

For In Vitro Diagnostic Use

CLIA Complexity: WAIVED*

*For US Only

For use with throat swab specimens.

A Certificate of Waiver is required to perform this test in a CLIA Waived setting. To obtain CLIA waiver information and a Certificate of Waiver, please contact your state health department. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA.

Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification.



Operator should carefully read this entire package insert before using the **cobas®** Strep A assay. Follow all instructions to ensure correct assay performance.

NOTE: For the remainder of this Package Insert, the **cobas®** Liat® Analyzer may be referred to as the Analyzer and the **cobas®** Liat® System may be referred to as the System.

I. Intended Use

The **cobas®** Strep A nucleic acid test for use on the **cobas®** Liat® System (**cobas®** Strep A) 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 with signs and symptoms of pharyngitis.

The **cobas®** Strep A assay utilizes nucleic acid purification and polymerase chain reaction (PCR) technology to detect *Streptococcus pyogenes* by targeting a segment of the *Streptococcus pyogenes* genome.

II. Summary and Explanation

Strep A causes a wide range of human infections, including pharyngitis, sinusitis, lymphadenitis, pyoderma, endocarditis, meningitis, septicemia, tonsillitis, impetigo, and upper respiratory tract infections. Accurate diagnosis of the pathogen is necessary to properly treat the disease using appropriate antibiotic therapy. If left untreated, Strep A infections can lead to serious, sometimes life-threatening conditions, such as rheumatic fever, scarlet fever, peritonsillar abscess, glomerulonephritis, necrotizing fasciitis, and streptococcal toxic shock syndrome.

Conventional methods for detection of Strep A involve 24-48 hour culture of throat swab specimens followed by identification using physiological, biochemical, or immunological traits. Faster results can be obtained using rapid antigen detection tests (RADT), but these tests generally have lower sensitivity. Reported sensitivities for RADTs vary widely and a review of nearly 100 studies reported an average sensitivity of 86% and specificity of 95%.¹ The **cobas®** Strep A assay is a rapid and easy to use molecular test that provides improved sensitivity over RADT, eliminating the need for confirmatory culture testing in adults and children.

III. Principle of the Procedure

The **cobas®** Strep A assay is an automated *in vitro* diagnostic test for the qualitative detection of Strep A DNA in throat swab specimens. The sample-to-result time is ~15 minutes.

The assay is performed on the **cobas®** Liat® System. The System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples using real-time PCR. The assay targets a well-conserved region of the Strep A genome. An Internal Process Control (IPC) is also included. The IPC is present to control for adequate processing of the target bacteria through all steps of the assay process and to monitor the presence of inhibitors in the sample preparation and PCR.

The **cobas®** Liat® Analyzer consists of an instrument with integrated software for running tests and analyzing the results. The System consists of the Analyzer and a single-use disposable **cobas®** Strep A assay tube that contains the nucleic acid purification and PCR reagents, and hosts the sample preparation and PCR processes. Other than adding the sample to the **cobas®** Strep A assay tube, no reagent preparation or additional steps are required. Because each **cobas®** Strep A assay tube is self-contained, the risk of cross-contamination between samples is reduced.

IV. Reagents and Instruments

A. Materials Provided



The **cobas®** Strep A assay tube pack (Cat # 07341911190) contains sufficient reagents to process 20 specimens or quality control samples. The pack contains 20 sets of a **cobas®** Strep A assay tube and a transfer pipette. A Package Insert Barcode Card (Cat # 07997060001) with a lot-specific barcode is also included.

B. Equipment

cobas® Liat® Analyzer, Cat # 07341920190

C. Materials Available but Not Provided

- Liquid Amies Swab Collection Kits, each kit containing:

Liquid Amies Medium, 1 mL

Collection Swab

Acceptable collection kits include Copan ESwab™ Collection Kit, Becton Dickinson Liquid Amies Elution Swab (ESwab) Collection and Transport System (BD Cat # 220245, Copan Cat # 480C). Some products may not be available in all countries

- CONTROL** **cobas®** Strep A Quality Control Kit, Cat # 07402678190, containing:

cobas® Strep A Positive Control (Positive Control), Cat # 07758502001

cobas® Strep A Dilution Amies (Dilution Amies), Cat # 07763808001

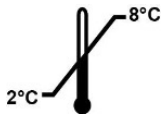
Transfer Pipette, Cat # 07898541001

Control Kit Barcode Card, Cat # 07945272001

Negative Control Barcode Label, Cat # 07945264001

Positive Control Barcode Label, Cat # 07945256001

V. Storage and Handling



- Store the **cobas**® Strep A assay tube and the **cobas**® Strep A Quality Control at 2 - 8°C.
- Do not use kits or reagents beyond their expiration dates.
- Do not open individual assay tube packaging until you are ready to perform testing.

VI. Warnings and Precautions



- Treat all biological specimens, including used **cobas**® Strep A assay tubes and pipettes, as if capable of transmitting infectious agents. Because it is often impossible to know which specimens might be infectious, all biological specimens should be treated with universal precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention and the Clinical and Laboratory Standards Institute.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not use a damaged **cobas**® Strep A assay tube. Do not use a **cobas**® Strep A assay tube that has been dropped after removal from its foil pouch.
- Do not open individual assay tube packaging until you are ready to perform testing.



- Each single-use **cobas**® Strep A assay tube is used to process one test. Do not reuse a spent **cobas**® Strep A assay tube. If a **cobas**® Strep A assay tube is not housed in a sleeve, or if the assay tube sample compartment already contains a liquid, this assay tube has been spent; do NOT use such assay tubes.
- Do not puncture the **cobas**® Strep A assay tube or the seal at the bottom of the sample compartment. If either is damaged while adding the sample, discard both the **cobas**® Strep A assay tube and the transfer pipette and repeat the test with a new transfer pipette and **cobas**® Strep A assay tube.
- Do not open the cap of the **cobas**® Strep A assay tube during or after the run on the Analyzer.
- Dispose of a used **cobas**® Strep A assay tube, pipette and specimen tubes according to your institution's safety guidelines for hazardous material.
- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- Due to the high sensitivity of the assays run on the Analyzer, contamination of the work area with previous positive samples may cause false positive results. Handle samples according to standard laboratory practices. Clean instruments and surrounding surfaces according to instructions provided in the cleaning section of the instrument User Manual using 5-10% bleach solution followed by isopropanol.
- Sample collection should be performed by specifically trained personnel using the recommended swabs. Inadequate or inappropriate sample collection, storage, and transport may yield false test results.
- Use only the transfer pipettes contained in the **cobas**® Strep A assay kit and **cobas**® Strep A Quality Control kit. Use of alternative transfer pipettes may lead to invalid results.

