Quest Diagnostics

TRAINING UPDATE

Lab Location:	GEC, SGAH & WAH	**Date Distributed:**	7/10/2012
Department:	Micro (Rapid Tests)	**Due Date:**	8/1/2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

***Streptococcus* Group A Antigen with Reflex to Culture** GEC/SGAH/WAH QDHOS706 v1.0A
STREP Group A QUALITY CONTROL LOG AG.F35.003

Description of change(s):

Adopting new BPT procedure - see blue highlight in attached SOP

- Defined process to add culture order
- Defined process if only one swab rec'd
- Change in external control frequency
- Section 10.6 – LIS resulting and reflex to culture or cancel culture as appropriate

Strep Group A QC Log revised for external control frequency

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	***Streptococcus*** **Group A Antigen with Reflex to Culture**
Prepared by	Ron Master Date: 5/12/2012

Laboratory Approval	**Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name and Title	**Signature**	**Date**

Corporate Approval	**Corporate Issue Date:** 5/25/2012	
Print Name and Title	**Signature**	**Date**
Lori Loffredo **Hospital BPT Chair**	*Approval on file*	**5/18/2012**
Dianne Zorka **NQA Manager (QC/ FDA Review)**	*Approval on file*	**5/17/2012**
R. Schlesinger, M.D. **BPT Medical Advisor**	*Approval on file*	**5/16/2012**
Stephen Suffin, M.D. **Chief Medical Officer, VP/Corporate**	*Approval on file*	**5/24/2012**

Retirement Date:	
Reason for retirement/replacement:	

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	*Streptococcus* Group A Antigen with Reflex to Culture
Method	Immunochromatography
Instrument	Not applicable
Synonyms	Rapid Strep, OSOM® Strep A, GAS Antigen
Department	Microbiology

Order Code	**Test Name**
QSTRP	*Streptococcus* Group A Ag with Reflex to Culture

Form revised 3/31/00

2. ANALYTICAL PRINCIPLE

The Genzyme OSOM® Strep A uses color immunochromatographic dipstick technology with rabbit antibodies coated on the nitrocellulose membrane. In the test procedure, a throat swab is subjected to a chemical extraction of a carbohydrate antigen unique to Group A *Streptococcus*. The Test Stick is then placed in the extraction mixture and the mixture migrates along the membrane. If Group A *Streptococcus* is present in the sample, it will form a complex with the anti-Group A *Streptococcus* antibody conjugated color particles. The complex will then be bound by the anti-group A streptococcus capture antibody and a visible blue test line will appear to indicate a positive result.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Two swabs should be submitted for this test. Use the sterile culture swabs to sample the back of the throat (posterior pharynx), tonsillar crypts, and between the tonsillar pillars and uvula. Avoid touching the lips, cheeks, tongue, and uvula.
Special Collection Procedures	Throat specimens should not be collected if the patient may have epiglottitis, a rapidly progressing infection with potential to cause complete airway obstruction. If epiglottitis is suspected, prompt otolaryngologic consultation for airway management is recommended.
Other	Refer to the Quest Diagnostics Incorporated *Directory of Services* for instructions on specimen collection and transport.

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	Two swabs in liquid media such as Amies (BD red cap) or modified Stuart's medium.
-Other Acceptable	None.
Collection Container	Swabs in liquid media such as Amies or modified Stuart's medium. Do not use a collection system containing charcoal or semisolid (gel) transport media.
Volume - Optimum	Two swabs are preferred. One is for the antigen test and the second for culture if necessary.
- Minimum	1 swab
Transport Container & Temperature	BD red cap rayon, in liquid bacterial transport medium. (This swab has been validated by Quest Diagnostics).

Form revised 03/06

Criteria		
Stability & Storage Requirements	Room Temperature:	Process swabs as soon as possible after collection. Swabs in liquid transport media may be stored up to 2 days.
	Refrigerated:	Swabs in liquid transport media up to 2 days.
	Frozen:	Not validated
Timing Considerations	None	
Unacceptable Specimens & Actions to Take	Reject • Specimens from other sources than the throat or nasopharynx • Swabs with wooden shafts, calcium alginate, or cotton tips • Frozen specimens • Specimens in expired transport devices • Specimens in viral transport medium or in Gen-Probe collection devices • Beyond stability (>48 hrs. old)	
Compromising Physical Characteristics	Throat specimens should not be collected if the patient may have epiglottitis, a rapidly progressing cellulitis with potential to cause complete airway obstruction. Epiglottitis is typically caused by *H. influenzae* type b, but occasionally by *S. aureus* or *S. pneumoniae.* Epiglottitis should be considered in a febrile patient who has a severe sore throat, dysphagia, high-pitched breathing noises or progressive respiratory distress, and minimal findings on visualization of the oropharynx. If epiglottitis is suspected, prompt otolaryngologic consultation for airway management is suggested.	
Other Considerations	If only one swab is received, first streak the culture plate (refer to Plating SOP) before starting the OSOM® Strep A procedure. The extraction reagent will render the specimen nonviable for culture. Alternatively, if two swabs are received, process one for the antigen test and use the second for culture.	

