1. Intended Use

The dedicio® Strep A Test is a rapid visual immunoassay for the qualitative, presumptive detection of group A *Streptococcus* antigens (Strep A) in human throat swab specimens. The test is intended for use as an aid in the diagnosis of Strep A infections in patients showing typical symptoms. The test is designed for professional use only.

2. Introduction and Clinical Significance

Beta-hemolytic group A *Streptococcus* is a major cause of upper respiratory infections such as tonsillitis, pharyngitis and scarlet fever. Early diagnosis and treatment of Strep A pharyngitis has been shown to reduce the severity of symptoms and further complications, such as rheumatic fever and glomerulonephritis.

Conventional methods for detecting Strep A infection are dependent on isolation and subsequent identification of the organism and often require 24-48 hours. Recent developments in immunological techniques to detect Strep A antigens directly from throat swabs support physicians in diagnosing Strep A infections and administering a therapy immediately.

3. Test Principle

The dedicio® Strep A Test enables the detection of group A *Streptococcus* antigens through visual interpretation of colour development on the internal test strip. Anti-Strep A antibodies are immobilised in the test line region of the membrane. During the test, the specimen reacts with the polyclonal anti-Strep A antibodies conjugated to coloured particles and precoated onto the sample pad of the test cassette. The mixture then migrates along the membrane by capillary action and interacts with the reagents on the membrane. If there are sufficient Strep A antigens in the specimen, a coloured line will form in the test line region of the membrane. The presence of this coloured line indicates a positive result, while its absence indicates a negative result. The appearance of a coloured line in the control line region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

4. Reagents and Materials Supplied

- 20 dedicio® Strep A test cassettes, incl. disposable droppers: Each test cassette contains coloured conjugates and reactive reagents precoated in the corresponding regions of the membrane
- 1 bottle Reagent 1: 1.0 M sodium nitrite (7 ml)

Danger
H301: Toxic if swallowed

- 1 bottle Reagent 2: 0.4 M acetic acid (7 ml)
- 1 bottle Positive Control +: non-viable Strep A; 0.09% sodium azide (1 ml)
- 20 extraction tubes incl. dropper-caps
- 1 reagent holder
- Provided additional material according to 93/42/EEC: 20 sterile throat swabs CE 0086

Puritan Medical Products Company LLC
31 School Street
Guilford, Maine 04443-0149 USA (authorised EU representative EMERGO EUROPE, The Hague, The Netherlands)

- 1 package insert

5. Additional Materials Required

- Timer

6. Storage & Stability

The test should be stored at 2-30°C until the expiry date printed on the sealed foil pouch. The test cassette must remain in the sealed foil pouch until use. Do not freeze the test. Care should be taken to protect components of the test kit from contamination. Do not use the test if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

7. Warnings and Precautions

- For professional *in-vitro* diagnostic use only.
- Carefully read through the test procedure prior to testing.
- Do not use the test beyond the expiration date indicated on the package.
- Do not use the test if the foil pouch is damaged.
- Do not reuse tests.
- Do not add samples to the reaction area (result area).
- In order to avoid contamination, do not touch the reaction area (result area).
- Avoid cross-contamination of specimens by using a new extraction tube for each specimen obtained.
- Do not substitute or mix components from different test kits. Do not mix dropper-caps.
- Do not swap caps between different extraction reagent bottles.
- Do not eat, drink or smoke in the area where specimens and test kits are handled.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being assayed.
- Handle all specimens as if they contain infectious agents. Observe established precautions for microbiological risks throughout all procedures and standard guidelines for the appropriate disposal of specimens.
- The test kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled in accordance with usual safety precautions (e.g., do not ingest or inhale).
- Use only dacron or rayon-tipped sterile swabs with plastic shafts such as those provided. Do not use calcium alginate, cotton-tipped or wooden-shafted swabs.
- Do not use swabs from damaged pouches.
- Reagents 1 & 2 are slightly caustic. Avoid contact with eyes or mucous membranes. In the event of accidental contact, wash thoroughly with water.
- The positive control contains sodium azide, which may react with lead or copper plumbing to form potentially explosive

metal azides. When disposing of this solution, always flush with copious amount of water to prevent azide build-up. Avoid contact with eyes or mucous membranes. In the event of accidental contact, wash thoroughly with water.

- Humidity and temperature can adversely affect test results.
- Used testing materials should be discarded according to local regulations.

8. Specimen Collection and Preparation

Collect throat swab specimens using standard clinical methods. Swab the posterior pharynx, tonsil and other inflamed areas. Avoid touching the tongue, cheeks or teeth with the swab.

It is recommended that swab specimens be processed as soon as possible after collection. If swabs are not processed immediately, they should be placed in a sterile, dry, tightly capped tube or bottle and refrigerated. Do not freeze swabs. Swabs can be stored at room temperature (15-30°C) for up to 4 hours or refrigerated (2-8°C) for up to 24 hours. All specimens should be brought to room temperature (15-30°C) prior to testing.

Do not place swabs in any transport device containing liquid transport media or transport media containing agar or charcoal. Transport media may interfere with the assay and viability of organisms. If transport medium is required we recommend using Modified Stuart's Transport Medium as outlined in the manufacture's instructions.

