GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® Xpress Strep A

REF **XPRSTREPA-CE-10**

Instructions for Use

CE IVD

Cepheid®

In Vitro Diagnostic Medical Device

301-6569, Rev. F
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Trademark, Patents and Copyright Statements

See Section 24, Revision History for a description of changes.

Xpert® Xpress Strep A

For *in vitro* diagnostic use only.

1 Proprietary Name

Xpert® Xpress Strep A

2 Common or Usual Name

Xpert Xpress Strep A test

3 Intended Use

The Xpert Xpress Strep A test, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test for the detection of *Streptococcus pyogenes* (Group A β-hemolytic Streptococcus, Strep A) in throat swab specimens from patients of all ages with signs and symptoms of pharyngitis.

The Xpert Xpress Strep A test utilizes an automated real-time polymerase chain reaction (PCR) to detect *Streptococcus pyogenes* DNA.

4 Summary and Explanation

Group A streptococci are gram-positive, beta-hemolytic bacterial pathogens that commonly cause infections in the throat (pharyngitis or "strep throat") and on skin (cellulitis and impetigo), but can cause a wide range of other infections (e.g., sepsis, pneumonia, and meningitis). 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.[1] 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 glomerular nephritis.[1,2]

The Xpert Xpress Strep A test is a rapid PCR test for the qualitative detection of Group A Streptococci from throat swab specimens. For negative samples, the time to result is 24 minutes. For positive samples, the time to result may be as early as 18 minutes.

5 Principle of the Procedure

The test is performed on the Cepheid GeneXpert Instrument Systems. With this platform, the operator can run the test by performing three simple steps: 1) transfer liquid sample to the cartridge with a transfer pipette, 2) run the test on the GeneXpert instrument, and 3) read the results. 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 system consists of a GeneXpert instrument, computer, and disposable fluidic cartridges that are designed to complete sample preparation and real-time PCR. The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR processes. Because the cartridges are self-contained, the risk of cross-contamination between samples is minimized.

The Xpert Xpress Strep A test includes reagents for the detection of Group A streptococcal bacterial DNA from throat swab specimens obtained from patients with signs and symptoms of pharyngitis. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR reaction. The PCC verifies reagent rehydration, PCR tube filling, and confirms presence of all reaction components in the cartridge including probe integrity and dye stability.

An Early Assay Termination function provides positive results if the signal from the target DNA reaches a predetermined threshold before the full 43 PCR cycles have been completed. When the Strep A target level is high enough to generate very early cycle thresholds (Cts) (≤ 30 Cts), the SPC amplification curve will be not seen and its results will not be reported as the SPC Ct may not reach the expected cycle threshold in high Strep A titer specimens.

6 Reagents and Instruments

6.1 Material Provided

The Xpert Xpress Strep A test kit contains sufficient reagents to process 10 specimens or quality control samples.

The kit contains the following:

Xpert Xpress Strep A Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2 and Bead 3 (freeze-dried)	1 of each per cartridge
• Lysis Reagent	1.5 mL per cartridge
◦ Guanidinium Thiocyanate	1.5 mL per cartridge
• Sodium Hydroxide	2.0 mL per cartridge
• Elution Reagent	
Disposable Transfer Pipettes	1 bag of 12 per kit
Compact Disk (CD)	1 per kit
• Assay Definition File (ADF)	
• Instructions to import ADF into GeneXpert software	
• Instructions for Use (IFU)	

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

7 Storage and Handling

- Store the Xpert Xpress Strep A cartridges at 2-28°C until the expiration date provided on the label.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use cartridges that have passed the expiration date.
- Do not use a cartridge that has leaked.

8 Materials Required but Not Provided

- Copan Liquid Amies Elution Swab (ESwab™) Collection and Transport System (Copan 480CE; Copan 480C)
- GeneXpert Dx Instrument or GeneXpert Infinity Systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, Operator Manual.

- For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher
- For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher

- Printer: If a printer is required, contact Cepheid Customer Support to arrange for the purchase of a recommended printer.

9 Warnings and Precautions

9.1 General

- For *in vitro* diagnostic use.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. All biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[3] and the Clinical and Laboratory Standards Institute[4].
- Follow safety procedures set by your institution for working with chemicals and handling biological samples.
- Performance characteristics of this test have been established with the specimen type listed in the Section 3. Intended Use only. The performance of this test with other specimen types or samples has not been evaluated.
- Reliable results are dependent on adequate specimen collection, transport, storage and processing. Incorrect test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the number of organisms in the specimen is below the limit of detection of the test. Careful compliance with the IFU instructions and the GeneXpert System User's Manual are necessary to avoid erroneous results.
- Performing the Xpert Xpress Strep A test outside the recommended storage temperature ranges and time may produce erroneous or invalid results.

9.2 Specimen

- For collection and transport of throat swab specimens, use the ESwab collection kit.
- Throat swab specimens must be collected and tested before the expiration date printed on the ESwab collection kit.
- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 11. Specimen Collection, Transport and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Do not freeze ESwab specimens.
- Proper sample collection, storage, and transport are essential for correct results.

9.3 Test/Reagent

- Do not open the Xpert Xpress Strep A cartridge lid except when adding specimen.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
- Do not place the sample ID label on the cartridge lid or on the barcode label of the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert Xpress Strep A cartridge is used to process one test. Do not reuse processed cartridges.
- Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens or reagents.
- In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a 1:10 dilution of household chlorine bleach and then repeat the cleaning of the work area with 70% denatured ethanol. Wipe work surfaces dry completely before proceeding.

10 Chemical Hazards[5,6]

- UN GHS Hazard Pictogram: 
- Signal Word: Warning
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - Causes skin irritation.
  - Causes serious eye irritation.
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash thoroughly after handling.
    - Wear protective gloves/protective clothing/eye protection/face protection.
  - **Response**
    - IF ON SKIN: Wash with plenty of soap and water.
    - Specific treatment, see supplemental first aid information.
    - Take off contaminated clothing and wash before reuse.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention.

11 Specimen Collection, Transport and Storage

Proper specimen collection, storage and transport are critical to ensure the integrity of the specimen and performance of the test. Inadequate specimen collection, improper specimen handling and/or transport may yield incorrect results. Follow your institution's guidelines for collecting swab specimens using a recommended collection and transport device (refer to Section 8. Materials Required but Not Provided section) and/or follow the instructions below:

11.1 Swab Collection Procedure

1. Use the ESwab Collection and Transport System (Copan 480CE; Copan 480C). Remove the swab from the envelope.
2. Swab the posterior pharynx, tonsils, and other inflamed areas. Avoid touching the tongue, cheeks, and teeth with the swab when collecting specimens.
3. Uncap the ESwab transport tube.
4. Place the specimen containing swab into the ESwab transport tube and break the swab at the indicated score line.
5. Cap the ESwab transport tube.

Note Do not place multiple swabs in the same ESwab transport tube.

