IFU

Instructions for Use

MODIFIED ALP AND NON-FERMENTER IDENTIFICATION MEDIA

Cat. no. Y21	FID (Fluorescence-Indole-Denitrification), 13x100mm Tube, 4.5ml Slant	10 tubes/box
Cat. no. Y23	Motility Nitrate, 13x100mm Tube, 3ml Deep	10 tubes/box
Cat. no. Y62	Modified ALP Arabinose, 13x100mm Tube, 3ml Slant	10 tubes/box
Cat. no. Y68	Modified ALP Lactose, 13x100mm Tube, 3ml Slant	10 tubes/box
Cat. no. Y69	Modified ALP Sucrose, 13x100mm Tube, 3ml Slant	10 tubes/box
Cat. no. Y70	Modified ALP Urea, 13x100mm Tube, 3ml Slant	10 tubes/box
Cat. no. Y71	Modified ALP Xylose, 13x100mm Tube, 3ml Slant	10 tubes/box

INTENDED USE

Hardy Diagnostics Modified ALP (Aerobic Low Peptone) Media, also known as OLP (Oxidative Low Peptone), are used to aid in the identification of non-fermentative gram-negative bacilli (NFB). When Modified ALP substrates are included with a battery of other properly selected tests or substrates, the most commonly encountered NFB can be identified within 24 hours.

Fluorescence-Denitrification (FN) Media are used to aid in the detection of fluorescein pigment and complete reduction of nitrate to nitrogen gas. The medium, if supplemented with tryptophane (FID), aides in the detection of indole-producing microorganisms.

Motility Nitrate is used to detect nitrate reduction and motility of NFB.

SUMMARY

Aerobic Low Peptone (ALP) Media, a modification of Oxidative-Fermentative (OF) Media and Buffered Single Substrate (BSS) Media, were originally formulated by Pickett and Greenwood to demonstrate the rapid acidification or alkalization of substrates by non-fermentative, gram-negative bacilli (NFB).[3,5]

As compared to OF Media, Pickett and Greenwood's media contains a reduced (25% less) amount of peptone. The limited amount of peptone minimizes the chance for alkalization to occur which can neutralize weak acid producers.

Hardy Diagnostics Modified ALP Media is a modification of the formula developed by Greenwood and Pickett. Like ALP Media, the modified formula employs phenol red as the color indicator, and depending upon the pH adjustment of the basal medium, Modified ALP Media can be used to detect acidification of carbohydrates or alkalization of organic salts and amides.

Fluorescence-Denitrification (FN) Media are formulated to detect fluorescein pigment and complete reduction of

nitrate to nitrogen gas. These two characteristics are important in the identification of the pseudomonads and other non-fermentative bacilli.

Fluorescence-Indole-Denitrification (FID) Media is a modification of FN Media. FID incorporates the use of tryptophane to allow the detection of organisms which possess the enzyme tryptophanase. Tryptophanase degrades tryptophane which results in the production of indole, pyruvic acid, and ammonia.

Motility Nitrate Media is used to detect motility and nitrate reduction of NFB. Motility is observed macroscopically by the appearance of a diffuse zone of growth spreading from a line of inoculation. Non-motile organisms grow only along the stab line and leave the surrounding medium clear. Nitrate reduction is manifested by cracks in the agar produced by the presence of gas bubbles.

FORMULA

Ingredients per liter of deionized water:*

Modified ALP
Modified ALP Media is a proprietary medium which includes a buffered medium of inorganic salts in a nutrient base with agar and phenol red.
The ALP Media used to determine alkalization of organic salts and amides (such as urea) consists of the basal medium, 1% salt or amide, and 0.1% dextrose. The final pH is adjusted to 6.5 +/- 0.2 at 25ºC.
The ALP Media used to determine acidification of carbohydrates consists of the basal medium and 1% carbohydrate. The final pH is adjusted to 7.6 +/- 0.2 at 25ºC.