4. REAGENTS

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
OSOM® Strep A	Genzyme Diagnostics Cat# 141

Form revised 3/31/00

4.2 Reagent Preparation and Storage

Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.

Genzyme Diagnostics OSOM® Strep A Kit	
Reagent 1	2 M Sodium Nitrite Caution: Toxic
Reagent 2	0.3 M Acetic Acid Caution: Corrosive
Positive Control	Nonviable Group A Streptococci, in 0.1% Sodium Azide.
Negative Control	Nonviable Group C Streptococci, in 0.1% Sodium Azide.
Container	Store in manufacturer's original container.
Storage	Store at Room Temperature (15^{o} to 30^{o}C)
Stability	Do not use Test Sticks or Reagents after expiration date.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Not applicable

5.2 Calibrator Preparation and Storage

Not applicable

5.3 Calibration Criteria and Procedure

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

Three levels of internal procedural controls are automatically performed with each test. The results of these internal controls must be documented each time the test is performed. These controls verify that:

1) The Extraction Reagents were mixed properly (indicated by a pink to light yellow color when mixed).
2) The Test Stick is working properly (indicated by the red Control Line), and
3) That there are no interfering substances in the specimen (indicated by a clear background).

Quality Control	Supplier
Internal Reagent Control	Reagents 1 & 2. Supplied in the kit.
Internal Positive Control	Each test device. Supplied in the kit.
Internal Negative Control	Each test device. Supplied in the kit.
External Positive Control (includes control of the extraction)	Supplied in the kit.
External Negative Control	Supplied in the kit.

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

Control	**Internal Procedural Controls**
Contents	Nitrocellulose membrane device coated with rabbit antibodies. Reagent 1 and Reagent 2 (Refer to Section 4.1 for contents).
Preparation	None
Storage	Store at 15-30° C
Stability	Stable until manufacturer's expiration date

Control	**External Positive Control**
Contents	Nonviable Group A Streptococci, 0.1% Sodium Azide
Preparation	None
Storage	Store at 15-30° C
Stability	Stable until manufacturer's expiration date

Control	**External Negative Control**
Contents	Nonviable Group C Streptococci, 0.1% Sodium Azide
Preparation	None
Storage	Store at 15-30° C
Stability	Stable until manufacturer's expiration date

6.3 Frequency

- The results of these internal controls must be documented each time the test is performed.
- *Positive and negative external controls are run and documented with each new kit lot number or shipment.* These controls verify the extraction step and test devices are working properly and that the analyst is performing the test correctly.

6.4 Tolerance Limits

Step	Tolerance Limits
Internal Reagent Control	Color change from pink to light yellow after the addition of Reagents 1 & 2, in each tube.
Internal Positive Control	Red line in the Control line area of each test device.
Internal Negative Control	Clear background in the Control Line area of each test device.
External Positive Control (includes extraction)	Blue test line and red control line.
External Negative Control	Red control line only.

- Refer to package insert illustration for test result interpretation.
- Each time the controls exceed the acceptable criteria specified above, the run is considered to be out of control (failed) and patient results must not be reported. The run must be brought to the attention of supervisor (or designee) for second review and further action.
- The following are guidelines for failed controls:

IF …	THEN…
The red (Internal Positive Control) line does not appear for an External Control	That QC test is invalid. Hold patient results until corrective action is performed.
If the background (Internal Negative Control) is not clear and interferes with the result of an External Control	That QC test is invalid. Hold patient results until corrective action is performed.
If one or both External Control tests are invalid BUT all patient tests are valid (i.e., presence of a red Control line and clear background on all patients). 1. If the repeat control test is valid … 2. If the repeat control test is invalid …	Repeat the failed External Control test to verify that the original test was performed correctly. 1. Report the patient results. 2. **DO NOT release patient results when controls do not meet tolerance limits.** Investigate and take corrective action for all unacceptable controls. Corrective action must be documented.

