

## Specification

Sterile selective supplement for the isolation of pathogenic *Neisserias*.

## Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials			
Vial	22±0.25 x 55±0.5 mm glass vials, tag labelled, White	49 months	2-25 °C
with: 3 ± 0.1 g	plastic cap - 10 vials per box.		

## Composition

Composition (g/vial)

Vancomycin.....	0.00100
Colistin sulphate.....	0.00375
Trimethoprim.....	0.00150
Anphotericin.....	0.00150

**Note :** Each vial is sufficient to supplement for 500 ml of medium Base GC + Enrichment Suppl.

Reconstitute the original freeze-dried vial by adding  
Sterile Distilled Water..... 6 ml

## Description /Technique

### Description:

The *Neisseria spp.* include a lot of commensal bacteria that colonize the mucosal surfaces of many animals. Between the 11 species that colonize humans, only two are pathogens *N. meningitidis* and *N. gonorrhoeae*. *N. gonorrhoeae* is the causative agent of gonorrhoea and is transmitted via sexual contact. *Neisseria meningitidis* is the responsible for septicemia and meningitis.

In media like Thayer Martin and Chocolate agar *N. gonorrhoeae* and *N. meningitidis* produce colourless and translucent colonies. Antibiotic incorporated in the medium with the inhibitory supplement avoid the growth of almost all the non pathogen micro organisms included in the sample, including the saprophytic species of *Neisseria*.

### Technique:

#### Thayer-Martin Agar:

Effective for the isolation of pathogen neisseria. It is prepared with GC Base Agar, haemoglobin and an inhibitor vial VCAT. It contains Vancomycin and Colistin to inhibit the oxidase-positive contaminants; Nystatin to prevent the growth of saprophytic fungi and trimethoprim that prevent the *Proteus* overgrowth.

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Reconstitute the vial with 6 ml sterile diluent in aseptic conditions and add it to 500 ml of melted Agar base cooled to 50°C.

Do not overheat once supplemented.

Pour the complete medium into Petri dishes and, once solidified on a flat surface, spread the plates either by streaking or by spiral method.

Incubate at 37°C, in a very moist, 10% CO<sub>2</sub> enriched atmosphere for 48h.

(Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample or the specifications).

After incubation, count all the colonies that have appeared onto the surface of the agar.

Presumptive isolation / recovery of *Neisserias spp.* must be confirmed by further microbiological and biochemical tests.

**Quality control****Physical/Chemical control**

Color : White-Gray

**Microbiological control**

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020

Distribute the complete medium, cooled to 50 °C, into 90 mm plates

Incubate according instructions for complete medium indicated in COMPOSITION.

5-10% CO<sub>2</sub> atmosphere. Incubation at 37 ±1 °C during 48 ± 2 h.

**Microorganism****Growth**

*Neisseria gonorrhoeae* ATCC® 19424

Good

*Neisseria meningitidis* ATCC® 13090

Good

*Staphylococcus aureus* ATCC® 6538, WDCM 00032

Inhibited

**Sterility control**

Add 5 ml of the sample to:

100 ml TSB and 100 ml Thioglycollate.

Incubation 48 hours at 30-35 °C and 48 hours at 20-25 °C: NO GROWTH.

**Bibliography**

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ODEGAARD, K. (1971) Trimethoprim for the prevention of overgrowth by swarming Proteus in the cultivation of gonococci. Acta. Path. Microbiol. Scand. Sect. (B) 79:545-548.

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