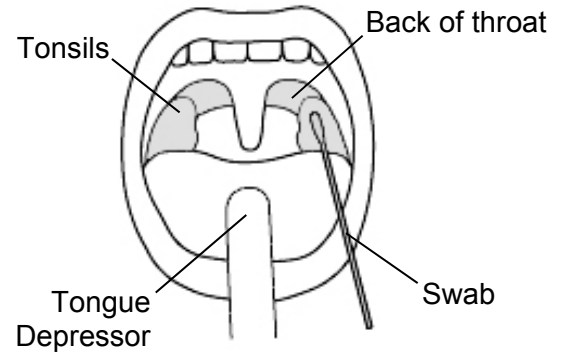
VII. Specimen Collection, Handling, and Storage

A. Throat Swab Collection

Materials: Liquid Amies Collection and Transport System, containing a sterile swab and a tube filled with 1 mL of Amies liquid transport medium. Do NOT use cotton or calcium alginate swabs, or swabs with wood shafts. After sample collection, place the swab into 1 mL of Amies transport medium.

Procedure:

1. Place the patient in a seated position facing the light.
2. Remove the swab from the protective packaging.
3. Instruct patient to open mouth as wide as possible. If needed, use tongue depressor to press the tongue downward to the floor of the mouth so that the throat can be seen.
4. Rub the swab tip up and down the back of the throat, the tonsils, and against any white patches in the tonsillar area. Remove swab from mouth.



Throat Swab Area

Note: Do NOT touch the tongue, cheeks, teeth, or gums with the swab.

5. Insert the swab into the Amies transport media tube. Swirl the swab against the inside wall of the tube 3 times. Break the swab shaft and leave the swab in the tube. Attach the cap securely.

B. Specimen Handling & Storage

- After the oropharyngeal swab sample from the patient has been collected in Amies Transport Medium, the specimens should be immediately added to the **cobas® Strep A** assay tube and the **cobas® Liat®** assay run on the **cobas® Liat®** Analyzer as soon as possible.
- If specimens cannot be immediately added to the **cobas® Strep A** assay tube for testing, oropharyngeal swab specimens collected in Amies Transport Medium are stable for up to 48 hours in refrigeration (2-8°C, preferred) or at room temperature (20-25°C).
- Refrigeration at 2-8°C is recommended for specimen transportation, Ensure all applicable regulations for the transport of biological agents are met.

VIII. Assay Procedure

Note:

- Consult the System User Guide (if software version 3.2 or lower, Operator's Manual) on the detailed operations of the cobas® Liat® System.
- To avoid error and sample cross contamination, change gloves between samples and work on one sample at a time. DO NOT add multiple samples into multiple cobas® Strep A assay tubes at the same time.

A. Add cobas® Strep A assay tube lot

Before using a new lot of cobas® Strep A assay tubes, the Add Lot procedure must be performed on the Analyzer to validate the cobas® Strep A assay tube lot at your site. The procedure comprises running a negative and a positive control sample.

The Analyzer will prompt you to add the lot if you try to run an assay from a new un-validated lot. You can also compare the lot number on the cobas® Strep A assay tube against the list of validated assay tube lots in step 1 below to check if the lot was previously added.



Helpful Hint: 4 barcodes are needed for this procedure. Make sure to scan the right barcode when prompted by the Analyzer.

- Package Insert Barcode: on the Package Insert Barcode Card contained in this cobas® Strep A assay tube pack. This barcode is lot-specific; match the lot number next to the barcode with the lot number on the cobas® Strep A assay tubes.
- cobas® Strep A assay tube barcode: on the cobas® Strep A assay tube sleeve.
- Negative Control Barcode: on the Control Kit Barcode Card contained in the QC Kit. Match the lot number next to the barcode with the lot number on the Dilution Amies tube.
- Positive Control Barcode: on the Control Kit Barcode Card contained in the QC Kit. Match the lot number next to the barcode with the lot number on the Positive Control tube.

Materials:

- From cobas® Strep A assay tube pack:
 - < Package Insert Barcode Card
 - < 2 cobas® Strep A assay tubes
- From the cobas® Strep A Quality Control (QC) Kit:
 - < Negative Control: Negative Control Barcode, (see Control Kit Barcode Card), 1 Dilution Amies tube (used as the negative control sample)
 - < Positive Control: Positive Control Barcode, (see Control Kit Barcode Card), 1 Positive Control tube, 1 Dilution Amies tube (used to mix with the positive control), 1 transfer pipette

Procedure:

1. Prepare and test Negative Control sample
 - a. Obtain:

Package Insert

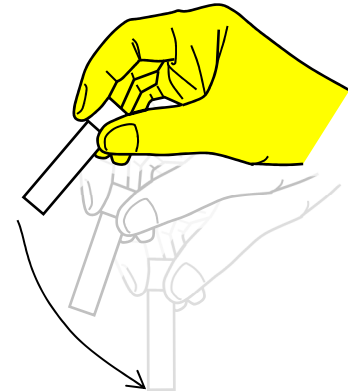
cobas® Strep A

Nucleic acid test for use on the cobas® Liat® System



- < Package Insert Barcode on the Package Insert Barcode Card contained in cobas® Strep A assay tube pack
- < Negative Control Barcode on the Control Kit Barcode Card
- < 1 Dilution Amies tube (used as the negative control sample)
- < 1 cobas® Strep A assay tube from this lot

- b. Select **Assay Menu** on the main menu of an Analyzer.
- c. Select **New Lot** at the bottom of the list.
- d. Select **Scan** and scan the Package Insert Barcode on the Package Insert Barcode Card from the cobas® Strep A assay tube pack.



- e. Select **Scan** and scan the Negative Control Barcode on the Control Kit Barcode Card. The Analyzer will prompt “Add negative control & scan tube ID”.
- f. Take a Dilution Amies tube from the QC Kit; this is used as the negative control sample. Hold the Dilution Amies tube by the tube cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion. Visually check that the Dilution Amies has pooled at the bottom of the tube. If not, repeat the shake down procedure.

Shake down the contents in the tube using a quick, sharp, downward wrist motion.

- g. Using the Dilution Amies as sample, run the assay following the Running cobas® Strep A assay procedure, step B.2.c-i (Add Sample) and B.3 (Insert cobas® Strep A assay tube).
- h. If “*Negative control result accepted.*” is displayed at the end of the run, select **Confirm** (If software version 3.2 or lower, select **OK**). If the result is rejected, repeat the negative control run (step A.1). If repeated control runs do not produce the expected results, contact your Roche Service Representative.
- i. Select **Confirm** (If software version 3.2 or lower, select **Back**).

2. Prepare Positive Control sample

- a. Take the following from the QC kit:

- < 1 transfer pipette
- < 1 Positive Control tube, containing a pellet of dried chemically-inactivated Strep A at the bottom of the tube
- < 1 Dilution Amies tube, containing a unit dose of Amies medium to be mixed with the positive control

- b. Hold the Dilution Amies tube by the tube cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion. Visually check that the liquid has pooled at the bottom of the tube. If not, repeat the shake down procedure.

- c. Using a transfer pipette, transfer the liquid from the Dilution Amies tube into the Positive Control tube:

- i. Check that the Positive Control pellet is at the bottom of the tube prior to addition of the Dilution Amies. Do not use the Positive Control if a pellet is not visible prior to rehydration.



