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).

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.

Solana Strep Complete

CLIA Complexity: Moderate

INTENDED USE

The Solana Strep Complete Assay is an *in vitro* diagnostic test, using isothermal amplification technology (helicase-dependent amplification, HDA), for the rapid qualitative detection and differentiation of *Streptococcus pyogenes* (Group A β-hemolytic Streptococcus) and *Streptococcus dysgalactiae* (pyogenic Group C and G β-hemolytic Streptococcus) nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis, such as sore throat. The Solana Strep Complete Assay is intended for use only with the Solana instrument.

SUMMARY AND EXPLANATION

Streptococcal pharyngitis, or "strep throat", is a common bacterial infection found in childhood. Strep throat affects all age groups but is most common in children between the ages of 5 to 15 years of age and is a costly disease to society due to medical care and absence from school.

Streptococcus pyogenes (Group A β-hemolytic Streptococcus, GAS) is the most common bacterial cause of acute pharyngitis, affecting approximately 1 in 10 children per year [1]. *Streptococcus dysgalactiae* (pyogenic Group C and G β-hemolytic Streptococcus, C/G) is an important human pathogen and cause the clinical spectrum of diseases that closely resemble GAS infections, including streptococcal pharyngitis. The Solana Strep Complete Assay detects and differentiates *Streptococcus pyogenes* and *Streptococcus dysgalactiae*.

Streptococcal throat infection has an incubation period of 2 to 4 days. Classic symptoms include the abrupt onset of sore throat accompanied by fever, malaise and headache. Physicians diagnose strep throat based on symptoms, physical findings and diagnostic procedures. When strep throat is suspected, prompt and accurate treatment is paramount in order to prevent the occurrence of non-suppurative disease, specifically acute rheumatic fever and post streptococcal acute glomerulonephritis. Traditional laboratory diagnosis is performed by culture, such as plating on sheep blood agar followed by Lancefield group differentiation with latex agglutination. The culture results may take 2 to 3 days. The Solana Strep Complete Assay allows for the rapid, accurate detection and differentiation of *Streptococcus pyogenes* and *Streptococcus dysgalactiae* without the need for culture confirmation.

Streptococci are classified by the production of hemolysis on blood agar and by the use of Lancefield group antigens. The beta-hemolytic isolates under Lancefield group A, C, F, and G are subdivided into large and small colony forming groups. The large colony groups possess numerous virulence mechanisms, and are labeled "pyogenic." *Streptococcus dysgalactiae* is a species of pyogenic β-hemolytic Streptococcus C/G commonly isolated from humans [1]. *Streptococcus dysgalactiae* is comprised of two subspecies: *Streptococcus dysgalactiae* subsp *dysgalactiae* (SDSD) and *Streptococcus dysgalactiae* subsp *equisimilis* (SDSE) [2, 3]. The

Solana Strep Complete Assay detects SDSE and SDSD that are pyogenic β-hemolytic Strep C/G and differentiates with GAS.

PRINCIPLE OF THE TEST

The Solana Strep Complete Assay amplifies, detects and differentiates *Streptococcus pyogenes* DNA and *Streptococcus dysgalactiae* DNA present in throat swab specimens obtained from symptomatic patients.

The assay consists of two major steps: 1) specimen preparation, and 2) amplification and detection of target sequence specific to *S. pyogenes* (GAS) and *S. dysgalactiae* (C/G) using isothermal Helicase-Dependent Amplification (HDA) in the presence of target-specific fluorescence probe.

Patient specimen on a throat swab is transferred to a Lysis Tube and subjected to heat-treatment at 95°C for 5 minutes. The heat-treated sample is added to a Dilution Tube, and then transferred to two Reaction Tubes, GAS Reaction Tube and Strep C/G Reaction Tube. GAS Reaction Tube contains white lyophilized HDA reagents, dNTPs, primers and probes specific for the amplification and detection of *S. pyogenes* target sequence, while C/G Reaction Tube contains blue lyophilized HDA reagents, dNTPs, primers and probes specific for the amplification and detection of *S. dysgalactiae* target sequence. Once rehydrated with the diluted sample, the Reaction Tubes are placed in a Solana Instrument for amplification and detection of the target sequences. In Solana, the target sequences are amplified by specific primers and detected by a specific fluorescence probe included in each Reaction Tube. Two (2) competitive process controls (PRCs) are included in the Lysis Tube to monitor sample processing, inhibitory substances in clinical samples, reagent failure or device failure for each target. PRCs are amplified by the target-specific primers and detected by a PRC specific fluorescence probe.

The target and PRC probes are labeled with a quencher on one end and a fluorophore on the other end. Upon annealing to target or PRC amplicons, the fluorescence signal increases due to physical separation of the fluorophore from the quencher. Solana measures and interprets the fluorescent signal for each Reaction Tube, using on-board method-specific algorithms. Solana then reports the test results for each Reaction Tube to the user on its display screen, and optionally prints out the results via a printer.

