#  *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
* pp: primer – probe
* PPE: personal protective equipment
* SEAC: *Simplexa* extraction and amplification control
* TE buffer: Tris – EDTA buffer

Area/Room 1: Clean roomArea/Room 2: Processing roomArea/Room 3: Amplification room |

## DOCUMENTATION/RECORDS

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

## SAFETY CONSIDERATIONS

* Standard precautions for infectious agents. Refer to [MB 2.02](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/Safety/212201.pdf), Biohazard containment
* Use of engineering controls: Refer to [MB 3.01](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212209.pdf) Engineering Controls to Prevent Nucleic Acid Contamination
* General Safety: [MB 2.01](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/Safety/212200.pdf) 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 -30⁰ C
* Refrigerator, 2 to 8⁰ 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 8⁰ C
* Freezer, ≥ - 70⁰C
* Nalgene cooling block
* Vortex
* 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* Liaison MDX

Room: Microbiology* McFarland densitometer (micro)
 | 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 process control (PCTL) | Universal disc |
| TA MasterMix | Universal disc sealer |
| Sani-Cloth Bleach wipes | Nitrile gloves (powder-free) |
| 70% alcohol | Sharps disposal container  |
| 5% Extran | Gripper rack, rm 2 |
|  | Orange barrier wipes |
|  | Culturette swabs |
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## QUALITY CONTROL

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

|  |  |
| --- | --- |
| **Control** | **Control Monitor** |
| Negative Control (NEGC) | Reagent and/or environmental contamination, cumulative effect  |
| Process Control (PCTL) | Elution and/or lysis failure; reagent failure |
| Internal Control (IC) | PCR inhibition in specimen, reagent failure or process error |

1. 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

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
|  | 1 | Call worksheet **GASD**; use this worksheet for sample identification throughout testing. | [MB 1.01](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/SpecMgt/212197.pdf)Specimen Management |
| **Sample order****Room 2** | 2 | Process patient samples plus one PCTL and NEGC per run. Position samples and controls in disc as follows:

|  |  |
| --- | --- |
| Sample | Position |
| Patient samples | 1 – nn |
| PCTL | 2rd to last position |
| NEGC | Last position |

 | [MB 3.01](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/EngCtl/212209.pdf) Engineering Controls[MB 2.01](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/Safety/212200.pdf) Safe Work Practices |
| **Organizing run****Room 2** | 3 | Using the GASD worksheet as a layout, organize patient samples and labels

|  |  |
| --- | --- |
| Step | Action |
| a | Color code worksheets and labels per run |
| b | Number patients on worksheet in consecutive order |
| c | Number corresponding patient labels according to worksheet, color coded by run |
| d | Number each primary patient specimen according to worksheet |

 |  |
| **Organizing run****Cont.** | 4 | Number and label one 250 µl TE buffer tube per patient sample and a PCTL per run

|  |  |
| --- | --- |
| Step | Action |
| a | Place required number of Sample buffer tubes in gripper rack |
| b | Number each cap consecutively  |
| c | Place corresponding label on each tube according to worksheet |

 |  |
|  | 5 | Number each patient swab according to GASD worksheet |  |
|  | 6 | Place numbered swabs in a rack in consecutive order |  |
| **Processing** | 7 | Loosen caps on each sample buffer tube, allowing the cap to sit lightly on tube* *Only one tube can be open at a time*
 |  |
| **Room 2** | 8 | Remove numbered swab from the Culturette container  |  |
|  | 9 | Lift cap on corresponding sample buffer tube  |  |
|  | 10 | Place swab into tube |  |
|  | 11 | Break swab as follows:

|  |  |
| --- | --- |
| Step | Action |
| a | Using an orange barrier protector, hold the swab near the rim of the tube |
| b | Lift the swab up 1 – 1.5 cm from the bottom of the tube |
| c | Bend the swab against the edge of the tube to break (final height: between the top and bottom of gripper threads) |
| d | Return swab shaft to original transport tube |
| e | Discard barrier protector |
| f | Screw cap tightly |

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| Change gloves | 12 | Change gloves when possible contamination is suspected or every 16 samples |  |

