|  |
| --- |
| GeneXpert Xpress Strep A Assay  |
| **Purpose** | This procedure provides instructions for performing the Xpert Xpress Strep A assay on the Cepheid GeneXpert system. |
| **Policy Statements** | This procedure applies to all technical staff performing testing on the GeneXpert. |
| **Principle and Clinical Significance** | The Xpert Xpress Strep A Assay is intended to aid in the diagnosis of Group A Streptococcal pharyngitis. The assay is not intended to monitor treatment for Group A *Streptococcus* infections. Group A streptococci are gram-positive, beta-hemolytic bacterial pathogens that commonly cause infections in the throat (pharyngitis or “strep throat”) or on skin (cellulitis and impetigo), and can cause a wide range of other infections (e.g., sepsis, pneumonia, and meningitis). Pharyngitis may also be caused by other bacteria including *Neisseria gonorrhoeae* and *Corynebacterium diphtheriae*, for which specific culture methods are required. If left untreated, mild infections can lead to more serious infections. The most severe but least common forms of invasive Group A streptococcal disease are necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). Approximately 9,000 to 11,500 cases of invasive Group A streptococcal (GAS) disease occur annually in the United States, resulting in 1,000 to 1,800 deaths, although several million cases of strep throat and impetigo occur each year. Treating an infected person with an appropriate antibiotic generally prevents the spread of the infection and reduces the risk of post-infectious complications, such as rheumatic fever and acute glomerular nephritis. The Xpert Xpress Strep A test is a qualitative assay that utilizes real-time polymerase chain reaction (PCR) for the detection of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*, Strep A) in throat swab specimens from patients with signs and symptoms of pharyngitis. The GeneXpert automates and integrates sample preparation, nucleic acid extraction, amplification, and detection of the target sequences in clinical specimens by using real-time PCR. The Xpert Xpress Strep A test includes reagents for the detection of Group A streptococcal bacterial DNA from throat swab specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for an adequate amplification process and to monitor for the presence of inhibitors in the PCR reaction. The PCC verifies reagent rehydration, PCR tube filling, and all reaction components, including probes and dyes, are present and functional in the cartridge.  |
| **Test Code** | **GASDN** |
| **Sample** | 1. **Acceptable specimens:**
* Throat swabs collected with ESwab collection kits
1. **SDES codes/Specimen type:**
* **THR** - Throat
1. **Specimen Collection and Transport:**
* Refer to *Lab Test Directory* on StarNet
1. **Specimen assessment:**
* Refer to the policy MCVI 2.1 *Specimen Rejection Criteria.*
1. **Specimen Storage**
* Room temp (2-28°C): 48 hours
* Refrigerated (2-15°C): 6 days
 |
| **Special Safety Precautions** | **Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology* and *Virology Policy Manual*:**1. ***Biohazard Containment***
2. ***Safety in the Microbiology/Virology Laboratory***
* ***Biohazardous Spills***
 |
| **Materials** |

|  |  |  |
| --- | --- | --- |
| Reagents | Supplies | Equipment |
| * 10% bleach
* 70% ethanol
 | * Xpert Xpress Strep A cartridges
* Transfer pipettes
* Simple racks
* Cartridge transfer tray

Store kits at 2-28°C. Kits are stable until the expiration date printed on the outer box.  | * Biosafety Hood
* Cepheid GeneXpert Instrument and computer
* Printer
 |

 |
|
| **Calibration** | Annual “Xpert Check Kit” calibration performed by Cepheid. |
| **Quality Control** | **Daily Quality Control:**Once an Xpert cartridge has been loaded and before the sample processing steps begin, the software checks the optics, the readiness of the module’s mechanical components, and the ambient temperature of the module to assure proper performance of PCR, and the physical integrity of the cartridge. **Quality Control**Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).* **Sample Processing Control (SPC):** Ensures the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the assigned acceptance criteria.
* **Probe Check Control (PCC):** Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the assigned acceptance criteria.

