Strep Identification Kit

Intended Use
A panel of 12 tests for identification of *Streptococcus* species (Kit contains sterile medium for Esculin Hydrolysis, Voges Proskauer Test, Arginine Dihydrolase Test, PYR Test, ONPG Test and 7 different carbohydrates-Glucose, Arabinose, Sorbitol, Mannitol, Sucrose, Raffinose, Ribose).

Summary
Streptococcus is a genus of Gram-positive coccus (plural cocci) or spherical bacteria that belongs to the family *Streptococcaceae*. It has broad significance in medicine and industry. The complete list of those organisms that is possible to identify with this system is given in the identification index provided at the end of this package insert.

Principle
Microxpress® Strep Identification Kit is a standardized identification system, comprising 12 miniature biochemical tests for identification of Streptococci. This kit contains sterile media for colorimetric identification using biochemical test and carbohydrate utilization tests based on principle of pH change and substrate utilization designed to identify various metabolic properties of different bacterial species. On incubation for an appropriate period, the media are examined for colour change. The results of these tests on the suspected organism are then compared to known standards to confirm its identification.

Kit Contents
1. 1 Kit of Strep Identification Kit
2. Technical Product Insert with Result Interpretation Chart, Result Entry Data Sheet and Identification Index
3. Barritt Reagent A (B-A) for Voges Proskauer Test
4. Barritt Reagent B (B-B) for Voges Proskauer Test
5. Creatine (CR) for Voges Proskauer Test
6. PYR Reagent (PYR) for PYR Test

Note: Microxpress® Strep Identification Kit contains sufficient material to perform one test.

Biochemical Tests
Microxpress® Strep Identification Kit is a reagent set for laboratory use only.
Kit comprises of sterile test medium for:

a) Esculin Hydrolysis (V4)
b) Voges Proskauer Test (V18)
c) Arginine Dihydrolase Test (V1)
d) PYR Test (V14)
e) ONPG Test (V11)
f) Glucose Utilization (V26)
g) Arabinose Utilization (V21)
h) Sorbitol Utilization (V36)
i) Mannitol Utilization (V30)
j) Sucrose Utilization (V 37)
k) Raffinose Utilization (V33)
l) Ribose Utilization (V35)

Additional Materials Required
0.9% saline, micropipettes, culture media, activated 2% glutaraldehyde solution, sterile test tubes, incubator/water bath at 37°C±2°C, sterile mineral oil.

Directions

Preparation of Inoculum:

1. Isolate the organism to be identified on Soyabean Casein Digest Agar (201190210500) or Nutrient Agar (201140030500).
2. Pick up 1-3 well isolated colonies and make a homogenous suspension in 2-3 mL sterile saline.
3. Match the turbidity of this suspension to McFarland standard number 0.5.

Note: Erroneous false negative results may be obtained if the inoculum turbidity is less than McFarland standard number 0.5.

Inoculation of the Vials:

1. Bring the kit components to room temperature before testing.
2. Open the kit aseptically.
3. Inoculate each vial with 100 µL of the above-prepared inoculum by surface inoculation method.
4. Overlay test vials V1 (for Arginine Dihydrolase Test) with sterile mineral oil.
5. Incubate at 35°C-37°C and read the result at 18-24 hours of incubation.
6. Alternatively, the kit can also be inoculated by stabbing each individual well with a loopful of inoculum.

Voges Proskauer Test

1. Add 1-2 drops of Creatine, 2-3 drops of Barritt Reagent A and 1-2 drops of Barritt Reagent B to the test vial V18.
2. Development of pinkish red colour within 5-10 minutes indicates a positive test.
3. No colour change or slight copper colour (due to reaction of Barritt reagent A and Barritt reagent B) indicates a negative test.

PYR Test

1. Add 2-3 drops of PYR reagent to the test vial V14.
2. Development of a cherry red colour indicates a positive test.
3. No change in colour indicates a negative reaction.

Identification Index

Organisms / Tests	Esculin Hydrolysis	Voges Proskauer Test	Arginine Dihydrolase Test	PYR Test	ONPG Test	Glucose Utilization	Arabinose Utilization	Sorbitol Utilization	Mannitol Utilization	Sucrose Utilization	Raffinose Utilization	Ribose Utilization
Streptococcus anginosus	+	+	+	-	V	+	-	-	(-)	+	V	-
Streptococcus mitis	-	-	(-)	-	V	+	-	-	-	+	V	V
Streptococcus oralis	V	-	-	ND	+	+	-	-	-	+	V	V
Streptococcus porcinus	(+)	(+)	+	(+)	-	+	-	+	V	V	-	+
Streptococcus pyogenes	-	-	+	+	ND	ND	ND	-	-	ND	-	-
Streptococcus salivarius	+	(+)	-	-	V	+	-	-	-	+	V	-
Streptococcus sanguinis	(+)	-	+	-	V	+	-	V	-	+	V	-
Streptococcus suis	+	-	+	-	V	+	-	-	-	+	V	-
Streptococcus equi	V	-	+	-	-	+	-	-	-	+	-	-

