

Lab Name:

Procedure #:

Procedure: CLIA Complexity: Waived

Prepared By	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete package insert in accordance with FDA labeling regulation (21 CFR 809.10). Prepared in accordance with the guidelines recommended by the Clinical and Laboratory Standards Institute, Wayne, PA 19087; CLSI Document GP2-A2.

Quidel Corporation provides CLSI procedures for your use. The procedures are required to include the same information as listed in the package insert. Any modifications to this document are the sole responsibility of the Laboratory.

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INTENDED USE

The QuickVue In-Line Strep A test allows for the rapid detection of Group A Streptococcal antigen directly from patient throat swab specimens. The test is intended for use as an aid in the diagnosis of Group A Streptococcal infection. For use by healthcare professionals.

SUMMARY AND EXPLANATION

Group A *Streptococci* are organisms that typically cause illnesses such as tonsillitis, pharyngitis and scarlet fever. These infections can lead to serious complications, including rheumatic fever and acute glomerulonephritis.¹ Rapid diagnosis and appropriate antibiotic therapy of Group A Streptococcal infections appear to be the best means of preventing these complications. The traditional means of detecting Group A Streptococcal infection involves 24–48 hour culture of throat swab specimens or other exudates, confirming beta-hemolysis, and showing susceptibility to bacitracin. Group A, but generally not other groups of beta-hemolytic *Streptococci*, is bacitracin susceptible which provides a presumptive diagnosis of Group A Streptococcal disease.²

The QuickVue In-Line Strep A test is a lateral-flow immunoassay utilizing an in-the-device antigen extraction. The test, containing a highly specific and sensitive antibody reactive to the Strep A antigen, is specific to group A with no cross-reactivity from other groups of *Streptococci*.

PRINCIPLE OF THE TEST

To perform the test, a throat swab specimen is collected and inserted into the Swab Chamber of the Test Cassette. The Extraction Solutions are mixed, resulting in a green color change, and added to the swab in the Swab Chamber in order for the antigenic component of the bacteria to be extracted.

Extraction begins instantaneously, after which the extracted solution flows from the Swab Chamber onto the test strip by capillary action. The extracted sample flows through a label pad consisting of a pink label containing rabbit polyclonal anti-Strep A antibody and a blue control label. If the extracted solution contains Strep A antigen, the antigen will bind to the antibody on the pink test label which, in turn, will bind a rabbit polyclonal anti-Strep A antibody spotted on the membrane, resulting in the formation of a pink-to-red Test Line. A blue Control Line will also appear next to the letter "C" on the

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Test Cassette indicating that the reagents were mixed and added properly, proper volume of fluid entered the Test Cassette and capillary flow occurred. A blue Control Line should always appear in a properly functioning Test Cassette. If Strep A is not present or present at very low levels, only a blue Control Line will be visible.

REAGENTS AND MATERIALS SUPPLIED

- Individually packaged Test Cassettes (25):
 - Membrane coated with rabbit polyclonal antibody to Strep A.
- Extraction Solution Bottles (25):
 - 4M Sodium Nitrite (0.38 mL), and 0.2M Acetic Acid (0.43 mL) inside glass ampule.
- Individually packaged sterile rayon-tipped swabs on green shafts (25).
 - Swab is sterile unless envelope is damaged or open.
- Positive Control Swab (+) (1):
 - Heat-inactivated Group A *Streptococcus*.
- Negative Control Swab (–) (1):
 - Heat-inactivated Group C *Streptococcus*.
- Package Insert (1)
- Procedure Card (1)
- Extraction Kit (1):
 - **5 Tubes and 5 Disposable Droppers for use with Proficiency Testing Samples only. Refer to the Proficiency Testing Section for instructions for use.**

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Do not use kit contents after the expiration date printed on the outside of the kit.
- Use appropriate precautions in the collection, storage, handling and disposal of patient samples and used kit contents.
- Use of Nitrile or Latex gloves is recommended when handling patient samples.³