MATERIALS PROVIDED

Cat. #M305

48 Tests per Kit

Component	Quantity	Storage
Strep Complete Lysis Buffer	48 tubes/kit 0.5 mL	2°C to 8°C
Strep Dilution Buffer	48 tubes/kit 0.5 mL	2°C to 8°C
GAS Reaction Tubes	48 tubes/kit	2°C to 8°C
Strep C/G Reaction Tubes	48 tubes/kit	2°C to 8°C

MATERIALS REQUIRED BUT NOT PROVIDED

- External controls for Group A Streptococcus (e.g. Quidel Molecular Strep A+G Control Set (M111), which contains positive and negative controls, serves as an external processing and extraction control)
- Sterile DNase-free filter-blocked or positive displacement micropipettor tips
- Micropipettor
- Stopwatch or timer
- Scissors or a blade
- Heat block capable of 95° ±2°C temperature
- Thermometer
- Solana workflow tray and transfer rack
- Solana instrument

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Refer to the Solana User Manual for further information regarding instrument installation and operation.
- Only use the protocol described in this package insert. Deviations from the protocol may give erroneous results.
- Performance characteristics of this test have been established with the specimen type listed in the Intended Use section only. The performance of this assay with other specimen types or samples has not been evaluated.
- All reagents are for *in vitro* diagnostic use only.
- Treat all specimen/samples as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
- All tubes should be capped tightly prior to vortexing.
- Proper sample collection, storage and transport are essential for correct results.
- Store assay reagents as indicated on their individual labels.
- Reagents are not interchangeable between lots.
- Never pool reagents from different tubes even if they are from the same lot.
- Do not use the reagents after their expiration date.
- Do not use kit components that appear to be broken or damaged.
- Do not interchange caps among reagents as contamination may occur and compromise test results.
- Only open the tubes when adding aliquots into tubes or removing aliquots from tubes. Keep the tubes closed at any other time to avoid contamination.
- To avoid contamination of the environment with amplicons, do not open the reaction tubes post-amplification.
- Avoid microbial and deoxyribonuclease (DNase) contamination of reagents when removing aliquots from tubes. The use of sterile DNase-free disposable filter-blocked or positive displacement pipettor tips is recommended.
- Use a new pipettor tip for each specimen or reagents.
- Performing the assay outside of the recommended time ranges can produce invalid results. Assays not completed within specified time ranges should be repeated.
- False negative results may occur if a specimen is improperly collected, transported or handled; or if inadequate quantities of the target nucleic acid are present in the specimen.
- Test results should be interpreted in conjunction with other laboratory and clinical data.
- Positive test results do not rule out co-infections with other pathogens.
- Negative test results do not rule out possible other infections besides those caused by *Streptococcus pyogenes* and *Streptococcus dysgalactiae*.
- To avoid exposure to excessive heat, care should be taken when inserting and removing tubes from the heat block, and when handling the heated tubes.
- Additional controls may be tested according to guidelines or requirements of local, state, provincial and/or federal regulations or accrediting organizations.
- Do not pipette by mouth.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Dispose of used devices, pipettes and specimen tubes according to your institution's safety guidelines for hazardous material.
- For accurate results, pipette carefully using only calibrated equipment.
- Maintenance and decontamination of workspace and equipment should follow and be performed according to established laboratory protocols and schedules.
- Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.

- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE AND HANDLING OF KIT REAGENTS

Store the Assay Kit at 2°C to 8°C until the expiration date listed on the outer kit box.

SPECIMEN COLLECTION, STORAGE AND HANDLING

During clinical studies, the Solana Strep Complete Assay was evaluated with Liquid Amies Single Plastic Applicator, Liquid Stuart Single Plastic Applicator, Puritan Liquid Amies Transport System, Copan eSwab Transport System and Sterile Rayon and Polyester Throat Swabs.

Analytical studies performed with contrived specimens containing *Streptococcus pyogenes* and *Streptococcus dysgalactiae*, near LOD (2x LOD) demonstrated that samples can be stored at 25°C ±2°C for 2 days and then at 2°C to 8°C for up to 6 more days before testing or at ≤ –15°C or ≤ –70°C for up to 32 days before testing with the Solana Strep Complete Assay. Specific requirements for shipping specimens should follow recommendations found in section 42 and 49 of the Code of Federal Regulation, CFR.