11.2 Specimen Transport and Storage

Specimen stability under shipping and storage conditions other than those listed in Table 1 have not been evaluated with the Xpert Xpress Strep A test.

Table 1. Specimen Transport and Storage Conditions

Specimen Collection Device	Specimen Transport and Storage Temperature (°C)	Specimen Storage Time
ESwab (Copan 480CE; Copan 480C)	15–30 °C	Up to 48 hours
	2–8 °C	Up to 6 days

12 Procedure

Important **Start the test within 30 minutes of adding the sample to the cartridge.**

12.1 Preparing the Cartridge

To add the specimen to the GeneXpert cartridge:

1. Obtain the following items: Xpert Xpress Strep A cartridge, 300 µL transfer pipette (provided), and an appropriately collected and labeled test sample.
2. Inspect the test cartridge for damage. If damaged, do not use it.
3. Mix the patient specimen by vigorously shaking the specimen transport tube for 5 seconds.
4. Open the cartridge by lifting the cartridge lid.
5. Remove the transfer pipette from the wrapper by opening the end next to the bulb. Follow the steps below in Option 1 or Option 2 according to the transfer pipette type included in the kit.

Note Do not place unwrapped pipette on the workbench.

Pipette Option 1:

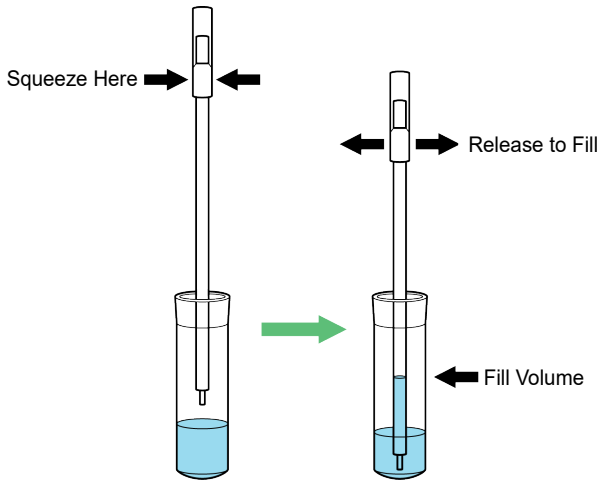

Figure 1. Transfer Pipette

1. Squeeze the bulb of the transfer pipette **completely** and place the pipette tip in the ESwab transport medium tube containing the patient specimen (see Figure 1).
2. Release the bulb of the pipette to fill the pipette with the patient specimen. Check that the pipette does not contain bubbles.
3. To transfer the patient specimen to the cartridge, squeeze the bulb of the transfer pipette completely again to empty the contents of the pipette into the large opening (Sample Chamber) in the cartridge shown in Figure 2.

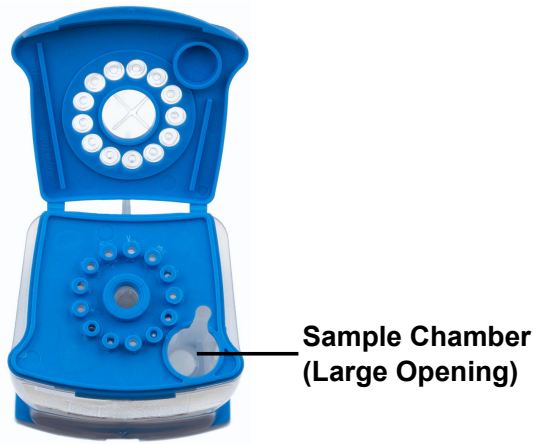

Figure 2. Cartridge (Top View)

Note Take care to dispense the **entire** volume of liquid into the sample chamber. False negative or indeterminate results may occur if insufficient sample is added to the cartridge.

4. Close the cartridge lid.
5. Dispose of the used pipette in an appropriate waste container.

Pipette Option 2:

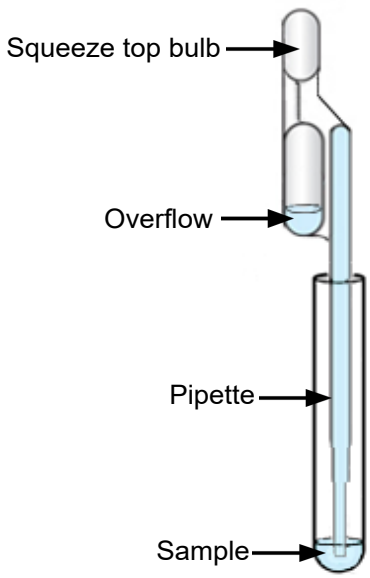

Figure 3. Transfer Pipette

1. Squeeze the top bulb of the transfer pipette **completely** and place the pipette tip in the ESwab transport medium tube containing the patient specimen (see Figure 3).
2. Release the bulb of the pipette to fill the pipette with the patient specimen. Check that the pipette does not contain bubbles.
3. To transfer the patient specimen to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette into the large opening (Sample Chamber) in the cartridge shown in Figure 4. It is okay to have excess specimen left in the overflow reservoir of the pipette (Figure 3).

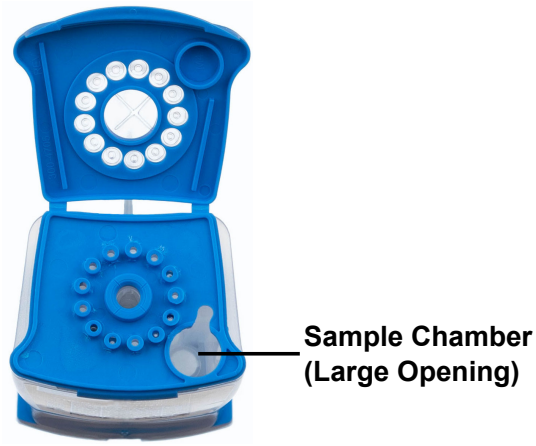

Figure 4. Cartridge (Top View)

Note Take care to dispense the **entire** volume of liquid into the sample chamber. False negative or indeterminate results may occur if insufficient sample is added to the cartridge.

4. Close the cartridge lid.
5. Dispose of the used pipette in an appropriate waste container.

12.2 Starting the Test

Note Before you start the test, make sure the system is running GeneXpert 4.7b software or higher and that the Xpert Xpress Strep A Assay Definition File (ADF) is imported into the software. This section lists the basic steps of running the test. For detailed instructions, see the GeneXpert Dx System Operator Manual or the GeneXpert Infinity System Operator Manual, depending on the model that is being used.

This section lists the default steps to operate the GeneXpert Instrument System. For detailed instructions, see the *GeneXpert DxSystem Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert Instrument System:
   - If using the GeneXpert Dx instrument, first turn on the instrument, and then turn on the computer. Log into the Windows operating system. The GeneXpert software may launch automatically or may require double-clicking on the GeneXpert Dx software shortcut icon on the Windows® desktop.

     or
   - If using the GeneXpert Infinity instrument, power up the instrument by turning the power switch clockwise to the **ON** position. Wait 2 minutes for the system to boot. Log into the Windows operating system. On the Windows desktop, double-click the Xpertise Software shortcut icon to launch the software.
2. Log on to the System software. The login screen appears. Type your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** followed by **Order Test** (Infinity).
4. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test result.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test result.
6. Scan the barcode on the Xpert Xpress Strep A cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date and Selected Assay.

