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| Effective Date: Revised: |  |
| Approval:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Date\_\_\_\_\_\_\_ Medical DirectorAuthor name/title: Don Biehl MT(ASCP) |  |

**Principle:**

 Group A *Streptococcus*, or *Streptococcus pyogenes*, is a bacterium commonly found in the human throat or on skin. *S. pyogenes* causes a wide variety of diseases in humans, the most common being acute pharyngitis or strep throat. Pharyngitis is diagnosed in approximately 11 million patients in the United States each year, with Group A *Streptococcus* accounting for 15% - 30% of cases in children and 5% - 20% of cases in adults.3 Streptococcal pharyngitis is often accompanied by sore throat, fever, tonsillar exudates and enlarged cervical lymph nodes.

Group A Streptococcus infections may result in mild illness (e.g. pharyngitis, impetigo) or may lead to invasive, life-threatening illness (e.g. cellulitis, bacteremia, necrotizing fasciitis, Streptococcal toxic shock syndrome). Approximately 25% of patients with necrotizing fasciitis will die from the infection; more than 35% of patients with streptococcal toxic shock syndrome will die from the infection.4 *Streptococcus pyogenes* may also present in healthy, asymptomatic patients. Throat culture surveys of healthy children taken during school outbreaks have shown a carrier prevalence of up to 20%.3

The***illumi****gene* Group A *Streptococcus* (Group A Strep) assay, performed on the ***illumi****pro-10™*, is a qualitative in vitro diagnostic test for the detection of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*) in throat swab specimens.

The***illumi****gene* Group A Strepassay utilizes loop-mediated isothermal DNA amplification (LAMP)1,2 technology to detect *Streptococcus pyogenes* by targeting a segment of the *Streptococcus pyogenes* genome. Results from the ***illumi****gene* Group A Strep assay can be used as an aid in the diagnosis of Group A Streptococcal pharyngitis. The assay is not intended to monitor treatment for Group A *Streptococcus* infections.

The ***illumi****gene* Group A Strep kit includes ***illumi****gene* Sample Preparation Apparatuses and ***illumi****gene* Group A *Streptococcus* Test Devices. The ***illumi****gene* Sample Preparation Apparatus II, used for specimen dilution and preparation, is filled with a buffered solution containing formalin treated *E. coli* harboring *Staphylococcus aureus* DNA. The ***illumi****gene* Group A Strep Test Device contains one lyophilized amplification reagent bead in each of two chambers: a TEST chamber with Group A *Streptococcus*-specific primers and a CONTROL chamber with *S. aureus*-specific primers. The *S. aureus* DNA in the Sample Preparation Apparatus and the *S. aureus*-specific primers in the CONTROL chamber function as the Internal Control for the assay. During specimen preparation, each patient specimen is added to the Sample Preparation Apparatus II and combined with the *S. aureus* DNA prior to amplification. Addition of *S. aureus* DNA to the patient sample allows for parallel processing of target DNA and Control DNA through amplification and detection. The Internal Control monitors amplification inhibition, assay reagent performance and sample processing effectiveness. The Control *S. aureus* target must be amplified and detected in the final reaction or the test is considered invalid and patient results are not reported.

**Specimen:**

**Preferred Sample Type:**

1. Throat swabs in Liquid Amies or Stuart’s transport media.
2. Acceptable swabs: Rayon, polyester, or flocked nylon with plastic shafts.

**Undesirable Specimens:** Do not use throat swabs with Liquid Amies with charcoal transport media.

**Interfering Substances:** Throat swabs that may contain Zincum Aceticum 2X, Zincum Gluonicum 1X, as found in Zicam® Homeopathic Cold Remedies.

**Collection and Storage:** Throat sample collection should be performed in accordance with institutional guidelines for collection of clinical specimens for culture of Group A *Streptococcus*. Samples should be collected by vigorously swabbing the tonsils and the posterior pharynx.

Place swab(s) in a non-nutritive transport medium (e.g. Liquid Amies, without charcoal or Liquid Stuart) and transport to the laboratory. Samples should be held at 2-27 C during transport.