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

Computer set-up

|  |  |  |  |
| --- | --- | --- | --- |
| **Activity** | Step | **Action** | **Related Doc** |
| **Computer Set-up****Room 3** | 1 | Set up Liaison MDX; take run specific patient labels into room 3

|  |  |  |
| --- | --- | --- |
| Step | Prompt | Action/Entry |
| a | ------ | Turn on the Liaison MDX (ABC) |
| b | ------ | Turn on the Liaison computer |
| c | ------ | Log on computer |
| d | User name | administrator |
| e | Password | focusIC#1 |
| f | ------ | Double-click on program icon to open  |
| g | User name | Enter personal user code  |
| h | Password | Enter personal password code  |
| i | ----- | Select **Setup Run** from Quick pick list |
| j | Assay definition | Select **GAS** from drop down box |
| k | Run Name Prefix | **GAS** |
| l | Lot information | PP lot: Add/deactivate reagent lot numbers as needed |
| m | Add Samples | Scan barcode ID from each label consecutively |
| n | Controls | Assign controls according to layout |
| o | ----- | Click **Move to Disc** button |
| p | ----- | Click **Save** to save the run for later use *or*  |
| q | ----- | Click **Run** to save the run and open the **Start Run** window |
| r | ----- | (Optional) Click the **Print Preview** button to generate a layout report, refer to Fig.1 |
| s | ----- | Recycle labels when run is complete; do not take back to room 2 |

 |  |
| **New user** | 2 | To switch users: Select **File: Switch Users;** cannot be done while instrument is running  |  |
| **Delete or Edit Segment** | 3 | To delete or edit segments, right click one of the wells in the segment

|  |  |
| --- | --- |
| Step | Action |
| a | 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
 |
| b | To move samples back to disc, click starting well location in Disc View |
| c | Click **Move to Disc** button |

 |  |
| **Change PPE** | 4 | Remove lab coat |  |
|  | 5 | Change gloves; move to room 1 |  |

**Figure 1:** Spoke 1 isidentified by theopen 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.

|  |  |
| --- | --- |
|  |  |

**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](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/GAS/212287.pdf)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](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/GAS/212286.pdf) 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 5 min (vortex speed 8); use within 20 min  |  |
|  | 2 | Remove Universal disc from package and set on disc cold block |  |
| **Load MM**  | 3 | Position spoke 1 over silver plate groove (refer to Fig. 1) |  |
| **Room 2** | 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

  |

 | [Simplexa Operator Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212340.pdf) |
| **Load samples** | 5 | Pipette 2 µl of each patient sample and each control into appropriate well* PCTL: swab elution
* NEGC: swab eliution

*Caution*: Do not go to second stop to avoid introduction of bubbles and producing aerosols |  |
|  | 6 | Apply the cover tape on the disc in horizontal position |  |
| **Seal disc** | 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** | 11 | Place disc into the instrument; close lid |  |
| **Start Run** | 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 |  |
|  | 19 | Remove disc from instrument; *check well volumes for pipetting accuracy* |  |
| **Run completion** | 20 | Place in bio-bag |  |
|  | 21 | Discard in red biohazard container |  |

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

Dye drop down box for GAS and IC

Amplification curve

 (Data acquisition)

Instrument drop down box

Progress bar shows estimated end 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 |  |
| Room 3**Review amplification curves** | 3 | Review IC Ct results and amplification curves for exponential growth and possible inhibition or low target amplification, refer to Figures 3 and 4

|  |  |
| --- | --- |
| Step | Action |
| a |  Select **Data** tab |
| b | Click on **Print Preview** |
| c | Check **Include Graphs** |
| d | Scroll through the report , reviewing comments, failures and amplification curves |
| e | A valid curve shows a smooth, exponential increase |
| f | Invalid curve may be linear or a curve with data “spikes” where the curve crosses the threshold |
| g | If curve is valid, the Ct values may be used to interpret the results |
| h | Confirm results by a second reviewer before releasing  |
| I | Positive results: Confirm name and accession number on primary sample/TE buffer before releasing |
| j | Select or deselect results to be released |

 |  |
| **Print Report** | 3 | Print report after review (optional)

|  |  |
| --- | --- |
| Step | Action |
| a | Click **Print Preview** button for multi-page analysis report |
| b | Checkbox: **Include Graphs**  |
| c | **Print** |

 |  |

**Figure 3:** Analysis Window

Export drop down box

Select and Deselect buttons

Review both channels

 by clicking dye box(es) to be reviewed

Data / Detail tabs

Print Preview

 **Figure 4:** Valid and invalid amplification curves

 **Valid Valid Invalid**

**PROCEDURE F:** Follow the activities below for evaluating QC acceptability

**Evaluating and Interpreting QC Results**

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
|  | 1 | Check QC for acceptability before reporting patient results |  |
|  | 2 | Failure indications: Review highlighted yellow results, QC notes and Ct values