TEST PROCEDURE

1. Turn on Solana by pressing the power button and wait until it completes self-testing.
   NOTE: Do not open the lid during the self-testing.
2. 25 minutes prior to the heat lysis step, warm a heating block to 95°C.
3. Place the required number of Lysis Tubes in a rack. Mark the Lysis Tubes on the cap and/or side of the tube.
   Note: One (1) Lysis Tube is required for each specimen or control to be tested.
   Note: A maximum of 12 tests can be performed in a single Solana instrument.
4. Place a throat swab in a patient-identified Lysis Tube and vigorously twirl the swab for 10 seconds to elute specimen material. When ESwab is used for specimen collection, vortex the ESwab collection device for 5 seconds and transfer 50 µL of the ESwab transport medium to a patient-identified Lysis Tube.
   Note: The specimens in Lysis Tubes may be stored at room temperature (20°C to 25°C) or at 2°C to 8°C for up to 24 hours.
5. Heat the Lysis Tubes at 95° ±2°C for 5 minutes and then vortex the Lysis Tubes for 5 seconds.
   Note: Begin 5-minute lysis procedure when the heat block measures 95° ± 2°C. The timer must be stopped if the temperature falls out of range at any time during the 5 minute period and cannot be restarted until the heat block returns to 95° ± 2°C.
   Note: The lysed specimens may be stored at room temperature (20°C to 25°C) or at 2°C to 8°C for up to 24 hours.
6. Place the required number of Dilution Tubes in a rack. Mark the Dilution Tubes on the cap and/or side of the tube.
   Note: One (1) Dilution Tube is required for each specimen or control to be tested.
7. Transfer 50 µL of each specimen to an identified Dilution Tube. Close the cap and mix the solution well by vortexing the tubes for 5 seconds.
   Note: Use a new pipette tip for each specimen.
   Note: The diluted specimen or control can be stored at room temperature (20°C to 25°C) or at 2°C to 8°C for up to 24 hours.
8. Remove the required number of GAS Reaction Tubes and Strep C/G Reaction Tubes from the protective pouch, remove the excess air and reseal the bag. Mark the Reaction Tubes on the cap.
   Note: GAS Reaction Tube contains white lyophilized reagents, while Strep C/G Reaction Tube contains blue lyophilized reagents.

9. Transfer 50 µL of the diluted specimen to the labeled GAS Reaction Tube, mix the solution by vigorously pipetting up and down 3 to 5 times and close the cap, and transfer 50 µL of the same specimen to the labeled Strep C/G Reaction Tube, mix the solution by vigorously pipetting up and down 3 to 5 times, close the cap. The solutions should be clear and free of solid material.
   Note: Use a new pipette tip for each diluted sample and for each Reaction Tube.
   Note: Proceed immediately to the next step. Do not allow reconstituted reaction mix to sit for longer than 15 minutes.
10. Open the lid and place the Reaction Tubes in Solana.
    Note: Be sure that all tubes are in tight contact with heat block.
11. Enter User ID and Password and press ↵ (ENTER).
12. Select "NEW TEST." If Solana displays a different screen, go to the home screen.
13. Select the tube positions to use.
14. Scan the assay barcode or select "Strep_Comp" from the Select Test drop-down menu and manually enter Lot ID/Exp Date, and press "▶."
15. Select sample type (patient or QC) from the drop-down menu and enter Sample IDs (optional; see 2nd Note in next step).
16. Close the lid and press "Start" to initiate the Solana Strep Complete Assay. Solana will display the progress and the count-down to assay completion. The test results will be displayed on the screen in approximately 25 minutes.
    Note: To avoid laboratory contamination, once the tube has been closed and the amplification reaction started, **DO NOT** open the Reaction Tube.
    Note: While the test is running, sample ID can be entered or edited by pressing the pencil icon.
17. After the run is completed the results can be printed by selecting the print button.
    Note: Do not navigate away from this screen before printing results. Once the screen is gone it cannot be revisited. If this occurs the results can be viewed individually by going Home then selecting Review Results.

INTERPRETATION OF RESULTS

Samples	Assay Result	Interpretation
Patient specimen	GAS POSITIVE	GAS tested, and GAS DNA detected
	GAS NEGATIVE	GAS tested, No GAS DNA detected and PRC detected
	GAS INVALID	GAS tested, No GAS DNA detected and No PRC detected; for invalid test results, retest the same processed sample first. If the test is invalid upon retesting with the processed sample, re-process another aliquot of the same sample or obtain a new sample and re-test.
	C/G POSITIVE	*S. dysgalactiae* Strep C/G tested, *S. dysgalactiae* Strep C/G DNA detected
	C/G NEGATIVE	*S. dysgalactiae* Strep C/G tested, No *S. dysgalactiae* Strep C/G DNA detected and PRC detected
	C/G INVALID	*S. dysgalactiae* Strep C/G tested, No *S. dysgalactiae* Strep C/G DNA detected and No PRC detected; for invalid test results, retest the same processed sample first. If the test is invalid upon retesting with the processed sample, re-process another aliquot of the same sample or obtain a new sample and re-test.

QUALITY CONTROL

The Solana Strep Complete Assay incorporates several controls to monitor assay performance.