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- Dispose of containers and unused contents in accordance with Federal, State and Local requirements.
- The Test Cassette must remain sealed in the protective foil pouch until just prior to use.
- The Extraction Solution Bottle contains an acidic solution. If the solution contacts the skin or eye, flush with large volumes of water.
- The Extraction Solution Bottle contains glass, break cautiously.
- If the Extraction Solution Bottle is missing the glass ampule, or the solution is green prior to the breaking of the ampule, discard and use another Extraction Solution Bottle.
- To obtain accurate results, Package Insert instructions must be followed.

KIT STORAGE AND STABILITY

Store kit at room temperature, 59–86°F (15–30°C), out of direct sunlight. Kit contents are stable until the expiration date printed on the outer box. Do not freeze.

SPECIMEN COLLECTION AND STORAGE

The sterile rayon-tipped swabs supplied with this kit must be the only swabs used for specimen collection. These special swabs have green print on the paper wrapper and green shafts.

Collect throat swab specimens by standard clinical methods. Depress the tongue with a tongue blade or spoon. Be careful not to touch the tongue, sides or top of the mouth with the swab. Rub the swab on the back of the throat, on the tonsils, and in any other area where there is redness, inflammation or pus. Consult standard reference procedures such as the collection method described by Facklam.⁴

Use **only** the rayon-tipped swabs on green plastic shafts supplied in the kit to collect throat specimens. Other swabs, including other rayon swabs, are incompatible with this test due to their small tip size.

It is recommended that swab specimens be processed as soon as possible after collection. Swabs can be held in any clean, dry plastic tube or sleeve up to 4 hours at room temperature (15–30°C), or 24 hours refrigerated (2–8°C) before processing. Performance with transport media has not been assessed, however the use of charcoal or agar medium is not recommended.

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If a culture is desired, lightly streak the swab on a 5% sheep blood agar plate before using the swab in the QuickVue In-Line Strep A test. Do not perform the QuickVue In-Line Strep A test before streaking the swab, as the Extraction Solution will destroy the bacteria on the swab, thereby rendering the organism incapable of successful culturing. Alternatively, throat swab specimens can be obtained by dual swabs or by two sequential swabs for the culture procedure.

QUALITY CONTROL***Built-in Control Features***

The QuickVue In-Line Strep A test contains built-in control features. The manufacturer's recommendation for daily quality control is to document these controls for the first sample tested each day.

A control of the extraction procedure is provided by a color change from clear to green as the extraction solutions are mixed. The color change is an indication of extraction reagent integrity and is also an indication that the extraction procedure was correctly performed.

The two-color result format provides a clear-cut readout for positive and negative results. The appearance of a blue Control Line next to the letter "C" provides several forms of control. First, detection components for the specimen and internal control are processed concurrently using identical procedures; therefore, the appearance of the Control Line ensures that functional activity of the detection component is maintained. Secondly, the appearance of the Control Line also ensures that the foil pouch integrity has been maintained and the Test Cassette has been stored in such a manner as not to compromise its functionality. Third, the appearance of the Control Line indicates that proper volume of fluid entered the Test Cassette and capillary flow occurred. This would indicate that the Test Cassette was assembled properly, by acting as a check for all membrane interfaces and proper positioning of components. If the Control Line does not develop within 5 minutes, the test result is invalid.

A negative background control is provided by the clearing of background color in the Result Window and indicates that there were no immunological interfering substances in the specimen. This area should be white to light pink within 5 minutes and not interfere with the reading of the test result. If background color remains in the Result Window which interferes with your ability to read the test result, your result may be invalid. In this case, contact Quidel Technical Support.

Positive and Negative Quality Control

External controls may also be used to demonstrate that the reagents and assay procedure perform properly.

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Quidel recommends that positive and negative controls be run once for each untrained operator, once for each new shipment of kits - provided that each different lot received in the shipment is tested - and as deemed additionally necessary by your internal quality control procedures, and in accordance with local, state, and federal regulations or accreditation requirements.