- ii. Squeeze the bulb of pipette until the bulb is fully flat. While holding the bulb fully flat, insert the pipette tip into the liquid just below the liquid surface in the Dilution Amies tube.
- iii. Slowly release the bulb completely while keeping the pipette tip below the liquid surface. You will see the liquid Amies medium rising into the pipette. After releasing the bulb completely, withdraw the pipette from the Dilution Amies tube. It is normal for a small volume of liquid to remain in the Dilution Amies tube after the pipette bulb is fully released.



- iv. Insert pipette into the Positive Control tube until the pipette tip is at the bottom of the tube.
- v. Slowly squeeze the bulb to empty the contents of pipette. Do not release the pipette bulb.
- vi. While still squeezing the pipette bulb, withdraw the pipette from the tube. Dispose of the transfer pipette according to your institution's guidelines for safe disposal of hazardous material. Do not reuse transfer pipettes.
- vii. Cap the Positive Control tube. Hold the Positive Control tube by the cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion.

- d. Let the Positive Control tube sit for 5 minutes. During this time, the dried positive control material within the tube will begin to dissolve.

3. Test Positive Control sample

a. Obtain:

- < Positive Control Barcode on the Control Kit Barcode Card
- < 1 cobas® Strep A assay tube from this lot

- b. On the Analyzer used for the Negative Control test, select **Scan** and scan the Positive Control Barcode on the Control Kit Barcode Card. The Analyzer will prompt “*Add positive control & scan tube ID*”.

- c. After the Positive Control tube from step A.2 has sat for 5 minutes, use the transfer pipette from the cobas® Strep A assay tube pouch to slowly pipette the sample up and down 10 times to dissolve and mix the positive control sample.

- d. Using the Positive Control as sample, run the assay following the Running cobas® Strep A assay procedure, step B.2.c-i (Add Sample) and B.3 (Insert cobas® Strep A assay tube).

- e. If “*Positive control result accepted.*” is displayed at the end of the run, select **Confirm** (If software version 3.2 or lower, select **OK**), select **Back** to return to the assay menu. If the result is rejected, repeat the positive control run (steps A.2 and A.3). If repeated control runs do not produce the expected results, contact your Roche Service Representative.

After Add Lot is completed on one Analyzer use the Advanced Tools (If software version 3.2 or lower, Advanced Tools Key) to transfer the lot information to the other Analyzers at your site. This allows the other Analyzers to use this cobas® Strep A assay tube lot without performing Add Lot on each Analyzer. Consult the software specific Advanced Tools guide for details of operation.

B. Running cobas® Strep A assay

The recommended environmental operating conditions for the cobas® Strep A assay are 15-32°C, 15-80% relative humidity, and ≤2,000m (6,500 feet) above sea level.

Materials: **cobas®** Strep A assay tube from a lot that has been added to the Analyzer. See section A for Add **cobas®** Strep A assay tube Lot instructions.

Procedure:

1. Scan Barcode

Tear open the foil packaging of the **cobas®** Strep A assay tube and remove the assay tube and the transfer pipette.

- a. Select **Run Assay** on the main menu using the touch screen or function button.
- b. Select **Scan** and scan the **cobas®** Strep A assay tube barcode on the assay tube sleeve by placing the assay tube on the table and sliding the assay tube towards the Analyzer until the red scan light is over the entire barcode.
- c. Select **Scan** again and scan the Patient or Sample barcode, or select **Enter** and type in a Sample or Patient ID.
- d. If patient verification is activated, the Analyzer will display the status of verification.
 - i. If patient verification is successful, the Analyzer may prompt confirmation of entered information before proceeding with running the assay.
 - ii. If patient verification fails, the Analyzer will display notification that verification failed:
 - < And may require acknowledgement before proceeding with running the assay or
 - < You cannot proceed with running the assay. Contact your lab administrator if this occurs.
- e. The Analyzer will prompt “*Add sample & rescan tube ID.*” (If software version 3.2 or lower, “*Add throat swab sample & rescan tube ID.*”).

2. Add Sample

Use the transfer pipette to load ~200 µL of sample into the **cobas®** Strep A assay tube. You do not need to measure the sample volume; the Analyzer will adjust the sample volume if more sample was loaded, or output an error if not enough sample was loaded.

- a. Vigorously shake the throat swab sample tube for 5 seconds to evenly disperse the patient specimen in the liquid medium.

Note: Failure to disperse the patient specimen thoroughly by vigorous shaking could adversely affect test performance and lead to the generation of false negative results.

- b. Unscrew the sample tube cap. Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube. Avoid lifting the swab completely out of the sample tube.



- c. Obtain the transfer pipette from the **cobas®** Liat® assay tube pouch. Firmly squeeze the bulb of the pipette until the bulb is fully flat.
- d. While holding the pipette bulb fully flat, insert the pipette tip into the sample just below the liquid surface.
- e. Slowly release the bulb while keeping the pipette tip below the liquid surface. This will draw up ~200 µL of sample into the pipette. After releasing the bulb completely, withdraw the pipette from the sample.
- f. Unscrew the cap from the **cobas®** Strep A assay tube.

- g. While watching through the viewing window in the sleeve, carefully insert the pipette into the **cobas®** Strep A assay tube. Place the pipette tip near the bottom of the sample compartment. Do not puncture the assay tube or the seal at the bottom.

Note: If you do puncture the seal at the bottom of the sample compartment, discard both the **cobas®** Strep A assay tube and the transfer pipette according to your institution's guidelines for safe disposal of hazardous material and repeat the test starting at Step 2.b. with a new transfer pipette and **cobas®** Strep A assay tube.



- h. Slowly squeeze the bulb to empty the contents of the pipette into the **cobas®** Strep A assay tube. Do not release the pipette bulb.
- i. While still squeezing the pipette bulb, withdraw the pipette from the assay tube. Screw the cap back on the **cobas®** Strep A assay tube. Dispose of the transfer pipette according to your institution's guidelines for safe disposal of hazardous material. Do not reuse transfer pipettes.

Note: Start the **cobas® Liat®** assay run on the Analyzer as soon as possible, but no later than 4 hours after adding the sample to the **cobas®** Strep A assay tube.

3. Insert **cobas®** Strep A assay tube

- a. Select **Scan** and re-scan the **cobas®** Strep A assay tube barcode. The assay tube entry door on top of the Analyzer will open automatically.
- b. Remove the **cobas®** Strep A assay tube sleeve.
- c. Immediately insert the **cobas®** Strep A assay tube into the Analyzer until the assay tube clicks into place. The **cobas®** Strep A assay tube only fits in one way. If the assay tube is not inserted by the time the door closes, re-scan the **cobas®** Strep A assay tube barcode (step 3a) and insert the **cobas®** Strep A assay tube again. Once the **cobas®** Strep A assay tube is properly inserted, the Analyzer will close the door automatically and begin the test.

4. View Result

During the test, the Analyzer displays the running status and estimated time remaining. Once the test is complete, the Analyzer displays the message, "Remove tube slowly and carefully." and opens the assay tube entry door automatically.

- a. Lift the **cobas®** Strep A assay tube out of the Analyzer.
- b. Select **Report** to see the Result Report.
- c. Select **Print** to print the report (if applicable).