FID (Fluorescence-Indole-Denitrification)	
Tryptone	10.0gm
Proteose Peptone	10.0gm
Potassium Nitrate	2.0gm
Potassium Phosphate	1.5gm
Magnesium Sulfate	1.5gm
Tryptophane	1.0gm
Glycerol	10.0ml
Agar	15.0gm
Final pH of 7.2 +/- 0.2 at 25ºC.	

Motility Nitrate	
Pancreatic Digest of Casein	10.0gm
Peptic Digest of Animal Tissue	5.0gm
Sodium Chloride	5.0gm
Yeast Extract	3.0gm
Potassium Nitrate	3.0gm
Beef Heart Infusion	2.0gm
Agar	3.0gm
Final pH of 7.4 +/- 0.2 at 25ºC.	

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

STORAGE AND SHELF LIFE

Upon receipt store media at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed.

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: These media are not intended for use as primary isolation media. They are for use in characterizing pure cultures. Consult listed references for information regarding the processing and inoculation of specimens.[2-5]

Method of Use:

1. Inoculate a KIA (Cat. no. L70) or TSI (Cat. no. L50) slant to determine if an isolate is an NFB.

> **Note:** Refer to the Instructions for Use (IFU) documents for KIA or TSI for procedural use and interpretation of results for each medium.

2. Following 24 hours of incubation of KIA or TSI, non-fermentative bacilli (NFB) present as either 'alkaline' over 'no change'; or 'no change' over 'no change'. Slow growing NFB may require an additional 24 hour incubation period before reactions can be interpreted. When KIA or TSI are used to determine whether or not the isolate is an NFB, there is no need to use the open or closed OF tubed method to determine the organism's ability to ferment or oxidize.

3. Perform an oxidase test (Cat. no. Z93) using an inoculum from the 24 hour growth on the KIA or TSI used in step 1 above.

4. Depending upon the results of the oxidase reaction, inoculate the media as listed below according to the following methods of inoculation:

METHOD OF INOCULATION

The following media should be inoculated for identification of:

OXIDASE-NEGATIVE NFB		OXIDASE-POSITIVE NFB	
Cat. no. Y23	Motility Nitrate	Cat. no. Y21	FID
Cat. no. Y62	Modified ALP Arabinose	Cat. no. Y23	Motility Nitrate
Cat. no. Y69	Modified ALP Sucrose		
Cat. no. Y70	Modified ALP Urea		

Modified ALP Media:

1. Using a heavy inoculum of test isolate, spot inoculate (do not streak) one area on the surface of the slant.

> **Note:** A spot application of the inoculum, rather than a streak across the entire surface, introduces a high concentration of preformed enzymes or other metabolic products than can be detected more quickly by the endpoint indicator.[3]

2. Incubate at 35ºC. for 24-48 hours*. Modified ALP Media with organic salts or amides, such as urea, should be incubated 72 hours before negative alkalization results are interpreted.

> **Note:** Some organisms such as *Burholderia cepacia* grow better (produce stronger reactions) when grown at room temperature on modified ALP with carbohydrates.

Motility Nitrate Media:

1. Using growth from an 18-24 hour pure culture, inoculate the medium by stabbing the agar to a depth of 2-3mm.

2. Incubate at 35ºC. for 24-48 hours.

FID (Fluorescence-Indole-Denitrification) Media:

1. Using growth from an 18-24 hour pure culture, stab to the bottom of the tube and streak the agar surface as the needle is withdrawn from the butt of the medium.

2. Incubate at room temperature (15-30ºC.) for 24-48 hours.

3. Add 4 drops of Indole Kovacs Reagent (Cat. no. Z67) to determine Indole reaction.

INTERPRETATION OF RESULTS

Test Media	Negative Reaction	Positive Reaction
Modified ALP Salts		
(e.g. Urea)	Yellow to yellow orange slant	Red slant
Modified ALP Sugars		
(e.g. Arabinose, Lactose, Sucrose, and Xylose)	Red slant	Yellow slant
Motility Nitrate		
Motility	No motility, growth confined	"Puff ball" of motility at 4 hours or turbid tube

Denitrification	Agar intact, no bubbles	Agar broken with gas bubbles
FID		
Fluorescence	No fluorescence with UV light	Fluorescence with UV light
Indole	No color change when 4 drops of indole reagent are added	Red reaction when 4 drops of indole reagent are added
Denitrification	Agar intact, no bubbles	Agar broken with gas bubbles

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.