**NOTE:** When Strep A levels are high enough to generate very early Cts, the SPC amplification curves may not be seen, and the results will not be reported.**External Quality Control:*** Perform QC using external positive and negative controls every 30 days. Record results in the GeneXpert assay binder on the Log.
* See IQCP document.
* See Quality Control Procedure.

**New Lot/Shipment Quality control:*** Perform QC using external positive and negative controls with each new lot or shipment before putting into service. Record results in the GeneXpert assay binder on the Log.
* See Quality Control Procedure

**Wipe testing control:*** Perform wipe testing every 30 days to monitor for contamination.
* See Quality Control Procedure.

**NOTE:** External quality control may be performed on an as needed basis if certain circumstances arise. Examples include:* Drift in results (e.g., increasing/decreasing positivity rates)
* Potential contamination (negative control)
* After drastic system maintenance
 |
| **Procedure** | **Cartridge preparation:**1. Clean hood with10% bleach (made daily) followed by 70% ethanol.
2. Change gloves.
3. Obtain an Xpert Xpress Strep A Assay cartridge, transfer pipette, and sample transport tube to be tested.
4. Label the side of the cartridge with a bar-coded foot-label.
5. Open the cartridge lid.
6. Vortex the ESwab tube 5-10 seconds.
7. Open the transfer pipette wrapper on the side of the squeeze bulb.
8. Carefully unscrew the ESwab lid, completely squeeze the pipette bulb, and draw up specimen in the transfer pipette (300 uL). See **Figure 1**.

1. Insert the pipette to the bottom of the well in the cartridge and empty the pipette’s content into the cartridge. See **Figure 2**.

1. Close the cartridge lid, and set onto the transfer tray.
2. Change gloves and proceed to prepare additional samples or start the test.

NOTES: -Hood surfaces must be cleaned between samples with 10% bleach followed with 70% ethanol if there were any splashes, spills, or uncertainty of cleanliness. -\*\*Start the test within 30 minutes of adding the sample to the cartridge**Starting the test:**1. Ensure clean gloves are on before stepping to the computer work space.
2. If instrument and computer are turned off: start up the instrument by flipping the power switch located in the back of the instrument. Turn on the computer next.
3. Log onto the appropriate Windows account:
	1. User: lab1
	2. Password: labstaff4
4. The GeneXpert software will launch automatically. If it doesn’t double-click the GeneXpert Dx software shortcut icon on the desktop.
5. Log onto the software.
	1. User: First 6 letters of your first and last name (combined)
	2. Password: First 6 letters of your first and last name (combined)
6. In the GeneXpert System window, click **Create Test.**
7. Navigate to the **Sample ID** box. Scan or type in the sample ID.
8. Scan the barcode on the cartridge.

NOTE: if the barcode on the cartridge does not scan, then repeat the test with a new cartridge.1. If prompted, select Xpert Strep A from the **Select Assay MENU.**
2. Select the appropriate test type for samples or controls.
3. Enter additional information in the “notes” field (day of QC, collect date, etc.) if needed.
4. Click **Start Test**.
5. Enter your username and password, if requested.
6. Open the instrument module door with the blinking green light.

NOTE: when setting up for testing you may opt to use any available module.1. With the barcode facing towards you, set the cartridge into the module and close the door.
2. Wait for the test to start and the light to stop blinking. The test will run for 24 minutes.

NOTE: Early assay positive call out can happen as early as 18 minutes into the run1. Turn printer on.
2. Remove the cartridge when testing is finished (the light will be off and the system will release the door lock).
3. Dispose of used cartridges into bio-bags and place into biohazard sharps bins.
4. Clean any equipment used (pipettes, racks, transfer tray, etc.), hood, and counters (including keyboard, scanner, and mouse) at the end of the day.

NOTE: Sample processing, testing, and cleaning should follow a unidirectional work-flow to avoid contamination.  |
| **Interpretation/ Results**  | 1. Click on **View Results** on the top drop-down menu bar and select **View Test**.
2. Select the result you would like to review: Click **OK**.
3. Review result interpretations and amplification curves for exponential growth. See **Figure 3**.
	1. NOTE: SPC does not need to pass for a positive result to be valid.
	2. NOTE: SPC does need to pass for a negative result to be valid.