Note If the barcode on the Xpert Xpress Strep A cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity) if Auto-Submit is not enabled. In the dialog box that appears, type your password, if required.

   For the GeneXpert Dx Instrument:

a. Locate the module with the blinking green light, open the instrument module door and load the cartridge.

b. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge.

c. Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.

or

For the GeneXpert Infinity System:

a. After clicking **Submit**, you will be asked to place the cartridge on the conveyor belt. After placing the cartridge, click **OK** to continue. The cartridge will be automatically loaded, the test will run and the used cartridge will be placed onto the waste shelf for disposal.

b. When all samples are loaded, click on the **End Order Test** icon.

Note Do not turn off or unplug the instruments while a test is in progress. Turning off or unplugging the GeneXpert instrument or computer will stop the test.

Note Time to result is 24 minutes. A strongly positive sample will have a time to result as early as 18 minutes.

12.3 Data Management and Archiving Tasks

For instructions on performing Database Management or archiving tasks, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the instrument model that is being used.

13 Viewing and Printing Results

For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

14 Quality Control

Each cartridge includes a Sample Processing Control (SPC) and a 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 test, 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.
- **External Controls** – External controls should be used in accordance with local, state, and federal accrediting organizations' requirements, as applicable.

15 Interpretation of Results

The results are interpreted automatically by the GeneXpert Instrument System and are shown in the **View Results** window. The possible results and interpretations are shown in Figures 3-8 and in Table 2.

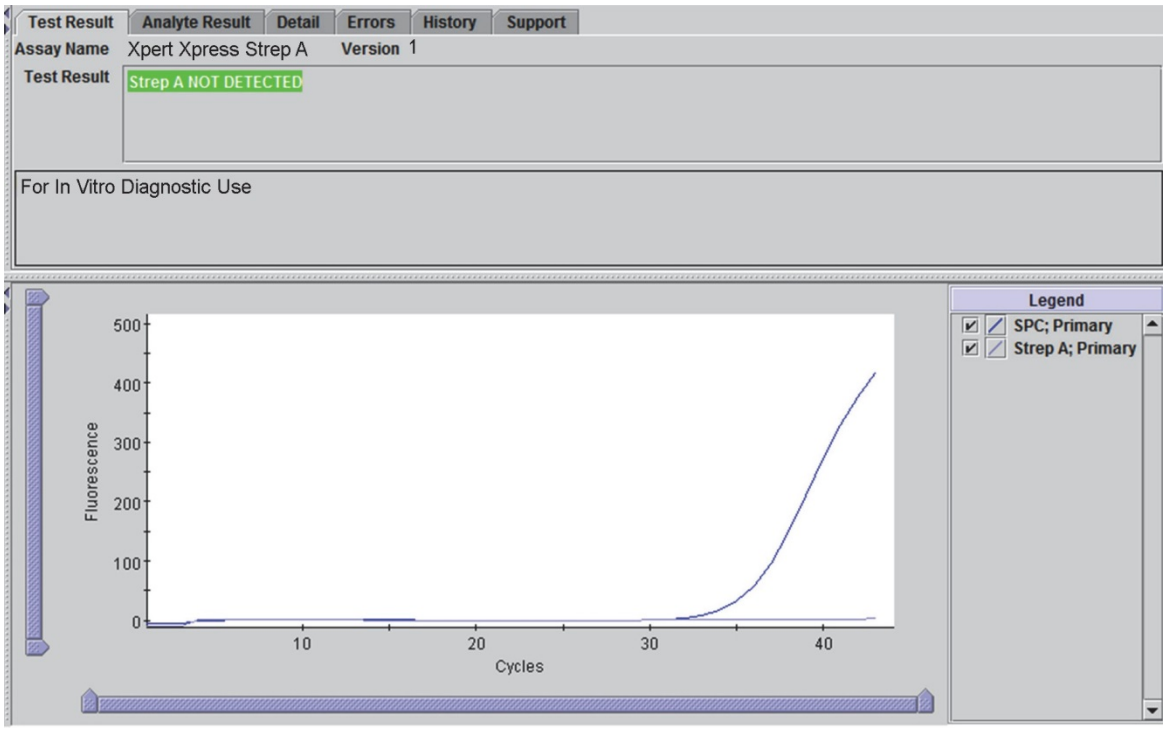

Figure 5. Example of Strep A Negative "Strep A NOT DETECTED" Test Result

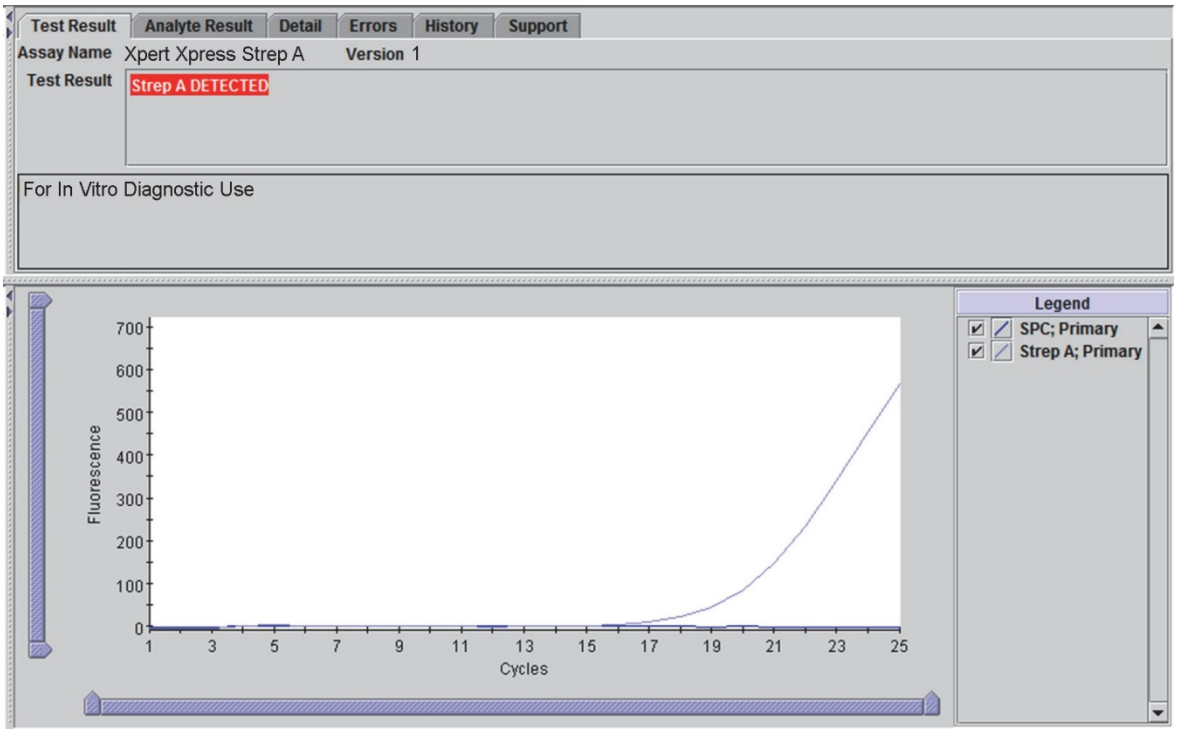

Figure 6. Example of Strep A Positive "Strep A DETECTED" Test Result (Early Assay Termination)

