

CARBOHYDRATE OXIDATION TABLETS

PRINCIPLE/DISCUSSION:

Carbohydrate Oxidation Test Tablets are used in identifying nonfermentative bacteria through their ability to utilize different carbohydrates in the presence of oxygen. This process uses oxygen as the final electron acceptor and causes a color change in the pH indicator. Key Fermentation tablets are not suitable for oxidation tests because of bacteriostatic compounds included in the formula. Since these are absent in the oxidation tablets, the substrate is heated to kill vegetative cells and contains a small amount of crystal violet to inhibit growth of resistant spores.

STORAGE:

Consult individual package labels for storage instructions.

MATERIALS REQUIRED:

Each carbohydrate is sold separately in bottles of 50 tablets. The complete carbohydrate list is given in the chart at the end. All tests require fresh 24 hour growth on culture media. Consult the Manual of Clinical Microbiology for recommended media for the specimen. In addition to the tablets, the following items are required but not provided:

Small glass test tubes with air permeable stoppers (e.g. 12 X 75), Inoculating loop, Sterile pipette, Sterile water, pH 6.5-7.5, Boiling water bath.

PROCEDURE:

(1) Add each tablet to 1 ml of water in a small test tube and heat in a boiling water bath for 10 minutes. Cool before using. The dry tablets in the tube may be microwaved on high for 10-15 seconds to achieve the same result.

- **NOTE:** This step is crucial, as results may not be accurate if the procedure is not followed.

(2) For multiple carbohydrate tests, inoculate with a heavy bacterial suspension made in 1-2 ml of sterile distilled water. Transfer 1-2 drops of this suspension to each tube with a sterile pipette. For a single test inoculate the tube with a loopful of the organism being tested without making a suspension.

(3) Stopper the tubes and incubate at 35-37°C for 24 hours.

- **NOTE:** The stoppers should permit gaseous exchange. eg.- cotton plugs.

INTERPRETATION OF RESULTS:

After 24 hours, observe the tube for color change from red to yellow, indicating acid production. In the absence of oxidation, the red color remains or intensifies. Negative tests should be kept for 4 days. Slight color changes may occasionally be observed within the first 24 hours, but these are not related to the oxidation process and will disappear within 48 hours if there is no oxidation.

QUALITY CONTROL:

Each lot of KEY Carbohydrate Oxidation tablets should be tested prior to use with organisms which produce known reactions. Some reactions are listed on the right. Consult a microbiology manual for a more complete list. Finished tests should be discarded in a manner appropriate for biohazardous materials. KEY Carbohydrate Oxidation Tablets are for INVITRO DIAGNOSTIC USE ONLY.

SELECTED COMMON REACTIONS:

1. *Pseudomonas aeruginosa*
2. *Acinetobacter anitratus*
3. *Pseudomonas maltophilia*
4. *Acinetobacter lwoffii*
5. *Pseudomonas stutzeri*
6. *Pseudomonas fluorescens*
7. *Pseudomonas alcaligenes*
8. *Pseudomonas cepacia*
9. *Pseudomonas pseudomallei*
10. *Alcaligenes odorans*

TABLET	POSITIVE	NEGATIVE
K060 Arabinose	1	3
K121 Cellobiose	3	1
K140 Dextrose	1	10
K220 Fructose	1	7
K340 Inositol	8	1
K370 Lactose	2	1
K400 Maltose	5	1
K420 Mannitol	1	3
K442 Melibiose	2	9
K560 Rhamnose	2	4
K620 Sucrose	6	2
K690 Xylose	2	7

REFERENCES:

- (1) Manual of Clinical Microbiology, Fifth Edition, Chapter 40, "Miscellaneous Gram-Negative Bacteria." and Chapter 41, "Pseudomonas and Related Genera."
- (2) Nonfermentative Gram Negative Bacilli: A syllabus for detection and identification, by Dr. M.J. Pickett



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