**Figure 3: Amplification Curve**1. Click on the **Errors** tab to ensure no errors occurred during testing. (Section 9.18.2 in Operator Manual provides error code descriptions)
2. Refer to **Table 1** for result interpretation.

**Table 1: Strep A Instrument Results and Interpretations**

|  |  |
| --- | --- |
| **Result** | **Interpretation** |
| **Strep A NOT DETECTED** | Strep A target DNA is not detected.* SPC – PASS; SPC has a Ct within the valid range and endpoint above the threshold setting
* PCC – PASS ; all probe check results pass
 |
| **Strep A DETECTED** | Strep A target DNA is detected. * SPC – N/A; SPC signal is not part of the result interpretation algorithm if Strep A is detected since SPC signal may be suppressed due to competition with Strep A
* PCC – PASS; all probe check results pass
 |
| **INVALID** | Presence or absence of the target DNA cannot be determined. Repeat testing. * Strep A: INVALID
* SPC: does not meet acceptance criteria
* PCC – PASS; all probe check results pass
 |
| **ERROR** | Presence or absence of the target DNA cannot be determined. Repeat testing. * Strep A: NO RESULT
* SPC: NO RESULT
* PCC – FAIL\*; all or one of the probe check results failed

\*If the probe check passes or shows NA, the error was caused by the maximum pressure limit exceeding the acceptable range or by a system component failure. |
| **NO RESULT** | Presence or absence of Strep A target DNA cannot be determined. A NO RESULT indicates that insufficient data were collected. For example, a cartridge integrity test failed, the operator stopped a test that was in progress or a power failure occurred. Repeat testing. * Strep A: NO RESULT
* SPC: NO RESULT
* PCC: N/A\*

\*If the probe check shows NA, the error caused by the maximum pressure limit exceeding the acceptable range terminates the run prior to probe check.  |

**Reasons to retest the original sample:**1. An INVALID result (SPC failure). This may indicate:
	1. The sample was not properly processed.
	2. PCR was inhibited.
2. An ERROR result. This may indicate:
	1. The reaction tube was filled improperly.
	2. A reagent probe integrity problem was detected.
	3. The maximum pressure limit was exceeded.
	4. A valve positioning error was detected.
3. NO RESULT:
	1. This result indicated that insufficient data were collected (e.g. test stopped while in progress or power failure occurred).

NOTE: Record any failures on the “GeneXpert Service and Error Log” log. **Retesting procedure:** 1. Obtain the original sample and a new cartridge.
2. Retest the sample according to the instructions in this SOP.
3. Report results according to **Table 2** below.

**Table 2: Retesting results and interpretation**

|  |  |  |
| --- | --- | --- |
| **Initial result** | **Repeat Result**  | **Report**  |
| **INVALID** | INVALID | Unresolved  |
| VALID | Valid results |
| **ERROR** | ERROR or INVALID | Unresolved |
| VALID | Valid results |
| **NO RESULT** | NO RESULT, ERROR or INVALID | N/A – repeat testing |

1. See the instructions below for reporting unresolved results.

NOTE: Record any failure, errors, and repeat testing on the “GeneXpert Service and Error Log” log.  |
| **Result Reporting** | 1. Ensure that the printer is turned on.
	1. Reports will print automatically.
2. Valid results will automatically transmit to the LIS and be auto-verified.