Select **Back**, and then **Main** to return to the main menu for the next test.

C. Viewing and Interpreting Results

The Analyzer reports results as Strep A "Detected", "Not Detected", "Indeterminate", or "Assay Invalid".

The manual data interpretation and reviewing PCR curves that you may be familiar with from other systems is no longer required. The Analyzer automatically interprets the results from measured fluorescent signals. Embedded calculation algorithms determine the PCR cycle threshold (Ct) and evaluate the Ct and fluorescence endpoint against the valid range to generate a positive or negative PCR result.

Additionally, pattern recognition algorithms inspect the PCR curves to determine if the curve pattern is within specification or abnormal. If Strep A is detected but its PCR curve is determined to be abnormal, the result is called “Strep A Indeterminate”.

Like the Strep A target, the IPC target is also evaluated in every assay run. In the case that Strep A target is not detected, the IPC target must be detected for the result to be called “Not Detected”; if the IPC is also not detected or if the IPC PCR curve is abnormal, the result is called “Assay Invalid” (If software version 3.2 or lower, “Assay Invalid. Repeat Assay.”). In some cases, high concentration of Strep A may inhibit the amplification of IPC; as such, IPC is not taken into consideration when Strep A is detected.

The table below shows the “Report Results” and the corresponding interpretation.

Interpretation of Results

Report Results	Interpretation
Strep A Not Detected	Negative test for Strep A (no Strep A DNA detected)
Strep A Detected	Positive test for Strep A (Strep A DNA present)
Strep A Indeterminate	Presence or absence of Strep A cannot be determined. Repeat assay with same sample or, if possible, new sample.
Assay Invalid (If software version 3.2 or lower, “Assay Invalid. Repeat Assay.”)	Presence or absence of Strep A cannot be determined. Repeat assay with same sample or, if possible, new sample.
[Error] Assay Aborted	Presence or absence of Strep A cannot be determined. Repeat assay with same sample or, if possible, new sample.

The following failure codes can be displayed in the result report upon completion of a run.

Failure Codes	Sample and External Controls	Negative Control (Add Lot)	Positive Control (Add Lot)
r0*	IPC Not Detected or Indeterminate. Repeat Run	IPC Not Detected or Indeterminate. Repeat Run	IPC Not Detected or Indeterminate. Repeat Run
r1			
r2			
r3			
r4			
x4	Target Indeterminate. Repeat Run	N/A	N/A
FP	N/A	Target Detected or Indeterminate. Repeat Run	N/A
b1	N/A	N/A	Target Not Detected or Indeterminate. Repeat Run
b2			
b3			
b4			

Note*: Failure code r0 goes not appear for Positive Control (Add Lot).

In addition, Pattern Codes may appear. For any questions, please contact your Roche Service representative.

D. Reasons to Repeat the Assay

If the test result is “Indeterminate” or “Invalid”, repeat the assay with the same patient specimen, or if possible, collect a new specimen from the patient and repeat the assay using the new specimen. Specimens that have repeat “Indeterminate” or “Invalid” results should be sent to a laboratory for confirmatory testing by an alternative method.

If an “Error” is reported by the Analyzer and/or the assay is aborted, repeat the test with the same patient specimen, or if possible, collect a new specimen from the patient and repeat the assay using the new specimen. Contact your Roche Service Representative if repeat “Errors” are reported.

E. Quality Control CONTROL

Internal Process Control (IPC): is a chemically-inactivated bacterium that is included in each cobas® Strep A assay tube to verify adequate processing of the Strep A. The IPC verifies that sample purification of Strep A has occurred and verifies that the specimen processing is adequate. Additionally, this control detects potential specimen-associated inhibition in the sample preparation or the PCR. The IPC should be positive in a negative sample and can be negative or positive in a Strep A positive sample. The IPC is valid if it meets the acceptance criteria.

External Controls: provide additional quality control to demonstrate positive or negative assay results using the System and cobas® Strep A assay tube. External Controls are run during the Add cobas® Strep A assay tube lot procedure (section A). Additional External Controls should be tested in accordance with local, state, federal and/or accrediting organization requirements as applicable. If testing either the Positive or Negative Control does not produce the expected results, repeat the affected control run with a fresh External Control. If repeated control runs do not produce the expected results, do not test patient specimens and contact your Roche Service Representative.

*Negative Control**Materials:*

- < 1 cobas® Strep A assay tube
- < From QC Kit: 1 Dilution Amies tube (used as the negative control sample), and Negative Control Barcode on the Control Kit Barcode Card

Procedure:

The Dilution Amies is used as the sample for the Negative Control run.

1. Take a Dilution Amies tube from the QC Kit.
2. Hold the Dilution Amies tube by the tube cap and shake down the liquid in the tube using a quick, sharp, downward wrist motion (as if shaking down a mercury thermometer). Visually check that the Dilution Amies has pooled at the bottom of the tube. If not, repeat the shake down procedure.
3. Using the Dilution Amies as sample, run the assay following the Running cobas® Strep A assay procedure (section B, skip step B.2.a-b). Scan the Negative Control Barcode on the Control Kit Barcode Card as the Sample ID.
4. View the Results Report by touching or clicking **Report** after the completion of the assay. The Report Result must be "*Strep A Not Detected*" for the negative control to pass.

Package Insert

cobas® Strep A

Nucleic acid test for use on the cobas® Liat® System



Positive Control

Materials:

- < 1 cobas® Strep A assay tube
- < From QC Kit: 1 Positive Control tube, 1 Dilution Amies tube, 1 transfer pipette, and Positive Control Barcode on the Control Kit Barcode Card

Procedure:

The Positive Control is a unit-dose of dried chemically-inactivated Strep A. Follow the directions below to dissolve the positive control in Dilution Amies and run it on the cobas® Strep A assay.

1. Follow step A.2. of the Add cobas® Strep A assay tube Lot procedure to prepare the Positive Control sample.
2. After the Positive Control tube from step A.2 has sat for 5 minutes, use a transfer pipette from the cobas® Strep A assay tube package to slowly pipette the sample up and down 10 times to dissolve and mix the positive control sample.
3. Using the Positive Control as sample, run the assay following the Running cobas® Strep A assay procedure (section B, skip step B.2.a-b). Scan the Positive Control Barcode on the Control Kit Barcode Card as the Sample ID.
4. View the Results Report by touching or clicking **Report** after the completion of the assay. The Report Result must be "*Strep A Detected*" for the positive control to pass.

IX. Limitations

- The performance of the cobas® Strep A assay was evaluated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- As with other tests, negative results do not preclude Strep A infection and should not be used as the sole basis for treatment or other patient management decisions. Results from the cobas® Strep A assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- Additional follow-up testing by culture is required if the cobas® Strep A assay result is negative and clinical symptoms persist, or in the event of an outbreak of acute rheumatic fever (ARF).
- Analyte targets (bacterial nucleic acid) may persist *in vivo*, independent of pathogen viability. Detection of analyte target does not imply that the corresponding pathogen is infectious, or is the causative agent for clinical symptoms.
- False negative results may occur if a specimen is improperly collected, transported or handled; or if inadequate numbers of organisms are present in the specimen.
- This test has not been evaluated for patients without signs and symptoms of Strep A infection.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- Cross-reactivity with organisms other than those tested can lead to erroneous results.
- This test cannot detect pathogens other than *Streptococcus pyogenes*.
- Good laboratory practices and changing gloves between handling patient specimens are recommended to avoid contamination of specimens or reagents.