6.5 Documentation

Refer to local policies and procedures for QC documentation and to Quest Diagnostics records management program for record retention requirements.

All steps taken in response to QC failures must be documented, including: a description of the QC failure, the root cause of the problem, actions taken to correct the problem, how patient samples were handled if applicable, and the date and initials of the person recording the information.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

- Test device supplied with kit

7.2 Equipment

- None

7.3 Supplies

- Timer or watch
- Marking pen

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used.

8.0	Ordering
1.	When a *Streptococcus* Group A Antigen test is ordered, the accessioner will order both the rapid antigen test and culture. Sunquest can not automatically reflex microbiology tests. If the Antigen test is positive, the culture must be cancelled by the technologist performing the antigen test.

8.1	Specimen Preparation
1.	Specimens received for this test should be sorted into two groups of accessions prior to beginning testing: (1) dual swab submissions, and (2) single swab submissions.
2.	**If a single swab is submitted, streak the culture plate before starting the OSOM® Strep A procedure. Once a specimen has been used for this test, it is no longer acceptable for culture.**
3.	If two swabs are submitted, use one for the OSOM® Strep A test and reserve the second for the reflex culture if necessary.

Form revised 12/31/00

8.2	Test Run
1.	Just before testing, add 3 drops of Reagent 1 (pink to light red) and 3 drops of Reagent 2 to one Test Tube for each patient and each external control. (The solution should turn light yellow).
2.	Immediately put each patient swab into a Test Tube.
3.	**Negative and Positive External Controls**: Vigorously mix the control contents. For each control, add 1 free falling drop from the dropper bottle to a tube containing reagents 1 and 2. Place a clean swab from the test kit into each of the control tubes.
4.	Vigorously mix each solution by rotating the swab forcefully against the side of the Tube at least ten (10) times. Best results are obtained when specimen is vigorously extracted in the solution. Let stand one minute.
5.	Express as much liquid as possible from the swab by squeezing the sides of the tube as the swab is withdrawn. Discard the swab into bio-hazard waste.
6.	Remove a Test Stick from the container; recap the container immediately.
7.	Place the Absorbent End of a Test Stick into each of the extracted samples.
8.	Read the results at 5 minutes. Positive results may be read as soon as the red Control Line appears.
9.	Results are invalid after the stated read time. The use of a timer is recommended.

9. CALCULATIONS

Not applicable

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

Note: A blue or red line which appears uneven in color density is considered a valid result. In cases of moderate or high positive specimens, some blue color behind the Test Line may be seen; as long as the Test Line and Control are visible, the results are valid.

Result	Interpretation
A blue Test Line and a red Control Line. Note that the blue line can be any shade of color.	Positive for Group A streptococcal antigen.
A red Control Line but no blue Test Line is visible.	Presumptive Negative for Group A streptococcal antigen.
No red Control Line appears or background color makes reading the red Control Line impossible.	Invalid result.

10.2 Rounding

Not applicable

10.3 Units of Measure

Not applicable

10.4 Analytical Measurement Range (AMR)

Not applicable. This is a qualitative test reported as Detected or Not Detected.

The OSOM® Strep A is a qualitative test for the detection of Group A Streptococcal antigen. This test does not differentiate between viable and nonviable Group A Streptococci.

10.5 Review Patient Data

- Review patient results for unusual patterns, trends or distribution.
- Report atypical or unexpected results or trends for this test to appropriate supervisory personnel, prior to releasing results.

10.6 Repeat Criteria and Resulting

IF the result is …	THEN…
Negative	1. Issue Final report as: "Not Detected" 2. Perform the Throat Culture / Group A *Streptococcus* Culture. **See also section 8.1**
Positive	1. Issue Final report as: " Detected" 2. Cancel the Throat Culture / Group A *Streptococcus* Culture order.
Invalid Result	1. Issue final report as: "invalid result" using code INVD. The comment "Please repeat test if clinically indicated" will be appended to the result by the LIS. 2. Perform the Group A *Streptococcus* Culture. **See also section 8.1**

Result Message	Sunquest Result Code
Detected	DET
Not Detected	NTD
Invalid	INVD

11. EXPECTED VALUES

11.1 Reference Ranges

Not Detected

Form revised 3/31/00

11.2 Critical Values

Refer to the Critical Values SOP (WAH.L43 and SGAH.L45, GEC.L40)

11.3 Standard Required Messages

Not applicable

12. CLINICAL SIGNIFICANCE

Group A *Streptococcus* (*S. pyogenes*) is the most common cause of bacterial pharyngitis ("Strep throat") causing local pharyngeal pain, adenopathy, and fever. In addition, it is important to treat infections caused by this virulent organism in order to avoid potential, morbid complications including peritonsillar and retropharyngeal abscesses, bacteremia, rheumatic fever, and acute glomerulonephritis. Group A *Streptococcus*, however, may also be isolated in small numbers as part of the oropharyngeal flora of asymptomatic carriers.

