination of samples
▪ Review the specimen handling/ preparation technique
▪ Repeat patient testing</td></tr>
<tr><td>IC not detected in the NEGC</td><td>▪ Failure caused by reagent or system failure
▪ Repeat patient testing</td></tr>
<tr><td>IC fails in negative patient sample but negative control acceptable</td><td>▪ Failure caused by inhibition, reagent or system failure
▪ Review disc well for proper volume
▪ Check sample for blood/mucus
▪ Repeat patient testing</td></tr>
<tr><td>Problem unresolved</td><td>▪ Call Focus technical service, 1-800-838-4548, option 3
▪ Notify section technical director or designee</td></tr>
</table>

Activity	Step	Action	Related doc
Problem Log	5	Do not report patient results until problem is resolved	
	6	Record problem/operator action in the ***QC and Equipment Failure Log***	

PROCEDURE G: Follow the activities below for evaluating the acceptability of patient results

Evaluating and Interpreting Patient Results

Activity	Step	Action		Related doc
Patient Results	1	Review amplification curves for each result for exponential growth and data spikes ▪ Review "QC statement/Note" on the Segment Report for failures ▪ Document operator action for failures on QC log and Segment report		
	2	If the amplification curve is valid, use Ct value to determine if GAS was detected		
Internal Control	3	**If**	**Then**	MB 8.07 Reporting and Archiving GAS Results
		IC is detected	▪ Negative results are valid ▪ Positive results are valid	
		IC is not detected	▪ Negative results are invalid ▪ Positive results are valid	
		Unresolved result	▪ Failure caused by inhibition, reagent or system failure ▪ F/T sample buffer to possibly reduce the PCR inhibitory substances ▪ Repeat testing after F/T ▪ If repeat testing remains unresolved, report UNR	
		GAS Ct value ≤ 39	▪ GAS detected	
	4	Refer to **Table 1** for interpretation of results.		

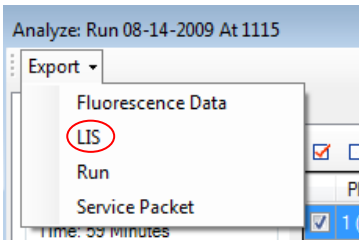
Table 1: Interpretation of Patient Results: Refer to MB 8.07 for *Reporting and Archiving Patient Results*

Sample Type	GAS Ct value	IC Ct value	Test Result	Sunquest Code	Repeat testing	Interpretation of result
Clinical Samples	0	20 - 38	NEG	NEG		Negative
	13 - 39	NA	POS	POS		Positive
	0	0	UNR	UNR	√	Unresolved – inhibitory specimen or reagent failure
POSC	29 - 35	NA	POS	-------		Valid POSC; valid run when EXC and NEGC are also valid.
	0	26 - 40	NEG	-------	√	Invalid POSC; invalid run. Patient results cannot be reported.
NEGC	0	26 - 35	NEG	-------		Valid NEGC; valid run when POSC and PCTL are also valid
	≤ 40	≤ 40 ≠ 0	POS	------	√	Invalid NEGC; invalid run. Patient results cannot be reported.
PCTL	26 - 34	NA	POS			Valid PCTL; valid run when POSC and NEGC are also valid.
	0	≤ 40 ≠ 0	NEG		√	Invalid PCTL; invalid run. Patient results cannot be reported

IC – Internal Control; NA – not applicable; PCTL – Process Control

PROCEDURE H: Follow the steps in the table below for exporting data to LIS from the analysis screen

Exporting Data to LIS

Activity	Step	Action	Related Doc
Select data	1	If all test results were valid upon review, select √ results to be exported on the **Data** tab, refer to Fig.3	MB 8.07 Reporting and Archiving Results
	2	*Do not* send failed patient results or PCTL, POSC and NEGC. Deselect by clicking on individual box(es)	
Export	3	From the Export drop down box, select **LIS** and then **LIS folder;** click **OK** 	
	4	A message that the run exported successfully will appear. Click **OK**	

