K490/K1490 ONPG TEST

PRINCIPLE/DISCUSSION:
Fermentation of lactose depends on the presence of two enzymes: permease, which allows the lactose to enter the bacterial cell, and beta-galactosidase, which splits lactose into glucose and galactose, which are subsequently metabolized. ONPG tests can be used to differentiate slow lactose fermenters, which are thought to be deficient in permease, and non-fermenters. It is also useful to differentiate *Pseudomonas cepacia* and *Pseudomonas maltophilia*, which are positive, from other pseudomonads, which are negative. The demonstration of beta-galactosidase is accomplished by the hydrolization of ortho-nitrophenol-beta-D-galacto-pyranoside liberating ortho-nitrophenol with its characteristic yellow color.

ACTIVE INGREDIENTS:
Each tablet contains 0.2 mg. of O-nitrophenyl-beta-D-galacto- pyranoside.

MATERIAL SAFETY DATA:
This product does not contain any materials known at this time to be hazardous.

STORAGE:
Store tightly covered, with dessicant, in a dry place at room temperature.

MATERIALS REQUIRED:
ONPG tablets require fresh 24 hour growth on culture media. Consult the Manual of Clinical Microbiology for recommended media for the specimen. The following items are required but not provided:

- Small test tubes (e.g. 12 X 75) (provided with WEE-TAB single test)
- Purified water pH 6.5-8.0

PROCEDURE:
(1) Dissolve one ONPG tablet in 1 ml. of water in a small test tube. For WEE-TABS use 0.5 ml.
(2) Inoculate heavily. A loopful of organism from a culture plate or slant should be sufficient.
(3) Incubate at 35-37C for up to 6 hours or until the yellow color of a positive test appears. WEE-TABS are finished at 2 hours.

INTERPRETATION:
The appearance of a yellow color at any time during the incubation is a positive result. A positive ONPG test shows that the organism being tested contains the enzymes necessary for the fermentation of lactose and therefore may be classified as a lactose fermenter.

QUALITY CONTROL:
Known positive and negative test organisms should be run with each batch. Key suggests Escherichia coli ATCC 25922 as positive and Proteus vulgaris ATCC 13315 as negative. Dispose of all used material in a manner appropriate for biohazardous material.

CHARACTERISTIC REACTIONS:

E. Coli	+
Enterobacter	+
Citrobacter	+
Klebsiella	+
S. arizona	+
Proteus	-
Salmonella	-
Pseudomonas (most)	-
P. cepacia	+
P. maltophilia	+
Shigella	var.

See the Manual of Clinical Microbiology for a more complete listing.

REFERENCES:
(1) Manual of Clinical Microbiology, Fifth Edition, Chapter 36, "Enterobacteriaceae"

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