X. Expected Values

In multicenter clinical studies for the cobas® Strep A assay, 570 throat swab specimens were analyzed. The number and percentage of positive cases per specified age group and gender, as determined by the cobas® Strep A assay, are presented in the table below:

Age	# Samples	% Samples	# Positive	% Positive
≤5 years	141	24.7%	59	41.8%
6-21 years	401	70.4%	130	32.4%
22-59 years	25	4.4%	4	16.0%
≥60 years	3	0.5%	0	0.0%
Total	570	100%	193	33.9%

Sex	# Samples	% Samples	# Positive	% Positive
M	268	47.0%	100	37.3%
F	302	53.0%	93	30.8%
Total	570	100%	193	33.9%

XI. Performance Characteristics

A. Clinical Performance

The **cobas®** Strep A assay was evaluated in December 2013 to April 2014 by 6 clinical sites, including 5 CLIA waived sites, representing geographically distinct regions throughout the United States. Clinical specimens were collected from patients with symptom characteristics of pharyngitis. Performance characteristics of the assay were determined by comparison to culture and latex agglutination for Strep A typing. Discordant results were investigated using PCR and bi-directional sequencing based on published methods.

The tables below summarize the clinical performance of the **cobas®** Strep A assay. Assay sensitivity was 98.3% (95% CI: 95.0% – 99.4%) and assay specificity was 94.2% (95% CI: 91.5% – 96.1%).

Strep A		Comparative Culture		Total
		Positive	Negative	
cobas® Liat®	Positive	170	23 ^a	193
	Negative	3 ^b	374	377
Total		173	397	570

^a Of 23 **cobas®** Liat® positive, culture negative specimens, all 23 were Strep A positive by PCR/sequencing.

^b Of 3 **cobas®** Liat® negative, culture positive specimens, 3 were Strep A positive by PCR/sequencing. All 3 were also positive when the **cobas®** Liat® assay was repeated using residual specimen after culture.

	#	%	95% CI
Sensitivity	170 / 173	98.3%	95.0% - 99.4%
Specificity	374 / 397	94.2%	91.5% - 96.1%
Accuracy	544 / 570	95.4%	93.4% - 96.9%
Prevalence	173 / 570	30.4%	26.7% - 34.2%
PPV	170 / 193	88.1%	82.8% - 91.9%
NPV	374 / 377	99.2%	97.7% - 99.7%
Invalid ^c	7 / 577	1.2%	0.6% - 2.5%

^c Rate includes all Invalid, Indeterminate and Assay Aborted results. In all cases, re-test of the same specimens gave a valid result.

B. Reproducibility

A Reproducibility Study was performed to assess the total variability of the **cobas®** Strep A assay across operators, study sites, testing days, Analyzers, and **cobas®** Strep A assay tube lots. The **cobas®** Strep A assay was evaluated at 3 sites. Two operators at each of the 3 sites tested a 4 member reproducibility panel in triplicate on 5 different days, for a total of 360 runs (4 panel members × 3 replicates × 2 operators × 5 days × 3 sites). Nine (9) Analyzers and 3 **cobas®** Strep A assay tube lots were used. The reproducibility panel comprises a negative, a high negative (C5: 0.03x LOD), a low positive (C95: 1x LOD) and a medium positive (C100: 3x LOD) Strep A sample.

The tables below show the reproducibility results for Strep A and the Internal Process Control (IPC). Total percent agreement was 99.7% for Strep A and 100% for IPC. For the negative and high negative samples, the expected result was negative; for the low positive and medium positive samples the expected result was positive.

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Strep A Reproducibility Results

Site	1					2					3					Total						
	Sample	Agree ment	Ct		Amp		Agree ment	Ct		Amp		Agree ment	Ct		Amp		Agree ment	Ct		Amp		95% CI
			Avg	%CV	Avg	%CV		Avg	%CV	Avg	%CV		Avg	%CV	Avg	%CV		Avg	%CV			
Neg.	30/30	-	-	-	-	30/30	-	-	-	-	30/30	-	-	-	-	90/90 (100%)	-	-	-	-	95.9% - 100.0%	
C5	30/30	-	-	-	-	30/30	-	-	-	-	30/30	-	-	-	-	90/90 (100%)	-	-	-	-	95.9% - 100.0%	
C95	29/30	29.4	2%	1.8	29%	30/30	29.8	4%	1.5	44%	30/30	29.2	3%	1.8	32%	89/90 (99%)	29.5	3%	1.7	35%	94.0% - 99.8%	
C100	30/30	27.2	2%	3.2	10%	30/30	27.9	2%	2.8	14%	30/30	26.8	2%	3.2	8%	90/90 (100%)	27.3	3%	3.0	12%	95.9% - 100.0%	
Total Agree- ment	119 / 120 (99.2%)					120 / 120 (100%)					120 / 120 (100%)					359 / 360 (99.7%)					98.4%- 100.0%	

Amp = Endpoint fluorescence value

IPC Reproducibility Results

Site	1					2					3					Total						
	Sample	Agree ment	Ct		Amp		Agree ment	Ct		Amp		Agree ment	Ct		Amp		Agree ment	Ct		Amp		95% CI
			Avg	%CV	Avg	%CV		Avg	%CV	Avg	%CV		Avg	%CV	Avg	%CV		Avg	%CV			
Neg.	30/30	29.0	2%	2.9	13%	30/30	29.0	2%	2.9	12%	30/30	29.1	2%	2.8	12%	90/90 (100%)	29.0	2%	2.9	13%	95.9% - 100.0%	
C5	30/30	28.8	2%	3.0	13%	30/30	29.1	2%	2.9	15%	30/30	29.1	2%	2.7	16%	90/90 (100%)	29.0	2%	2.9	15%	95.9% - 100.0%	
C95	30/30	28.9	2%	3.0	11%	30/30	28.8	2%	2.9	10%	30/30	29.1	1%	2.6	10%	90/90 (100%)	28.9	2%	2.8	12%	95.9% - 100.0%	
C100	30/30	28.5	2%	2.7	12%	30/30	28.8	2%	2.7	11%	30/30	28.7	2%	2.2	16%	90/90 (100%)	28.7	2%	2.5	15%	95.9% - 100.0%	
Total Agree- ment	120 / 120 (100%)					120 / 120 (100%)					120 / 120 (100%)					360 / 360 (100%)					98.9- 100.0%	

Amp = Endpoint fluorescence value

C. Limit of Detection

The Limit of Detection (LOD) of the **cobas®** Strep A assay was determined by limiting dilution studies using titered bacteria of 4 Strep A strains. The bacteria were spiked into throat swab sample matrix, and then tested using the **cobas®** Strep A assay. The LOD was determined as the lowest bacterial concentration that was detected $\geq 95\%$ of the time (i.e. at least 19 out of 20 replicates tested positive). The **cobas®** Strep A assay detected all strains tested, with an LOD in the range of 5 - 20 CFU/mL, or 1 - 4 CFU/test.

