1. Description

Rapid Test Strep A is a qualitative, lateral flow immunoassay designed to detect the presence or absence of Group A Streptococcal antigen in throat swab specimens directly collected.

2. Principal of the assay

In this test, antibodies specific to Streptococcus A are coated on the test line region (Test line) of the nitrocellulose membrane. During testing, antigens in the specimen react with the antibodies that are coated onto gold nanoparticles. The mixture migrates up the membrane to react with the antibodies immobilized on the membrane and generate a colored line in the test region T. The presence of the colored Test line indicates a positive result. To serve as a procedural control, a colored line will always appear in the control region if the test has been performed properly.

3. Introduction

Group A Streptococcus (S.pyogenes) is one of the main causes of respiratory infections in the human upper respiratory tract, and the most significant pharyngitis causing pathogen. During childhood, pharyngitis and tonsillitis are really common and heamolytic Streptococcus group A (S. pyogenes) is observed mostly at the ages of 3-10 years old and it is transmitted by talking, coughing, or sneezing, which creates respiratory droplets that contain the bacteria. There is a need to discriminate between virus infection (such as RSV) because Streptococcus A should be treated with antibiotics otherwise it could lead to complications such as rheumatic fever, toxic shock syndrome or glomerulonephritis.

4. Reagents Provided

	V2001	V2005	V2020
Reaction device	1	5	20
Extraction Buffer A	1	1	1
Extraction Buffer B	1	1	1
Sterile swabs	1	5	20
Extraction tubes	-	5	20
Carton case	-	-	1
Instruction of use	1	1	1

5. Materials required but not provided

- Clock or Timer
- Gloves and face protection shield
- Container for biohazardous waste

6. Storage Instructions

Store kit components between 4 and 30°C (39.2 - 96°F). Do not freeze any components provided. Expiry of the kit and reagents is stated on their labels and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly and the reagent is not contaminated prior handling.

7. Safety and Precautions for use

7.1 Health and safety precautions

- Use gloves, protective clothing and eye/face protection and handle appropriately with the requisite Good Laboratory Practices. The product must only be used by qualified personnel.
- WASTE MANAGEMENT: Dispose of all specimens and materials used to perform the test as biohazard waste. Laboratory chemical and biohazard wastes must be handled and discarded in accordance with all local, state, and national regulations.
- During the specimen collection human source material spills should be also treated as potentially infectious. Spills should be immediately decontaminated, including the spill area, materials and any contaminated surfaces, with an appropriate chemical disinfectant and should be wiped away.
- Do not re-use any of the kit components as they are single-use only.
- Sterile swabs must be used only for throat specimen. Avoid to touch the tip of the swab.
- Do not eat, drink or smoke in the area where the specimens and the kit are stored and handled.
- All positive results should be processed following local news and regulations.

7.2 Precautions related to the procedure

- In accordance with Article 1, Paragraph 2b of European Directive 98/79/EC, the use of in-vitro diagnostic medical devices is envisaged by the manufacturer to ensure the suitability, performance, and safety of these products. Consequently, the testing procedure, information, precautions, and warnings in the instructions for use must be followed rigorously. No changes to the test procedure are permitted, nor is any use in combination with other products not approved by the manufacturer. The user is solely responsible for any such changes. The manufacturer is not responsible for false results nor incidents arising as a result of these.
- Do not use the kit if the packaging of components is damaged, if there is an expired reagent or if the desiccant bag is absent inside the foil containing the sticks.
- All reagents should be warmed in room temperature before use (15°C - 25°C).
- Cover or cap all reagents when not in use.
- Do not mix and interchange different specimens.
- Do not interchange individual reagents between kits of different lot numbers.

8. Specimen collection

Collect the samples from the throat using standard clinical methods and the swab supplied with the kit. To obtain the sample, lower the tongue using a depressor and move the swab across the tonsils or any area with inflammation, redness and pus. (Figure 1.) Take care not to touch the tongue, sides or top of the mouth with the swab.

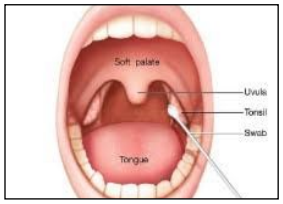


Figure 1. Throat swab procedure

9. Method Procedure

9.1 Throat swab method

1 Add 6 drops of Extraction buffer A and 4 drops of Extraction buffer B into the extraction tube.

Note: *In case of V2001 add all the liquid from the bottle with RED foil (extraction Buffer B) into the bottle with GREEN foil (extraction buffer A)*

2 After the specimen collection (see Chapter 8), place the swab in the extraction tube, rotate the swab forcefully against the side of the tube for 1min. Best results are obtained when the specimen is vigorously extracted in the solution.

