

## Neisseria Identification Kit

### Intended Use

A panel of 12 tests for identification of *Neisseria* species (Kit contains sterile medium for Urease Detection, Voges Proskauer Test, Oxidase Detection, Catalase Detection, Nitrate Reduction Test, ONPG Test and 6 different carbohydrates- Glucose, Maltose, Lactose, Sucrose, Fructose, Mannose).

### Summary

Gram-negative rods inhabit the mucous membranes of humans. The kit can be used for screening pathogenic *Neisseria* from nasopharyngeal, urethral, cervical exudates, rectal and pharyngeal specimens. 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

Micropress® *Neisseria* Identification Kit is a standardized identification system, comprising 12 miniature biochemical tests for identification of *Neisseria*. 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 *Neisseria* Identification Kit
2. Technical Product Insert with Result Interpretation Chart, Result Entry Datasheet 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. Gordon McLeod (Oxidase) Reagent for Oxidase Detection
7. Nitrite Detection Strip for Nitrate Reduction Test
8. Zinc Dust for Nitrate Reduction Test

**Note:** Micropress® *Neisseria* Identification Kit contains sufficient material to perform one test.

### Biochemical Tests

Micropress® *Neisseria* Identification Kit is a reagent set for laboratory use only.

Kit comprises of sterile test medium for:

- a) Urease Detection (V17)
- b) Voges Proskauer Test (V18)
- c) Oxidase Detection (V13)
- d) Catalase Detection (V3)
- e) Nitrate Reduction Test (V10)
- f) ONPG Test (V11)
- g) Glucose Utilization (V26)
- h) Maltose Utilization (V29)
- i) Lactose Utilization (V28)
- j) Sucrose Utilization (V37)
- k) Fructose Utilization (V24)
- l) Mannose Utilization (V31)

### 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 Brain Heart Infusion Agar (BHI) (201020230500).
2. Pick up a single well isolated colony and streak on to BHI agar slant for enrichment and incubate at 37°C for 18-24 hours.
3. Make a homogenous suspension in 2-3 mL sterile saline and 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. Incubate at 35°C-37°C and read the result at 18-24 hours of incubation.
5. 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 copper colour indicates a negative test.

### Oxidase Test

1. Add 1-2 drops of Gordon McLeod reagent (oxidase reagent) to the test vial V13.
2. Development of purplish blue colour within 5-10 seconds indicates a positive test.
3. No change in colour or delayed colour development after 60 seconds indicates negative test.

**Note:** Excess reagent should be decanted.

### Catalase Test

1. Take a loopful (using a platinum loop) of the inoculated culture from the vial V3 and dip in 3 % H<sub>2</sub>O<sub>2</sub> contained in a small, clean test tube.
2. Positive test is seen as effervescence coming out of the loop.
3. No effervescence is observed in case of negative test.

**Note:** 3 % H<sub>2</sub>O<sub>2</sub> has to be freshly prepared.

### Nitrate Reduction Test

1. Dip the nitrite detection strip in the test vial V10 for the solution to be just absorbed on the reaction pad.
2. Alternatively put one drop of the inoculated broth on the reaction pad and observe for colour change. If no colour change is observed, add a pinch of zinc dust (addition of too much zinc dust may result in false negative reaction)
3. Formation of pink, red or violet colour upon addition of nitrite detection strip is a positive reaction. No colour change upon addition of a pinch of zinc dust is a positive reaction.

## Identification Index

Organisms / Tests	Urease Detection	ONPG Test	Voges Proskauer Test	Oxidase Detection	Catalase Detection	Nitrate Reduction Test	Glucose Utilization	Maltose Utilization	Lactose Utilization	Sucrose Utilization	Fructose Utilization	Mannose Utilization
<i>Neisseria animalis</i>	-	-	ND	+	+	-	-	-	-	-	-	ND
<i>Neisseria canis</i>	-	-	ND	+	+	+	-	-	-	-	-	-