Samples may be held at 21-27 C for up to 48 hours prior to testing. When testing will not be initiated within this time, the sample may be stored at 2-8 C for up to seven days.

**Materials and Equipment:**

**REAGENTS/MATERIALS PROVIDED:**

1. ***illumi****gene* **Sample Preparation Apparatus II/Negative Control III (SMP PREP):** Tris-buffered solution containing formalin-treated *E. coli* harboring *S. aureus* DNA and sodium azide (0.09%) as a preservative.
2. ***illumi****gene* **Group A Strep Test Device:** Two-chambered device containing lyophilized amplification reagents (DNA polymerase, deoxynucleotide triphosphates) and either Group A *Streptococcus*-specific primers (TEST Chamber) or *Staphylococcus aureus*-specific primers (CONTROL Chamber)
3. ***illumi****gene* **Heat Treatment Tubes**

**MATERIALS PROVIDED SEPARATELY:**

* 1. ***illumi****gene* Group A *Streptococcus* (Group A Strep) External Control Kit, Catalog Number: 279910

**MATERIALS NOT PROVIDED:**

1. Disposable latex gloves, powder free
2. DNase/RNase-free, aerosol resistant pipette tips
3. Specimen collection and transport system
4. Swabs, breakable plastic shaft: Rayon, Polyester or Flocked Nylon
5. Non-nutritive Transport Medium: Liquid Amies, without charcoal; Liquid Stuart

**EQUIPMENT NOT PROVIDED:**

1. Dry-bath with 12 mm heat block capable of 95 C
2. Digital thermometer with Max/Min Temperature Memory (eg, Traceable® Lollipop™ Waterproof/Shockproof Thermometer)
3. Vortex mixer
4. Interval timer
5. Micropipette capable of dispensing 50 μL
6. ***iIlumi****pro-10™,* Meridian Bioscience, Inc. Catalog Number: 610172

**PERFORMANCE CONSIDERATIONS:**

1. All reagents are for in vitro diagnostic use only.
2. Do not interchange Sample Preparation Apparatuses or Test Devices between lots. Heat Treatment Tubes are interchangeable provided they are within assigned expiration date when used.
3. Follow Biosafety Level 2 and Good Laboratory practices during testing.6 Treat all specimens and used Test Devices as capable of transmitting infectious agents. Do not eat, drink or smoke in areas where specimens or kit reagents are handled.
4. Wear disposable gloves while handling specimens and thoroughly wash hands afterwards.
5. Quality Control Programs for Molecular Testing Laboratories, including proper use and care of equipment, should be employed.7
6. Th*e* ***illumi****gene* Group A Strep Test Device contains lyophilized reagents. The protective pouch should not be opened until ready to perform the assay.
7. The***illumi****gene* Group A Strep Test Device includes a latch feature that is designed to prevent contamination of the test area with amplification product. Do NOT use Test Devices with broken latches.
8. Dispose of used ***illumi****gene* Test Devices immediately after processing, leaving the device latch securely in place. Do NOT open the Test Device after processing. Opening the device after amplification may result in contamination of the test area with amplification product.

**STORAGE REQUIREMENTS:**

The expiration date is indicated on the kit label. Store the kit at 2-27 C.

Do not use the devices or reagents after their expiration dates.

At this facility, kits are stored at room temperature.

**Calibration:**

 There are no calibrations associated with this procedure.

**Quality Control:**

1. Each device contains an internal control chamber that controls for amplification inhibition, assay reagents and sample processing effectiveness.
2. The heat treatment step is monitored with an external thermometer and interval timer. Use the max/min temperature memory of the thermometer to ensure that a temperature of 95 ± 5 C is maintained. Use the interval timer to ensure that heat-treatment duration is 10 ± 2 minutes.
3. Good laboratory practice recommends the use of control materials. Users should follow the appropriate federal, state and local guidelines concerning the running of external quality controls.
4. ***illumi****gene* Group A *Streptococcus* External Control Reagents are supplied separately (Catalog 279910). It is recommended that the reactivity of each new lot and each new shipment of ***illumi****gene* Group A Strep be verified on receipt and before use. External control tests should be performed thereafter in accordance with appropriate federal, state and local guidelines. The ***illumi****gene* Group A Strep test kit should not be used in patient testing if the external controls do not produce the correct results.
5. A separate device must be used for each external control reagent.