If controls do not perform as expected, do not use the test results. Repeat the test or contact Quidel Technical Support.

Positive and Negative Control Swabs are supplied in the kit. Additional Control Swabs may be obtained by ordering Quidel Catalog Number 00345. To test using a Positive or Negative Control Swab, remove the Control Swab from its container and insert it into the QuickVue In-Line Strep A Test Cassette Swab Chamber. Continue with the assay as instructed in the **TEST PROCEDURE** Section.

To test using a liquid control (Catalog #00354), shake the Control Solution Bottle vigorously. Hold the bottle vertically and place one free falling drop of liquid control on a sterile rayon-tipped swab provided in the kit. Insert the Swab into the QuickVue In-Line Strep A Test Cassette Swab Chamber. Continue with the assay as instructed in the **TEST PROCEDURE** Section.

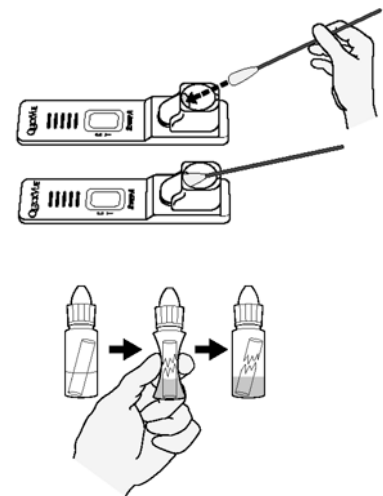
TEST PROCEDURE

Important:

- Gloves should be worn when handling human samples.
- Do not use the extraction solution if it is green prior to breaking the ampule.
- **For proficiency testing, follow the alternate test procedure under PROFICIENCY TESTING SURVEY PROCEDURE.**

BEFORE TESTING:

- **MUST** use the swabs provided in the kit.
- Remove the Test Cassette from foil pouch and place on a clean, dry, level surface. Using the notch at the back of the chamber as a guide, insert the swab **completely** into the Swab Chamber.
- Squeeze **ONCE** to break the glass ampule inside the extraction solution bottle.



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PERFORM THE ASSAY:

- Vigorously shake the Bottle five times to mix the solutions. Solution should turn green after the ampule is broken.

Solution must be used immediately.

- Remove the cap. Holding bottle vertically, quickly fill the chamber to the rim (approximately 8 drops).

Begin timing.

If liquid has not moved across the Result Window in 1 minute, completely remove the swab and re-insert. If liquid still does not move across, retest with a new specimen, Test Cassette and Extraction Solution Bottle.

The Test Cassette should not be moved until the assay is complete.

READ RESULTS AT 5 MINUTES.

SOME POSITIVE RESULTS MAY BE SEEN EARLIER.



INTERPRETATION OF RESULTS

Positive Result:

The appearance of any pink-to-red line next to the letter “T” in the Result Window, along with a blue Control Line next to the letter “C”, means that the test is positive for Group A *Streptococcus*.



*Look closely! This is a positive result. Even if you see a very faint, pink Test Line and a blue Control Line, you must report the results as POSITIVE. The positive test line is usually very prominent, but test line intensity can vary.

Negative Result:

The appearance of only the blue Control Line next to the letter “C” in the Result Window means that the test is negative. A negative QuickVue result means that the swab is presumptive negative for Group A *Streptococcus*.



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Invalid Result:

If the blue Control Line does not appear next to the letter “C” at 5 minutes, the test is considered INVALID, and the test result cannot be used. If this occurs, retest using a fresh swab and a new QuickVue Test Cassette or contact Technical Support.



For a photographic example of the test results, please see the procedure card.

PROFICIENCY TESTING SURVEY PROCEDURE

The testing procedure for Proficiency Survey swab specimens is outlined below. **This procedure must be followed to ensure accuracy** with the QuickVue test on Proficiency Survey swab specimens because Proficiency Testing swab tips are smaller in size than the swabs provided for use with the kit.

