KS5400 Flow-Card Strep

INTENDED USE:

Flow-card Strep test kit aids in the identification of gram-positive, catalase-negative cocci. Hydroylsis of the substrates pyroglutamate aminopeptidase (PYR), leucine aminopeptidase (LAP), and esculin (ESC) simplifies the identification of *Enterococcus* spp. and group A streptococcus (*Streptococcus pyogenes*).

SUMMARY:

Flow-Card Strep rapidly determines three biochemical reactions for the differentiation and identification of gram-positive, catalase-negative cocci. L-pyroglutamic acid b-naphthylamide (PYR), impregnated into well 1, is a rapid chromogenic test for the detection of pyroglutamate aminopeptidase . L-leucine-naphthylamide (LAP) is impregnated into the filter paper in well 2. This substrate is hydrolyzed by the enzyme leucine aminopeptidase. Released naphthylamide in either of the above combines with the cinnamaldehyde reagent to form a pink to cherry-red color. Esculin and ferric ammonium citrate are contained within well 3. Organisms capable of producing the enzyme esculinase (Esculetin) combine with ferric ions to produce a dark brown color in this well. (1) *Enterococcus* spp. and group D streptococci are positive for esculin hydrolysis. Well 4 is filter paper only without any chemicals to be used as a negative control.

FORMULA:

Each well contains the respective substrate with enrichments and buffers as needed for performance:

WELL 1: Pyroglutamic aminopeptidase (PYR): L-pyroglutamic acid beta-naphthylamide

WELL 2: Leucine aminopeptidase (LAP): L-leucine-beta-naphthylamide

WELL 3: Esculin hydrolysis (ESC): Esculin, Ferric Ammonium Citrate

WELL 4: Negative control (NEG)

PEP Developer:p-dimethylaminocinnamaldehyde

STORAGE AND SHELF LIFE:

Storage: Upon receipt store at 2-8 degrees C. away from direct light. Do not freeze - the reagent must be discarded if ever frozen. Tests do not have to be restored to room temperature to use. Do not use if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Protect from light, excessive heat and freezing. The expiration date applies to the product in its intact packaging when stored as directed.

PRECAUTIONS:

For *in vitro* diagnostic use only. Observe approved biohazard techniques and precautions. Flow-card Strep is to be used only by adequately trained and qualified laboratory personnel. Sterilize all biohazard waste before disposal. LAP, PYR,and ESC substrates are carcinogenic. PEP reagent contains hydrochloric acid. MSDS for each chemical is available on request.

PROCEDURE:

Note: Perform gram stain and catalase test prior to inoculating. Test organisms should be gram-positive cocci that are aerobic or facultatively anaerobic and catalase-negative.

1) Moisten each test circle slightly with 1-2 drops of distilled or deionized water. **Do not saturate.**
2) Using a sterile plastic loop, pick 2-3 well isolated colonies (see "MATERIALS REQUIRED") and rub into a small area of the first well.
3) Using a new loop for each well, repeat step 2 for the LAP, ESC, and Neg test circles.
4) Cover with a glass slide and allow to develop for 10 minutes, preferably in a 35-38C incubator.

INTERPRETATION:

After the 10 minute incubation period:

1) Observe for a dark brown or black color to form in the ESC circle. For pigmented colonies, the NEG well serves as a color check. Look for contrast between the 2 wells.
2) After determining the ESC reaction, add one drop of PEP developer to the PYR, LAP, and NEG circles, observing for an immediate color change to dark pink or red. Any color development in the PYR and LAP circles appearing after 1 minute should be disregarded. See "LIMITATIONS" if a burgundy color is noted.

LIMITATIONS:

Biochemical and/or serological tests may be performed on colonies from pure culture for complete identification. Consult listed references for more information.Many other species (e.g. Staphylyococcus, Corynebacterium, *Enterobacteriaceae* and other gram-negative bacilli are are positive for the substrates so it is critical to use only pure cultures or isolated colonies of gram-positive, catalase-negative cocci. (1,2) For PYR and LAP tests color development after adding reagent is immediate. Disregard changes after 1 minute. The last well is a control well only. If this well turns red on adding the reagent, the entire test should be redone. **False-negative results can occur for any of the reactions if inadequate inoculum is used.** Bile tolerance is not included in the esculin hydrolysis test, so results obtained may not correlate with results obtained from Bile Esculin Media.

MATERIALS REQUIRED:

Flow-card Strep requires a pure, fresh 18-24 hour growth of isolated colonies on blood agar. This product is used in conjunction with other biochemical or serological tests to identify cultures of isolated organism. Other standard microbiological supplies and equipment such as loops, other culture media, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL:

The following organisms are routinely used for testing at KEY Scientific Products. However other organisms of known reactivity may be used to perform quality control.

Test Organisms		**PYR**	**LAP**	**ESC**
Streptococcus agalactiae	ATCC® 12386	Neg	Pos	Neg
Enterococcus faecalis	ATCC® 29212	Pos	Pos	Pos
Aerococcus viridans	ATCC® 11563	Pos	Neg	Neg

ORGANISMS	RESULTS			
	PYR	LAP	ESC	VANCO
Aerococcus urinae	-	+	-	S
Aerococcus viridans	+	-	V	S
Enterococcus faecium	-	+	+	V
Enterococcus faecalis	+	+	+	V
Gemella	+	V(1)	-	S
Globicatella	V	-	+	S
Lactobacillus	-	V	V	V
Lactococcus	-(2)	+	+	S
Leuconostoc	-	V	V	R
Pediococcus	-	+	V(3)	R
Streptococcus Gp A	+	+	V	S
Streptococcus Gp B	-	+	-	S
Streptococcus Gp D	-	+	+	S
Streptococcus viridans group	-	+	V	S
Streptococcus pneumoniae	V	+	V	S

+, most strains positive; -, most strains negative; V, variable S, susceptible; R, resistant

(1) G. haemolysans and G. sanguinis are LAP negative(2) L. garvieae is PYR positive

(2) L. garvieae is PYR positive

(3)check control well for pigment

REFERENCES

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, R., et al. Evaluation of three disk tests for identification of enterococci, leuconostocs, and pediococci. J. Clin. Microbiol. 1995; 33:885-887.6. Ruoff, K.L., D.R. Kuritzkes, J.S. Wolfson and M.J. Ferraro. J. Clin. Microbiol.1988; 26:2064-2068.

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