Strain	LOD	
	CFU/mL	CFU/test
ATCC BAA-946	5	1
ATCC BAA-1066	10	2
ATCC 12370	10	2
ATCC 700294	20	4

D. Analytical Reactivity

A Reactivity Study was performed to evaluate the ability of the **cobas®** Strep A assay to detect Strep A strains representing temporal and geographical diversity. In addition to those strains tested in LOD study, the **cobas®** Strep A assay was evaluated for reactivity with 5 Strep A strains at 20 - 80 CFU/mL, or 4 - 16 CFU/test. The bacteria were spiked into throat swab sample matrix, and then tested using the **cobas®** Strep A assay. The assay detected all strains tested.

Strain	Test Concentration		Strep A Result
	CFU/mL	CFU/test	
ATCC 700497	20	4	+
ATCC 700949	20	4	+
ATCC 700499	40	8	+
ATCC 21548	40	8	+
ATCC 10403	80	16	+

E. Cross Reactivity

A Cross-reactivity Study was performed to evaluate the potential of the **cobas®** Strep A assay to cross-react with other microorganisms that may be present in throat swab samples. The **cobas®** Strep A assay was evaluated against a panel of 72 microorganisms. Bacteria were tested at $\geq 10^6$ CFU/mL. Viruses were tested at $\geq 10^5$ TCID₅₀/mL or the highest available concentration. The **cobas®** Strep A assay showed no cross reactivity with the tested microorganisms.

Microorganism	Test Concentration	Strep A Result
<i>Acinetobacter baumannii</i>	1.25×10 ⁶ CFU/mL	-
<i>Arcanobacterium haemolyticum</i>	4.40×10 ⁶ CFU/mL	-
<i>Bacillus cereus</i>	2.90×10 ⁶ CFU/mL	-
<i>Bacteroides oralis</i>	1.55×10 ⁶ CFU/mL	-
<i>Bordetella bronchiseptica</i>	1.25×10 ⁶ CFU/mL	-
<i>Bordetella parapertussis</i>	1.25×10 ⁶ CFU/mL	-
<i>Bordetella pertussis</i>	1.25×10 ⁶ CFU/mL	-
<i>Burkholderia cepacia</i>	1.25×10 ⁶ CFU/mL	-
<i>Campylobacter rectus</i>	1.45×10 ⁶ CFU/mL	-
<i>Candida albicans</i>	1.25×10 ⁶ CFU/mL	-
<i>Chlamydia pneumoniae</i>	1.40×10 ⁵ TCID ₅₀ /mL	-
<i>Chlamydia trachomatis</i>	1.25×10 ⁶ EB/mL	-
<i>Corynebacterium diphtheriae</i>	1.25×10 ⁶ CFU/mL	-
<i>Corynebacterium pseudodiphtheriticum</i>	1.25×10 ⁶ CFU/mL	-
<i>Enterococcus faecalis</i>	1.25×10 ⁶ CFU/mL	-
<i>Enterococcus faecium</i>	1.25×10 ⁶ CFU/mL	-
<i>Escherichia coli</i>	1.25×10 ⁶ CFU/mL	-
<i>Haemophilus influenzae</i>	1.25×10 ⁶ CFU/mL	-
<i>Haemophilus parahaemolyticus</i>	1.25×10 ⁶ CFU/mL	-
<i>Haemophilus parainfluenzae</i>	1.25×10 ⁶ CFU/mL	-
<i>Klebsiella pneumoniae</i>	1.25×10 ⁶ CFU/mL	-
<i>Lactobacillus acidophilus</i>	1.20×10 ⁶ CFU/mL	-
<i>Lactococcus lactis</i>	1.25×10 ⁶ CFU/mL	-
<i>Legionella jordanis</i>	1.25×10 ⁶ CFU/mL	-
<i>Legionella micdadei</i>	1.25×10 ⁶ CFU/mL	-
<i>Legionella pneumophila</i>	1.25×10 ⁶ CFU/mL	-
<i>Listeria monocytogenes</i>	1.25×10 ⁶ CFU/mL	-
<i>Moraxella catarrhalis</i> (2 strains)	1.25×10 ⁶ CFU/mL	-
<i>Moraxella lacunata</i>	1.25×10 ⁶ CFU/mL	-

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Microorganism	Test Concentration	Strep A Result
<i>Mycoplasma pneumoniae</i>	1.25×10 ⁶ copies/mL [†]	-
<i>Neisseria gonorrhoeae</i>	1.25×10 ⁶ copies/mL [†]	-
<i>Neisseria lactamica</i>	1.25×10 ⁶ CFU/mL	-
<i>Neisseria meningitidis</i>	1.25×10 ⁶ CFU/mL	-
<i>Neisseria mucosa</i>	1.25×10 ⁶ CFU/mL	-
<i>Neisseria sicca</i>	1.25×10 ⁶ CFU/mL	-
<i>Neisseria subflava</i>	1.25×10 ⁶ CFU/mL	-
<i>Proteus mirabilis</i>	1.25×10 ⁶ CFU/mL	-
<i>Proteus vulgaris</i>	1.25×10 ⁶ CFU/mL	-
<i>Pseudomonas aeruginosa</i>	1.25×10 ⁶ CFU/mL	-
<i>Pseudomonas fluorescens</i>	1.25×10 ⁶ CFU/mL	-
<i>Serratia marcescens</i>	1.25×10 ⁶ CFU/mL	-
<i>Staphylococcus aureus</i>	1.25×10 ⁶ CFU/mL	-
<i>Staphylococcus epidermidis</i>	1.25×10 ⁶ CFU/mL	-
<i>Staphylococcus haemolyticus</i>	1.25×10 ⁶ CFU/mL	-
<i>Stenotrophomonas maltophilia</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus agalactiae</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus anginosus</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus bovis</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus canis</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus constellatus</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus dysgalactiae</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus equi</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus gallolyticus</i>	1.60×10 ⁶ CFU/mL	-
<i>Streptococcus intermedius</i>	1.10×10 ⁶ CFU/mL	-
<i>Streptococcus mitis</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus mutans</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus oralis</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus pneumoniae</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus salivarius</i>	1.25×10 ⁶ CFU/mL	-
<i>Streptococcus sanguis</i>	1.25×10 ⁶ CFU/mL	-
<i>Treponema denticola</i>	1.63×10 ⁶ copies/mL [†]	-
<i>Veillonella parvula</i>	2.13×10 ⁶ CFU/mL	-
<i>Yersinia enterocolitica</i>	1.25×10 ⁶ CFU/mL	-
Adenovirus, Type 1	4.45×10 ⁵ TCID ₅₀ /mL	-

Microorganism	Test Concentration	Strep A Result
Adenovirus, Type 7	4.45×10 ⁴ TCID ₅₀ /mL	-
Cytomegalovirus	1.00×10 ⁵ TCID ₅₀ /mL	-
Epstein-Barr virus	2.15×10 ⁵ copies/mL	-
Hepatitis B virus	5.00×10 ⁵ copies/mL	-
Herpes simplex virus 1	2.80×10 ⁵ TCID ₅₀ /mL	-
Human papilloma virus, Type 11	2.50×10 ⁵ copies/mL	-
Human papilloma virus, Type 6	2.50×10 ⁵ copies/mL	-

† Testing was performed with genomic DNA due to difficulties in propagation of these bacteria.