- Place a clean tube from the Extraction Kit in a test tube rack.
- Squeeze to crush the glass ampule inside the Extraction Solution Bottle as described in the **TEST PROCEDURE** Section.
- Dispense **8 DROPS** from the Extraction Solution Bottle into the tube. Place the proficiency swab into the tube. Hold the bottom of the tube so that the swab head is slightly compressed. Rotate the swab three (3) times.
- WAIT ONE (1) MINUTE
- Express **all** liquid from the swab head in the tube by rolling the swab against the inside of the tube and pressing slightly as it is withdrawn from the tube. Discard the swab.
- Fill the Disposable Dropper **to the fill line** with the solution from the tube and add the contents into the Test Cassette Swab Chamber.
- Read the result at 5 minutes. See **INTERPRETATION OF RESULTS** Section.

LIMITATIONS

The contents of this kit are for use in the **qualitative** detection of Group A Streptococcal antigen from throat swab specimens only. Failure to follow the test procedure and interpretation of test results may adversely affect performance and/or produce invalid results.

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Respiratory infections, including pharyngitis, can be caused by *Streptococcus* from serogroups other than group A as well as other pathogens. The QuickVue In-Line Strep A test will not differentiate asymptomatic carriers of Group A *Streptococcus* from those exhibiting Streptococcal infection.⁵ In rare cases, test specimens heavily colonized with *Staphylococcus aureus* ($>10^{10}$) can yield false positive results.

Test results must always be evaluated with other data available to the physician. A negative test result might occur if the level of extracted antigen in a sample is below the sensitivity of the test. Additional follow-up testing using the culture method is recommended if the QuickVue test result is negative.

EXPECTED VALUES

Group A *Streptococci* are responsible for about 19% of all upper respiratory tract infections, but the incidence varies by clinical setting.⁶ Streptococcal pharyngitis is seasonal in nature with the highest prevalence found during the winter and early spring. The highest incidence of this disease is found in crowded populations such as military bases and in school-aged children, and is evenly distributed between males and females.⁷

PERFORMANCE CHARACTERISTICS***Clinical Sensitivity and Specificity***

The QuickVue In-Line Strep A test will yield positive test results with specimens containing 5×10^5 Group A *Streptococci* organisms per test.

A multi-center evaluation of the QuickVue test was conducted to determine the clinical performance of the test relative to standard culture techniques. A total of 537 throat swab specimens were collected from patients presenting with pharyngitis. Prior to performance of the QuickVue test, each swab specimen was inoculated onto a sheep blood agar plate containing a bacitracin disk and incubated at 37°C for 48 hours for culture evaluation. All cultures were confirmed for the presence of Group A Strep using commercial latex agglutination assays.

Swabs were either tested in the QuickVue test immediately upon collection at the field site (fresh specimens) or frozen and shipped overnight to Quidel. Testing with the QuickVue test was performed by trained technicians and by users in the field with various levels of work experience and educational backgrounds.

Of the 537 total specimens, 301 fresh specimens were tested by field users while the other 236 frozen specimens were tested by trained technicians at Quidel. Ten (10) additional specimens tested resulted in uninterpretable results and were eliminated from the analysis.

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In the field study, 240 specimens were found to be negative by SBA culture and 225 were also negative by the QuickVue test; similarly, 61 specimens were found to be positive by SBA culture and 53 were also positive by the QuickVue test. Based on this data, specificity was 94% and sensitivity was 87% for the QuickVue test, 95% confidence intervals were calculated to be 91–97% and 78–95% for specificity and sensitivity, respectively. Overall agreement between SBA culture and QuickVue was 92% (278/301).

