IFU

Instructions for Use

OXIDATIVE-FERMENTATIVE MEDIUM (OF BASAL MEDIUM)

Cat. no. Y51	OF Base Medium, 13x100mm Tube, 3ml	20 or 100 tubes/box
Cat. no. Y57	OF Dextrose, 13x100mm Tube, 3ml	20 or 100 tubes/box
Cat. no. Y58	OF Fructose, 13x100mm Tube, 3ml	20 or 100 tubes/box
Cat. no. Y54	OF Lactose, 13x100mm Tube, 3ml	20 or 100 tubes/box
Cat. no. Y52	OF Maltose, 13x100mm Tube, 3ml	20 or 100 tubes/box
Cat. no. Y53	OF Mannitol, 13x100mm Tube, 3ml	20 or 100 tubes/box
Cat. no. Y55	OF Sucrose, 13x100mm Tube, 3ml	20 or 100 tubes/box
Cat. no. Y56	OF Xylose, 13x100mm Tube, 3ml	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics OF Basal Medium is recommended for the detection of oxidation or fermentation of carbohydrates by bacteria.

SUMMARY

OF Basal Medium was developed by Hugh and Leifson to aid in the identification of gram-negative bacteria on the basis of their ability to oxidize or ferment a specific carbohydrate.[6]

As compared to other OF Media, Hugh and Leifson's formula employs a low peptone/carbohydrate ratio and a minimal amount of agar. The decreased amount of peptone reduces the formation of alkaline amines which can ultimately mask the small quantities of acid that may be produced from oxidative metabolism.[5] The increased carbohydrate results in an increase in the amount of acid that may be formed. The small amount of agar added to the medium provides a semi-solid structure which concentrates the acid at the point of reaction, thereby facilitating visual interpretation of the pH shift.

Proper performance of the OF test requires an organism to be inoculated to two tubes of each OF Medium. Once inoculated, one tube is overlaid with mineral oil or melted paraffin. The other tube is left open to the air.

Oxidative utilization of the carbohydrate will result in acid production (yellow) in the open tube only. Fermentative utilization of the carbohydrate will result in acid production (yellow) in both the open and closed tubes. Acidic changes in the overlaid tubes are considered to be a result of true fermentation, while acidic development in the open tubes are due to oxidative utilization of the carbohydrate present. Asaccharolytic organisms will not produce acid in either tube.

FORMULA

Ingredients per liter of deionized water:*

Sodium Chloride	5.0gm
Pancreatic Digest of Casein	2.0gm
Dipotassium Phosphate	0.3gm
Bromothymol Blue	0.08gm
Agar	2.0gm

Additionally, OF Media with carbohydrate contains 10.0gm/L of specific carbohydrate.

Final pH 6.8 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2-8ºC. (except Cat. no. Y51, store at 2-30ºC.) away from direct light. Media should not be used if there are any signs of contamination, deterioration or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Specimen collection is not applicable since this medium is not intended for primary isolation from clinical specimens. As a general rule, infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation. Consult listed references for information on specimen collection.[1-5]

Method of Use:

1. Allow media to equilibrate to room temperature.

2. Obtain pure, isolated colonies from an 18-24 hour culture.

3. For each test organism, inoculate tubes in duplicate. Inoculate by stabbing the agar to approximately 1/4 inch from the bottom.

4. Apply sterile mineral oil, sterile melted paraffin, or sterile melted petrolatum to one of each duplicate tubes. Tighten the cap of the overlaid tube, and loosen the cap of the non-overlaid tube.

5. Incubate both tubes aerobically at 35ºC. for up to 14 days.

6. Examine tubes daily.

7. A control tube of OF Base Medium (Cat. no. Y51) should be inoculated and incubated in parallel with the OF tests.

INTERPRETATION OF RESULTS

A positive carbohydrate utilization test is indicated by the development of a yellow color in the medium.

A negative carbohydrate utilization test is indicated by the absence of a yellow color (media remains green or turns blue).