1. The process control is used to monitor sample processing, to detect HDA inhibitory specimens and to confirm the integrity of assay reagents and Solana. The process control is included in the Lysis Buffer tube.
2. The external positive control may be treated as a patient specimen. The control should be sampled and tested as if it were a specimen and processed as described above in the Assay Procedure. The external positive control is intended to monitor substantial reagent and instrument failure.
3. The external negative control may be treated as a patient specimen. The control should be sampled and tested as if it were a specimen and processed as described above in the Assay Procedure. The external negative control is used to detect reagent or environmental contamination (or carry-over) by *Streptococcus pyogenes* or *Streptococcus dysgalactiae* DNA or amplicon.

It is recommended that the reactivity of each new lot and each new shipment of the Solana Strep Complete Assay be verified on receipt and before use. External control tests should be performed thereafter in accordance with appropriate federal, state and local guidelines. The Solana Strep Complete Assay should not be used in patient testing if the external controls do not produce the correct results.

LIMITATIONS

- Additional follow-up testing using the culture method is required if the result is negative and clinical symptoms persist, or in the event of an acute rheumatic fever (ARF) outbreak.
- *Klebsiella pneumoniae, Serratia marcescens* and *Enterococcus faecalis* each cross-reacted once out of six times tested.
- The main laboratory technique required is pipetting. Good laboratory technique is essential for the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all reagents, especially in cases where multiple aliquots are taken from a tube.
- The Solana Strep Complete Assay does not distinguish between viable and non-viable organisms and may produce a positive result in the absence of living organisms.
- The Solana Strep Complete Assay does not differentiate asymptomatic carriers of *Streptococcus pyogenes* or *Streptococcus dysgalactiae* from those exhibiting streptococcal infection.
- Positive test results do not rule out the possibility of co-infection with other pathogens, including other forms of Group C or G streptococci, such as *Streptococcus canis or Streptococcus equi*.
- As with other assays of this type, there is a risk of false negative results due to the presence of sequence variants in the amplification targets.

EXPECTED VALUES

Performance characteristics of the Solana Strep Complete Assay were established during a prospective study during the winter through summer of 2016 (February to July). Two thousand six hundred eighty-eight (2688) fresh throat swab specimens were included in this study at four (4) external and one (1) internal laboratory sites across the United States using the same swab that was plated for the culture. A single specimen was collected per patient. Samples were collected on Polyester or Rayon Swab with liquid Amies, Polyester Swab or Rayon with liquid Stuart's or nylon swab with liquid Amies.

The gender and age demographics for each category are listed below.

Combined Study – Age and Gender Distribution		
Gender	Female	Male
Total	1526	1162
Age		
$\leq$ 2 years	74	84

Combined Study – Age and Gender Distribution		
3 to 12 years	590	599
13 to 21 years	330	227
≥ 22 years	532	252

The prevalence of *Streptococcus pyogenes* (Group A β-hemolytic Streptococcus) and *Streptococcus dysgalactiae* (pyogenic Group C and G β-hemolytic Streptococcus) with the Solana Strep Complete Assay has been calculated based on the age of the patient. Two (2) specimens were invalid when tested with the Solana Strep Complete Assay (0.07%) (in both the initial and repeat test no internal control was detected) and have been removed from the Expected Values table. The table below presents the data for the remaining two thousand six hundred eighty-six (2686) specimens.

The overall prevalence of *Streptococcus pyogenes* or *Streptococcus dysgalactiae* in patients tested during this study based on culture results alone was 16.0% (431/2686) for *Streptococcus pyogenes* and 2.4%(65/2686) for *Streptococcus dysgalactiae*. The overall incidence of *Streptococcus pyogenes* or *Streptococcus dysgalactiae* in patients tested during this study based on a combination of culture results and another FDA-cleared NAAT assay was 17.9% (481/2686) for *Streptococcus pyogenes* and 2.9% (78/2686) for *Streptococcus dysgalactiae*.

Combined Study Prevalence (n=2686)						
Age	*Streptococcus pyogenes*			*Streptococcus dysgalactiae*		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤ 2 years	158	11	7.0%	158	3	1.9%
3 to 12 years	1189	336	28.3%	1189	12	1.0%
13 to 21 years	556	50	9.0%	556	38	6.8%
≥ 22 years	783	103	13.2%	783	39	5.0%
Overall	2686	481	17.9%	2686	78	2.9%

CLINICAL PERFORMANCE

Performance characteristics of the Solana Strep Complete Assay were established during a prospective study during the winter through summer of 2016 (February to July). Two thousand six hundred eighty-eight (2688) fresh throat swab specimens were included in this study at four (4) external and one (1) internal laboratory using the same swab that was plated for the culture at sites across the United States. A single specimen was collected per patient. Samples were collected on Polyester or Rayon Swab with liquid Amies, Polyester Swab or Rayon with liquid Stuart's or nylon swab with liquid Amies.