3 Remove the swab, squeezing the sides of the tube to extract as much liquid as possible.

4 Discard the swab.

5 Close the extraction tube with the dropper cup. Add 3 drops in the circular window of the cassette.

6 After 5 minutes, the test stick can be visually read and interpreted according to the corresponding figure.

Note: The test result should not be read and interpreted after 10 minutes.

9.2 Suspected Group A Streptococcus colonies

Blood agar is the method of choice for the isolation of Group A Streptococcus. After 72 hours of incubation in anaerobic atmosphere/37°C, typical colonies will be translucent white showing beta haemolysis.

1 Examine Blood plates after incubation for typical Streptococcus colonies. Using a sterile swab pick 1-3 suspected colonies.

2 Follow the steps 9.1 (1-6)

Streptococcus pyogenes ATCC 19615

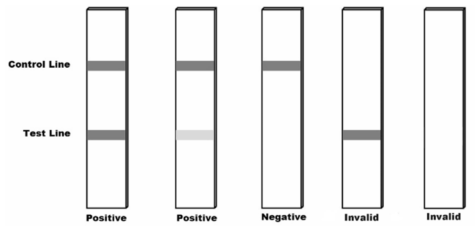
10. Interpretation of results

Note: *For internal procedure purposes two colored lines are present on the result window of the Rapid Test Strep A. The colored lines have no effect on the product's performance since they are washed away during the experiment.*

Positive: Two visible colored bands appear at both Test line (T) and Control (C) line. It indicates a positive result for Group A Streptococcal antigen in the specimen.

Negative: One visible colored band appears at Control line. It indicates that the concentration of the antigen is zero or below the detection limit of the test.

Invalid: No colored band appears at Control line no matter whether it appears at Test line or not.



Interpretation of results

11. Limitations

- The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
- After specimen collection the swab must be extracted as soon as possible. Otherwise they may be stored dry in their original packaging for up to 2 hours at RT.
- After the extraction specimens should be tested as soon as possible. Otherwise they can be stored at room temperature 20-25°C (68-77°F) for two hours.
- The test should be used for the detection of Strep A antigen ONLY in throat swab specimens.
- Failure to follow the guidelines for proper specimen collection, test procedure and interpretation of test results may adversely affect test performance and/or produce invalid result.
- USE ONLY the sterile swabs that are provided in the kit for the specimen collection.
- During specimen collection avoid soaking the swab with saliva. may give a false positive result due to interference with the test performance.
- Positive results indicate the presence of Strep A antigens but a diagnosis of an infection should only be made by a physician evaluating all clinical and laboratory findings and must be based in the correlation of the results with further clinical observations.
- A negative test result may occur if the level of extracted antigen in a specimen is below the sensitivity of the test or if a poor quality specimen is obtained.
- Positive test results do not rule out co-infection with other pathogens.

12. Immunoassay Performance

12.1 Cross-reactivity

In order to determine the cross reactivity of Rapid Test Strep A , an evaluation was performed; no cross reactivity against organism, pathogens that could cause infections was observed.

Microorganism	Concentration	Result
Adenovirus Type 1 (Species C)	2.57 x 10^8 TCID50/mL	Negative
Adenovirus Type 3 (Species B)	3.39 x 10^7 TCID50/mL	Negative
Adenovirus Type 7A (Species B)	1.02 x 10^8 TCID50/mL	Negative
Alpha coronavirus 229E	4.68 x 10^4 TCID50/mL	Negative
Alpha coronavirus NL63	1.70 x 10^5 TCID50/mL	Negative
Beta coronavirus OC43	5.01 x 10^5 TCID50/mL	Negative
Escherichia Coli O157	6.4x10^6 CFU/ml	Negative
Influenza A virus	1.51 x 10^6 TCID50/mL	Negative
Influenza B virus	5.01 x 10^5 TCID50/mL	Negative
Listeria monocytogenes	2.5x10^6 CFU/ml	Negative
Salmonella enteritidis	3.6x10^6 CFU/ml	Negative
SARS-Cov-2	1.15 x 10^7 TCID50/mL TCID50/mL TCID50/mL	Negative
Streptococcus pneumococcal	4.2x10^6 CFU/ml	Negative

12.2 Limit of Detection

The lowest detectable concentration of an analyte in a method is known as LOD. In this case, the LOD was established by testing a known number of organisms (ATCC 19615) in serial dilutions. The LOD is the level at which 95% of the replicates are characterized as positive. The results of 20 replicates are shown below.

LOD : 1.5 x 10^5 CFU/mL

Cell number in CFU/mL	Positive Replicates	Visual Interpretation of results
6 x 10^6	20 / 20	Strong positive
6 x 10^5	20 / 20	Strong positive
3 x 10^5	20 / 20	Positive
1.5 x 10^5	20 / 20	Positive
7.7 x 10^4	1 / 20	Negative

12.3 High Dose Hook Effect

No high dose hook effect was observed up to 6 x 10^6 CFU/mL.

12.4 Clinical performance characteristics

In order to determine the clinical performance of the Rapid Test Strep A, 43 negative and 25 positive specimens (total amount 68) confirmed by blood agar culture, were tested. The results are presented below.

Rapid Test Strep A	Blood agar culture		
	Positive	*Negative*	*Total*
Positive	24	1	25
Negative	1	42	43
Total	25	43	68

Clinical Diagnostic Specificity: 97.6%
Clinical Diagnostic Sensitivity: 96%

The same specimens were tested with a commercially available rapid test for detecting group A Streptococcus (BIOSYNEX® STREP A). The compared results are presented below.

BIOSYNEX® STREP A	Blood agar culture		
	Positive	*Negative*	*Total*
Positive	24	1	25
Negative	1	42	43
Total	25	43	68

	Sensitivity	*Specificity*	*PPV*	*NPV*
Rapid Test Strep A (Prognosis Biotech) vs BIOSYNEX® STREP A	**>99%**	**>99%**	**>99%**	**>99%**

13. Method Summary

Total procedure time (after specimens preparation): 5 min.

Specimen collection according to specific instruction (chapter 8)

⇩

Add 6 drops of Extraction buffer A and 4 drops of Extraction buffer B into the extraction tube

In case of V2001 add all the liquid from the bottle with RED foil (extraction Buffer B) into the bottle with GREEN foil (extraction buffer A)

⇩

Insert the swab into the extraction tube and rotate it forcefully against the side of the tube for 1min

⇩

Remove the swab, squeezing the sides of the tube to extract as much liquid as possible

⇩

Close the extraction tube with the dropper cup. Add 3 drops in the circular window of the device.

⇩

(Wait 5 mins)
Positive: Formation of 2 lines Test and Control line
Negative: Formation of Control line ONLY
Invalid: No Control line even if there is a Test line

14. References

1. Clinical microbiology procedures handbook. Procedures Guidelines for the Microbiology Laboratory Henry D. Isenberg, American Society for Microbiology. 2010.
2. Miller, J.M., 1999. A Guide to Specimen Management in Clinical Microbiology, 2nd Edition. American Society for Microbiologists, Washington D.C. Health ptotection Agency, 2012
3. Miller, J.M., 1999. A Guide to Specimen Management in Clinical Microbiology, 2nd Edition. American Society for Microbiologists, Washington D.C. Health ptotection Agency, 2012
4. Henningham A, Barnett TC, Maamary PG, Walker MJ. Pathogenesis of group A strepto-coccal infections. Discov Med 2012; 13:329-342.

IVD In Vitro Diagnostics Medical Device
Content sufficient for tests
REF Catalog number
Manufacturer
Storage Conditions
Instructions for use
EXP Expiration Date

CE

VERSION 2/ 2023-11-01

REF V20XX

RAPID TEST STREP A

Lateral Flow Test | IVD

For the detection of Group A Streptococcal antigen directly from human throat swab specimens.

This Lateral Flow test kit is manufactured by ProGnosis Biotech S.A. and complies with the specifications on the Standard EN ISO 13485:2016 Use only the current version of Product Data Sheet enclosed with the kit.

Rapid Test Strep A, V20XX, is a qualitative Lateral Flow test for the detection of Group A Streptococcal antigen directly from human throat swab specimens.

The Lateral flow kit contains all reagents required for the immunoassay method.

Specimen: throat swab.

- For professional use only.
- For in vitro diagnostic use only
- Rapid Test Strep A is a sensitive screening assay for the detection of Streptococcus Group A. Results should not be used as the only source to diagnose or to determine infection status.
- Negative result do not rule out Streptococcus group A infection
- Test should only be conducted by medical personnel
- Test time (incubation time after specimens preparation): 10 min
- Shelf life: 2 years
- Storage: 4-30°C

www.prognosis-biotech.com
e: info@prognosis-biotech.com
t: +30 2410 623922
f: +30 700 700 6262
Farsalon 153 | 41335 Larissa, Greece

PROGNOSIS BIOTECH