|  |  |
| --- | --- |
| Step | Action |
| a | Click the Print Preview button to review the “Data Quality message” on the Segment report under QC Notes |
| b | Review associated amplification curves and Ct values |
| c | Click the **Print** button to generate a report for the ***QC and Equipment Failure* *Log*** documentation |
| d | Record corrective action on QC log |
| e | Record number of failed samples on **Failed Run** log |

 | [Simplexa Operator Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212340.pdf)Appendix B: Troubleshooting |
| QC / Valid assay | 3 | For a valid run, the following QC conditions must be met: *ranges are subject to change based on periodic re-calculated values*

|  |  |  |  |
| --- | --- | --- | --- |
| Control | GAS Ct | IC Ct | Assay Result |
| PCTL | 26 – 33  | NA | Positive |
| NEGC  | 0 | 25 – 31 | Negative |

 | 3SD ranges periodically determined in EP Evaluator and programmed into the Liaison MDX |
| **QC conditions not met****Invalid assay** | 4 | If | Then | Refer to MB 8.05, Proc. I for repeat testing |
| **Valid assay:** Controls as expected | * Report patient results
 |
| **Invalid assay conditions:**PCTL/ NEGC fail | * Do not report patient results
* Failure caused by inhibition, reagent or system failure
* Repeat patient testing
 |
| PCTL negative or out of range | * Review the specimen handling/ preparation technique
* Repeat patient testing
 |
| NEGC positive | * Possible contamination of samples
* Review the specimen handling/ preparation technique
* Repeat patient testing
 |
| IC not detected in the NEGC | * Failure caused by reagent or system failure
* Repeat patient testing
 |
|  |  | IC fails in negative patient sample but negative control acceptable | * Failure caused by inhibition, reagent or system failure
* Review disc well for proper volume
* Check sample for blood/mucus
* Repeat patient testing
 |  |
|  |  | Problem unresolved | * Call DiaSorin technical service, **1-800-838-4548, opt. 3**
* Notify section technical director or designee
 |  |
| **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 spikesReview “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](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/GAS/212290.pdf)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 |
| PCTL | 26 - 33 | NA | POS |  |  | Valid PCTL; valid run when NEGC is also valid. |
| 0 | ≤ 40 ≠ 0 | NEG |  | √ | Invalid PCTL; invalid run. Patient results cannot be reported |
| NEGC | 0 | 26 - 35 | NEG | ------- |  | Valid NEGC; valid run when PCTL is also valid |
| ≤ 40 | ≤ 40 ≠ 0 | POS | ------ | √ | Invalid NEGC; 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 onthe **Data** tab, refer to Fig.3  | [MB 8.07](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/GAS/212290.pdf)Reporting and Archiving Results |
|  | 2 | *Do not* send failed patient results or PCTL 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** |
|  | 1 | Perform repeat testing from sample TE buffer tube  | Refer toMB 8.05 Proc. D |
| **Timeframe** | 2 | Repeat within 48 h if stored at 2 – 8⁰ 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 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
 |
| System error | * Repeat run from PCTL and patient sample buffer tubes
* Include NEGC
 |
| Failure unresolved | * Call DiaSorin technical service, **1-800-838-4548, option 3**
* Notify section technical director or designee
 |

 | [Simplexa Operator Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212340.pdf)Appendix B: Troubleshooting[MB 8.06](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/GAS/212289.pdf) Troubleshooting Guide |

**PROCEDURE J:** Follow the steps in the table below for Liaison MDX 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** |
| --- | --- | --- |
|  | 1 | Store test samples in -70⁰ C freezer, shelf 3, for 1 week |
| **Storage** | 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: 104 CFU/ml

**PROFICIENCY TESTING**

* Wisconsin State Laboratory of Hygiene (WSLH): 2 shipments per year, 5 samples each

#### 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, DiaSorin Molecular 2009-2012, DiaSorin Molecular, 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; prev GAS 005 |
|  | 3 | P. Ackerman | 03.29.17 | Instrument name change from Focus Simplexa to DiaSorin Liaison MDX; fixed hyperlinks for SharePoint upload |
|  | 4 | J. Laramie | 02.12.18 | -Eliminated steps and notes regarding the Positive Control (manufactured)-Changed from alternate proficiency testing to WSLH  |
|  | 5 | J. Laramie | 02.12.18 | -Edited negative control notes to reflect use of a negative control swab |