QC Testing Frequency and Documentation:

For this facility, External QC is run every 30 days, each new lot, and each new delivery.

Results of External QC and action(s) taken when control results are unacceptable are documented.

**Procedure:**

**NOTE:** Ensure that the ***illumi****pro-10* is powered on and required performance verifications have been completed prior to initiation of SPECIMEN PREPARATION. Refer to the ***illumi****pro-10* Operator’s Manual for further information regarding instrument set-up and operation.

**Specimen Preparation:**

Add the throat swab to the ***illumi****gene* **SMP PREP**. Break off the handle of the swab. Replace and secure **SMP PREP** cap. Vortex the **SMP PREP** for a minimum of 10 seconds. Specimens in the ***illumi****gene* **SMP PREP** may be held at 2-29 C for up to 2 hours prior to heat treatment.

Remove the tip cap from the**SMP PREP** and squeeze five to 10 drops of sample into a clean ***illumi****gene*Heat Treatment Tube.

Repeat Sample Preparation Steps for all samples to be processed.

Heat each Sample/Control mixture in a dry-bath/heat block at 95 ± 5 C for 10 ±2minutes. Monitor heat-treatment step with digital thermometer and interval timer.

Remove each Heat Treatment Tube from the dry-bath/heat. Heat-treated samples may be held at 21-29 C for up to one hour prior to testing.

**NOTE:** A maximum of 10 samples can be processed in a single ***illumi****pro-10* run.

6. Vortex heat-treated samples for approximately 10 seconds.

7. Remove 1 ***illumi****gene* Group A Strep Test Device from its protective pouch per sample. Carefully open the device, holding the chambers such that the lyophilized reagent will not fall out upon opening. Place device on a flat surface or in a rack that can accommodate the device.

8. Transfer 50 µL of the heat-treated sample to the TEST chamber (White Bead) of the ***illumi****gene* Test Device. Take care to not introduce extraneous air to the reaction mixture. Using a new pipette tip, transfer 50 µL of the heat-treated sample to the CONTROL chamber (Yellow Bead) of the ***illumi****gene* Test Device. Take care to not introduce extraneous air to the reaction mixture. Close the ***illumi****gene* Test Device and fasten the latch securely.

9. Tap device on the bench top or mix to remove air bubbles. Carefully examine the test device for dissolution of the Control/Test Bead, for air bubbles left in the tube and liquid in the top of the device. If undissolved beads, air bubbles or liquid is noted, tap the device on the bench top and repeat the visual inspection.

10. Insert the ***illumi****gene* Test Device into the ***illumi****pro-10* and initiate amplification reaction and detection. Results will be displayed at the conclusion of the run.

**Interpretation of Results:**

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| **Sample ID**  | **Reported Result** | **Interpretation** |
| Patient Specimen | POSITIVE | Sample contains *Streptococcus pyogenes* target DNA. |
| NEGATIVE | No *Streptococcus pyogenes* DNA detected. |
| INVALID | **No reportable result. Repeat the test using the original sample.**Inhibitory patient specimen, improper sample preparation, reagent failure, instrument failure or internal control failure.  |
| Positive Control | POSITIVE | Valid positive control result. Reagents active at time of use, ***illumi****pro-10* performing correctly. |
| NEGATIVE | **Incorrect control result.** Repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian’s Technical Services at 1-800-343-3858 (US) or your local distributor. |
| INVALID | **No reportable result. Repeat entire assay run using original samples.** Improper sample preparation, reagent failure, instrument failure or internal control failure. |
| Negative Control | POSITIVE | **Incorrect control result.** Repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian’s Technical Services at 1-800-343-3858 (US) or your local distributor. |
| NEGATIVE | Valid negative control result. Reagents active at time of use, ***illumi****pro-10* performing correctly. |
| INVALID | **No reportable result. Repeat entire assay run using original samples.** Improper sample preparation, reagent failure, instrument failure or internal control failure. |
| EMPTY WELL | NONE | No ***illumi****gene* Test Device in the ***illumi****pro-10* Well.**OR**The ***illumi****gene* Test Device present is compromised due to sample preparation failure, dirty device or improperly seated device. **Repeat the test using original sample.** |

**Reporting of Results:**

**Positive Test:** Group A *Streptococcus (Streptococcus pyogenes*) detected by NAAT or molecular method.

**Negative Test:** No Group A *Streptococcus* (*Streptococcus pyogenes*) detected.

**EXPECTED VALUES:**

Overall incidence of *Streptococcus pyogenes* in patients tested during the 2012 clinical study was 14.6% (116/796).

