Strep-A-Chek™ Kit

Reagent Strips and Color Developer
Cat. No.: 13-050-00

Intended Use

Strep-A-Chek™ Kit is intended for use in the detection of pyrrolidonyl arylamidase (PYR) from beta-hemolytic colonies grown on blood agar plates, as an aid in the presumptive identification of Group A *Streptococcus*.

Description

Strep-A-Chek™ consists of **Strep-A-Chek™ Reagent Strips** impregnated with a chromogenic substrate for the detection of pyrrolidonyl arylamidase (PYR), an enzyme reported to be present in Group A beta-hemolytic *Streptococcus*, and **EY-20™ Reagent Tubes** which contain a diazo dye color developer, Fast Garnet. The PYR enzyme has been shown to be accurate in differentiating Group A streptococci and enterococci from other *Streptococcus* species. **Strep-A-Chek™ Kit** when used in conjunction with other tests such as CAMP, hippurate, and bile-esculin, may be used for the presumptive identification of beta-hemolytic streptococci or enterococci from any source.

Chemical Principle

Hydrolysis of the chromogenic substrate impregnated on the **Strep-A-Chek™ Reagent Strips** by pyrrolidonyl arylamidase (PYR) releases a free beta-naphthylamine derivative. This complexes with a diazo dye, Fast Garnet, the color developer present in **EY-20™ Reagent Tubes**, to produce a PINK/RED color, which is indicative of a positive result.

Materials Supplied

100. **Strep-A-Chek™ Reagent Strips** impregnated with 0.1% L-Pyroglutamyl-β-naphthylamide.
20. **EY-20™ Reagent Tubes** containing 0.35% Fast Garnet.

Materials Needed but not Supplied

Inoculation loop or applicator stick Pipette or dropper Distilled or Deionized water

Recommended Quality Control Organisms and Expected Results

Good laboratory practices include the use of control specimens to ensure proper kit performance. Positive and negative organisms should be tested according to the laboratory's established Quality Control program.

ORGANISM (not supplied)	ATCC#	EXPECTED RESULTS
Streptococcus pyogenes	19615	PINK/RED color change
Group C streptococci	12449	No color change

Precautions

Strep-A-Chek™ is intended for *IN VITRO* DIAGNOSTIC USE only and should be used by properly trained, qualified laboratory personnel. Normal precautions should be taken against dangers of microbial hazards. Sterilization of all materials used during testing is recommended. The active ingredient in the **EY-20™ Reagent Tubes**, Fast Garnet, is a suspected carcinogen. Avoid contact with skin. Refer to enclosed Material Safety Data Sheet for further information. DO NOT use **EY-20™ Reagent Tubes** if visibly wet.

Storage and Stability

Store **Strep-A-Chek™ Reagent Strips** and **EY-20™ Reagent Tubes** desiccated and in the original box at 2-8°C. This product should not be used passed the expiration date. Allow **Strep-A-Chek™ Kit** components to come to room temperature (20°-28°C) before using. Protect **EY-20™ Reagent Tubes** from light and moisture. DO NOT use **EY-20™ Reagent Tubes** if visibly wet. Store reconstituted **EY-20™ Reagent** at room temperature (20°-28°C) protected from light. Use within 8 hours of reconstitution.

Specimen Collection

1. A GRAM STAIN and CATALASE TEST **MUST** be performed on the specimen before using **Strep-A-Chek™** Group A *Streptococcus* are gram positive and catalase negative.
2. Only beta-hemolytic colonies should be selected from blood agar plates.
   NOTE: Group A streptococci colonies are surrounded by a well-defined zone of complete hemolysis, usually two to four times the diameter of the colony. However, the appearance of the colonies may vary greatly depending on the medium used.

Procedure

1. Allow the **Strep-A-Chek™ Kit** components to come to room temperature (20°-28°C) before using.
2. Reconstitute the contents of an **EY-20™ Reagent Tube** by adding 1.0 ml of distilled or deionized water to the tube and agitate. 1 ml of **EY-20™** solution is sufficient for more than 5 tests.

   Note: Store reconstituted **EY-20™ Reagent** at room temperature (20°-28°C) protected from light. Use within 8 hours of reconstitution.

3. Remove **Reagent Strip** from its container. Remove at least 5 well isolated beta-hemolytic streptococci colonies from the blood agar plate using a wooden applicator stick or inoculation loop.
4. Inoculate reagent strip by rubbing colonies onto filter paper area of strip.
5. Add 1 drop of **EY-20™** solution to the inoculated area. Incubate at room temperature (20°-28°C) for up to 10 minutes.
6. View for color formation. Formation of a PINK/RED color in the test area indicates the detection of pyrrolidonyl arylamidase (PYR), a POSITIVE result for the presumptive identification of Group A *Streptococcus*. A NEGATIVE result should be recorded if there is no color change after 10 minutes.

Interpretation of Results

OBSERVATION	INTERPRETATION	RESULT
PINK/RED color change	Pyrrolidonyl arylamidase (PYR) detected	Presumptive identification of Group A *Streptococcus*
No color change	Pyrrolidonyl arylamidase (PYR) NOT detected	NEGATIVE

Limitations of Test

It must be emphasized that only pure cultures with characteristics listed in SPECIMEN COLLECTION should be tested with the **Strep-A-Chek™** system. Some Leuconostoc and Streptococcus strains may appear coccobacillary, even rod shaped, and are often confused with members of the genus Lactobacillus. These strains may also be gram positive and catalase negative. The source of the specimen and clinical symptoms are important. Further biochemical and serological testing is necessary for definitive identification.

Performance Characteristics

In a clinical trial by Yajko, *et al.* comparing **Strep-A-Chek™** with bacitracin disk susceptibility test for accuracy in the presumptive identification of *Streptococcus pyogenes* (Group A *Streptococcus*) from a primary blood agar plate the sensitivity and specificity was 100%. **Strep-A-Chek™** was evaluated using a total of 320 clinical isolates of beta- hemolytic streptococci (See table). These included 169 group A, 42 group B, 38 Group C, 21 group F, 39 group G and 11 beta-hemolytic streptococci which did not agglutinate with antisera to groups A,B,C,D,F, or G with the Streptex Latex agglutination test.

Comparison of Bacitracin with Strep-A-Chek™

	NO.	NO. BACITRACIN SENSITIVE	(%)	NO. PYR	(%)
S.Pyogenes	167	167	(100)	167	100
GROUP B	42	0	(0)	0	(0)
GROUP C	38	16	(42)	0	(0)
GROUP F	21	0	(0)	0	(0)
GROUP G	39	7	(18)	0	(0)
NON-GROUPABLE	11	0	(0)	0	(0)
(GROUP A)	2	0	(0)	0	(0)
	320	190	(59)	167	52

False positive rate for Bacitracin = 15%

In another clinical trial by Daly, *et al*. comparing **Strep-A-Chek™** with Streptex and Litmus milk reduction for identification of Streptococci the sensitivity and specificity was also 100%. A total of 311 isolates were evaluated and included 176 group A, 43 group B, 8 group C, 9 group F and 9 group G. 100% of 52 group D enterococci and 100% of 14 group D non-enterococci were identified by **Strep-A-Chek™**.

Bibliography

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