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Section: Technical Manual	Subject Title: ONPG Test	
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ONPG TEST

Principle

This test is used to demonstrate the presence or absence of the enzyme B-galactosidase using the substrate ortho-nitrophenyl-D-galactopyranoside in order to differentiate lactose-fermenting from non lactose-fermenting organisms and in the identification of *B. cepacia*.

Reagents

ONPG disks (Store refrigerated)
Sterile saline

Other materials

Sterile tube (13 x 100 mm)
Bacteriology loop
Sterile graduated Pasteur pipette

Procedure

1. Place an ONPG disk into a sterile tube and add 0.2 mL saline.
2. Heavily inoculate the tube with a loopful of the test isolate.
3. Incubate at 35°C for up to 4 hours.

Interpretation

Positive: yellow colour within 4 hours

Negative: colourless at 4 hours

Precautions

1. A heavy inoculum is necessary to obtain a high concentration of enzyme.

Quality Control

Test with known positive and negative controls each time the test is performed.

Positive: *E. coli* (ATCC 25922)
Negative: *P. vulgaris* (ATCC 13315)

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References

1. MacFaddin JF, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore MD., 1980, p120-128.