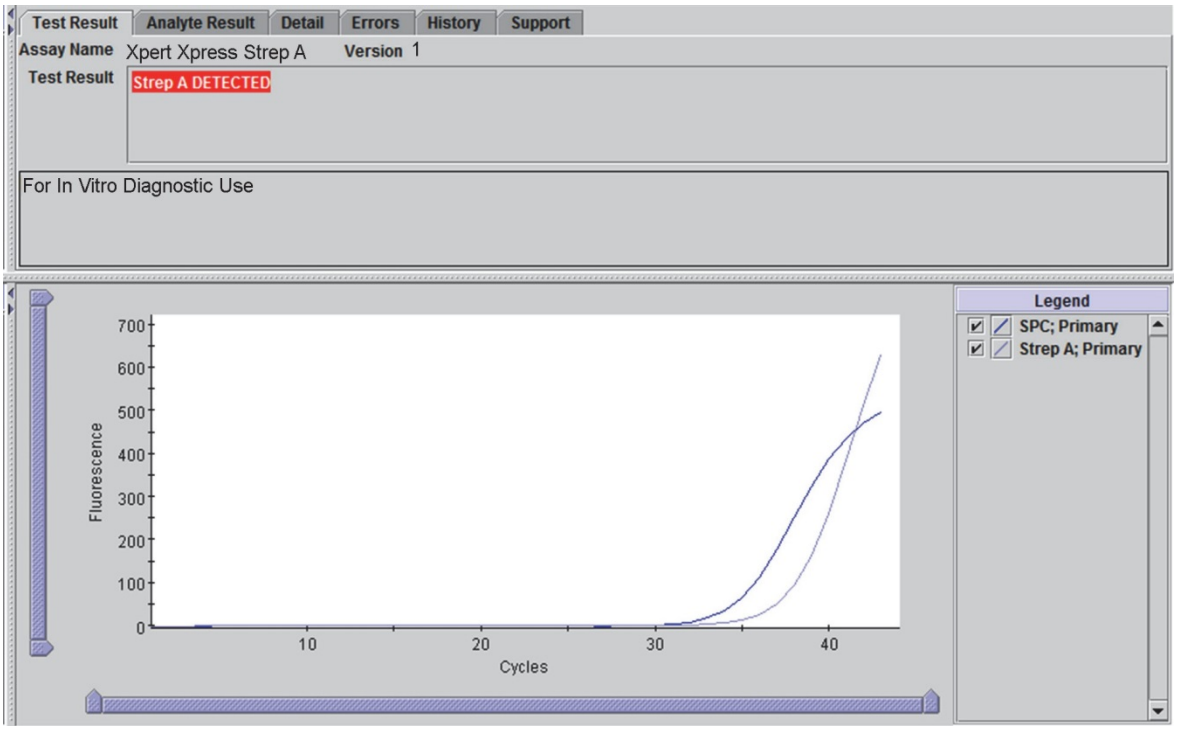

Figure 7. Example of Strep A Positive "Strep A DETECTED" Test Result

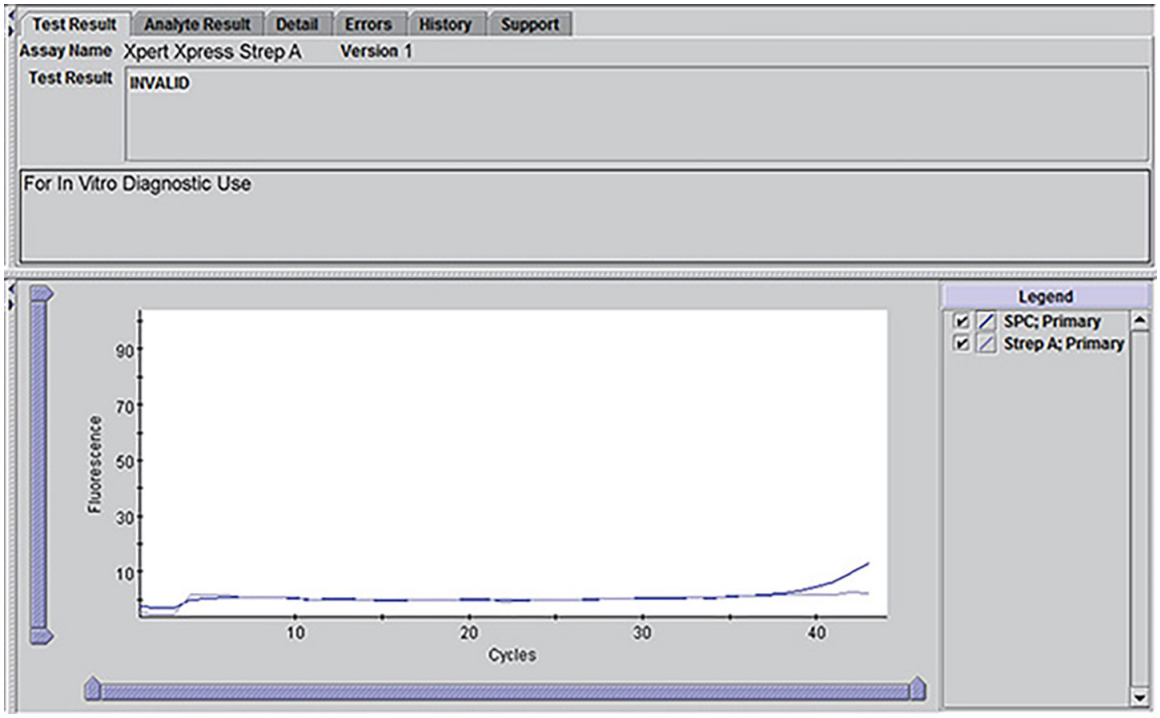

Figure 8. Example of "INVALID" Test Result

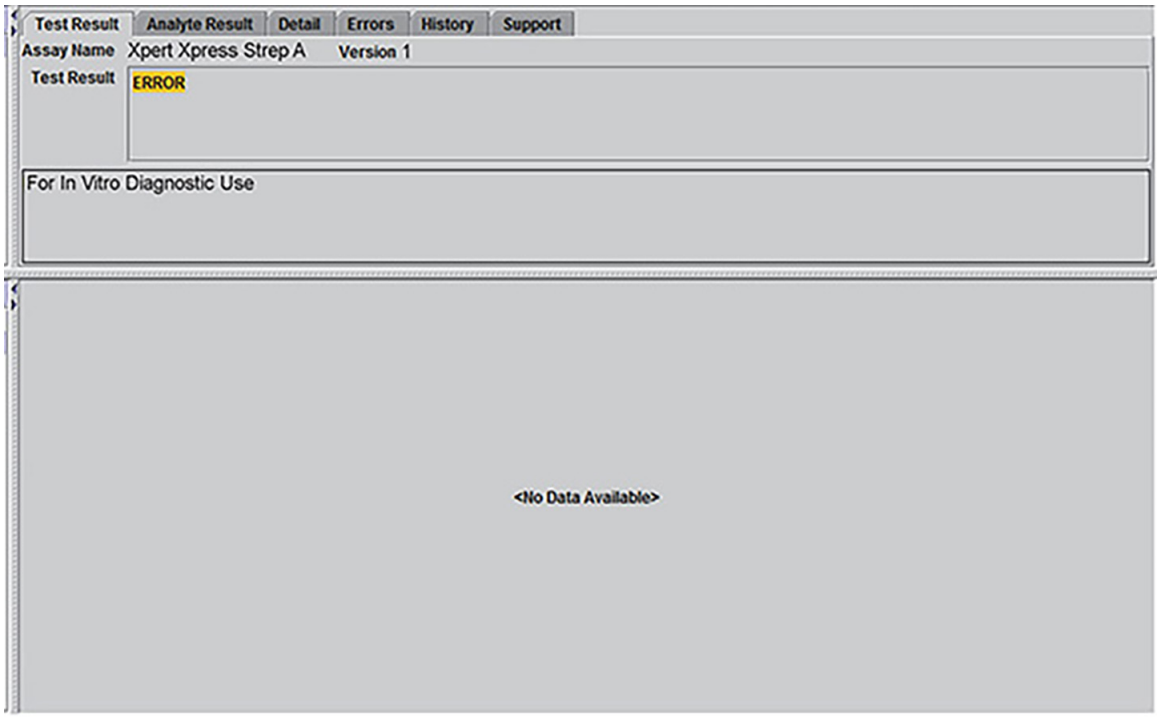

Figure 9. Example of "ERROR" Test Result

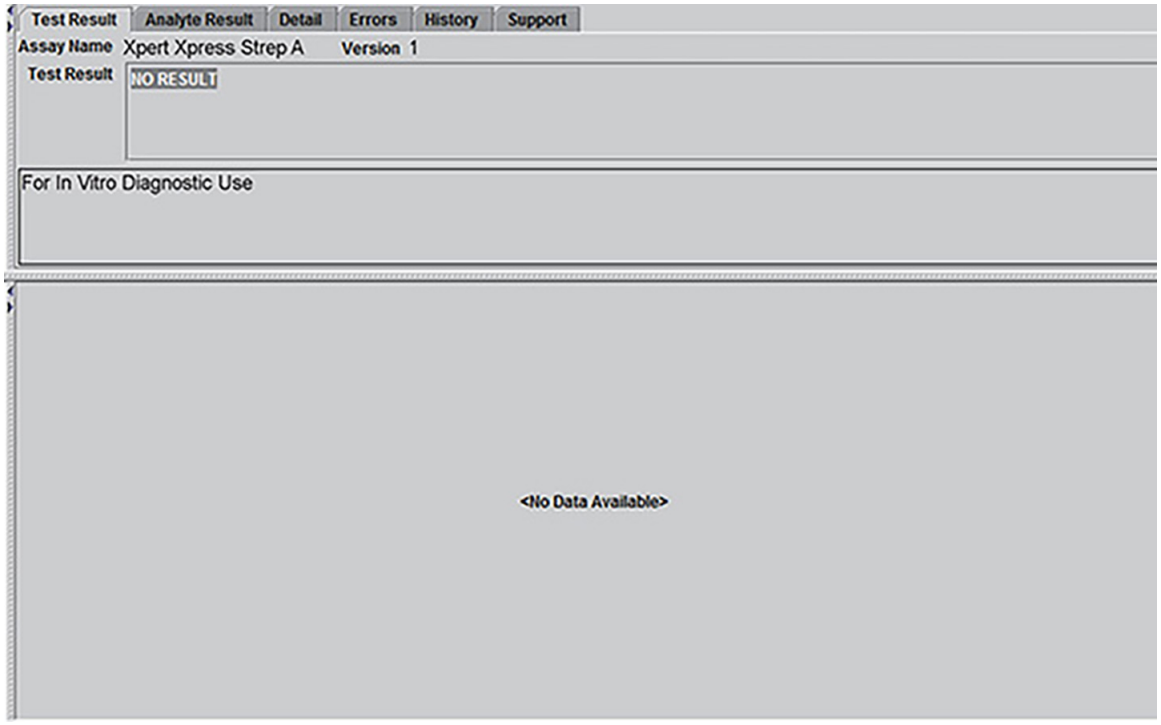

Figure 10. Example of "NO RESULT" Test Result

Table 2. Xpert Xpress Strep A Test Results and Interpretations

Result	Interpretation
Strep A NOT DETECTED (See Figure 5)	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 (See Figure 6 and Figure 7)	Strep A target DNA is detected. ● Strep A – Ct is within the valid range. ● SPC – NA (not applicable); 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 (See Figure 8)	Presence or absence of the Strep A target DNA cannot be determined. ● Strep A – INVALID ● SPC – does not meet acceptance criteria. ● PCC – PASS; all probe check results pass.
ERROR (See Figure 9)	Presence or absence of Strep A target DNA cannot be determined. ● Strep A – NO RESULT ● SPC – NO RESULT ● PCC – FAIL*; all or one of the probe check results fail. * If the probe check passed or shows **NA**, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure. Repeat test according to the instructions in Section 16.2. Retest Procedure, below.
NO RESULT (See Figure 10)	Presence or absence of Strep A target DNA cannot be determined. A **NO RESULT** indicates that insufficient data were collected. For example, cartridge integrity test failed, the operator stopped a test that was in progress or a power failure occurred. ● Strep A – NO RESULT ● SPC – NO RESULT ● PCC – NA (not applicable)* * If the probe check shows **NA**, the error is caused by the maximum pressure limit exceeding the acceptable range that terminates the run prior to probe check. Repeat test according to the instructions in Section 16.2. Retest Procedure, below.

16 Retests

16.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 16.2. Retest Procedure.

- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
- An **ERROR** result could be due to, but is not limited to, Probe Check Control failure, system component failure, or the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.
- If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

16.2 Retest Procedure

To retest an **INVALID**, **NO RESULT**, or **ERROR** result (non-determinate result), use a new cartridge.

Use the leftover sample from the original ESwab transport medium tube.