13. PROCEDURE NOTES

- **FDA Status:** FDA Waived /Cleared or Approved
- **Validated Test Modifications:** None

- Approximately 19% of all upper respiratory tract infections are caused by Group A Streptococci. Streptococcal pharyngitis displays a seasonal variation and is most prevalent during winter and early spring. The highest incidence of this disease is found in crowded populations such as military bases and in school-age children.
- A negative result may be obtained if the specimen is inadequate or if the antigen concentration is below the sensitivity of this test.
- The American Academy of Pediatrics states [(5)]. "Several rapid diagnostic tests for GAS pharyngitis are available. The specificities of these tests generally are very high, but the reported sensitivities vary considerably. As with Throat cultures, the accuracy of these tests is most dependent on the quality of the throat specimen, which must contain tonsillar and pharyngeal secretions. Therefore, when a patient suspected of having GAS pharyngitis has a negative rapid streptococcal test, a throat culture should be obtained to ensure that the patient does not have GAS infection".
- The Genzyme OSOM® Strep A has been categorized as CLIA waived only for the application of qualitative detection of Group A Streptococcal Antigen from throat swabs. The application for the confirmation of presumptive Group A Streptococcal colonies recovered from culture is not waived.
- The use of swab specimens taken from sites other than throat or the use of other samples such as saliva, sputum or urine has not been established.
- This test does not differentiate between carriers and acute infection. Pharyngitis may be caused by organisms other than Group A *Streptococcus*.
- In the event that two swabs were submitted and the OSOM® Strep A test results were invalid from one swab, the test may be repeated using the second swab, rather than

reporting the test with the TNP message. Appropriate culture plate(s) must be setup prior to using the second swab for antigen testing. The extraction step in the OSOM® Strep A test makes the swab nonviable for culture. All criteria for a valid test must be obtained with the second swab in order to report that test result.

- This is an FDA cleared assay.

14. LIMITATIONS OF METHOD

14.1 Precision

Not applicable

14.2 Interfering Substances

- Gel transport medium may interfere with test results.
- The following organisms were tested at levels of approximately $1x10^8$ organisms/test and found to be negative when tested with the OSOM® Strep A kit.

Streptococcus Group B	*Cornybacterium diphtheria*
Streptococcus Group C	*Serratia marcescens*
Streptococcus Group F	*Candida albicans*
Streptococcus Group G	*Klebsiella pneumoniae*
Streptococcus pneumoniae	*Pseudomonas aeruginosa*
Streptococcus sanguis	*Bordetella pertussis*
Streptococcus mutans	*Neisseria meningitides*
Haemophilus influenzae	*Neisseria gonorrhoeae*
Enterococcus faecalis	*Neisseria sicca*
Staphylococcus aureus	*Neisseria subflava*
Staphylococcus epidermidis	*Branhamella catarrhalis*

14.3 Clinical Sensitivity/Specificity/Predictive Values

- In a multi-center study, OSOM® with 639 specimens from patients with pharyngitis. 464 specimens were culture negative and 454 negative by the OSOM® test for a specificity of 97.8%. Of the 175 culture positives, 168 were also positive by the OSOM® test for a sensitivity of 96.0%. Overall agreement was 97.3%
- A negative result may be obtained if the specimen is inadequate or antigen concentration is below the sensitivity of the test. Therefore, all specimens yielding negative OSOM® Group A Strep results should undergo confirmatory testing using the culture method.
- The Genzyme OSOM® Strep A should be used only with throat swabs. The use of swab specimens taken from the other sites or the use of other samples such as saliva, sputum or urine has not been established. The quality of the test depends on the quality of the sample; proper throat swab specimens must be obtained.

15. SAFETY

You, the employee, have direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Refer to your local and corporate safety manuals for detailed information on safety practices and procedures.

Report all accidents and injuries immediately to your supervisor or to the business unit Environmental Health and Safety Manager or Specialist.