A composite result of directly cultured patients' throat swabs combined with the culture. Cultured isolates were typed by latex agglutination. β-hemolytic isolates that were typed as Group C or G were subcultured and the species were determined using an FDA-cleared MALDI TOF assay. Swab transport fluid was also tested using another FDA cleared nucleic acid amplification test (NAAT) and cultured at a central reference laboratory. Results from culture and NAAT were used to calculate assay sensitivity and specificity. Each site cultured the swabs prior to performing the Solana Strep Complete Assay. The swab specimens were processed and tested with Solana Strep Complete Assay. The leftover swab transport media was shipped to the central location for an additional culture and NAAT testing.

Two thousand six hundred eighty-eight (2688) fresh throat swab specimens were tested using the algorithm described above (dual culture, FDA-cleared NAAT and Solana Strep Complete Assay). Two (2) specimens were repeatedly invalid when tested with the Solana Strep Complete Assay (0.07%). These specimens have been

removed from additional analysis. The table below details the combined results for *Streptococcus pyogenes* for the remaining two thousand six hundred eighty-six (2686).

Combined Clinical Sites' Results for *Streptococcus pyogenes*			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	475	25	500
Negative	6	2180	2186
Total	481	2205	2686
			95% CI
Sensitivity	475/481	98.8%	97.3% to 99.4%
Specificity	2180/2205	98.9%	98.3% to 99.2%

Site 1 – *Streptococcus pyogenes* Results			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	90	4	94
Negative	2	679	681
Total	92	683	775
			95% CI
Sensitivity	90/92	97.8%	92.4% to 99.4%
Specificity	679/683	99.4%	98.5% to 99.8%
Site 2 – *Streptococcus pyogenes* Results			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	84	6	90
Negative	1	510	511
Total	85	516	601
			95% CI
Sensitivity	84/85	98.8%	93.6% to 99.8%
Specificity	510/516	98.8%	97.5% to 99.5%
Site 3 – *Streptococcus pyogenes* Results			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	100	3	103
Negative	3	492	495
Total	103	495	598
			95% CI
Sensitivity	100/103	97.1	91.8% to 99.0%
Specificity	492/495	99.4%	98.2% to 99.8%

Site 4 – *Streptococcus pyogenes* Results			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	83	12	95
Negative	0	254	254
Total	83	266	349
			95% CI
Sensitivity	83/83	100%	95.6% to 100%
Specificity	254/266	95.5%	92.3% to 97.4%
Site 5 – *Streptococcus pyogenes* Results			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	118	0	118
Negative	0	245	245
Total	118	245	363
			95% CI
Sensitivity	118/118	100%	96.8% to 100%
Specificity	245/245	100%	98.5% to 100%

Two thousand six hundred eighty-eight (2688) fresh throat swab specimens were tested using the algorithm described above (dual culture, FDA-cleared NAAT and Solana Strep Complete Assay). Two (2) specimens were repeatedly invalid when tested with the Solana Strep Complete Assay (0.07%). The table below details the combined results for *Streptococcus dysgalactiae* for the remaining two thousand six hundred eighty-six (2686).

Combined Clinical Sites' Results for *Streptococcus dysgalactiae*			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	78	14	92
Negative	0	2594	2594
Total	78	2608	2686
			95% CI
Sensitivity	78/78	100%	95.3% to 100%
Specificity	2594/2608	99.5%	99.1% to 99.7%
Site 1 – *Streptococcus dysgalactiae* Results			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	32	4	36
Negative	0	739	739
Total	32	743	775
			95% CI
Sensitivity	32/32	100%	89.3% to 100%
Specificity	739/743	99.5	98.6% to 99.8%

Site 2 – *Streptococcus dysgalactiae* Results			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	16	5	21
Negative	0	580	580
Total	16	585	601
			95% CI
Sensitivity	16/16	100%	80.6% to 100%
Specificity	580/585	99.1%	98.0% to 99.6%
Site 3 – *Streptococcus dysgalactiae* Results			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	26	4	30
Negative	0	568	568
Total	26	572	598
			95% CI
Sensitivity	26/26	100%	87.1% to 100%
Specificity	568/572	99.3%	98.2% to 99.7%
Site 4 – *Streptococcus dysgalactiae* Results			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	2	0	2
Negative	0	347	347
Total	2	347	349
			95% CI
Sensitivity	2/2	100%	34.2% to 100%
Specificity	347/347	100%	98.9% to 100%
Site 5 – *Streptococcus dysgalactiae* Results			
	Combined Culture and NAAT Result		
Solana Strep Complete Assay	Positive	Negative	Total
Positive	2	1	3
Negative	0	360	360
Total	2	361	363
			95% CI
Sensitivity	2/2	100%	34.2% to 100%
Specificity	360/361	99.7%	98.4% to 100%

ANALYTICAL PERFORMANCE

Limit of Detection

The analytical sensitivity (limit of detection or LOD) of the Solana Strep Complete Assay was determined using quantified (CFU/mL) cultures of two (2) *Streptococcus pyogenes* and two (2) *Streptococcus dysgalactiae* subsp *equisimilis* strains by serial dilution. Analytical sensitivity (LOD) is defined as the lowest concentration at which 95% of all replicates tested positive.