1. Mix the leftover patient specimen by vigorously shaking the specimen transport tube for 5 seconds.
2. Open the cartridge by lifting the cartridge lid.
3. Remove the transfer pipette from the wrapper by opening the end next to the bulb.
4. Squeeze the bulb of the transfer pipette completely and place the pipette tip in the transport medium tube containing the patient specimen (Figure 1).
5. Release the bulb of the pipette to fill the pipette with the patient specimen.
6. To transfer the patient specimen to the cartridge, squeeze the bulb of the transfer pipette completely again to empty the contents of the pipette into the large opening (Sample Chamber) shown in Figure 2.
7. Close the cartridge lid.
8. Dispose of the used pipette in an appropriate waste container.

17 Limitations

- The performance of the Xpert Xpress Strep A test was evaluated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Careful compliance with the instructions in this IFU and in the Copan Liquid Amies Elution Swab (ESwab) Collection and Transport System package insert is necessary to avoid erroneous results.
- The Xpert Xpress Strep A test has been validated only with Copan Liquid Amies Elution Swab (ESwab) Collection Kit (Copan 480CE; Copan 480C). Because the detection of *Streptococcus pyogenes* is dependent on the intact organism 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 *S. 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 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 10 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.

18 Performance Characteristics

18.1 Clinical Performance

Clinical specimens were collected from two multi-center investigational studies using throat ESwab specimens (flocked swab in Liquid Amies medium) from patients presenting with signs and symptoms of pharyngitis. One study enrolled consented subjects from whom a second prospective throat swab specimen was collected following the collection of a standard of care (SOC) throat swab. Another study tested specimens from subjects for which leftover excess SOC throat swab specimens were available. Across the two studies, the Xpert Xpress Strep A test was evaluated by nine clinical sites from geographically diverse regions within the United States between December 2016 and March 2017.

Among the 583 tests performed, 96.9% (565/583) were successful on the initial test and upon retest 99.0% (577/583) gave valid results.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Xpert Xpress Strep A test were established relative to culture and latex agglutination for Strep A typing. The overall performance of the Xpert Xpress Strep test from both studies combined are presented in Table 3. Results of the first study (second swab specimens) and the second study (SOC throat swab, i.e., first swab) are presented separately in Table 4. Discordant results between Xpert Xpress Strep A and culture were resolved by bidirectional sequencing and results are footnoted in Table 3 and Table 4.

Table 3. Overall Performance of the Xpert Xpress Strep A Test vs. Reference Method (First and Second Swab Data Combined)

		Reference Method		
	Strep A	Pos	Neg	Total
Xpert Xpress Strep A Test	Pos	138	26[a]	164
	Neg	0	413	413
	Total	138	439	577
Sensitivity		100% (95%CI: 97.3-100)		
Specificity		94.1% (95%CI: 91.5-95.9)		
PPV		84.1% (95%CI: 77.8-88.9)		
NPV		100% (95%CI: 99.1-100)		

[a] Testing results by sequencing: 21 of 26 were Strep A positive by sequencing; 4 of 26 were Strep A negative by sequencing; 1 of 26 samples was not sequenced.

Table 4. Performance of the Xpert Xpress Strep A Test vs. Reference Method (Data for First and Second Swab)

	First Swab		Second Swab	
	N	% (95% CI)	N	% (95% CI)
Sensitivity	65/65	100% (94.4-100)	73/73	100% (95.0-100)
Specificity	244/253[a]	96.4% (93.4-98.1)	169/186[b]	90.9% (85.9-94.2)
NPV	244/244	100% (98.5-100)	169/169	100% (97.8-100)
PPV	65/74	87.8% (78.5-93.5)	73/90	81.1% (71.8-87.9)

[a] Testing results by sequencing: 7 of 9 were Strep A positive by sequencing; 1 of 9 was Strep A negative by sequencing; 1 of 9 samples was not sequenced.
[b] Testing results by sequencing: 14 of 17 were Strep A positive by sequencing; 3 of 17 were Strep A negative by sequencing.

18.2 Reproducibility

A three member reproducibility panel with varying concentrations of *Streptococcus pyogenes* was tested 4 times per day on six different days by two different operators at three sites (3 specimens x 4 times/day x 6 days x 2 operators x 3 sites). Three lots of Xpert Xpress Strep A test cartridges were used, with each representing two days of testing. The samples were prepared in simulated throat swab matrix at the different concentration levels and are presented in Table 5. Results of the reproducibility study by percent agreement and by study site/ operator are summarized in Table 6.

Table 5. Reproducibility Panel

Strain	Panel Member
Not applicable	Negative
ATCC19615 (*Streptococcus pyogenes*)	Low Positive (~1X LoD)
ATCC19615 (*Streptococcus pyogenes*)	Moderate Positive (~3X LoD)

Table 6. Summary of Reproducibility Results: % Agreement by Study Site/Operator

Sample	Site 1			Site 2			Site 3			% Total Agreement by Sample
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
Neg	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
Low Pos	92% (22/24)	100% (24/24)	96% (46/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	98.6% (142/144)
Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)

The reproducibility of the Xpert Xpress Strep A test was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-lots, between-days, between-operators and within-test for each panel member are presented in Table 7.

Table 7. Summary of Reproducibility Data

Sample	N[a]	Between-Site		Between-Lot		Between-Day		Between Operator		Within-Test		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Strep A - Low Pos	142	0.2	0.6	0	0	0.1	0.4	0.1	0.2	1.0	2.7	1.1	2.8
Strep A - Mod Pos	144	0	0	0.3	0.8	0	0	0.1	0.3	0.9	2.3	0.9	2.5
NEG	144	0	0	1.9	5.3	0.3	1.0	0	0	1.3	3.7	2.3	6.6

[a] Results with non-zero Ct values out of 144.

19 Analytical Performance

19.1 Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical sensitivity or Limit of Detection (LoD) of the Xpert Xpress Strep A test using the ESwab collection kit (Copan 480CE, Copan P/N 480C, referred to in Section 8 as the "ESwab"). The LOD is the lowest concentration of sample (reported as CFU/mL in ESwab transport medium or CFU/test) that can be reproducibly distinguished from negative samples 95% of the time with 95% confidence, or the lowest concentration of organisms at which 19 of 20 replicates were positive. This study determined the lowest concentration of *Streptococcus pyogenes* cells diluted into pooled clinical throat swab matrix that can be detected using the Xpert Xpress Strep A test.

The analytical sensitivity of the Xpert Xpress Strep A test was assessed following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2 using two lots of reagents tested across three testing days with two *Streptococcus pyogenes* strains: ATCC BAA-946 encoding M6 protein and ATCC 19615 encoding M5 and M49 protein. The *emm5* and *emm6* are associated with throat infections and rheumatic fever, whereas *emm49* is found in pyoderma and acute glomerulonephritis.[7]

The LoD was established by testing six concentration levels with two reagent lots across three testing days in replicates of 20. The LoD and 95% confidence interval (CI) were then estimated for each lot using probit regression analysis. The probit regression analysis does not rely on a single concentration but utilizes the probit function to incorporate all of the information (concentrations) in the model. The point estimates were calculated using a method of maximum likelihood estimates (MLE) on the probit regression model parameters. The maximum estimated LoD observed per strain from the probit regression analysis was used to establish the LoD claim. The LoD point estimates and 95% upper and lower confidence intervals for each Strep A strain tested are summarized in Table 8.

The results of this study indicate that the Xpert Xpress Strep A test will produce a positive Strep A result 95% of the time with 95% confidence for a throat swab containing 9–18 CFU/mL in ESwab transport medium, or 3–6 CFU/test.

Table 8. Strep A LoD and Confidence Intervals

Strep A Strain	Reagent Lot	LoD Estimate by Probit Analysis (CFU/mL in ESwab transport medium)			LoD Claim (CFU/mL in ESwab transport medium)	LoD Estimate (CFU/test)
		Lower 95% CI	LoD Point Estimate	Upper 95% CI		
ATCC BAA-946	Lot 1	7.0	8.4	10.7	9	3
	Lot 2	5.9	7.2	9.3		
ATCC 19615	Lot 1	14.5	17.1	21.0	18	6
	Lot 2	12.9	15.3	19.0		

19.2 Analytical Reactivity (Inclusivity)

Twenty-four *Streptococcus pyogenes* strains were tested at 3X LoD using the Xpert Xpress Strep A test in replicates of three. The strains tested represent M-types 1, 3, 4, 6, 11, 12,18, 22, 25, 27, 38, 75, 77, 89, 94, 95, chromosomal patterns associated with pharyngitis, prevalence and geographical locations. The list of strains tested is shown in Table 9 in ESwab medium containing simulated throat swab matrix. All 24 strains were correctly reported as **Strep A DETECTED** with the Xpert Xpress Strep A test.

Table 9. Analytical Reactivity (Inclusivity) of the Xpert Xpress Strep A Test

Strep A Strain ID	*emm* type	Strain
ATCC 12202	1	NCTC 8370
ATCC 12344	1	T1
ATCC 700294	1	SF370
ATCC 12383	3	D58X
ATCC 12384	3	C203
ATCC 12385	4	J17A4
ATCC 12203	6	NCTC 8709
ATCC 12352	11	T11
ATCC BAA-1065	12	MGAS 2096
ATCC BAA-1315	12	MGAS9429
ATCC 12357	18	J17C
ATCC 10403	22	T22
ATCC 12204	25	A25
ATCC 8135	27	T27
ATCC 12365	38	C107
ATCC 12370	38	C94
ATCC 700497	75	CDC-SS-1147
ATCC 700499	77	CDC-SS-1149
ATCC 700949	89	CDC-SS-1397
ATCC BAA-355	94	N/A
ATCC BAA-356	95	N/A

Strep A Strain ID	*emm* type	Strain
ATCC 14289	M protein-deficient *S. pyogenes*	C203 S
ATCC 49399	*emm* type not available	QC A62
ATCC 51339	*emm* type not available	1805

19.3 Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert Xpress Strep A test was evaluated by testing a panel of 70 potentially cross-reactive microorganisms, phylogenetically related to *Streptococcus pyogenes* and members of the throat commensal microflora (e.g., other bacteria, viruses, and yeast) with the potential to cross-react in the Xpert Xpress Strep A test. The 70 organisms tested were identified as either Gram-positive (27), Gram-negative (33), or Gram-indeterminate (3), yeast (1), and viruses (6). Streptococcus Group B, Streptococcus Group C, and Streptococcus Group G strains were also included in this study. All strains were tested in triplicate in ESwab transport medium containing simulated throat swab matrix at $\geq 10^6$ CFU/mL for bacteria and yeast and $\geq 10^5$ $TCID_{50}$/mL for viruses. All 70 organisms were reported as **Strep A NOT DETECTED** by the Xpert Xpress Strep A test (Table 10). The analytical specificity of the Xpert Xpress Strep A test was 100%.

Table 10. Analytical Specificity of the Xpert Xpress Strep A Test

Organism	Results
Acinetobacter baumannii	Strep A NOT DETECTED
Arcanobacterium haemolyticum	Strep A NOT DETECTED
Adenovirus, Type 1	Strep A NOT DETECTED
Adenovirus, Type 7	Strep A NOT DETECTED
Bacillus cereus	Strep A NOT DETECTED
Bordetella bronchiseptica	Strep A NOT DETECTED
Bordetella parapertussis	Strep A NOT DETECTED
Bordetella pertussis	Strep A NOT DETECTED
Burkholderia cepacia	Strep A NOT DETECTED
Campylobacter rectus	Strep A NOT DETECTED
Candida albicans	Strep A NOT DETECTED
Corynebacterium diphtheriae	Strep A NOT DETECTED
Corynebacterium pseudodiphtheriticum	Strep A NOT DETECTED
Cytomegalovirus AD-169	Strep A NOT DETECTED
Enterococcus faecalis	Strep A NOT DETECTED
Enterococcus faecium	Strep A NOT DETECTED
Epstein-Barr Virus 4	Strep A NOT DETECTED
Escherichia coli	Strep A NOT DETECTED
Fusobacterium necrophorum	Strep A NOT DETECTED
Haemophilus influenzae type A	Strep A NOT DETECTED
Haemophilus parahaemolyticus	Strep A NOT DETECTED
Haemophilus parainfluenzae	Strep A NOT DETECTED
Hepatitis B Virus	Strep A NOT DETECTED
Herpes Simplex Virus	Strep A NOT DETECTED

Organism	Results
Klebsiella pneumoniae	Strep A NOT DETECTED
Lactobacillus acidophilus	Strep A NOT DETECTED
Lactococcus lactis subsp. *lactis*	Strep A NOT DETECTED
Legionella jordanis	Strep A NOT DETECTED
Legionella micdadei	Strep A NOT DETECTED
Legionella pneumophila	Strep A NOT DETECTED
Listeria monocytogenes	Strep A NOT DETECTED
Moraxella catarrhalis (two strains)	Strep A NOT DETECTED
Moraxella lacunata	Strep A NOT DETECTED
Mycoplasma pneumoniae	Strep A NOT DETECTED
Neisseria gonorrhoeae	Strep A NOT DETECTED
Neisseria lactamica	Strep A NOT DETECTED
Neisseria meningitidis	Strep A NOT DETECTED
Neisseria mucosa	Strep A NOT DETECTED
Neisseria sicca	Strep A NOT DETECTED
Neisseria subflava	Strep A NOT DETECTED
Peptostreptococcus micros	Strep A NOT DETECTED
Prevotella (Bacteroides) oralis	Strep A NOT DETECTED
Proteus mirabilis	Strep A NOT DETECTED
Proteus vulgaris	Strep A NOT DETECTED
Pseudomonas aeruginosa	Strep A NOT DETECTED
Pseudomonas fluorescens	Strep A NOT DETECTED
Serratia marcescens	Strep A NOT DETECTED
Staphylococcus aureus	Strep A NOT DETECTED
Staphylococcus epidermidis	Strep A NOT DETECTED
Staphylococcus haemolyticus	Strep A NOT DETECTED
Stenotrophomonas maltophilia	Strep A NOT DETECTED
Streptococcus agalactiae	Strep A NOT DETECTED
Streptococcus anginosus	Strep A NOT DETECTED
Streptococcus bovis	Strep A NOT DETECTED
Streptococcus canis	Strep A NOT DETECTED
Streptococcus constellatus	Strep A NOT DETECTED
Streptococcus dysgalactiae	Strep A NOT DETECTED
Streptococcus equi	Strep A NOT DETECTED
Streptococcus gallolyticus	Strep A NOT DETECTED
Streptococcus intermedius	Strep A NOT DETECTED
Streptococcus mitis	Strep A NOT DETECTED

Organism	Results
Streptococcus mutans	Strep A NOT DETECTED
Streptococcus oralis	Strep A NOT DETECTED
Streptococcus pneumoniae	Strep A NOT DETECTED
Streptococcus salivarius	Strep A NOT DETECTED
Streptococcus sanguinus	Strep A NOT DETECTED
Treponema denticola	Strep A NOT DETECTED
Veillonella parvula	Strep A NOT DETECTED
Yersinia enterocolitica	Strep A NOT DETECTED

19.4 Carry-Over Contamination

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent specimen and amplicon carry-over contamination from very high titer positive samples (*S. pyogenes*) into successively run negative samples when processed in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module immediately after processing a very high titer positive sample at a concentration ≥1 X 10^6 CFU/mL in ESwab transport medium containing simulated throat swab matrix.

The testing scheme was repeated 40 times between 2 GeneXpert instruments (one module per instrument) for a total of 41 runs per instrument (20 high positive samples per instrument and 21 negative samples per instrument). There was no evidence of any carry-over contamination. All 42 negative samples were correctly reported as **Strep A NOT DETECTED**. All 40 positive samples were correctly reported as **Strep A DETECTED**.

19.5 Potentially Interfering Substances

Nine potential interfering substances that may be present in clinical throat specimens with the potential to interfere with the performance of the Xpert Xpress Strep A test were evaluated. The potentially interfering substances included blood, mucus, human saliva, sugar-containing cold and flu remedies, cough medicine, antiseptics, salt-modifying remedies, pH-modifying remedies, and food or drinks that increase salivary viscosity. The substances, active ingredients, and concentrations tested are listed in Table 11. All interfering substances with the exception of mucin, blood, and cough medicine were tested at 6.5% (v/v) in ESwab medium containing simulated throat swab matrix for negative (simulated matrix only) and Strep A-positive samples. Mucin, blood, and cough medicine were tested at 2.5% (w/v), 5.0% (v/v), and 5 mg/mL, respectively, in simulated throat swab matrix for negative (simulated matrix only) and Strep A-positive samples.

Simulated throat swab matrix in ESwab medium without interfering substances (negative and positive) were included as controls.

Positive samples were tested with interfering substances with one *S. pyogenes* strain at 3X LoD in ESwab medium containing simulated throat swab matrix.

Replicates of eight positive and negative samples with each interfering substance were evaluated in this study. Negative samples in the presence of a potentially interfering substance were tested to determine the impact on the performance of the sample processing control (SPC).

The effect of each potentially interfering substance on positive and negative samples was assessed by comparing the target cycle threshold (Ct) values generated in the presence of the potentially interfering substance to the Ct values of the controls in the absence of the potentially interfering substance.

There was no test interference in the presence of the substances at the concentrations tested in this study. All positive and negative samples were correctly identified using the Xpert Xpress Strep A test.

Table 11. Potential Interfering Substances Tested

Substance/Class	Description/Active Ingredient	Concentration Tested
Saliva	100% Human Saliva	6.5% (v/v)
Mucin	Bound sialic acid, 0.5-1.5%	2.5% (w/v)
Blood	Whole human blood	5.0% (v/v)
Antiseptic	0.092% Eucalyptol, 0.042% menthol, 0.060% methyl salicylate, 0.064% thymol	6.5% (v/v)
Cough Medicine	Dextromethorphan HBr USP 10 mg, Guaifenesin USP 200 mg	5 mg/mL
Sugar-containing cold and flu remedies	Acetaminophen 650 mg, Dextromethorphan HBr 20 mg, Doxylamine Succinate 12.5 mg, Phenylephrine HCl 10 mg	6.5% (v/v)
Salt-modifying remedies	Sodium Chloride (0.65%)	6.5% (v/v)
Foods/drinks that increase salivary viscosity	Milk	6.5% (v/v)
pH Modifying Remedies	100% Orange juice	6.5% (v/v)

19.6 Microbial Interference

An interfering microorganism study was performed to assess the inhibitory effects of commensal microorganisms in throat swab samples on the performance of the Xpert Xpress Strep A test. Twenty-seven microorganisms were tested for potential interference on Strep A detection (Table 12). The microorganisms were tested at $\geq 10^6$ CFU/mL in the presence of Strep A at 3X LoD concentration in ESwab medium containing simulated throat swab matrix. The results showed that the presence of the tested microorganisms did not interfere with the detection of Strep A target DNA.

Table 12. Commensal Microorganisms Tested

Organism
Acinetobacter baumannii
Candida albicans
Enterococcus faecalis
Fusobacterium necrophorum
*Haemophilus influenzae*type A
Lactobacillus acidophilus
Neisseria lactamica
Peptostreptococcus micros
Prevotella (Bacteroides) oralis
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus bovis
Streptococcus canis
Streptococcus constellatus

Organism
Streptococcus dysgalactiae
Streptococcus equi
Streptococcus gallolyticus
Streptococcus intermedius
Streptococcus mitis
Streptococcus mutans
Streptococcus oralis
Streptococcus pneumoniae
Streptococcus salivarius
Streptococcus sanguinus
Treponema denticola
Veillonella parvula

20 References

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21 Cepheid Headquarters Locations

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22 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

United States Technical Support

Telephone: + 1 888 838 3222
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Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/support/contact-us.

23 Table of Symbols

Symbol	Meaning
	Catalog number
	CE marking – European Conformity
	*In vitro* diagnostic medical device
	Authorized Representative in the European Community
	Do not reuse
	Batch code
	Consult instructions for use
	Warning
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
	Control
	Expiration date
	Temperature limitation
	Biological risks
	Authorized Representative in Switzerland
	Importer

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IVD CE

24 Revision History

Description of Changes: 301-6569, Rev. E to Rev. F

Purpose: To add a new pipette procedure, CH REP and Importer information, and make continuous improvement updates

Section	Description of Change
Preparing the Cartridge	Updated the Preparing the Cartridge section to include steps for two types of pipettes.
Table of Symbols	Added CH REP and Importer symbols and definitions to Table of Symbols. Added CH REP and Importer information with Switzerland address.
Revision History	Updated revision history table.