If a bacterial culture is required, lightly roll the swab on a 5% sheep blood agar plate before using it in the test. The extraction reagents in the test will kill bacteria on the swabs and make them impossible to culture.

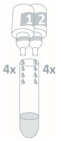
9. Test Procedure

Bring tests, specimens, reagents and/or controls to room temperature (15-30°C) prior to testing.

To avoid cross contamination, do not allow the tips of the reagent bottles to come into contact with sample material.

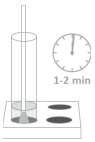
1. **Prepare swab specimens:**

- Place a clean extraction tube onto the designated area of the reagent holder. Add 4 drops of reagent 1 to the extraction tube, followed by 4 drops of reagent 2. In order to ensure reliable drop size when adding the reagents, hold the dropper bottles vertically. Mix the solution by gently swirling the extraction tube.

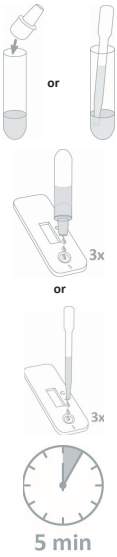


- Immediately immerse the swab into the extraction tube. Using circular motions, roll the swab against the side of the extraction tube so that the liquid is expressed from the swab and can reabsorb. Repeat at least 5 times.

- Let the solution stand for 1-2 minutes at room temperature, then squeeze the swab firmly against the tube to expel as much liquid as possible from the swab. Discard the swab in accordance with the guidelines for handling infectious agents.



2. Remove the test cassette from the sealed foil pouch and place it on a clean and level surface. The test cassette should be used immediately or within one hour at the latest after opening the foil pouch. Label the test cassette with the patient or control identification. For the best results, the assay should be performed within one hour.
3. Transfer 3 drops (approximately 120 µL) of the extracted solution, with the included disposable pipette or dropper cap, from the extraction tube to the sample well (S) of the test cassette.
   Avoid trapping air bubbles in the specimen well (S) and do not add any solution to the result area.
4. As the test begins to run, you will observe a coloured liquid migrate along the membrane. Wait for the coloured line(s) to appear. The test result should be read after 5 minutes. Do not interpret the result after more than 10 minutes.



10. Result Interpretation

Positive:

Two coloured lines appear on the membrane. One line appears in the control line region (C) and the other line appears in the test line region (T). This indicates that Strep A antigen has been detected in the sample.

Negative:

Only one coloured line appears in the control line region (C). No apparent coloured line appears in the test lineregion (T). No Strep A antigen has been detected.

Invalid:

The control line fails to appear. Results from any test which has not produced a control line at the specified reading time must be discarded. Please review the procedure and repeat he test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Note:

The colour intensity in the test line region (T) may vary depending on the concentration of the analyte present in the specimen. Therefore, any shade of colour in the test line region should be considered positive. Note that this is a qualitative test only and it cannot determine the concentration of the analyte in the specimen.

Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for the control line failure.

After the results have been interpreted, used tests should be discarded immediately in accordance with local regulations for potentially infectious materials.

11. Quality Control

An internal procedural control is included in the test cassette: A coloured line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Good laboratory practice (GLP) recommends the use of control materials to ensure proper test kit performance. A positive control containing heat-killed group A *Streptococcus* is provided with each test kit.

Operating Procedure for External Quality Control Testing

1. Add 4 drops of reagent 1 and 4 drops of reagent 2 to an extraction tube.
2. Thoroughly mix the positive control by shaking the bottle vigorously. Add 1 drop of the positive control to the tube.
3. Place a clean, sterile swab into the tube and swirl it. Leave the swab in the extraction tube for 1 minute. Then express the liquid from the swab head by rolling the swab against the inside of the extraction tube and squeezing the extraction tube as the swab is withdrawn. Discard the swab.
4. Continue as described in the Step 2 of the "Procedure of the Test".
   If the control does not yield a positive result, do not use the tests with samples. Repeat the Quality Control Testing or contact your distributor.

12. Limitations

- The dedicio® Strep A Test is for professional *in-vitro* diagnostic use only and should only be used for the qualitative detection of group A *Streptococcus*. No meaning should be inferred from the colour intensity or width of any apparent lines.
- The accuracy of the test depends on the quality of the swab specimen. False negative results may occur due to improper specimen collection or storage. A negative result may also be obtained from patients at the onset of the disease due to low antigen concentration.
- The dedicio® Strep A Test does not differentiate asymptomatic carriers of group A *Streptococcus* from those with symptomatic infection. If clinical signs and symptoms are not consistent with laboratory test results, a follow-up throat culture is recommended.
- In few cases, swab specimens heavily colonized with *Staphylococcus aureus* can yield false positive results.
- Respiratory infections, including pharyngitis, can be caused by streptococci of serogroups other than group A as well as other pathogens. A negative Strep A test result does not exclude infection with other pathogenic microorganisms.
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

13. Expected values

It is known that approximately 19% of all upper respiratory tract infections are caused by group A streptococci. Such infections are most prevalent in winter and early spring, with most cases arising in patients living in highly populated areas.