**ASSAY REACTIVITY:**

The following *S. pyogenes* strains were tested and produced positive reactions at or below stated assay limit of detect of 400 CFU/Test with ***illumi****gene* Group A Strep: ATCC12384, NCIMB 13285, CCUG 33061, CCUG 33409, CCUG 39158, ATCC 49399, CCUG 53553.

**TESTS FOR INTERFERING SUBSTANCES:**

Interfering substance testing was performed by adding potentially interfering substances diluted in sterile saline or contrived positive samples (ATCC 12344, ATCC 19615) to the ***illumi****gene* **SMP PREP**. Rayon swabs inoculated with negative throat flora and Liquid Amies Transport Media were added to the ***illumi****gene* **SMP PREP** containing potentially interfering substances and tested.

The following biological substances, at the saturated solvent/diluent concentrations indicated, do not interfere with test results: Mucus (5.0mg/mL), Human saliva (10% v/v), and Whole Blood (2.5% v/v). Whole Blood at concentrations greater than 2.5% v/v may interfere with the ***illumi****gene* Group A Strep assay.

The following chemical substances, at the saturated solvent/diluents concentrations indicated, do not interfere with test results:

Acetaminophen (19.5 mg/mL), Aspirin (12.3 mg/mL), Cepacol® Mouthwash, [Cetyopridinium Chloride (0.005% v/v)], Cepacol® Sore Throat Lozenges [Benzocaine (0.09 mg/mL), Menthol (0.02 mg/mL)], Chloraseptic® Oral Anesthetic/Analgesic [Phenol (0.07% v/v)], Contac® Cold + Flu Tablets [Acetaminophen (16.2 mg/mL), Chlorpheniramine maleate (0.06 mg/mL), Phenylephrine HCl (0.16 mg/mL)], Crest® Complete Toothpaste [Sodium fluoride (0.1 mg/mL)], Diphenhydramine HCl (2.7 mg/mL), HALLS® Cough Drops [Menthol (0.08 mg/mL)], Ibuprofen (15.6 mg/mL), Listerine® Antiseptic Mouthwash [Eucalyptol (0.0092% v/v), Menthol (0.0042% v/v), Methyl salicylate (0.0060% v/v), Thymol (0.0064% v/v)], Robitussin® Cough/Chest Congestion Cough Syrup [Dextromethorphan HBr (0.2 mg/mL), Guaifenesin (2.0 mg/mL)].

Zicam® Oral Mist [Zincum Aceticum 2X, Zincum Gluonicum 1X (0.625% v/v)] produced invalid results in all replicates tested.

**LIMITATIONS:**

1. The ***illumi****gene* Group AStrep assay does not distinguish between viable and non-viable organisms.
2. Respiratory infections can be caused by Streptococci of serogroups other than A as well as other pathogens. This device does not differentiate between carriers and infected individuals.
3. The use of antibiotics or over-the-counter medications may suppress the *Streptococcus* growth in culture despite the presence of organisms detectable by nucleic acid tests.
4. As will all diagnostic tests, results should be interpreted together with other information available to the physician.
5. Zincum Aceticum 2X, Zincum Gluonicum 1X, as found in Zicam® Homeopathic Cold Remedies, interfere with the ***illumi****gene* Group A Strep Assay.

**PERFORMANCE CHARACTERISTICS:**

Refer to Directional Insert- Meridian Bioscience **illumi**gene Group A *Streptococcus*

**REFERENCES:**

Refer to Directional Insert- Meridian Bioscience **illumi**gene Group A *Streptococcus*

Reviewed (No Changes):

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