OF KING MEDIUM w/ and w/o CARBOHYDRATES

INTENDED USE
Remel OF King Medium w/ and w/o Carbohydrates are semisolid media recommended for use in qualitative procedures to determine the oxidative and fermentative metabolism of carbohydrates by microorganisms.

SUMMARY AND EXPLANATION
Hugh and Leifson first reported that microorganisms which utilize carbohydrates oxidatively (i.e., via an aerobic metabolic pathway) produce only a minimal amount of acid.[1] In media containing large amounts of peptones, oxidative microorganisms utilize the peptones and produce enough alkaline by-products to neutralize the acid resulting from carbohydrate metabolism. Oxidative-Fermentative (OF) Medium was developed by Hugh and Leifson with a ratio of peptone to carbohydrate which minimized the formation of alkaline by-products and enhanced acid production by oxidative organisms. OF King Medium is a modification of the Hugh and Leifson formulation which uses phenol red as the indicator of acid production. OF King Medium is recommended by the Centers for Disease Control and Prevention (CDC) for identification of gram-negative nonfermentative bacilli according to the protocol of Clark et al.[2]

PRINCIPLE
OF King Medium is a semisolid medium with a low peptone to carbohydrate ratio and contains the pH indicator phenol red. Oxidation usually begins at the top of the tube; whereas, fermentation results in acid production equally throughout the tube. Oxidative organisms which metabolize the carbohydrate in OF King Medium produce acid high enough in concentration to prevent neutralization by alkaline by-products of peptone utilization. The agar allows for detection of motility and aids in even distribution of acid produced at the surface of the medium helping to distinguish between oxidative and fermentative organisms. In the presence of acid, phenol red indicator changes from red to yellow.

REAGENTS (CLASSICAL FORMULA)*
Base Medium:

Casein Peptone	2.0 g	Agar	3.0 g
Phenol Red	30.0 mg	Demineralized Water	1000.0 ml

pH 6.8 ± 0.2 @ 25°C (w/o carbohydrates)

The following carbohydrates are available per liter of medium:

Dextrose	10.0 g	Mannitol	10.0 g
Lactose	10.0 g	Sucrose	10.0 g
Maltose	10.0 g	Xylose	10.0 g

*Adjusted as required to meet performance standards.

PROCEDURE
1. The performance of this medium is dependent on a properly prepared inoculum. The test isolate should be 18-24 hours old and in pure culture growing on an agar plate. Growth from a slant such as Kligler Iron Agar or Triple Sugar Iron Agar may also be used, provided the inoculum is in pure culture. For each test isolate, two tubes of OF King Medium Base (w/o carbohydrate) should also be inoculated and incubated in parallel with the carbohydrate tubes.
2. Inoculate duplicate tubes of selected OF King Medium w/ Carbohydrate for each isolate to be tested. Touch the colony using an inoculating needle and stab once down the center of the medium to within approximately one-fourth inch of the bottom of the tube. Overlay one tube with sterile mineral oil.
3. Incubate the open tube (without mineral oil overlay) with cap loosened and the closed tube (with mineral oil overlay) aerobically at 33-37°C for up to 7 days.
4. Examine tubes daily for a color change from red to yellow, indicating acid production.

INTERPRETATION OF THE TEST
Acid Production:
Positive Test - Yellow color development
Negative Test - Red color remains

Note: Acid production in the open tube indicates oxidative metabolism. Acid production in both tubes indicates fermentative metabolism. No acid production in either tube indicates the organism does not metabolize the carbohydrate.

Motility:
Positive Test - Growth diffused away from the stab line of inoculation
Negative Test - Growth confined to stab line

QUALITY CONTROL
All lot numbers of OF King Medium w/ and w/o Carbohydrates have been tested for performance and found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. Control organisms should be selected that demonstrate a positive and negative reaction for each carbohydrate tested. If aberrant quality control results are noted, patient results should not be reported.

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LIMITATIONS

1. Organisms which fail to spread in OF King Medium w/ Dextrose should not be assumed to be nonmotile; such organisms may spread in standard semisolid motility medium. Acidity has been found to inhibit spreading by motile bacteria.[3]
2. A heavy inoculum, such as that delivered by a Pasteur pipette, may alter the composition and pH of the medium and inhibit growth of the organism.[3]
3. Organisms that only oxidize glucose will not ferment any other carbohydrate.[1] Therefore, when testing with other carbohydrates, the mineral oil overlay tube may be omitted.
4. Acid production by an oxidative organism first appears at the surface of the open tube and gradually spreads throughout the tube. Prolonged incubation may be required for organisms which are weakly oxidative.[3]
5. Some organisms may grow in OF King Medium and fail to produce acid. Such negative reactions should be confirmed using another basal medium containing dextrose.[3]
6. A heavy inoculum may be necessary to detect biochemical reactions with nonfermenters that are strict aerobes because the environment within the tube may not support optimal growth.[4]

BIBLIOGRAPHY

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2. Clark, W.A., D.G. Hollis, R.E. Weaver, and P. Riley. 1984. *Identification of Unusual Pathogenic Gram-Negative Aerobic and Facultatively Anaerobic Bacteria*. Centers for Disease Control and Prevention, Atlanta, GA.
3. MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA.
4. 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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