**NOTE**: you must check your results upon completion of testing to ensure validity of results 1. At the end of the shift call a completed worksheet for GASD, check results, and staple to GeneXpert Report. Place in the GeneXpert Strep A result binder.
2. Store samples in fridge:
	1. Mark positive samples on top of caps.
3. Discard old samples after 1 week.
 |
| **Critical Results** | No critical result values.  |
| **Reporting Invalid (unresolved) Results** | 1. Notify the care provider of the unresolved result.
2. Log into Sunquest to release results.
3. Select Result Entry from Menu options
4. In the Configuration field select CGX from the dropdown box.
5. Click on the  button located in the lower right corner to populate the transmitted results.
6. Review messages located on the top and results. Compare results to the GeneXpert report.
7. The result will be reported as **unresolved** (UNRE) and the following code SIA will automatically append: “This sample is inhibitory to amplification and the results are inconclusive. Consider repeat collection if clinically indicated.”
8. Add the code CAL, press tab, enter semi-colon record who the result was relayed to and the date/time.
9. Check the release box.
10. Click  button located on the lower left corner. Click  when the “Verify Release Destination” window opens.
 |
| **Correcting Results** | 1. Open Result Entry, select the Manual resulting mode (top left corner), from the configuration drop down select the appropriate test. Click  in the lower right corner.
2. Enter the Specimen ID, enter Tab and click Yes to modify the result.
3. Change the incorrect result. The corrected result comment will automatically append. Add the CAL comment, press tab, enter a semi-colon and record who was called and the time/date.

 1. Click . Click  when the “Verify Release Destination” window opens.
 |
|  |
| **Limitations** | * Additional follow-up testing by culture is required if the Xpert Xpress Strep A test result is negative and clinical symptoms persist, or there is an outbreak of acute rheumatic fever (ARF).
* The performance of the Xpert Xpress Strep A test was evaluated using the procedures provided in the package insert only.
* Careful compliance with the instructions in the Copan Liquid Amies Elution Swab (ESwab) Collection and Transport System package insert is necessary to avoid erroneous results.
* Because the detection of *Streptococcus pyogenes* is dependent on the organism’s DNA present in the sample, reliable results are dependent on proper sample collection, handling, and storage.
* The Xpert Xpress Strep A test provides qualitative results and does not provide the quantitative value of the organism detected in the specimen.
* Mutations or nucleotide polymorphisms in primer or probe binding regions may affect detection of new or unknown *Streptococcus pyogenes* strains resulting in a false negative result.
* A negative test result does not exclude the possibility of infection because the test result may be affected by improper specimen collection, technical error, sample mix-up, or because the number of organisms in the sample is below the limit of detection of the test.
* As with many diagnostic tests, negative results from the Xpert Xpress Strep A test do not preclude a Strep A infection and should not be used as the sole basis for treatment or other patient management decisions.
* The Xpert Xpress Strep A test does not differentiate asymptomatic carriers of Group A streptococci from those exhibiting streptococcal infection.
* The results from the Xpert Xpress Strep A test should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
* This test has not been evaluated for patients without signs and symptoms of pharyngitis.
* This test cannot rule out pharyngitis caused by other bacterial or viral pathogens besides Group A streptococci.
* Cross-reactivity with organisms other than those listed in the Exclusivity Table 11 may lead to erroneous results.
* The analyte target (bacterial nucleic acid) may persist in vivo, independent of pathogen viability. Detection of the analyte target does not imply that the corresponding pathogen is infectious, or is the causative agent of the clinical symptoms.
 |
| **Method Performance Specifications** | According to the manufacturer (per the package insert) – Overall specifications are shown in **Table 3**:**Table 3: Overall Specifications**NOTE: * “First swabs” were swabs that were collected and used for Standard Of Care testing prior to Xpert testing
* “Second swabs” were swabs collected alongside initial patient swabs
 |
| **References** | Xpert Xpress Strep A Package Insert, 301-6574, Rev. B, May 2018. Sunnyvale, CA: Cepheid. |
| **Alternate Methods** | 1. Bacterial culture
 |
| **Proficiency Testing** | WSLH materials (ST): 3 shipments a year with 5 samples. (Item: PT05170) |
| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure.
2. Employee will demonstrate the ability to perform procedure, record results, and document corrective action after instruction by the trainer.
 | 1. Direct observation
 |
| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Julie Laramie | 9/30/2019 | Initial Version |
|  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |
| **Archived by:** |  | **Archived Date:** |  |