14. Performance Characteristics

Correlation Study

Table: dedicio® Strep A Test vs. culture

A correlation study between the dedicio® Strep A Test and conventional culture was performed. Throat swab specimens were taken from children and adults exhibiting symptoms of pharyngitis. The swabs were then used for the inoculation of cultures (blood agar plates) and for testing with the dedicio® Strep A Test.

Beta-hemolytic colonies from the blood agar plates were determined as group A *Streptococcus* using serologic streptococcal grouping methods. Strep A was recorded as present or not present. Quantification was not performed during the testing of clinical samples.

The results are presented in the following table:

		dedicio® Strep A Test		
		+	–	Total
Culture	+	**82**	**2**	**84**
	–	**4**	**156**	**160**
	Total	**86**	**158**	**244**

Relative sensitivity: 97.6% (91.7%-99.7%)*
Relative specificity: 97.5% (93.7%-99.3%)*
Overall agreement: 97.5% (94.7%-99.1%)*
*95% Confidence Interval

Sensitivity Study

8 different strains of Strep A (ATCC Numbers: 12202, 12203, 12204, 12365, 14289, 19615, 49399, 51399) were examined at different levels with the dedicio® Strep A Test. The detection limit of the assay was at least 1.5×10^5 organisms/swab for all strains. This indicates that dedicio® Strep A Test detects multiple Strep A strains with reliable sensitivity.

Prozone Effect Study

No adverse effect on T-line formation was recorded for Strep A concentration up to $1.0\text{x}10^9$ organisms per swab.

Specificity Study

Cross-reactivity studies with organisms likely to be found in the respiratory tract were performed using the dedicio® Strep A Test. The following organisms were tested at 1×10^7 organisms/swab and showed negative results

Organism	ATCC No.	Organism	ATCC No.
Bordetella pertussis	**8467**	***Strep B***	**12386**
Branhamella catarrhalis	**25238**	***Strep C***	**12401**
Candida albicans	**1106**	***Strep F***	**12392**
Corynebacterium diphtheria	**13812**	***Strep G***	**12394**
Enterococcus durans	**19432**	***Streptococcus canis***	**43496**

Organism	ATCC No.	Organism	ATCC No.
Enterococcus faecalis	19433	*Streptococcus equisimilis*	9528
Haemophilus influenzae	9006	*Streptococcus equisimilis*	9542
Klebsiella pneumoniae	9987	*Streptococcus equisimilis*	12388
Neisseria gonorrhoeae	27633	*Streptococcus mutans*	25175
Neisseria meningitidis	13077	*Streptococcus pneumonie*	27338
Neisseria sicca	9913	*Streptococcus sanguis*	10556
Neisseria subflava	14799	*Streptococcus oralis*	9811
Pseudomonas aeruginosa	9721	*Streptococcus mitis*	903
Serratia marcescens	8100	*Streptococcus anginosus*	33397
*Staphylococcus aureus**	12598	*Streptococcus intermedius*	27335
Stapylococcus epidermidis	1228	*Streptococcus agalactiae*	13813

* In rare cases a heavy colonization with *Staphylococcus aureus* might lead to false positive test results (see "12. Limitations").

Physician Office Laboratory (POL) Studies

An evaluation of the dedicio® Strep A Test was conducted at three physicians' office laboratory sites, using a panel of coded samples containing negative control, low positive and medium positive specimens. Each specimen level was tested at each site in replicates of 20 over a period of five days. The study showed >99.9% agreement with the expected results.

Interference Study

A variety of sore throat medication (cough drops) and mouthwashes were tested at concentrations of 1%. None of them interfered with the generation of correct test results.

Inter-lot and intra-lot variability

Three independent lots were tested with negative, low, medium and high positive controls in 10-fold determinations. No unexpected or inconsistent results were obtained, indicating that inter-lot and intra-lot variability is low.

15. References

1. Facklam RR, Carey RB. Streptococci and Aerococci. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ, editors. Manual of Clinical Microbiology. 4th ed. Washington DC: American Society for Microbiology; 1985.
2. Levinson ML, Frank PF. Differentiation of group A from other beta hemolytic streptococci with bacitracin. J Bacteriol. 1955 Mar;69(3):284-7.
3. Edwards EA, Phillips IA, Suiter WC. Diagnosis of group A streptococcal infections directly from throat secretions. J Clin Microbiol. 1982 Mar; 15(3): 481-3.
4. Gupta R, Talwar GP, Gupta SK. Rapid antibody capture assay for detection of group-A streptococci using monoclonal antibody and colloidal gold-monospecific polyvalent antibody conjugate. J Immunoassay. 1992; 13(3): 441-55.
5. Ross PW. Throat swabs and swabbing technique. Practitioner. 1971 Dec; 207(242): 791-6.
6. Lauer BA, Reller LB, Mirrett S. Effect of atmosphere and duration of incubation on primary isolation of group A streptococci from throat cultures. J Clin Microbiol. 1983 Feb; 17(2): 338-40.

Rev. 4, 2017-10-24 OM/JB