F. Interfering Microorganisms

An Interfering Microorganism Study was performed to evaluate whether other microorganisms that may be present in throat swab samples can interfere with the detection of Strep A by the **cobas®** Strep A assay. The 72 microorganisms were tested for potential interference on Strep A detection. Bacteria were tested at ≥10⁶ CFU/mL, and viruses were tested at ≥10⁵ TCID₅₀/mL, or the highest available concentration, in the presence of Strep A at 3x LOD concentration in throat swab matrix. Results show that the presence of the tested microorganisms did not interfere with the detection of Strep A.

Microorganism	Test Concentration	Strep A Result
<i>Acinetobacter baumannii</i>	1.25×10 ⁶ CFU/mL	+
<i>Arcanobacterium haemolyticum</i>	4.40×10 ⁶ CFU/mL	+
<i>Bacillus cereus</i>	2.90×10 ⁶ CFU/mL	+
<i>Bacteroides oralis</i>	1.55×10 ⁶ CFU/mL	+
<i>Bordetella bronchiseptica</i>	1.25×10 ⁶ CFU/mL	+
<i>Bordetella parapertussis</i>	1.25×10 ⁶ CFU/mL	+
<i>Bordetella pertussis</i>	1.25×10 ⁶ CFU/mL	+
<i>Burkholderia cepacia</i>	1.25×10 ⁶ CFU/mL	+
<i>Campylobacter rectus</i>	1.45×10 ⁶ CFU/mL	+
<i>Candida albicans</i>	1.25×10 ⁶ CFU/mL	+
<i>Chlamydia pneumoniae</i>	1.40×10 ⁵ TCID ₅₀ /mL	+
<i>Chlamydia trachomatis</i>	1.25×10 ⁶ EB/mL	+
<i>Corynebacterium diphtheriae</i>	1.25×10 ⁶ CFU/mL	+
<i>Corynebacterium pseudodiphtheriticum</i>	1.25×10 ⁶ CFU/mL	+
<i>Enterococcus faecalis</i>	1.25×10 ⁶ CFU/mL	+
<i>Enterococcus faecium</i>	1.25×10 ⁶ CFU/mL	+
<i>Escherichia coli</i>	1.25×10 ⁶ CFU/mL	+

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Microorganism	Test Concentration	Strep A Result
<i>Haemophilus influenzae</i>	1.25×10 ⁶ CFU/mL	+
<i>Haemophilus parahaemolyticus</i>	1.25×10 ⁶ CFU/mL	+
<i>Haemophilus parainfluenzae</i>	1.25×10 ⁶ CFU/mL	+
<i>Klebsiella pneumoniae</i>	1.25×10 ⁶ CFU/mL	+
<i>Lactobacillus acidophilus</i>	1.20×10 ⁶ CFU/mL	+
<i>Lactococcus lactis</i>	1.25×10 ⁶ CFU/mL	+
<i>Legionella jordanis</i>	1.25×10 ⁶ CFU/mL	+
<i>Legionella micdadei</i>	1.25×10 ⁶ CFU/mL	+
<i>Legionella pneumophila</i>	1.25×10 ⁶ CFU/mL	+
<i>Listeria monocytogenes</i>	1.25×10 ⁶ CFU/mL	+
<i>Moraxella catarrhalis</i> (2 strains)	1.25×10 ⁶ CFU/mL	+
<i>Moraxella lacunata</i>	1.25×10 ⁶ CFU/mL	+
<i>Mycoplasma pneumoniae</i>	1.25×10 ⁶ copies/mL [†]	+
<i>Neisseria gonorrhoeae</i>	1.25×10 ⁶ copies/mL [†]	+
<i>Neisseria lactamica</i>	1.25×10 ⁶ CFU/mL	+
<i>Neisseria meningitidis</i>	1.25×10 ⁶ CFU/mL	+
<i>Neisseria mucosa</i>	1.25×10 ⁶ CFU/mL	+
<i>Neisseria sicca</i>	1.25×10 ⁶ CFU/mL	+
<i>Neisseria subflava</i>	1.25×10 ⁶ CFU/mL	+
<i>Proteus mirabilis</i>	1.25×10 ⁶ CFU/mL	+
<i>Proteus vulgaris</i>	1.25×10 ⁶ CFU/mL	+
<i>Pseudomonas aeruginosa</i>	1.25×10 ⁶ CFU/mL	+
<i>Pseudomonas fluorescens</i>	1.25×10 ⁶ CFU/mL	+
<i>Serratia marcescens</i>	1.25×10 ⁶ CFU/mL	+
<i>Staphylococcus aureus</i>	1.25×10 ⁶ CFU/mL	+
<i>Staphylococcus epidermidis</i>	1.25×10 ⁶ CFU/mL	+
<i>Staphylococcus haemolyticus</i>	1.25×10 ⁶ CFU/mL	+
<i>Stenotrophomonas maltophilia</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus agalactiae</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus anginosus</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus bovis</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus canis</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus constellatus</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus dysgalactiae</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus equi</i>	1.25×10 ⁶ CFU/mL	+

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Microorganism	Test Concentration	Strep A Result
<i>Streptococcus gallolyticus</i>	1.60×10 ⁶ CFU/mL	+
<i>Streptococcus intermedius</i>	1.10×10 ⁶ CFU/mL	+
<i>Streptococcus mitis</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus mutans</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus oralis</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus pneumoniae</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus salivarius</i>	1.25×10 ⁶ CFU/mL	+
<i>Streptococcus sanguis</i>	1.25×10 ⁶ CFU/mL	+
<i>Treponema denticola</i>	1.63×10 ⁶ copies/mL [†]	+
<i>Veillonella parvula</i>	2.13×10 ⁶ CFU/mL	+
<i>Yersinia enterocolitica</i>	1.25×10 ⁶ CFU/mL	+
Adenovirus, Type 1	4.45×10 ⁵ TCID ₅₀ /mL	+
Adenovirus, Type 7	4.45×10 ⁴ TCID ₅₀ /mL	+
Cytomegalovirus	1.00×10 ⁵ TCID ₅₀ /mL	+
Epstein-Barr virus	2.15×10 ⁵ copies/mL	+
Hepatitis B virus	5.00×10 ⁵ copies/mL	+
Herpes simplex virus 1	2.80×10 ⁵ TCID ₅₀ /mL	+
Human papilloma virus, Type 11	2.50×10 ⁵ copies/mL	+
Human papilloma virus, Type 6	2.50×10 ⁵ copies/mL	+

[†] Testing was performed with genomic DNA due to difficulties in propagation of these bacteria.