In a separate study conducted at Quidel, 136 specimens were found to be negative by SBA culture and 135 were also negative by the QuickVue test; similarly, 100 specimens were found to be positive by SBA culture and 92 were also positive by the QuickVue test. Based on this data, **specificity was 99%** and **sensitivity was 92%** for the QuickVue test, 95% confidence intervals were calculated to be 96–100% and 87–97% for specificity and sensitivity, respectively. **Overall agreement between SBA culture and QuickVue was 96% (227/236).**

Culture Classification	Specimen Mix In Population			
	Field Users		Trained Technicians	
	<u>Correct Calls</u>	<u>% Correct</u>	<u>Correct Calls</u>	<u>% Correct</u>
Negative	225/240	94	135/136	99
1+	3/9	33	2/5	40
2+	7/9	78	6/6	100
3+	18/18	100	39/39	100
4+	25/25	100	45/50	90
Overall Agreement to Culture	278/301	92%	227/236	96%

Physician’s Office Laboratory (POL) Studies

An evaluation of the QuickVue In-Line Strep A test was conducted at four Physicians’ Offices using a panel of coded specimens. Testing was performed by physician’s office personnel with diverse educational backgrounds and work experience at different locations. The proficiency panel contained negative, low positive, moderate positive and high positive specimens. Each specimen level was tested in replicates of five at each site over a period of three days.

The results obtained at each site ranged from 88 to 100% agreement with the expected results. No significant differences were observed within run (five replicates), between runs (three different assay days), or between sites (four POL sites).

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Cross-Reactivity

Group C *Streptococcus*, Group G *Streptococcus*, *S. aureus*, *N. subflava*, *H. influenza*, *C. albicans*, *N. meningitidis*, *N. gonorrhoea*, *B. catarrhalis*, *E. faecalis*, *S. pneumoniae*, and *S. mutans* were tested in the QuickVue In-Line Strep A test at levels exceeding 10^7 /test and did not affect the expected test results.

ASSISTANCE

If you have any questions regarding the use of this product, please call Quidel's Technical Support Number, (800) 874-1517 (toll-free in the U.S.A.) or (858) 552-1100, Monday through Friday, between 7:00 a.m. and 5:00 p.m., Pacific Time, U.S.A. If outside the United States, contact your local distributor or technicalsupport@quidel.com.

REFERENCES

1. Rammelkamp C.H., Jr. Principles of Internal Medicine, 8th Edition, 1977, pp. 814–820.
2. Youmans G.P., Paterson P.Y., and Sommers H.M., The Biological and Clinical Basis of Infectious Disease, 1975, pp. 172–185.
3. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, U.S. Department of Health and Human Services, CDC, NIH, Washington, D.C. (2007).
4. Facklam, R.R. and Washington II, J.A., Manual of Clinical Microbiology, Balows, A. ed. 5th Edition, 1991, 29:241.
5. Rammelkamp C.H., Jr., Ibid., pp. 814.
6. Lauer B.A., Reller L.B., and Mirrett S., Journal of Clinical Microbiology, 17:338–340, 1983.
7. Wannamaker L.W., New England Journal of Medicine, 282: 23–31, 78–85, 1970.

Covered by U.S. Patents 4,943,522, 5,415,994, 5,763,262, 5,766,961, and 5,770,460; European Patents 0 296 724, 0 566 695 and 0 712 495; Japanese Patent Nos. 2868900, and 3394539; other patents pending.

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REF

00343 - QuickVue In-Line Strep A 25 Test Kit
00345 - QuickVue Strep A Control Swab Set
00354 - QuickVue Strep A Liquid Control Set

IVD

CE

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For *In Vitro* Diagnostic Use



Temperature Limit

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LOG SHEET



Record Built-in Procedural Controls on the first patient tested each day.

	Date	Patient Name	Positive Procedural Control (Blue Line)	Negative Procedural Control (White to light pink background)	Test Results At 5 Minutes	Lot Number and Exp. Date	Technician
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

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LOG SHEET



	Date/Date Received	External Positive Controls	External Negative Controls	Lot Number and Exp. Date	Technician
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					

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