PROCEDURE I: Follow the activities below for repeat testing

Repeat Testing

Activity	Step	Action	Related doc
Timeframe	1	Perform repeat testing from sample TE buffer tube	Refer to MB 8.05 Proc. D
	2	Repeat within 48 h if stored at 2 – 8° C	
	3	Repeat samples may be retested in the same run as new samples	
Vortex	4	Vortex the sample buffer tube for 1 min prior to retesting; vortex setting 9	
Type of Failure	5	Review type of failure (not all inclusive) **Failure** / **Action** Inhibition: ▪ Perform one F/T prior to retesting; vortex 1 min PCTL: ▪ Prepare new PCTL; vortex sample buffer tubes and repeat testing ▪ Include POSC and NEGC ▪ If PCTL fails on repeat, thaw new PCTL NEGC: ▪ Repeat run from patient sample buffer tubes ▪ Replace NEGC if contamination is indicated; review patient results ▪ Pipette carefully to avoid possible aerosol contamination POSC: ▪ Repeat run from patient sample buffer tubes ▪ Vortex POSC and sample tubes before repeat testing ▪ If POSC fails on repeat, thaw new POSC System error: ▪ Repeat run from PCTL and patient sample buffer tubes ▪ Include POSC and NEGC Failure unresolved: ▪ Call Focus technical service, **1-800-838-4548, option 3** ▪ Notify section technical director or designee	Simplexa Operator Manual Appendix B: Troubleshooting MB 8.06 Troubleshooting Guide

PROCEDURE J: Follow the steps in the table below for Simplexa instrument shutdown in room 3

Computer and Instrument Shutdown

Activity	Step	Action
CBA	1	Shut down computer and then the analyzers when all runs are completed (Computer before analyzer)
	2	Click on the **Close** button or "X" out of the program
Shutdown menu	3	Click on the **Start** button (Windows icon)
	4	Next to **Restart**, click on ▶
	5	Select **Shutdown** from the drop down menu
CBA	6	After the computer has shutdown, turn off the analyzers

PROCEDURE K: Follow the steps in the table below for storing test specimens

Storage and Retention of test specimens

Activity	Step	Action
Storage	1	Store test samples in -70^{o} C freezer, shelf 3, for 1 week
	2	Number freezer boxes 1 – 6
	3	Rotate boxes once filled; discard box after rotation is complete starting with box 1

METHOD PERFORMANCE

- Clinical Sensitivity/Specificity: 98% / 100%
- Analytical Sensitivity: 10^4 CFU/ml

PROFICIENCY TESTING

- Alternate proficiency: split sample analysis

ALTERNATE METHOD

1. Throat Culture, Strep, CHC Microbiology department
2. Sunquest Order code: TCS
3. Logistics:
   - Swab in sample transport medium
   - Transport at RT ≤ 24 h

LIMITATIONS

1. This assay does not detect other beta-hemolytic streptococci including group C or group G. If suspected, order *Throat Culture, Routine*. Group C and G have been associated with pharyngitis and, occasionally, acute nephritis but do not cause rheumatic fever.
2. Negative results do not rule out Group A strep completely and should not be used as the sole basis for diagnosis. Interpretation of assay results should be made in conjunction with clinical symptoms and results of other diagnostic tests.
3. False negative results may occur due to loss of nucleic acid. Detection of Group A strep is dependent upon adequate specimen collection, transport, and handling.
4. There is a risk of false negatives due to sequence variation in the target.
5. This assay detects both viable and nonviable organisms.

REFERENCES

1. Simplexa™ 3M™ Integrated Cycler Studio 5.0 , 3M™ Integrated Cycler Operator Manual Reference 34-8710-8382-9, PI.MOL1101.UD_REV. F for use with user defined assays, Focus Diagnostics 2009-2012, Focus Diagnostics, Inc. Cypress, CA
2. Clinical Verification and Validation Study performed at Children's Hospitals and Clinics of MN August 2014
3. *Red Book* 2012: 668-680: Group A Streptococcal Infections, American Academy of Pediatrics

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1	P. Ackerman	08.16.14	Initial Version
2	P. Ackerman	07.29.16	Reformatted for CMS upload