Neisseria PET
(Preformed Enzyme Test)
CK3025

Outline
Neisseria PET uses chromogenic substrates to detect the presence of preformed enzymes in fresh cultures of Neisseria. The test can be used to confirm *Neisseria meningitidis* and *Neisseria lactamica* and presumptively identify *Neisseria gonorrhoeae.*

Principle of test
Neisseria PET contains three non-interfering substrates: gamma-glutamyl-nitroanalide, bromo-chloro-indol-B-D-galactopyranoside and proline napthylamide. These substrates are bound to chromogens, coloured end products are released when substrates are hydrolysed.

Neisseria lactamica produces beta galactosidase, which hydrolyses bromo-chloro-indol-B-D-galactopyranoside to produce a blue coloured end product.

Neisseria meningitidis produces gamma-glutamylaminopeptidase, which hydrolyses gamma-glutamyl nitroanalide to produce a yellow coloured end product. A second reaction gives a blue/purple colour on the addition of the PEP reagent.

Neisseria gonorrhoeae produces hydroxyprolineaminopeptidase which hydrolyses proline napthylamide, the free napthylamide produces a red colour on addition of the PEP reagent.

Limitations
1 This test is for use only on organisms that are oxidase positive, Gram negative cocci.
2 This test is designed for identifying organisms isolated on GC Selective media. Strains isolated on non-selective media may be saprophytic Neisseria and give misleading results. Saprophytic Neisseria will grow on nutrient agar whilst *Neisseria gonorrhoeae* will not.
3 It is possible that when subculturing *Neisseria gonorrhoeae* from selective medium to a non-selective medium saprophytic Neisseria that had been inhibited will grow through. Other tests for differentiating saprophytic Neisseria from pathogenic Neisseria include acid production tests, nitrate reduction and colistin resistance. .
4 Proline aminopeptidase negative strains of *Neisseria gonorrhoeae* have been reported. **These strains will give false negative results.**
5 This test should not be used on cultures of more than 48 hours or on cultures that have been sat at room temperature for several hours as the enzyme activity will be diminished.
6 Some strains of *Neisseria mucosa* and *Neisseria perflava* may produce a pale yellow reaction suggesting they are producing gamma-glutamylaminopeptidase. Pigmented strains producing gamma-glutamylaminopeptidase should be tested for classic acid production patterns before being identified as *Neisseria meningitidis.*
7 Whilst no colour change in the three tests may indicate *Moraxella catarrhalis* this should be confirmed with a Tributyrin/butyrate test.
8 **Do not run blank negative controls**. Doing so will result in false positive results. Negative controls must be run with organisms negative for this screen. The bacteria convert the pH of the test allowing for accurate results.
9 *M. catarrhalis* isolates may produce a yellow/orange colour after the addition of the PEP reagent which should be confirmed with a Tributyrin/butyrate test. This colour development is due to unreacted reagent rather than hydrolysis of the substrate.

Method
Use fresh 24 hour cultures. Test only cultures which are pure, Gram negative and oxidase positive and have been isolated on GC Selective agar.

Add 3 drops of distilled water to the tube containing the tablet. It is not necessary to bring the reagents to room temperature before use.

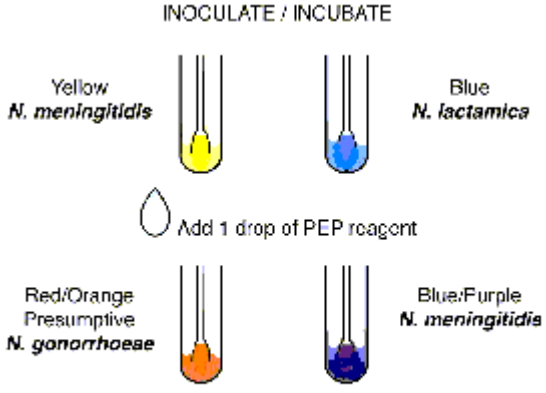
Step 1. Using a swab harvest 5-10 colonies and mix into the tube. It is not necessary for the tablet to dissolve. Leave the swab in the tube during incubation for best results.
Incubate for 30 minutes at 37°C. Test may be held for up to 2 hours but no longer as false reactions may occur.