The LOD for the 2 *Streptococcus pyogenes* strains tested were $1.5x10^4$ CFU/mL (ATCC #19615) and $8.5x10^4$ CFU/mL (ATCC #12344). The LOD for the two (2) *Streptococcus dysgalactiae* subsp *equisimilis* strains were $5.7x10^5$ CFU/mL (ATCC 12394) and $7.1x10^5$ CFU/mL (ATCC #10009).

Based on this data the reported LOD for *Streptococcus pyogenes* and *Streptococcus dysgalactiae* using the Solana Strep Complete Assay is $8.5x10^4$ CFU/mL and $7.1x10^5$ CFU/mL, respectively.

Analytical Reactivity (Inclusivity)

The inclusivity of the Solana Strep Complete Assay was further evaluated by functional testing of organisms in addition to those strains used in the LOD study. Seven (7) strains of *Streptococcus pyogenes* (GAS) and twenty-five (25) *Streptococcus dysgalactiae* (C/G) strains were tested at concentrations at a LOD of $8.5x10^4$ CFU/mL and $7.1x10^5$ CFU/mL, respectively.

Bacterial species	Bacterial Strain*	Concentration CFU/mL	Strain Detected (Yes/No)
Streptococcus pyogenes	ATCC 12384	$8.48x10^4$	Yes
Streptococcus pyogenes	NCIMB 13285	$8.48x10^4$	Yes
Streptococcus pyogenes	CCUG 33061	$8.48x10^4$	Yes
Streptococcus pyogenes	CCUG 33409	$8.48x10^4$	Yes
Streptococcus pyogenes	CCUG 39158	$8.48x10^4$	Yes
Streptococcus pyogenes	ATCC 49399	$8.48x10^4$	Yes
Streptococcus pyogenes	CCUG 53553	$8.48x10^4$	Yes
S. dysgalactiae subspecies equisimilis group G	ATCC 6644	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group C	ATCC 9542	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group C	ATCC 12388	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group C	ATCC 35666	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group G	CCUG 502	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group C	CCUG 1483	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group C	CCUG 6713	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group G	CCUG 15679	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group G	CCUG 15680	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group G	CCUG 21557	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group G	CCUG 24070	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group G	CCUG 26147	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group G	CCUG 27477	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group C	CCUG 27479	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group C	CCUG 27480	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group G	CCUG 27482	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group G	CCUG 27483	$7.07x10^5$	Yes
S. dysgalactiae subspecies dysgalactiae group C	CCUG 27658	$7.07x10^5$	Yes
S. dysgalactiae subspecies dysgalactiae group C	CCUG 27659	$7.07x10^5$	Yes
S. dysgalactiae subspecies dysgalactiae group C	CCUG 27664	$7.07x10^5$	Yes
S. dysgalactiae subspecies dysgalactiae group C	CCUG 28115	$7.07x10^5$	Yes
S. dysgalactiae subspecies dysgalactiae group C	CCUG 28116	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group C	CCUG 28238	$7.07x10^5$	Yes
S. dysgalactiae subspecies equisimilis group G	CCUG 33802	$7.07x10^5$	Yes
S. dysgalactiae subspecies dysgalactiae group C	CCUG 48477	$7.07x10^5$	Yes

*ATCC: American Type Culture Collection; CCUG: Culture Collection, University of Göteborg

Repeatability Study
The Precision/Within Laboratory Repeatability was determined via a study, where a four-member panel (3x, 1x, 0.3x LOD of both *Streptococcus pyogenes* and *Streptococcus dysgalactiae* and a negative sample) was tested by two (2) operators for twelve (12) days.

The Solana Strep Complete Assay produces results that are highly reproducible. This observation is based on the following findings:

- All negative samples generated negative results for both *Streptococcus pyogenes* and *Streptococcus dysgalactiae*.
- The percentage of positive High Negative (0.3x LOD) *Streptococcus pyogenes* samples is 43%, this is within the target range of 20% to 80%.
- The percentage of positive High Negative (0.3x LOD) *Streptococcus dysgalactiae* samples is 28%, this is within the target range of 20% to 80%.
- The percentage of positive of the Low Positive for both *Streptococcus pyogenes* and *Streptococcus dysgalactiae* (1x LOD) samples was 100%.
- The percentage of positive of the Moderate Positive for both *Streptococcus pyogenes* and *Streptococcus dysgalactiae* (3x LOD) samples was 100%.