The method of metabolism is determined as follows:

Carbohydrate Utilization	**Open Tube (No overlay)**	**Closed Tube (Oil overlay)**
Oxidation	Positive (Yellow)	Negative (Green)
Fermentation	Positive (Yellow)	Positive (Yellow)
Non-oxidizer/Non-fermenter	Negative (Green or Blue)	Negative (Green)
Both oxidation and fermentation	Positive (Yellow)	Positive (Yellow)

Note: Color reactions should be compared to the OF Base Medium (Cat. no. Y51) control tube.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Organisms that only oxidize dextrose will not ferment any other carbohydrate. Other carbohydrates will only be oxidized. The overlaid (closed) tube, therefore, may be omitted when determining other carbohydrate utilization of such organisms.

Some microorganisms do not grow in OF Basal Medium. It may be necessary to use another basal medium containing dextrose to confirm the negative reaction.

Some mineral oils are acidic and may result in erroneous results.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, sterile mineral oil,

applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
OF Base Medium:					
Escherichia coli ATCC® 25922	D	18-48hr	35°C	Aerobic	Growth; no color change (top may turn blue at 48 hours) in open tube and in oil overlaid tube
OF Dextrose:					
Escherichia coli ATCC® 25922	D	18-24hr	35°C	Aerobic	Growth; acid (yellow) reaction and gas in open tube and in oil overlaid tube
Pseudomonas aeruginosa ATCC® 27853	D	18-24hr	35°C	Aerobic	Growth; acid (yellow) reaction in open tube; no change in oil overlaid tube
OF Fructose:					
Klebsiella pneumoniae ATCC® 13883	D	18-24hr	35°C	Aerobic	Growth; acid (yellow) reaction in open and oil overlaid tube
Branhamella (Moraxella) catarrhalis ATCC® 25240	D	18-24hr	35°C	Aerobic	Growth, negative fermentation and oxidation - green
OF Lactose:					
Enterobacter cloacae ATCC® 23355	D	18-24hr	35°C	Aerobic	Growth; acid (yellow) reaction in open and oil overlaid tube
Branhamella (Moraxella) catarrhalis ATCC® 25240	D	18-24hr	35°C	Aerobic	Growth; negative oxidation and fermentation-green
OF Maltose:					
Klebsiella pneumoniae ATCC® 13883	D	18-24hr	35°C	Aerobic	Growth; acid (yellow) reaction in open and oil overlaid tube
Branhamella (Moraxella) catarrhalis ATCC® 25240	D	18-24hr	35°C	Aerobic	Growth; no color change in either tube
OF Mannitol:					
Enterococcus faecalis ATCC® 29212	D	18-24hr	35°C	Aerobic	Growth: acid (yellow) reaction in open and oil overlaid tube

Escherichia coli ATCC® 25922	D	18-24hr	35°C	Aerobic	Growth; acid (yellow) reaction in open and oil overlaid tube
Branhamella (Moraxella) catarrhalis ATCC® 25240	D	18-24hr	35°C	Aerobic	Growth, negative oxidation and fermentation reactions - green
OF Sucrose:					
Enterobacter aerogenes ATCC® 13048	D	18-24hr	35°C	Aerobic	Growth; acid (yellow) reaction in open and oil overlaid tube
Branhamella (Moraxella) catarrhalis ATCC® 25240	D	18-24hr	35°C	Aerobic	Growth; negative oxidation and fermentation - green
OF Xylose:					
Enterobacter aerogenes ATCC® 13048	D	18-24hr	35°C	Aerobic	Growth; acid (yellow) reaction in open and oil overlaid tube
Branhamella (Moraxella) catarrhalis ATCC® 25240	D	18-24hr	35°C	Aerobic	Growth; negative oxidation and fermentation - green

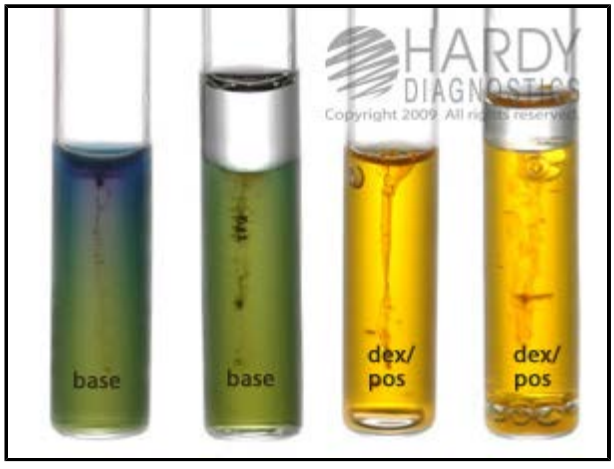
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