Organisms / Tests	Urease Detection	ONPG Test	Voges Proskauer Test	Oxidase Detection	Catalase Detection	Nitrate Reduction Test	Glucose Utilization	Maltose Utilization	Lactose Utilization	Sucrose Utilization	Fructose Utilization	Mannose Utilization
<i>Neisseria cinerea</i>	-	-	ND	+	+	-	-	-	-	-	-	-
<i>Neisseria denitrificans</i>	-	-	ND	+	+	-	+	-	-	+	+	+
<i>Neisseria elongata</i>	-	-	ND	+	-	-	d	-	-	-	-	-
<i>Neisseria flavescens</i>	-	-	ND	+	+	-	-	-	-	-	-	-
<i>Neisseria gonorrhoeae</i>	-	-	ND	+	+	-	+	-	-	-	-	-
<i>Neisseria iguanae</i>	-	-	ND	+	+	+	V	-	-	V	-	ND
<i>Neisseria lactamica</i>	-	+	ND	+	+	-	+	+	+	-	-	-
<i>Neisseria macacae</i>	-	-	ND	+	+	-	+	+	ND	+	+	ND
<i>Neisseria meningitidis</i>	-	-	ND	+	+	-	+	+	-	-	-	-
<i>Neisseria mucosa</i>	-	-	ND	+	+	+	+	+	-	+	+	-
<i>Neisseria subflava</i> biovar <i>perflava</i>	-	-	ND	+	+	-	+	+	-	d	d	-
<i>Neisseria polysaccharea</i>	-	-	ND	+	+	+	+	+	-	-	-	ND
<i>Neisseria sicca</i>	-	-	ND	+	+	-	+	+	-	+	+	-
<i>Neisseria subflava</i>	-	-	ND	+	+	-	+	+	-	d	d	-
<i>Neisseria weaveri</i>	-	-	ND	+	+	-	-	-	-	-	-	-

### 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; V: Variable; ND: Not Detected; d: 11-89% strains are positive.

### Result Interpretation Chart

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V17	Urease Detection	-	Detects urease activity	Orangish yellow	Pink	Orangish yellow
V11	ONPG Test	-	Detects $\beta$ -galactosidase activity	Colourless to cream	Yellow	Colourless to cream
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 to cream	Pinkish red within 5-10 minutes	Colourless / Slight copper
V13	Oxidase Detection	1-2 drops of Gordon McLeod reagent	Detects cytochrome oxidase production	Colourless to cream	Purplish blue	Colourless to cream
V3	Catalase Detection	3% H <sub>2</sub> O <sub>2</sub> Solution	Detects catalase activity	Colourless to cream	Effervescence seen	No Effervescence

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V10	Nitrate Reduction Test	Nitrite Detection Strip and a pinch of Zinc dust	Detects nitrate reduction	Colourless to cream	Pinkish red	Colourless
V26	Glucose Utilization	-	Detects glucose utilization	Red	Yellow	Red / Pink
V29	Maltose Utilization	-	Detects maltose utilization	Red	Yellow	Red / Pink
V28	Lactose Utilization	-	Detects lactose utilization	Red	Yellow	Red / Pink
V37	Sucrose Utilization	-	Detects sucrose utilization	Red	Yellow	Red / Pink
V24	Fructose Utilization	-	Detects fructose utilization	Red	Yellow	Red / Pink
V31	Mannose Utilization	-	Detects mannose utilization	Red	Yellow	Red / Pink

### Result Entry Data Sheet

Sample Number	V17 Urease Detection	V11 ONPG Test	V18 Voges Proskauer Test	V13 Oxidase Detection	V3 Catalase Detection	V10 Nitrate Reduction Test
Sample Number	V26 Glucose Utilization	V29 Maltose Utilization	V28 Lactose Utilization	V37 Sucrose Utilization	V24 Fructose Utilization	V31 Mannose 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. Microexpress® Neisseria 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

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4. Coblenz, L.H 1943, Rapid detection of the production of acetyl-methyl-carbinol, Am. J. Pub. Health 33:815-817.
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8. Murray, P. R. and *et al.*, Manual of Clinical Microbiology Vol. 1, ASM, 8th Edition, 2003.
9. Koneman. E. W and *et al.*, Color Atlas and Textbook of Diagnostic Microbiology lippincoh, 6th Edition, 2006.
10. Bergey's Manual of Systematic Bacteriology, Proteobacteria (Part C), 2nd edition, Vol. 2
11. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

### Product Presentation:

Cat. No.	Product Description	Pack Size
203140180001	Biochemical Identification Kit	1 Kit (1 Test)

### Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.

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