Reproducibility Study
In order to confirm the reproducibility of the Solana Strep Complete Assay a blinded and randomized study panel containing both *Streptococcus pyogenes* and *Streptococcus dysgalactiae* negative and positive samples (3x, 1x, 0.3x LOD) were tested at three (3) test sites (one in-house laboratory and two (2) clinical sites) with three (3) instruments. Each site tested a reproducibility panel and Assay Controls for five (5) days in triplicate. Testing was done by two operators at each site. Each operator ran the panel once a day using one lot of Solana Strep Complete Assay. A total of five hundred forty (540) specimens were tested (including controls). The Solana Strep Complete Assay generated reproducible results in this study.

Streptococcus pyogenes **Category**	**SITE**						**Overall Percent Positive**		**95% Confidence Interval**
	Site #1		**Site #2**		**Site #3**				
	Detected: #positive /# tested	*% Positive*	*Detected: #positive /# tested*	*% Positive*	*Detected: #positive /# tested*	*% Positive*			
GAS High Negative	13/30	43%	10/30	33%	13/30	43%	36/90	40%	27% to 47%
GAS Low Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
GAS Moderate Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
GAS Negative	0/30	0%	0/30	0%	0/30	0%	0/90	0%	0% to 4%
GAS Positive Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
GAS Negative Control	0/30	100%	0/30	0%	0/30	100%	0/90	0%	0% to 4%

***Streptococcus dysgalactiae* Category**	**SITE**						**Overall Percent Positive**		**95% Confidence Interval**
	Site #1		**Site #2**		**Site #3**				
	Detected: #positive /# tested	*% Positive*	*Detected: #positive /# tested*	*% Positive*	*Detected: #positive /# tested*	*% Positive*			
C/G High Negative	10/30	33%	6/30	20%	5/30	17%	21/90	23%	16% to 33%
C/G Low Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
C/G Moderate Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
C/G Negative	0/30	0%	0/30	0%	0/30	0%	0/90	0%	0% to 4%
C/G Positive Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	96% to 100%
C/G Negative Control	0/30	100%	0/30	0%	0/30	100%	0/90	0%	0% to 4%

Analytical Specificity – Cross-reactivity and Microbial Interference

An *in silico* BLAST analysis of primers used in the Solana Strep Complete Assay against sixty-one (61) potential interfering organisms (see below) did not show evidence of cross-reactivity.

Arcanobacterium sp.	Human adenovirus F	*Lactobacillus* sp.[1]
Bacillus sp.	Human adenovirus G	*Legionella pneumophila*
Bacteroides sp.[2]	Human coronavirus 229E	Measles virus
Bordetella sp.	Human coronavirus HKU1	Human Metapneumovirus
Branhamella sp.	Human coronavirus NL63	*Moraxella* sp.
Burkholderia sp.	Human enterovirus A	Mumps virus
Campylobacter sp.[3]	Human enterovirus B	*Mycoplasma pneumoniae*
Candida sp.	Human enterovirus C	*Neisseria* sp.
Corynebacterium sp.	Human enterovirus D	*Peptostreptococcus* sp.
Cytomegalovirus	Human herpesvirus 1	*Proteus* sp.
Enterobacterio phage MS2	Human herpesvirus 2	*Pseudomonas* sp.
Enterococcus sp.	Human herpesvirus 4	Respiratory syncytial virus Type B
Escherichia coli	Human parainfluenza virus 1	*Saccharomyces cerevisiae*
Fusobacterium sp.	Human parainfluenza virus 2	*Serratia* sp.
Haemophilus sp.	Human parainfluenza virus 3	*Staphylococcus* sp.
Human adenovirus A	Human parainfluenza virus 4a and 4b	*Treponema* sp.
Human adenovirus B	Influenza virus A	*Veillonella* sp.
Human adenovirus C	Influenza virus B	*Yersinia* sp.
Human adenovirus D	Influenza virus C	*Prevotella oralis*[4]
Human adenovirus E	*Klebsiella* sp.	*Parvimonas micra*[5]
Veillonella parvula		

A study was performed to evaluate the performance of the Solana Strep Complete Assay in the presence of forty-five (45) microorganisms commonly found in throat specimens. Each potentially interfering

[1] Includes *L. acidophilus*

[2] Includes *B. ovatus*

[3] Includes *C. rectus*

[4] In NCBI, *Bacteroides oralis* is *Prevotella oralis*.

[5] In NCBI, *Peptostreptococcus micros* is *Parvimonas micra*.

microorganism was tested in the presence of 2 x LOD *Streptococcus pyogenes* and *Streptococcus dysgalactiae* (2 strains each) in the presence of clinically relevant levels of viruses (10^5pfu/ml) and bacteria (10^6cfu/mL) or higher. All strain combinations were spiked on to swabs. The strains included in the cross-reactivity study are shown in the table below.