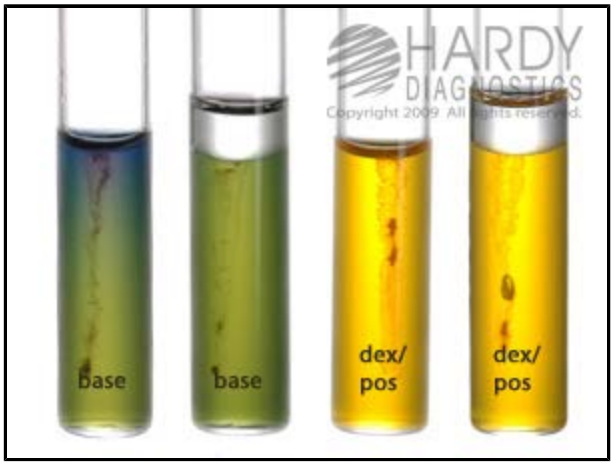
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

OF Basal Media should appear slightly opalescent, and green in color.



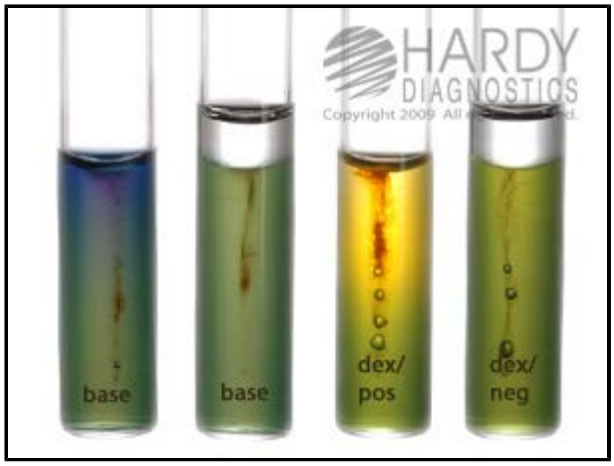
Escherichia coli (ATCC® 25922) growing in Oxidative-



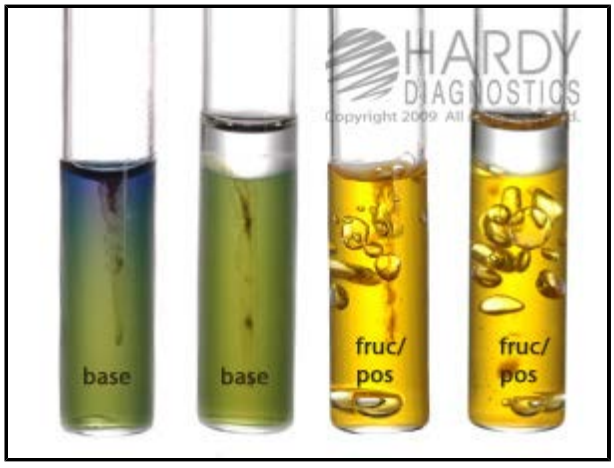
Shigella flexneri (ATCC® 12022) growing in Oxidative-

Fermentative Base (Cat. no. Y51) and OF Dextrose (Cat. no. Y57) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.

Fermentative Base (Cat. no. Y51) and OF Dextrose (Cat. no. Y57) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.

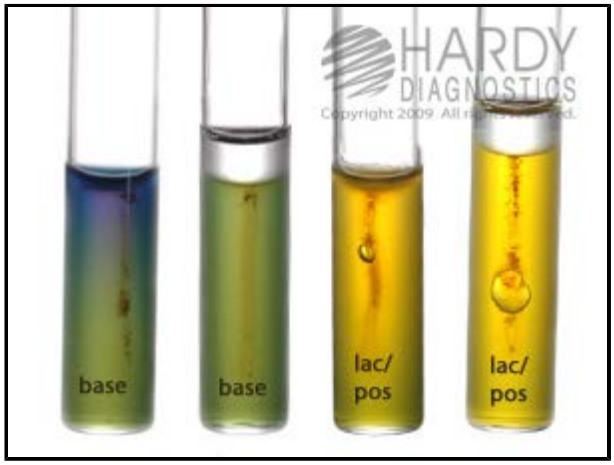


Pseudomonas aeruginosa (ATCC® 27853) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Dextrose (Cat. no. Y57) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.

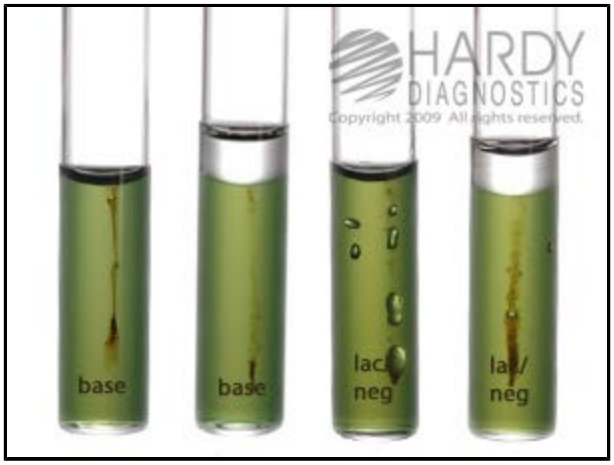


Klebsiella pneumoniae (ATCC® 13883) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Fructose (Cat. no. Y58) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.

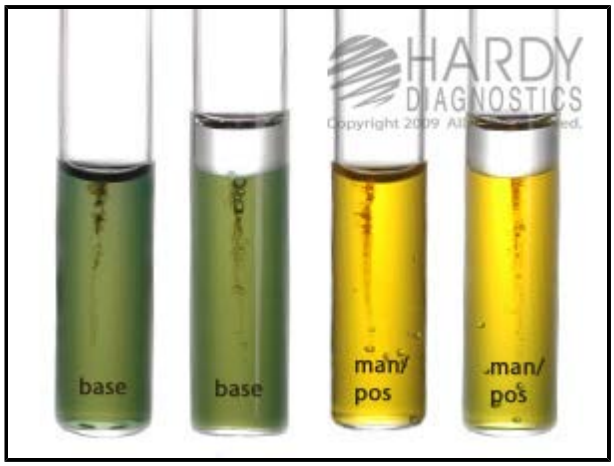
Branhamella (Moraxella) catarrhalis (ATCC® 25240) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Fructose (Cat. no. Y58) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.



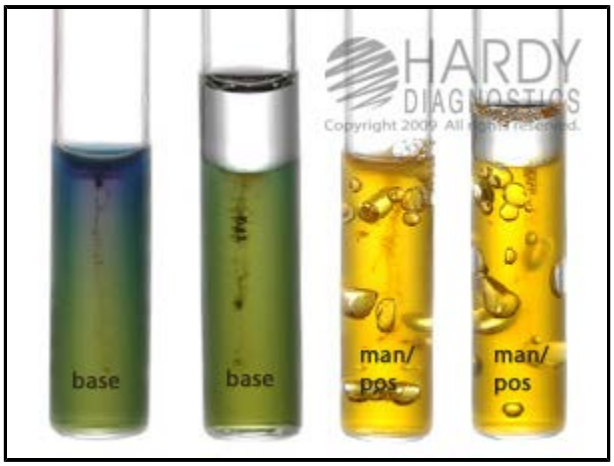
Enterobacter cloacae (ATCC® 23355) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Lactose (Cat. no. Y54) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.



Branhamella (Moraxella) catarrhalis (ATCC® 25240) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Lactose (Cat. no. Y54) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.



Enterococcus faecalis (ATCC® 29212) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Mannitol (Cat. no. Y53) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.



Escherichia coli (ATCC® 25922) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Mannitol (Cat. no. Y53) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.