Streptococcus agalactiae	-	(-)	-	-	-	+	-	-	-	+	-	+
Organisms / Tests	**Esculin Hydrolysis**	**Voges Proskauer Test**	**Arginine Dihydrolase Test**	**PYR Test**	**ONPG Test**	**Glucose Utilization**	**Arabinose Utilization**	**Sorbitol Utilization**	**Mannitol Utilization**	**Sucrose Utilization**	**Raffinose Utilization**	**Ribose Utilization**
Streptococcus acidominimus	+	-	-	+	ND	+	-	-	+	+	ND	-
Streptococcus bovis	+	+	-	ND	ND	ND	ND	-	V	ND	ND	ND
Streptococcus equinus	+	+	+	-	-	+	-	-	-	+	V	-
Streptococcus dysgalactiae	V	-	+	-	-	+	-	V	V	+	-	+
Streptococcus canis	+	-	+	-	+	+	-	-	-	+	-	+
Streptococcus mutans	+	+	-	-	-	+	-	+	+	+	+	-
Streptococcus uberis	+	V	V	+	-	+	-	+	+	+	-	+
Streptococcus constellatus	+	+	+	-	-	+	-	-	-	ND	-	-
Enterococcus faecalis	+	+	+	+	-	+	-	V	+	+	-	+
Enterococcus faecium	+	+	+	+	+	+	-	V	+	+	-	+
Streptococcus intermedius	+	+	+	-	+	+	-	-	-	+	-	-

Key:
Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.
+: 90% or more strains are positive; -: 90% or more strains are negative; ND: Not detected; (+): 76-89% strains are positive; (-): 11-25% of strains are positive; V: Variable

Result Interpretation Chart

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V4	Esculin Hydrolysis	-	Detects esculin hydrolysis	Brownish yellow	Black	Olive Green
V18	Voges Proskauer Test	1-2 drops of Creatine, 2-3 drops of Barritt reagent A and 1-2 drops of Barritt reagent B	Detects acetoin production	Colourless	Pinkish red within 5-10 minutes	Colourless / slight copper
V1	Arginine Dihydrolase Test	-	Detects arginine decarboxylation	Reddish purple	Purple	Yellow

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V14	PYR Test	2-3 drops of PYR reagent	Detects PYR enzyme activity	Cream	Cherry red	Cream
V11	ONPG Test	-	Detects β-galactosidase activity	Colourless	Yellow	Colourless
V26	Glucose Utilization	-	Detects glucose utilization	Red	Yellow	Red / Pink
V21	Arabinose Utilization	-	Detects arabinose utilization	Red	Yellow	Red / Pink
V36	Sorbitol Utilization	-	Detects sorbitol utilization	Red	Yellow	Red / Pink
V30	Mannitol Utilization	-	Detects mannitol utilization	Red	Yellow	Red / Pink
V37	Sucrose Utilization	-	Detects sucrose utilization	Red	Yellow	Red / Pink
V33	Raffinose Utilization	-	Detects raffinose utilization	Red	Yellow	Red / Pink
V35	Ribose Utilization	-	Detects ribose utilization	Red	Yellow	Red / Pink

Important points to be taken into consideration while interpreting the result

1. Allow the reagents to come to room temperature after removal from the refrigerator.
2. In case of carbohydrate fermentation test some microorganisms show weak reaction. In this case record the reaction as ± and incubate further up to 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
3. In case of arginine dihydrolase test, incubation up to 48 hours may be required.
4. At times organisms give conflicting result because of mutation or the media used for isolation, cultivation and maintenance.
5. The identification index has been compiled from standard references and results of tests carried out in the laboratory.

Result Entry Data Sheet

Sample Number	V4 Esculin Hydrolysis	V18 Voges Proskauer Test	V1 Arginine Dihydrolase Test	V14 PYR Test	V11 ONPG Test	V26 Glucose Utilization
Sample Number	V21 Arabinose Utilization	V36 Sorbitol Utilization	V30 Mannitol Utilization	V37 Sucrose Utilization	V33 Raffinose Utilization	V35 Ribose Utilization

Interpretation of Results

1. Add the reagents in the required vials at the end of incubation period.
2. Interpret results as per the standards given in the result interpretation chart.

Remarks

1. Microxpress® Strep Identification Kit is an *In vitro* diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. This kit cannot be used directly on clinical specimens. Only pure cultures should be used to obtain optimum results.
3. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.
4. Erroneous false negative results may be obtained if inoculum turbidity is less than McFarland standard number 0.5.
5. At times, the organism may give contradictory results because of mutation or media used for isolation, cultivation and maintenance. Results are prominent when fresh and enriched culture is used.
6. In case of carbohydrate fermentation some microorganisms may show weak reaction. Incubate further for 48 hours. Orange colour seen after 48 hours should be a negative reaction.
7. Identification index has been compiled based on standard references and results of tests obtained in the laboratory.
8. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.

Storage and Stability

1. Store the kit at 2°C-8°C. Do Not Freeze.
2. Stability of the kit is as per the expiry date mentioned on the label.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Practical Medical Microbiology, Mackie & McCartney, 13th edition 1989, Edited by J. G. Collee, J. P. Duguid.
2. Clarke P.H. And S.T. Cowan, Biochemical Methods for Bacteriology, J. Gen. Microbiol., 1952, Vol. 6: 187-197.
3. A. L. Barry and K. L. Feeney, Two quick methods for Voges-Proskauer test, Applied Microbiology, Sept. 1967, p.: 1138-1141.
4. Coblentz, L.H 1943, Rapid detection of the production of acetyl-methyl-carbinol, Am. J. Pub. Health 33:815-817.
5. Rapid Arginine Decarboxylase Test For The Identification Of Enterobacteriaceae, 1972, Applied Microbiol; 23, p 710-713.
6. Facklam.R.R. *et al.,* 1982, Presumptive identification of streptococci with a new test system, J. Clin.Microbiol; 15: p-987990.
7. M. Manafi, New approaches for the fast detection of indicators, in particular enzyme detection methods (EDM) OECD Workshop molecular methods for safe drinking water, 1998
8. Bergey's Manual of Determinative Bacteriology, 9th edition 1994; Edited by John G. Holt, Noel R. Krieg.
9. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203190860001	Biochemical Identification Kit	1 Kit (1 Test)