Acinetobacter lwoffii	*Staphylococcus epidermidis* MRSE
Arcanobacterium haemolyticum	*Stenotrophomonas maltophilia*
Bacillus cereus	*Streptococcus agalactiae*
Bordetella pertussis	*Streptococcus anginosus*
Burkholderia cepacia	*Streptococcus bovis*
Corynebacterium diphtheria	*Streptococcus canis*
Enterococcus faecalis	*Streptococcus gordonii (Virdans type)*
Escherichia coli	*Streptococcus intermedius*
Fusobacterium necrophorum	*Streptococcus mitis*
Haemophilus influenza type A	*Streptococcus mutans*
Klebsiella pneumonia	*Streptococcus oralis*
Lactobacillus acidophilus	*Streptococcus pneumoniae*
Lactococcus lactis	*Streptococcus salivarius*
Legionella jordanis	*Streptococcus sanguinis*
Legionella micdadei	*Streptococcus suis*
Legionella pneumophila	*Candida albicans*
Moraxella cartarrhalis	Adenovirus Type 1
Neisseria gonorrhoeae	Adenovirus Type 11 (Slobitski)
Neisseria subflava	Influenza A
Peptostreptococcus micros (aka Parvimonas micra)	Influenza B
Pseudomonas aeruginosa	Parainfluenza Type 4B (VR-1377)
Serratia marcescens	Rhinovirus Type 15 (1734)
Staphylococcus aureus MRSA	

Of the 45 microorganisms tested that might be found in throat specimens, *Klebsiella pneumoniae, Serratia marcescens* and *Enterococcus faecalis* each cross-reacted once out of six times tested (triplicate testing was repeated for each cross-reactive strain) with the Solana Strep Complete Assay.

Analytical Specificity – Interfering Substances

A study was conducted using two strains of *Streptococcus pyogenes* (ATCC 19615 and 12344) and *Streptococcus dysgalactiae* strains (ATCC 12394 and ATCC 10009) tested near LOD to evaluate the Solana Strep Complete Assay for potential interference using a panel consisting of twenty-eight (28) common biological and chemical substances found in throat samples. Substances were introduced into the swabs at concentrations which were medically relevant. Each of the strains was tested for each substance. None of the substances tested were found to interfere `with the Solana Strep Complete Assay.

Substance Name	**Test Concentration**	**Interference? (Y/N)**
Children's Dimetapp DM Cold & Cough Elixir	25% v/v	No
Chloraseptic Max: Sore Throat Relief	10% v/v	No
BreathSavers 3 Hour Mint-Spearmint	10% w/v	No

Substance Name	Test Concentration	Interference? (Y/N)
Cepacol Sore Throat: Cherry Flavor	5% w/v	No
Robitussin Cough & Cold-CF Max	10% v/v	No
Ricola Mountain Herb Throat Drops-Sugar Free	15% w/v	No
Human Saliva	10% v/v	No
Robitussin Nighttime Cold, & Flu	10% v/v	No
Crest Pro-Health Night Mint	25% v/v	No
CVS Tussin CF	15% v/v	No
Chloraseptic Throat Cherry lozenge	10% w/v	No
Halls Cherry Mentholyptus	15% w/v	No
Tic Tac Freshmints	10% w/v	No
Zicam® Oral Mist	0.625% v/v	No
Sucrets Complete-Vapor Cherry	5% w/v	No
Acetaminophen	19.5 mg/mL	No
Aspirin	12.3 mg/mL	No
Ibuprofen	15.6 mg/mL	No
Benadryl	2.7 mg/mL	No
Crest® Complete Toothpaste	5% w/v	No
Contac® Cold + Flu Caplets Night	10% w/v	No
Children's Wal-Tap Elixir Cold & Allergy (Dimetap Children's Cold and Allergy)	25% v/v	No
Children's Wal-Tap DM Elixir Cold & Cough	25% v/v	No
Robitussin Nighttime Cough, Cold, & Flu (peak cold)	10% v/v	No
Halls Mentholyptus (not cherry flavor)	15% w/v	No
Listerine Cool Mint Antiseptic	15% v/v	No
Whole Blood	5% v/v	No
Mucin (Bovine Submaxillary Gland, type I-S)	5.0 mg/mL	No

Carryover – Cross Contamination

A study was performed where three (3) operators tested a total of 50 high *S. pyogenes/S. dysgalactiae* positive (1.0×10^6 CFU/mL) and 50 negative swabs in multiple runs. In each run, 5 positive and 5 negative swabs were tested in an alternating order and also included a positive and negative control assays.

All positive *S. pyogenes/S. dysgalactiae* samples were positive and all negative *S. pyogenes/S. dysgalactiae* samples were negative. No carryover/cross contamination was observed when the assay was performed in accordance with the Package Insert.

CUSTOMER AND TECHNICAL ASSISTANCE

To place an order or for Technical Support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

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