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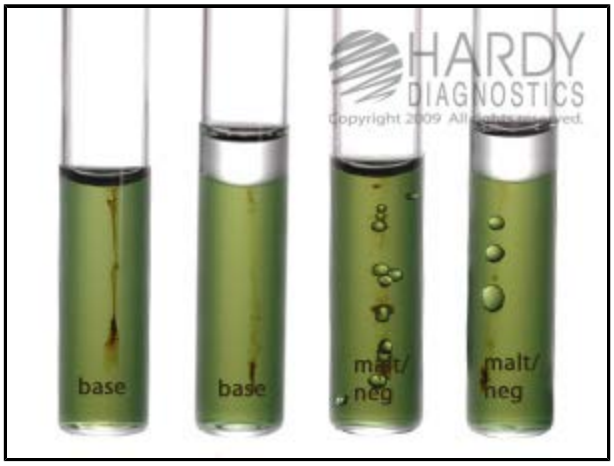
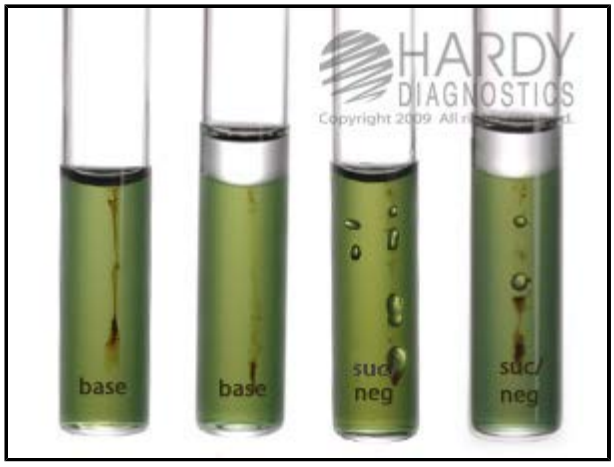
Branhamella (Moraxella) catarrhalis (ATCC 25240) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Mannitol (Cat. no. Y53) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.

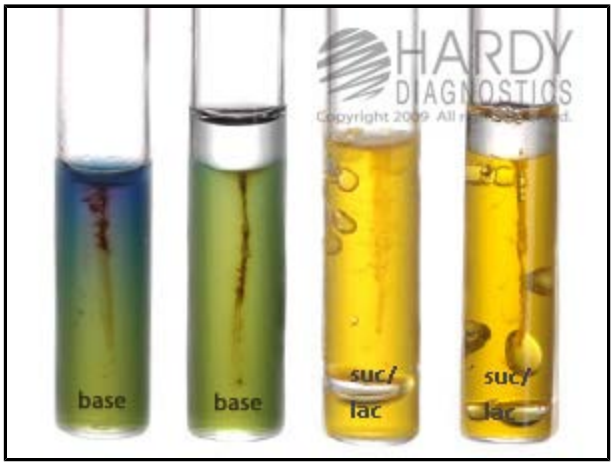
Klebsiella pneumoniae (ATCC® 13883) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Maltose (Cat. no. Y52) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.



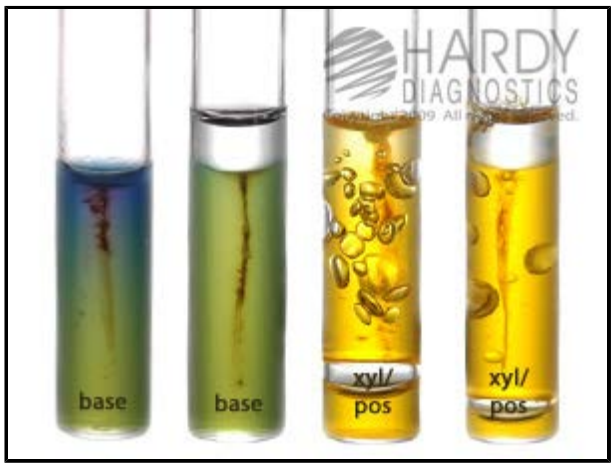
Branhamella (Moraxella) catarrhalis (ATCC® 25240) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Maltose (Cat. no. Y52) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.



Branhamella (Moraxella) catarrhalis (ATCC® 25240) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Sucrose (Cat. no. Y55) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.



Enterobacter aerogenes (ATCC® 13048) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Sucrose (Cat. no. Y55) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC



Enterobacter aerogenes (ATCC® 13048) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Xylose (Cat. no. Y56) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.

Branhamella (Moraxella) catarrhalis (ATCC® 25240) growing in Oxidative-Fermentative Base (Cat. no. Y51) and OF Xylose (Cat. no. Y56) media. Inoculated in duplicate, with Mineral Oil (Cat. no. Z79) overlay applied to one of each duplicate. Incubated aerobically (overlay tubes had tightened caps) for 24 hours at 35ºC.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

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5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. Hugh, R. and Leifson, E.J. 1953. *Bacteriol.*; 66:24-26.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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