Reading
Follow this exact sequence
If a blue colour is produced the organism is *Neisseria lactamica.* **DO NOT CONTINUE.**

If a yellow colour is produced the organism is *Neisseria meningitidis*. Weakly reacting *Neisseria meningitidis* can be confirmed at step 2.

Step 2. Perform the aminopeptidase test by adding 1 drop of PEP reagent to the swab/tube. *Neisseria meningitidis* may give a purple reaction immediately on addition of the PEP reagent. **DO NOT CONTINUE IF A BLUE/PURPLE OR RED/ORANGE COLOUR IS PRODUCED**

Observe at room temperature for 2 minutes. If a red/orange colour is produced the organism is identified as *Neisseria gonorrhoeae*. If a blue purple colour is produced the organism is *Neisseria meningitidis*.
N.B. After addition of the PEP reagent interpretation is made on the first colour produced, for example if a blue/purple colour is produced and then fades to a muddy orange the interpretation is *Neisseria meningitidis*. Any colour changes occurring after 5 minutes may be misleading.



Quality Control Organisms	Colour reaction - Step 1	Colour reaction - Step 2
Neisseria meningitidis ATCC 13077	Yellow	Blue/Purple
Neisseria lactamica ATCC 23970	Blue	-
Neisseria gonorrhoeae ATCC 19424	-	Red/Orange
Moraxella catarrhalis ATCC 25238	None	None

Performance Characteristics

	Total	Neisseria PET
Positive	642	642
Negative	144	138

Sensitivity - 100% Specificity - 99.1%

Storage - In airtight container at 2-8°C **Shelf Life** - Expiry date on packaging. 18 months from date of manufacture.

Health & Safety Information

Each tablet contains approximately 0.5 milligrams of each substrate with inert fillers and tableting compounds. None of the ingredients are hazardous in this form. The PEP reagent contains hydrochloric acid, which will stain surfaces and hands and is corrosive.

References

D'amato et al 1978. Rapid identification of *Neisseria gonorrhoea* and *Neisseria meningitidis* using enzymatic profiles. J.Clin. Micro. 7:77-8

Dillon et al 1988. Evaluation of eight methods for identification of pathogenic Neisseria species. J.Clin Microbiol. 26:680-8

The following table is helpful in distinguishing saprophytic *Neisseria* spp. from *N. gonorrhoea* (source www.cdc.gov).

Species that Produce Hydroxy-prolylaminopeptidase	Acid Production Patterns				Gram stain*	Nitrate Reduction	Polysacc-haride from Sucrose	Superoxol	Colistin Resistance
	G	M	L	S					
N. gonorrhoeae *"N. kochii"**	+	-	-	-	GND	-	-	Strong (4+)	R
K. denitrificans	+	-	-	-	GNC	+	-	-	R
N. cinerea	+	-	-	-	GND	-	-	Weak (2+)	(R)
N. polysaccharea	+	+	-	-	GND	-	+	Weak (2+)	(R)
N. subflava biovars *subflava/flava*	+	+	-	-	GND	-	-	Weak (2+)	S
N. subflava biovar *perflava*	+	+	-	+	GND	-	-	Weak (2+) positive	S
N. sicca	+	+	-	+	GND	-	-	Weak (2+) positive	S
N. mucosa	+	+	-	+	GND	+	-	Weak (2+) positive	S
N. elongata	-	+	-	-	GNR	-	-	Weak (2+) positive	S
N. flavescens	-	-	-	-	GND	-	-	Weak (2+) positive	S

Whilst the use of a single test method and clinical findings may be considered sufficient for a presumptive identification, we would advise the use of two test methods involving different principles (e.g. biochemical, antigenic or molecular) before issuing a definitive/confirmed identification of *Neisseria gonorrhoeae*. This advice is given on the CDC website: http://www.cdc.gov/std/Gonorrhea/

Other products from BioConnections for Neisseria identification:

CK7240, GonGen II Antigen detection (no boiling)
CK2001, Nitrocefin Discs
CK3520, Oxidase Test
CK1265, Gamma Glutamyl aminopeptidase

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