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G. Interfering Substances

The **cobas®** Strep A assay was evaluated with 28 substances that may be encountered in throat swab specimens. Medically and/or physiologically relevant concentrations of potential interferents were tested in throat swab matrix in the presence and absence of Strep A at 3x LOD. Results showed that none of the substances tested interfered with the **cobas®** Strep A assay.

Potential Interferent	Concentration	Strep A Result	
		Strep A 3x LOD	Negative Matrix
Acetaminophen (Tylenol)	100 µg/mL	+	-
Adult Robitussin Peak Cold, Maximum Strength, Cough + Chest	5% v/v	+	-
Adult Robitussin Peak Cold, Nighttime, Multi-symptom cold	5% v/v	+	-
Amoxicillin	25 µg/mL	+	-
Blood (human)	5% v/v	+	-
Brompheniramine Maleate	60 ng/mL	+	-
Cepacol Sore Throat	5 mg/mL	+	-
Cepacol Ultra Sore Throat Spray	5% v/v	+	-
Children's Dimetapp Cold & Cough	5% v/v	+	-
Children's Robitussin Cough & Cold	5% v/v	+	-
Children's Dimetapp Nighttime Cold & Congestion	5% v/v	+	-
Chloraseptic Max	5% v/v	+	-
Chlorpheniramine Maleate	25 ng/mL	+	-
Cool Mint Listerine, antiseptic	5% v/v	+	-
Crest Pro-Health	5% v/v	+	-
Dextromethorphan HBr	20 ng/mL	+	-
Diphenhydramine HCl	350 ng/mL	+	-
Doxylamine Succinate	300 ng/mL	+	-
Erythromycin	15 µg/mL	+	-
Guaifenesin (Guaiaicol glyceryl)	5 mg/mL	+	-
Halls Mentho-lyptus Cherry	5 mg/mL	+	-
Halls Mentho-lyptus Sugar Free	5 mg/mL	+	-
Ibuprofen (Advil)	25 µg/mL	+	-
Mucin: bovine submaxillary gland, type I-S	25 mg/mL [†]	+	-
Penicillin G	1.2 mg/mL	+	-
Sucrets Complete	5 mg/mL	+	-
Tussin Adult Chest Congestion	5% v/v	+	-
Tylenol Cold Sore Throat	5% v/v	+	-

[†] In the presence of mucin at 25 mg/mL, Strep A Ct was delayed and endpoint fluorescence was suppressed, though all Strep A 3x LOD samples were detected as positive.

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H. CLIA Waiver Study

As part of the prospective study, as described in section A above, the accuracy of the cobas® Strep A assay was evaluated at 5 CLIA waived sites. A total of 17 untrained operators representative of CLIA waived site intended operators participated in the study. The invalid rate was 1.4%.

This CLIA waiver study demonstrated assay sensitivity was 97.7% (95% CI: 93.4% - 99.2%) and assay specificity was 93.3% (95% CI: 89.9% - 95.6%).

Strep A (5 CLIA Waived Sites)		Comparative Culture		Total
		Positive	Negative	
cobas® Liat®	Positive	126	20 ^a	146
	Negative	3 ^b	278	281
	Total	129	298	427

^a Of 20 cobas® Liat® positive, culture negative specimens, all 20 were Strep A positive by PCR/sequencing.

^b Of 3 cobas® Liat® negative, culture positive specimens, 3 were Strep A positive by PCR/sequencing. All 3 were also positive when the cobas® Liat® assay was repeated using residual specimen after culture.

	#	%	95% CI
Sensitivity	126 / 129	97.7%	93.4% - 99.2%
Specificity	278 / 298	93.3%	89.9% - 95.6%
Accuracy	404 / 427	94.6%	92.1% - 96.4%
Prevalence	129 / 427	30.2%	26.1% - 34.7%
PPV	126 / 146	86.3%	79.8% - 91.0%
NPV	278 / 281	98.9%	96.9% - 99.6%
Invalid ^c	6 / 433	1.4%	0.6% - 3.0%

^c Rate includes all Invalid, Indeterminate and Assay Aborted results. In all cases, re-test of the same specimens gave a valid result.

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cobas® Strep A

Nucleic acid test for use on the **cobas® Liat®** System



A Device Performance with Analyte Concentrations Near Cutoff study was performed to assess the capability of CLIA waived site intended operators to test weak negative and weak positive samples and obtain accurate results. Weak positive and weak negative sample concentrations of *Streptococcus pyogenes* ATCC# BAA-946 were determined by dilution studies performed by professional operators using the Comparative Culture method. Sixty (60) weak positive and 60 weak negative samples were distributed equally among 3 CLIA waived sites and tested by intended operators (2 per site) using the **cobas®** Strep A assay. The **cobas®** Strep A assay yielded “Strep A Detected” result for 100% of the weak positive samples, and “Strep A Not Detected” result for 100% of the weak negative samples. The results show that untrained operators are able to perform the **cobas®** Strep A assay accurately with samples that contain a low concentration of *S. pyogenes* organisms.




















Sample Level	Untrained Intended Operators using cobas® Liat® assay		
	#	% Agreement	95% CI
Weak Positive*	60 / 60	100%	94.0%-100%
Weak Negative*	60 / 60	100%	94.0%-100%

The expected results for the Weak Positive and Weak Negative samples were “Strep A Detected” and “Strep A Not Detected”, respectively.

* Guidance for Industry and Food and Drug Administration Staff: Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices January 30, 2008.

Using risk analysis as a guide, analytical flex studies were conducted. The studies demonstrated that the test is insensitive to stresses of environmental conditions and potential user errors.

XII. Table of Symbols

Symbol	Used For	Symbol	Used For
	Consult instructions for use		Serial number
	<i>In Vitro</i> diagnostic medical device		Use-by date
	Temperature limit		Control
	Biological risks		Negative Control
	Do not reuse		Positive Control
	Contains sufficient for <n> tests		Authorized representative in the European community
	Catalogue number		Distributed by
	Batch code		Manufacturer
	Global Trade Item Number		Date of manufacture
	This product fulfills the requirements of Directive 98/79/EC on <i>in vitro</i> diagnostic medical devices.	Rx Only	US Only: Federal law restricts this device to sale by or on the order of a physician.

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XIII. Manufacturer

Technical Support

If you have any questions or problems, please contact your Roche Service representative.

Test system problems may also be reported to the FDA through the MedWatch medical products reporting program (phone: 1-800-FDA-1088; fax: 1-800-FDA-0178; <http://www.fda.gov/medwatch>).

Trademarks and Patents

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The cobas® Strep A nucleic acid test for use on the cobas® Liat System is covered by US Patent Nos. U.S. Patent Nos. 6,780,617; 6,964,862; 7,337,072; 7,718,421; 7,833,489; 7,799,521; 7,935,504; 8,936,933; and 9,005,551; corresponding foreign patents. Other patents pending.

See <http://www.roche-diagnostics.us/patents>

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XIV. References

1. JF Cohen, et. al. Rapid antigen detection test for group A streptococcus in children with pharyngitis. Cochrane Database Syst Rev. 2016 Jul 4.

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Notes