Lactobacillus Identification Kit

Intended Use
A panel of 12 tests for identification of *Lactobacillus* species (Kit contains sterile medium for Esculin Hydrolysis, Catalase Detection and 10 different carbohydrates- Xylose, Cellobiose, Arabinose, Maltose, Galactose, Mannose, Melibiose, Raffinose, Sucrose, Trehalose).

Summary
Carbohydrate utilization studies are an offshoot technology related to biochemical analysis. The types of compounds that an organism can ferment and the end products that are produced depend on the enzymes the microorganism can make. Among the end products commonly generated by fermentation are organic acids and gases such as carbon dioxide and hydrogen. A positive fermentation reaction is indicated by a change in the colour of the indicator present in the medium. The complete list of organisms that is possible to identify with this system is given in the identification index at the end of this package insert.

Principle
Microxpress® Lactobacillus Identification Kit is a standardized identification system, comprising 12 miniature biochemical tests for the identification of *Lactobacillus*. 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, that can be used to differentiate even closely related organisms. The media are inoculated by adding a bacterial suspension. After incubating for an appropriate period, the media are examined when the various indicator systems present are affected by the metabolites or added reagents to give a 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 Lactobacillus Identification Kit
2. Technical Product Insert with Result Interpretation Chart, Result Entry Datasheet and Identification Index

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

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

a) Esculin Hydrolysis (V4)
b) Catalase Detection (V3)
c) Xylose Utilization (V39)
d) Cellobiose Utilization (V22)
e) Arabinose Utilization (V21)
f) Maltose Utilization (V29)
g) Galactose Utilization (V25)
h) Mannose Utilization (V31)
i) Melibiose Utilization (V32)
j) Raffinose Utilization (V33)
k) Sucrose Utilization (V37)
l) Trehalose Utilization (V38)

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

Directions

Preparation of Inoculum:

1. Isolate the organism to be identified on Lactobacillus MRS Agar (201120020500) or Soyabean Casein Digest Agar (201190210500).
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 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.
4. Alternatively, the kit can also be inoculated by stabbing each individual well with loopful of inoculum.
5. Incubate at 35°C-37°C with or without 5-10 % CO_2 and read the result at 24-48 hours of incubation.

Identification Index

Organisms / Tests	Esculin Hydrolysis	Catalase Detection	Xylose Utilization	Cellobiose Utilization	Arabinose Utilization	Maltose Utilization	Galactose Utilization	Mannose Utilization	Melibiose Utilization	Raffinose Utilization	Sucrose Utilization	Trehalose Utilization
Lactobacillus acidophilus	ND	-	+	+	d	+	+	+	d	d	+	d
Lactobacillus acidifarinae	-	-	-	-	+	+	+	-	-	-	-	-
Lactobacillus antri	W	-	d	-	+	+	+	-	+	+	+	-
Lactobacillus brevis	d	-	d	-	+	+	d	-	+	d	d	-
Lactobacillus buchneri	d	-	d	-	+	+	d	-	+	d	d	-
Lactobacillus coleohominis	-	-	-	-	-	d	-	-	-	-	-	-
Lactobacillus collinoides	+	-	+	-	+	+	+	-	+	-	-	-
Lactobacillus diolivorans	-	-	+	-	+	+	+	-	+	-	-	-
Lactobacillus durianis	d	-	+	-	+	-	d	-	-	-	-	-
Lactobacillus ferintoshensis	+	-	+	d	+	+	+	+	d	d	+	+
Lactobacillus fermentum	-	-	d	d	d	+	+	W	+	+	+	d
Lactobacillus fructivorans	-	-	-	-	-	d	-	-	-	-	d	-
Lactobacillus frumenti	+	-	-	+	d	+	+	+	+	+	+	+
Lactobacillus gastricus	-	-	-	+	d	+	+	+	+	+	+	+
Lactobacillus hammesii	-	-	+	+	+	+	+	+	-	-	-	+
Lactobacillus hilgardii	-	-	+	-	-	+	d	-	-	-	d	-
Lactobacillus ingluviei	d	-	+	-	+	d	-	d	-	d	+	-
Lactobacillus kefiri	-	-	-	-	d	+	-	-	+	-	-	-
Lactobacillus kunkeei	-	-	-	-	ND	-	-	-	-	W	+	-
Lactobacillus leichmanii	-	-	+	+	+	+	+	+	+	+	+	+
Lactobacillus lindneri	-	-	-	-	-	+	-	-	-	-	-	-
Lactobacillus malefermentans	-	-	-	-	-	+	-	-	-	-	-	-
Lactobacillus mucosae	+	-	d	-	d	+	d	-	d	d	+	-
Lactobacillus oris	d	-	+	d	+	+	+	d	+	+	+	d
Lactobacillus panis	+	-	+	-	+	+	+	+	+	+	+	-
Lactobacillus parabuchneri	-	-	-	-	+	+	+	ND	+	+	+	-
Lactobacillus paracollinoides	ND	-	+	-	d	+	ND	-	+	-	-	-

