



ONPG Broth

M1930

Recommended for the differentiation of microorganisms on the basis of beta-galactosidase activity.

Composition**

Ingredients	Gms / Litre
Casein Peptone	7.500
Sodium Phosphate Dibasic	0.350
O-Nitrophenyl-β-D-galactopyranoside	1.500
Sodium chloride	3.750
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 13.10 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Cool to 45-50°C and dispense into sterile tubes. DO NOT AUTOCLAVE.

Principle And Interpretation

ONPG (Ortho-nitrophenyl beta-D-galactopyranoside) is a synthetic colourless compound (galactoside) structurally similar to lactose(1).

beta-galactosidase positive organisms cleaves ONPG to galactose and o-nitrophenyl, a yellow compound. The ONPG test is specially useful in the rapid identification of cryptic lactose fermenters (late fermenters). Since members of family *Enterobacteriaceae* are routinely grouped according to their lactose fermenting ability the ONPG test is significant here.

ONPG Broth is used to differentiation of organisms based on their ability to utilize Ortho-nitrophenyl beta-D-galactopyranoside. ONPG is similar in structure to lactose. The presence of two enzymes is required to demonstrate lactose fermentation in a conventional test. The first enzyme permease, facilitates the entry of lactose molecules into the bacterial cell while the second enzyme, b-galactosidase, hydrolyzes the lactose to yield glucose and galactose. True non-lactose fermenters lack both enzymes; however some organisms lack permease but possess beta-galactosidase. These organisms are late lactose fermenters.

Casein peptone serves as a source of nitrogenous compounds and other growth factors. Phosphate buffers the medium. Sodium chloride maintains the osmotic balance. Ortho-nitrophenyl beta-D-galactopyranoside serves as a substrate for the presence of beta galactosidase enzyme.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured clear solution in tubes

Reaction

Reaction of 1.3% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH

7.30-7.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	ONPG test
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Cultural Response

<i>Salmonella Choleraesuis</i> ATCC 12011	50-100	luxuriant	Positive reaction: yellow colour
<i>Citrobacter freundii</i> ATCC 8090	50-100	luxuriant	Positive reaction: yellow colour
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	Positive reaction: yellow colour
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	Positive reaction: yellow colour
<i>Proteus vulgaris</i> ATCC 13315	50-100	luxuriant	Negative reaction: no colour change
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	Negative reaction: no colour change

Storage and Shelf Life

Store dehydrated medium and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1. Lowe G.H., 1962., J. Med. Lab. Technol., 19:21

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