ONPG BROTH

INTENDED USE
Remel ONPG Broth is a liquid medium recommended for use in qualitative procedures for determination of β-D-galactosidase activity of members of the *Enterobacteriaceae* and other microorganisms.

SUMMARY AND EXPLANATION
Seidman and Link developed σ-nitrophenyl-β-D-galactopyranoside (ONPG) substrate.[1] Lederberg used ONPG as a substrate in his studies of the β-D-galactosidase enzyme produced by *Escherichia coli*.[2] LeMinor and Ben Hamida reported, detection of β-D-galactosidase activity was essential for rapid biochemical identification of enteric gram-negative bacilli.[3] The ONPG test is recommended to aid in the differentiation of certain enteric gram-negative bacilli which may at first appear to be nonlactose fermenters, including *Citrobacter* spp. Such organisms lack the enzyme permease, but do possess β-galactosidase and are positive for ONPG.[4]

PRINCIPLE
Peptone provides nitrogenous compounds and amino acids essential for bacterial growth. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. The fermentation of lactose depends on the action of two enzymes, permease and β-galactosidase. Permease allows lactose to enter the cell wall and β-galactosidase breaks down the lactose to glucose and galactose which can then be metabolized. In slow lactose-fermenting organisms, lactose enters the cells slowly due to perrmease deficiency. ONPG is the substrate which is hydrolyzed by β-galactosidase, resulting in the liberation of σ-nitrophenol which yields a yellow color in alkaline solution.

REAGENTS (CLASSICAL FORMULA)*

Peptone	7.5 g	ONPG	1.5 g
Sodium Chloride	3.75 g	Sodium Phosphate Buffer	250.0 ml
		Demineralized Water	750.0 ml

pH 7.0 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Heavily inoculate ONPG Broth using isolated colonies from a pure, 18-24 hour culture.
2. Incubate aerobically at 33-37°C for 1 to 24 hours.
3. Examine for a yellow color development at 1 hour and up to 24 hours.

INTERPRETATION OF THE TEST
Positive Test - A yellow color development
Negative Test - No color change

QUALITY CONTROL
All lot numbers of ONPG Broth have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Escherichia coli ATCC® 25922	Aerobic, 18-24 h @ 33-37°C	Positive
Salmonella enterica serovar Typhimurium ATCC® 14028	Aerobic, 18-24 h @ 33-37°C	Negative

BIBLIOGRAPHY

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4. W.H. Ewing. 1986. Edwards and Ewing's Identification of Enterobacteriaceae. 4th ed. Elsevier, New York, N.Y.
5. Winn, W., S. Allen, J. William, E. Koneman, G. Procop, P. Schreckenberger, and G. Woods. 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams and Wilkins, Baltimore, MD.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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