EY-20™ Reagent Tube

Color Developer
Cat. No.: 13-020-50

Intended Use

The **EY-20™ Reagent Tubes** are a component of the **Strep-A-Chek™ kit** (Cat. No: 13-050-00) and are to be used with **Strep-A-Chek™ Reagent Strips**. They are 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

EY-20™ Reagent Tubes contain a color developer, which when used with the **Strep-A-Chek™ Reagent Strips** detect pyrrolidonyl arylamidase (PYR), an enzyme present in Group A beta-hemolytic *Streptococcus*. The PYR enzyme has been shown to be accurate in differentiating Group A streptococci and enterococci from other *Streptococcus* species. The **Strep-A-Chek™** test system, when used in conjunction with other tests such as CAMP, hippurate, and bile-esculin, may be used for the presumptive identification of 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

50 **EY-20™ Reagent Tubes** containing 0.35% Fast Garnet.
1 Material Safety Data Sheet

Materials Needed but not Supplied

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

Strep-A-Chek™ Reagent Strips (Cat. No.: 13-051-00)

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 Quality Control program.

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

Precautions

EY-20™ Reagent Tubes are 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 **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 **EY-20™ Reagent Tubes** 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 the **Strep-A-Chek™** system. 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 **EY-20™ Reagent Tubes** and **Strep-A-Chek™ Reagent Strips** (not supplied) 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.

Procedure (continued)

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. TESTED	NO. BACITRACIN SENSITIVE	(%)	NO. PYR POSITIVE	(%)
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)
S. MILLERI (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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2. Daly, J.A., and J.L. Rufener, 1985. Comparison of Strep-A-Chek™, Streptex and Litmus Milk Reduction for Identification of Streptococci. Abstracts of the 1985 Annual Meeting of the American Society for Microbiology.
3. Facklam, R.R., L.G. Thacker, B. Fox & L. Eriquez, 1982. Presumptive Identification of Streptococci by a New System. J. Clin. Microbiol. 15:987-990.
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6. Lawrence, J., D.M. Yajko, & W.K. Hadley, 1984. Comparison of Strep-A-Chek™ with Bacitracin for the Presumptive Identification of *Streptococcus pyogenes* (Group A Strep). Abstracts of the 1984 Annual Meeting of Interscience Conference on Antimicrobial Agents and Chemotherapy.
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8. Yajko, David M., J. Lawrence, P. Nassos, J. Young, & W.K. Hadley, 1986. Clinical Trial Comparing Bacitracin with Strep-A-Chek™ for Accuracy and Turnaround Time in the Presumptive Identification of *Streptococcus pyrogenes*. J. Clin. Microbiol. 24:431-434.

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Rev. 3 (3/06)

MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006
Revision 5

PRODUCT IDENTIFICATION

Name	EY-20™ Reagent Tubes
Catalog Number	13-020-50

EMERGENCY INFORMATION

EY Laboratories, Inc.
107 North Amphlett Blvd.
San Mateo, CA 94401

EMERGENCY PHONE: 650-342-3296

HAZARDOUS COMPONENTS

MATERIAL	CONCENTRATION
Fast Garnet GBC salt CAS # : 101-89-3	0.35% (w:w) Fast Garnet:Glucose

HEALTH HAZARD INFORMATION

EXPOSURE LIMITS	None established. The toxicological properties of these chemicals have not been thoroughly investigated.
EFFECTS OF OVEREXPOSURE	The chemical may cause local irritation if allowed to contact skin. Irritation may result if affected skin is allowed to contact the eyes or mucous membranes of the nose or mouth.
ROUTES OF EXPOSURE	Fast Garnet may be harmful by inhalation, ingestion, or absorption through the skin. The chemical is supplied as a powder in the tube. The primary route of exposure would be by inhalation of the powder or by contact with the solution after reconstitution.

PHYSICAL CHARACTERISTICS

APPEARANCE	Light orange / brown powder.
FORM	Fast Gamet powder mixed with glucose.
SOLUBILITY in H_2O	100%.

FIRE AND EXPLOSION HAZARDS

Not considered to be a fire hazard.

EXTINGUISHING MEDIA	Water spray, CO_2, or dry chemical powder.
SPECIAL FIRE FIGHTING NOTE	Wear protective equipment to prevent contact with skin, eyes, and respiratory tract.

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REACTIVITY DATA

STABILITY	Stable. Decomposition products are not known to be hazardous.
HAZARDOUS POLYMERIZATION	Will **NOT** occur.
INCOMPATIBILITY	Strong oxidizing agents, moisture, light, and alkaline conditions.

SPILL / LEAK PROCEDURES

MATERIAL RELEASE / SPILL	Avoid contact with material. Clean up spill and place all waste in a bag for disposal. Ventilate area.
WASTE DISPOSAL	Dissolve powder in water or buffer. Autoclave for 1 hour. Dispose of in accordance with all Local, State, and Federal regulation.

EMERGENCY FIRST AID PROCEDURES

May be harmful if swallowed, inhaled, or allowed to absorb through the skin. Wash contacted area with water for 15 minutes. If inhaled remove to fresh air. Report exposure to the appropriate safety official. Consult physician as necessary.

SPECIAL HANDLING PRECAUTIONS

VENTILATION	Mechanical exhaust recommended.
EYE PROTECTION	Safety glasses recommended.
RESPIRATORY PROTECTION	OSHA approved respirator.
PROTECTIVE GLOVES	Required.
ADDITIONAL INFORMATION	Avoid skin contact.

SPECIAL PRECAUTIONS

This material is for in vitro diagnostic use only. It is not intended for food, drug, household, agricultural, or cosmetic use. All material should be handled only by technically qualified individuals experienced with working with potentially hazardous chemicals. The above information is correct to the best of our knowledge. The user should make independent decisions regarding completeness of the information, based on all sources available. EY Laboratories, Inc. shall not be held liable for any damage resulting from handling or contact with the above product.

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