16. RELATED DOCUMENTS

- Quest Diagnostics Incorporated Corporate Safety Manual.
- Quest Diagnostics Incorporated Critical and Priority Result Policy and Procedure SOP (QDMED704).
- Material Safety Data Sheets.
- Quest Diagnostics Incorporated *Directory of Services*, Specimen Collection section.
- Quest Diagnostics Incorporated Training Verification (QDNQA600) and Competency Assessment (QDNQA601) SOPs.
- Quest Diagnostics Incorporated Proficiency Test Handling and Result Submission SOP (QDNQA711).
- Quest Diagnostics Incorporated Records Management Program for Record Retention Requirements.
- *Streptococcus Group A Antigen with Reflex to Culture* (QDMI700)
- Local Quality Control policies and procedures
- Current package insert for OSOM® Strep A

17. REFERENCES

1. Youmans G.P., Paterson, P.Y., and Sommers, H.M., Upper Respiratory Tract Infections: General Considerations, in The Biologic and Clinical Basis of Infectious Diseases, W.B. Saunders Co., Philadelphia, 177-183, 1980.
2. Facklam, R.R., and Washington, J.A., Streptococcus and Related Catalase-Negative Gram-Positive Cocci, in Manual of Clinical Microbiology, 5th Edition, Balows, A., Hausler, W.J., Hermann, K.L., Isengerg, H.D., and Shadomy, H.J., Eds., Am. Society of Microbiology, Washington, D.C., 238-257, 1991.
3. CDC, Biosafety in Microbiological and Biomedical Laboratories, 2nd Ed., HHS Publication No. 8808395, 4-6, 1988.
4. Committee on Infectious Diseases, American Academy of Pediatrics. Group A Streptococcal Infections. In: Peter,G. ed. 1994 Red Book: Report of the Committee on Infectious Diseases. 23rd ed. Elk Grove Village, IL: American Academy of Pediatrics. 1994: 430 – 437.
5. Pickering LK.ed., Group A Streptococcal Infections, in 2000 RedBook; Report of Committee on Infectious Diseases, 25th ed. American Academy of Pediatrics, Elk Grove Village, IL., 528-530, 2000.
6. Lauer, B.A., Reller, L.B., and Mirrett, S., Effect of Atmosphere and Duration of Incubation on Primary Isolation of Group A Streptococci from Throat Cultures, J. Clin. Microb., 17:338-340, 1983.

7. Wannamaker, L.W., Differences Between Streptococcal Infections of the Throat and of the Skin, N. Eng. J. Med., 282:23-31, 78-85, 1970.
8. OSOM® Strep A Package Insert, June, 2011 Revision 3096-0. Sekisui Diagnostics
9. Assay and BD swab in liquid Amies Evaluation data, Quest Diagnostics, Collegeville, PA.

18. DOCUMENT HISTORY

Version	Date	Section	Revision	Revised By	Approved By
1.0	5/2012		New SOP, supersedes WAH.M26.001, SGAH.M26.001, GEC.M07.001	R. Master	R. Schlesinger
1.0	5/25/12	Title pg	Update to local format	L. Barrett	R. Master
1.0	5/25/12	1	Added local test code	R. Master	R. Master
1.0	5/25/12	3.1	Add 'reject' to list of unacceptable	L. Barrett	R. Master
1.0	5/25/12	8.0	Add instruction for ordering	R. Master	R. Master
1.0	5/25/12	10.6	Add local LIS codes	R. Master	R. Master
1.0	5/25/12	11.2	Update heading to local terminology, add local policy, removed corporate policy	L. Barrett	R. Master
1.0	5/25/12	16	Add current package insert	L. Barrett	R. Master
1.0	5/25/12	19	Add QC log	L. Barrett	R. Master

19. ADDENDA

Strep Group A Quality Control Log (see Attachment Tab of Infocard)

Quest Diagnostics

☐ Germantown Emergency Center
☐ Shady Grove Adventist Hospital
☐ Washington Adventist Hospital

STREP Group A QUALITY CONTROL LOG

1. **External Controls** are tested and documented with each new kit lot number or shipment.
2. Internal controls must be documented each time the test is performed (Y or Yes indicates acceptable performance, N or No indicates unacceptable).

Date	**Patient Name / MR#**	**Patient Result**	**Kit**	**Internal Negative Control**	**Internal Positive Control**	**Internal Reagent Control**	**External Positive Control**		**External Negative Control**		**Tech**
			Lot # / Expire	Clear (**Y**es or **N**o)	Red (**Y**es or **N**o)	Pink to Yellow (**Y**es or **N**o)	**Lot # / Expire**	**Result**	**Lot # / Expire**	**Result**	

Weekly review:	Weekly review:	Weekly review:
Weekly review:	Weekly review:	Monthly review: