Flowflex® PLUS

Strep A Rapid Test Cassette Package Insert

L03A-R0115	English

For prescription use only. For in vitro diagnostic use.

CLIA Complexity: WAIVED

IVD | ℞ ONLY

A Certificate of Waiver is required to perform this test in a CLIA waived setting and all applicable state and local laws must be met.

Laboratories with a Certificate of Waiver must follow the test system instructions for use. 42 CFR 493.15(e)(1). Failure to follow the instructions or modifications to the test instructions will result in the test no longer meeting the requirements for waived classification (i.e., test will be high-complexity).

INTENDED USE

The Flow*flex* Plus Strep A Rapid Test Cassette is a rapid chromatographic immunoassay for the qualitative detection of Group A Streptococcus antigen from throat swab specimens from symptomatic patients. The test is used to aid in the diagnosis of Group A Streptococcal infection. All negative test results should be confirmed by bacterial culture because negative results do not preclude infection with Group A Streptococcus and should not be used as the sole basis for treatment.

SUMMARY AND EXPLANATION

Streptococcus pyogenes is non-motile gram-positive cocci, which contains the Lancefield group A antigen that can cause serious infections such as pharyngitis, respiratory infection, impetigo, endocarditis, meningitis, puerperal sepsis, and arthritis.[1] Left untreated, these infections can lead to serious complications, including rheumatic fever and peritonsillar abscess.[2] Traditional identification procedures for Group A Streptococci infection involve the isolation and identification of viable organisms using techniques that require 24 to 48 hours or longer.[3,4]

The Flow*flex* Plus Strep A Rapid Test Cassette is a rapid test to qualitatively detect the presence of Strep A antigen in throat swab specimens, providing results within 5 minutes. The test utilizes antibodies specifically for whole cell Lancefield Group A Streptococcus to detect Strep A antigen in a throat swab specimen.

PRINCIPLE

The Flow*flex* Plus Strep A Rapid Test Cassette is a qualitative, lateral flow immunoassay for the detection of Strep A carbohydrate antigen in a throat swab. In this test, an antibody specific to Strep A carbohydrate antigen is coated on the test line region of the test strip. During testing, the extracted throat swab specimen reacts with an antibody to Strep A that is coated onto particles. This mixture migrates up the membrane to react with the antibody to Strep A on the membrane and generate a colored line in the test line region. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a blue colored line will always appear at the control line region, after proper volume of specimen has been added and membrane wicking has occurred.

WARNINGS, PRECAUTIONS, AND SAFETY INFORMATION

- This kit is for prescription, *in vitro* diagnostic use only.
- Do not use the kit contents after the expiration date printed on the outside of the kit.
- Do not eat, drink, or smoke in the area where the specimens and kits are handled.
- Do not use kit contents if packaging is damaged.
- Follow your laboratory safety guidelines in the collection, handling, storage and disposal of patient specimens and used kit contents.
- Wear protective clothing such as laboratory coats, disposable gloves, and eye protection when specimens are being tested.
- All specimens and the used test should be considered potentially infectious and be discarded according to Federal, State and Local regulatory requirements.
- Humidity and temperature can adversely affect results.
- Reagent B contains an acidic solution. If the solution comes in contact with the skin or eye, immediately flush with a large amount of water.
- Do not interchange reagent and control solution bottle caps.
- This package insert must be read before performing the test. Failure to follow instructions may yield inaccurate test results.
- Swabs, tubes, and cassettes are for single use only. Do not re-use.
- Testing should only be performed using the swabs provided within the kit. Do not touch the swab tip.
- Inaccurate results may occur if the swab is rotated less than 5 times or removed before 1 minute.

STORAGE AND STABILITY

- The kit can be stored at temperatures between 36-86°F (2-30°C) when not in use.
- The test is stable until the expiration date printed on the sealed pouch or canister.
- The test must remain sealed in the protective pouch until just prior to use.
- DO NOT FREEZE.
- The test cassettes are stable through the expiration date printed on the box.
- Once the reagent bottle has been opened, it is stable for 24 months at room temperature.
- Do not use kit contents beyond the expiration date.

MATERIALS

Materials Provided

- 27 Test Cassettes
- 27 Test Tubes
- 27 Dropper Tips
- 27 Sterile Throat Swabs
- 1 Package Insert
- 1 Quick Reference Instructions
- 1 Reagent A (2M Sodium Nitrite)
- 1 Reagent B (0.2M Acetic Acid)
- 1 Positive Control (Heat-inactivated Group A Streptococcus)
- 1 Negative Control (Heat-inactivated Group C Streptococcus)
- 1 Tube Holder

Materials Required But Not Provided

- Timer

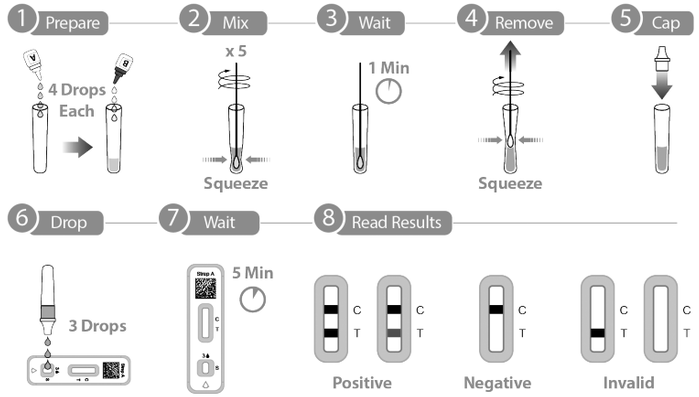
SPECIMEN COLLECTION AND PREPARATION

- The Flow*flex* Plus Strep A Rapid Test Cassette can be performed using throat swab specimens.
- For best results, testing should ideally be performed after specimen collection. If immediate testing is not possible, swab specimens may also be stored in a clean, dry plastic tube for up to 72 hours at room temperature or refrigerated, 36-46°F (2-8°C), prior to testing.
- Throat swab specimens should be collected by healthcare professionals only. Collect the throat swab specimen using the sterile swab provided in the kit. When swabbing the throat, be careful not to touch the teeth, gums, tongue, or cheek surfaces with the swab.[5] Rub the swab on the back of the throat, on the tonsils and in any other area where there is redness or inflammation.

DIRECTIONS FOR USE

Allow the test, reagents, specimens, and/or controls to reach room temperature, 59-86°F (15-30°C), prior to testing.

1. Remove the test cassette from the sealed pouch and use it as soon as possible. Place the test cassette on a clean, dry, level surface.
2. Hold the Reagent A bottle upside down vertically and **add 4 full drops** (approximately 160 µL) to the test tube. Hold the Reagent B bottle upside down vertically and **add 4 full drops** (approximately 160µL) of Reagent B. Reagent B is yellow in color. Mix the solution by gently swirling the tube. Adding Reagent B to Reagent A changes the color of the solution from colorless to light blue. See illustration 1.
3. Immediately place the swab into the test tube with light blue solution and **rotate at least 5 times** while squeezing the tube. Leave the swab in the tube for **1 minute**. Remove the swab while squeezing the tube to extract as much liquid as possible. Discard the swab. See illustrations 2 to 4.
4. Attach the dropper tip firmly onto the tube. Mix thoroughly by swirling or flicking the bottom of the tube. See illustration 5.
5. **Add 3 full drops of solution** (approx. 90µL) to the sample well (S) of the test cassette and then start the timer. See illustration 6.
6. **Read results at 5 minutes.** Do not read results after 8 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

POSITIVE: *

If the blue Control (C) line and the red Test (T) line are visible, the test is positive. Any visible faint red or pink test (T) line with a visible blue control (C) line should be read as positive. A positive result indicates that Strep A was detected in the sample.

*NOTE: The color intensity of the test line may vary depending on the concentration of the Strep A antigen present in the sample. Therefore, any shade of color in the test line region (T) should be considered positive.

NEGATIVE:

If the blue Control (C) line is visible, but the red Test (T) line is not visible, the test is negative. A negative result indicates that Strep A antigen is not present in the sample or the levels of antigen in the sample were below the detectable level of the test. It is recommended that a negative patient sample be cultured to confirm the absence of Strep A infection. If clinical symptoms are not consistent with results, obtain another sample for culture.

INVALID:

If the blue control (C) line is not visible, the test is invalid. Insufficient specimen volume or incorrect procedural technique are the most likely reasons for control line failure. Review the procedure and repeat the test with a new swab and a new test cassette. If the problem persists, discontinue using the test and contact Customer Support.

QUALITY CONTROL

Internal Quality Control

Internal procedural controls are included in the test. A blue line appearing in the control line region (C) is an internal procedural control. The appearance of the procedural control line indicates that proper volume of specimen has been added, and capillary flow occurred. If the procedural control line does not develop in 5 minutes, the test result is considered invalid, and retesting with a new cassette and sample is recommended.

External Quality Control

Positive and Negative control solutions are supplied with each kit. Positive control solution is non-infectious heat-inactivated Strep A bacteria with buffer and stabilizer solution. Negative control solution is heat-inactivated Strep C bacteria with buffer solution.

In addition to your laboratory's standard quality control procedures, it is recommended that positive and negative controls be tested once for each untrained operator and once for each new shipment of kits—provided that each different lot in the shipment is tested. This will be to ensure that the test cassette and that the test procedure is performed correctly. Commercial controls may contain interfering preservatives; therefore, other commercial controls are not recommended.

Procedure for External Quality Control Testing

1. Add 4 full drops of Reagent A and 4 full drops of Reagent B into a test tube. Mix the solution by gently swirling the tube.
2. Shake the control solution bottle vigorously. Hold the bottle upside down vertically, then add 1 full drop of positive or negative control solution into the test tube.
3. Place a new swab into the tube and swirl at least 5 times. Leave the swab in the tube for 1 minute. Remove the swab while squeezing the tube to extract as much liquid as possible. Discard the swab.
4. Follow Step 4 of the "DIRECTIONS FOR USE" section.

Note: If the controls do not perform as expected, repeat the test or contact Customer Support before testing patient specimens.

LIMITATIONS

1. The Flow*flex* Plus Strep A Rapid Test Cassette is for *in vitro* diagnostic use only. The test should be used for the detection of Strep A antigen in throat swab specimens only. Neither the quantitative value nor the rate of increase in Strep A antigen concentration can be determined by this qualitative test.
2. This test will only indicate the presence of Strep A antigen in the specimen from both viable and non-viable Group A Streptococcus bacterium.
3. The accuracy of the test depends on the quality of the throat sample. Avoid touching the tongue, cheeks, and teeth[5] and any bleeding areas of the mouth with the swab when collecting specimens.
4. 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 specimen is below the detectable level of the test. Additional follow-up testing by culture is required if the test result is negative and clinical symptoms persist, or in the event of an outbreak of acute rheumatic fever (ARF).
5. False negative results may occur if bacteria are present at levels below the test's limit of detection.
6. False negative results may occur if mutations are present in the regions targeted by the test.
7. Positive and negative predicted values are dependent upon prevalence. The performance was established for the 2022-2024 season. Performance may vary depending on the prevalence and the population tested.
8. The sterile swabs provided with this test must be used for specimen collection. Other swabs have

not been validated with this test.

9. Do not interchange materials from different lots of products.

EXPECTED VALUES

Approximately 15% of pharyngitis in children ages 3 months to 5 years is caused by Group A beta-hemolytic Streptococcus.[6] In school-aged children and adults, the incidence of Strep throat infection is about 40%.[7] This disease usually occurs in the winter and early spring in temperate climates.[3] In the multi-center clinical study conducted from 2022-2024, 27.5% of the patients presenting with pharyngitis were found to be culture positive for Strep A.

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity and Specificity

In a multi-center field evaluation, two swabs were collected from each patient presenting symptoms of pharyngitis. One swab was used for the Flow*flex* Plus Strep A Rapid Test Cassette, and the other was used for a Strep A bacterial culture. Testing with the Flow*flex* Plus Strep A Rapid Test Cassette was performed by operators who had no prior experience in the laboratory and were representative of the intended users. The culture plates were incubated by a CLIA-certified laboratory following standard procedures for the routine culture of Group A Streptococcus (GAS). The presence of beta-hemolytic colonies confirmed a positive result for Group A streptococci. A total of 396 patients participated in the evaluation. The results are summarized below.

Clinical Study Test Results		Lab Culture	
		Positive	Negative
Flow*flex* Plus Strep A Rapid Test Cassette	Positive	107	14
	Negative	2	273
	Total	109	287

Sensitivity: 107/109 = 98.2% (95% confidence interval: 93.6% - 99.5%)

Specificity: 273/287 = 95.1% (95% confidence interval: 92.0% - 97.1%)

Clinical Performance Stratified by Age

Age (in years)	Sensitivity	95% CI	Specificity	95% CI
0-5 (excluding 5)	100.0%	67.6% - 100.0%	96.7%	83.3% - 99.4%
5-21(excluding 21)	96.3%	87.5% - 99.0%	95.2%	90.5% - 97.7%
21 and older	100.0%	92.4% - 100.0%	94.5%	88.6% - 97.5%
All	98.2%	93.6% - 99.5%	95.1%	92.0% - 97.1%

There were no statistical differences in the Flow*flex* Plus Strep A Rapid Test Cassette performance between the age groups.

Analytical Sensitivity: Limit of Detection (LoD)

The limit of detection of the test is 7.4×10^5 CFU/mL (3.7×10^4 CFU/swab). This was established by testing limiting dilutions of Streptococcus pyogenes (ATCC 49399) spiked into negative clinical matrix and added to swabs. The LoD was confirmed by testing 20 replicates at a specific concentration.

Analytical Specificity: Cross-Reactivity and Microbial Interference

Organisms likely to be found in the respiratory tract were tested for cross-reactivity and microbial interference with the Flow*flex* Plus Strep A Rapid Test Cassette in replicates of five. While bacteria were tested at $1x10^7$ CFU/ml, viruses were tested at concentrations ranging from 4.64×10^4 $TCID_{50}$/ml to 1.0×10^6 $TCID_{50}$/ml. No cross-reactivity or microbial interference were found for each microorganism listed in the table below.

Adeno virus Type 1	Measles virus culture fluid	Streptococcus Anginosus Z199
Arcanobacterium Haemolyticum	Moraxella catarrhalis Ne 11	Streptococcus aureus CoNS
Bordetella pertussis A639	Mycobacterium tuberculosis	Streptococcus bovis Z167
Candida albicans Z006	Mycoplasma pneumoniae	Streptococcus constellatus Z124
Corynebacterium diphtheriae Z116	Mumps virus	Streptococcus dysgalactiae subso. Equisimilis Z068
Cytomegalo-virus	Neisseria Gonorrhoeae Z017	Staphylococcus haemolyticus
Echovirus Type 09	Neisseria Lactamica Z041	Streptococcus intermedius Z216
Enterococcus faecium Z265	Neisseria Meningitidis Serogroup A	Streptococcus Mitis Clinical Isolate
Enterococcus Faecalis Z346 VSE,	Neisseria Mucosa	Streptococcus mutans Z072
Epstein-Barr virus	Neisseria Sicca Z043, T	Streptococcus oralis Z307
Escherichia coli clinical isolate,	Neisseria Subflava Biovar Flava Z119	Streptococcus salivarius Z127
Fusobacterium necrophorum Z239	Proteus vulgaris Z129	Streptococcus sanguinis Z089
Haemophilus parainfluenzar Z492	Pseudomonas aeruginosa Z139	Strep B
Human metapneumo-virus (hMPV) 16 Type A1	Respiratory syncytial virus Type A (RSV-A) (1/2015 isolated 1)	Strep C
Influenza A	Rhinovirus Type 1A culture fluid	Strep D
Influenza A H3N2	Serratia marcescens Z053	Strep F
klebsiella pneumoniae KPC-2	Staphylococcus aureus MRSA	Strep G
Klebsiella pneumoniae Z026,	Staphylococcus epidermidis MRSE, RP62A	
Lactobacillus Salivarius	Streptococcus Agalactiae Z019	

Interfering Substances

The potentially interfering substances were evaluated in samples spiked with and without Streptococcus pyogenes. Endogenous and exogenous substances were tested, and their concentrations are listed below. No interference was observed with any of the tested substances at the specified concentrations.

Item No.	Substances Tested for Interference	Concen-tration	Item No.	Substances Tested for Interference	Concen-tration
1	Mucin (Bovine Submaxillary Gland, type I-S)	0.4 mg/ml	16	Robitussin Nighttime Cough DM	25% v/v
2	Blood	20% v/v	17	Robitussin Cough & Cold-CF Max	25% v/v
3	Sheep Blood Agar (5% Sheep Blood)	Sweeping	18	Halls Cherry Mentholyptus	20% w/v
4	Ethanol	99.5%	19	Halls Mentholyptus (Menthol)	20% w/v
	OTC Mouthwashes:		20	Ricola LemonMint Herb Throat Drops-Sugar Free	20% w/v
5	Listerine Antiseptic	25% v/v	21	Cepacol Cherry Sore Throat	20% w/v
6	Listerine Cool Mint	25% v/v	22	BreathSavers 3 Hour Mint-Spearmint	20% w/v
7	Crest Pro-Health Clean Night Mint	25% v/v	23	Tic Tac wintergreen	20% w/v
	OTC Throat Sprays:			Active Ingredients:	
8	Chloraseptic Max	25% v/v	24	Acetaminophen (Tylenol),	10 mg/ml
	OTC Cough Syrups:		25	Brompheniramine Maleate	5 mg/mL
9	Tylenol Cold, Cough and Sore Throat	25% v/v	26	Chlorpheniramine Maleate	5 mg/mL
10	Rite Aid Tussin CF	25% v/v	27	Dextromethorphan HBr	5 mg/ml
11	Children's Dimetapp Cold & Cough	25% v/v	28	Diphenhydramine HCl	5 mg/mL
12	Children's Dimetapp Cold & Allergy	25% v/v	29	Doxylamine Succinate	5mg/mL
13	Children's Dimetapp Nighttime Cold & Congestion	25% v/v	30	Guaifenesin (Guaiacol Glyceryl)	20 mg/ml
14	Children's Wal-Tap DM Elixir Cold & Cough	25% v/v	31	Ibuprofen (Advil)	10 mg/ml
15	Robitussin Cough &Chest Congestion DM	25% v/v	32	Phenylephrine HCl	5 mg/mL

Precision/Reproducibility Studies

An evaluation of the Flow*flex* Plus Strep A Rapid Test Cassette was conducted at three external, CLIA-waived testing sites, where testing was performed by three personnel at each site. Over a period of five days, each site tested a panel of randomly coded specimens, including 3 true negative, 3 low negative (0.4× LoD), 3 low positive (1x LoD), and 3 medium positive (3x LoD) samples. The results showed 100% agreement (135/135) for negative and medium positive specimens, 97.8% agreement (132/135) for low positive specimens, and 51.9% negative agreement (70/135) for low negative specimens when compared to the expected results.

High Dose Hook Effect

An evaluation of the Flow*flex* Plus Strep A Rapid Test Cassette was conducted using high concentrations of Group A Streptococcus. No hook effect was observed up to a concentration of 1.48×10^8CFU/ml.

Inclusivity

The Flow*flex* Plus Strep A Rapid Test Cassette was able to detect various Strep A strains at the listed concentrations below.

Strep A Pathogens	Strain (ATCC #)	Detection Conc. in sample (CFU/ml)	Detection Conc. in Reagent (CFU/ml)
Streptococcus pyogenes typing strain T1 [NCIB 11841, SF 130]	12344	2.85×10^5	3.85×10^4
Streptococcus pyogenes typing strain C94	12370	3.68×10^5	4.97×10^4
Streptococcus pyogenes strain Bruno [CIP 104226]	19615	2.71×10^5	3.66×10^4

ASSISTANCE

If you have any questions regarding the use of this product, please contact Customer Support at 1-800-838-9502. Test system problems may also be reported to the FDA through the MedWatch medical products reporting program

(phone: 800.332.1088; fax: 800.FDA.0178; http://www.fda.gov/medwatch).

BIBLIOGRAPHY

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2. Webb, KH. Does Culture Confirmation of High-sensitivity Rapid Streptococcal Tests Make Sense? A Medical Decision Analysis. Pediatrics (Feb 1998), 101:2, 2.
3. Bisno AL, Gerber MA, Gwaltney JM, Kaplan EL, Schwartz RH. Diagnosis and Management of Group A Streptococcal Pharyngitis. Clinical Infectious Diseases (1997), 25: 574-83.
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6. Nussinovitch, M, Finkelstein Y, Amir J, Varsano, I. Group A beta-hemolytic streptococcal pharyngitis in preschool children aged 3 months to 5 years. Clinical Pediatrics (June 1999), 38: 357-360.
7. Woods WA, Carter CT, Stack M, Connors Jr AF, Schlager TA. Group A Streptococcal Pharyngitis in Adults 30 to 65 years of age. Southern Medical Journal (May 1999), 491-492.

Index of Symbols

	Manufacturer		Date of manufacture
Σ	Contains sufficient for <*n*> tests	REF	Catalogue number
IVD	*In vitro* diagnostic medical device		Use-by date
Rx ONLY	Prescription use only	LOT	Batch code
	Consult instructions for use		Do not reuse
2°C 36°F 30°C 86°F	Store between 36-86°F (2-30°C)		

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Customer Support: 1-800-838-9502

Number: 1150000294
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