Lactobacillus parakefiri	-	-	-	-	+	+	+	-	D	-	-	-
Lactobacillus pontis	-	-	-	-	-	+	d	-	d	d	+	ND
Lactobacillus reuteri	ND	-	-	-	+	+	+	-	+	+	+	-
Organisms / Tests	**Esculin Hydrolysis**	**Catalase Detection**	**Xylose Utilization**	**Cellobiose Utilization**	**Arabinose Utilization**	**Maltose Utilization**	**Galactose Utilization**	**Mannose Utilization**	**Melibiose Utilization**	**Raffinose Utilization**	**Sucrose Utilization**	**Trehalose Utilization**
Lactobacillus rossiae	-	-	d	-	+	+	W	W	d	-	-	-
Lactobacillus sanfranciscensis	ND	-	-	-	-	+	d	-	-	-	d	-
Lactobacillus spicheri	-	-	+	-	-	+	-	-	-	-	-	-
Lactobacillus suebicus	-	-	+	d	+	+	+	ND	d	-	d	-
Lactobacillus thermotolerans	+	-	+	ND	+	ND	-	ND	d	d	ND	ND
Lactobacillus vaccinostercus	-	-	+	W	+	+	W	-	-	-	-	-
Lactobacillus vaginalis	d	-	-	-	-	+	+	+	+	+	+	-
Lactobacillus zymae	-	-	d	-	+	+	d	-	-	W	-	-

Key:
Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.
+: Positive reaction; -: Negative reaction; ND: Not Detected; d: Variable; W: Weak fermentation

Result Interpretation Chart

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V4	Esculin Hydrolysis	-	Detects esculin hydrolysis	Cream / Yellow	Black	Cream / Yellow
V3	Catalase Detection	3% H_2O_2 solution	Detects catalase activity	Colourless to cream	Effervescence seen	No effervescence seen
V39	Xylose Utilization	-	Detects xylose utilization	Red	Yellow	Red
V22	Cellobiose Utilization	-	Detects cellobiose utilization	Red	Yellow	Red
V21	Arabinose Utilization	-	Detects arabinose utilization	Red	Yellow	Red
V29	Maltose Utilization	-	Detects maltose utilization	Red	Yellow	Red
V25	Galactose Utilization	-	Detects galactose utilization	Red	Yellow	Red
V31	Mannose Utilization	-	Detects mannose utilization	Red	Yellow	Red

V32	Melibiose Utilization	-	Detects melibiose utilization	Red	Yellow	Red
Code	**Test**	**Reagent to be added**	**Principle**	**Original colour of medium**	**Positive reaction**	**Negative reaction**
V33	Raffinose Utilization	-	Detects raffinose utilization	Red	Yellow	Red
V37	Sucrose Utilization	-	Detects sucrose utilization	Red	Yellow	Red
V38	Trehalose Utilization	-	Detects trehalose utilization	Red	Yellow	Red

Result Entry Data Sheet

Sample Number	V4 Esculin Hydrolysis	V3 Catalase Detection	V39 Xylose Utilization	V22 Cellobiose Utilization	V21 Arabinose Utilization	V29 Maltose Utilization
Sample Number	V25 Galactose Utilization	V31 Mannose Utilization	V32 Melibiose Utilization	V33 Raffinose Utilization	V37 Sucrose Utilization	V38 Trehalose Utilization

Interpretation of Results

1. Interpret results as per the standards given in the result interpretation chart.

Remarks

1. Microxpress® Lactobacillus 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 24-48 hours. Orange colour seen after 24-48 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.
9. 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. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203120370001	Biochemical Identification Kit	1 Kit (1 Test)