Some NFB are slow acid producers. Modified ALP Media with carbohydrates should be incubated 48 hours before negative acidification results are interpreted.

Modified ALP Media with organic salts or amides, such as urea, should be incubated 72 hours before negative alkalization results are interpreted.

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, incinerator, incubators, pasteur pipets, as well as serological and biochemical reagents, are not provided. Additionally, the identification method described in this technical information sheet requires OxiStrips (Cat. no. Z93), Kovacs Indole Reagent (Cat. no. Z67), and a long-wave UV lamp (Cat. no. UVL56).

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	
Modified ALP with Urea					
Pseudomonas aeruginosa ATCC® 27853	E	24-48hr	35°C	Aerobic	Growth; color change from yellow to red
Burkholderia cepacia ATCC® 25416	E	24-48hr	35°C	Aerobic	Growth; no color change
Modified ALP with Arabinose and Xylose					
Pseudomonas aeruginosa ATCC® 27853	E	24-48hr	35°C	Aerobic	Growth; color change from red to yellow
Moraxella catarrhalis ATCC® 25240	E	24-48hr	35°C	Aerobic	Growth; no color change
Modified ALP with Lactose, and Sucrose					

Burkholderia cepacia ATCC® 25416	E	24-48hr	35°C**	Aerobic	Growth; color change from red to yellow
Pseudomonas aeruginosa ATCC® 27853	E	24-48hr	35°C	Aerobic	Growth; no color change
Motility Nitrate					
Pseudomonas aeruginosa ATCC® 27853	D	24-48hr	35°C	Aerobic	Growth; positive motility and denitrification
Moraxella catarrhalis ATCC® 25240	D	24-48hr	35°C	Aerobic	Growth; negative motility and denitrification
FID (Fluorescence-Indole-Denitrification)					
Pseudomonas aeruginosa ATCC® 27853	C	24-48hr	15-30°C	Aerobic	Growth; positive fluorescence (blue) and denitrification (gas bubbles formed), negative for indole
Burkholderia cepacia ATCC® 25416	C	24-48hr	15-30°C	Aerobic	Growth; negative fluorescence, denitrification, and indole
Elizabethkingia meningoseptica ATCC® 13253	C	24-48hr	15-30°C	Aerobic	Growth; indole-positive (turns red when 4 drops of indole reagent are added), fluorescence and denitrification-negative

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

** Some organisms such as *Burholderia cepacia* grow better (produce stronger reactions) when grown at room temperature on modified ALP with carbohydrates.

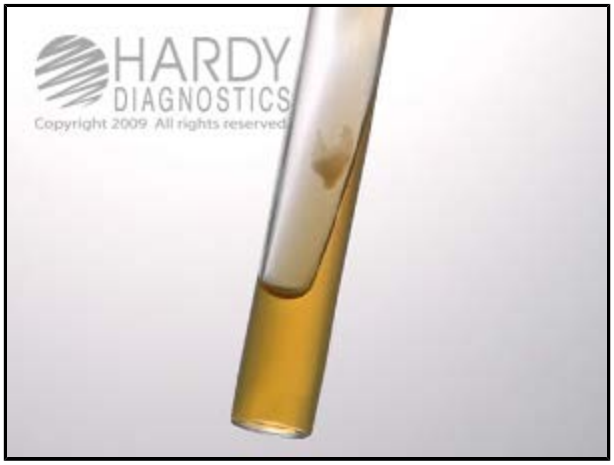
USER QUALITY CONTROL

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.

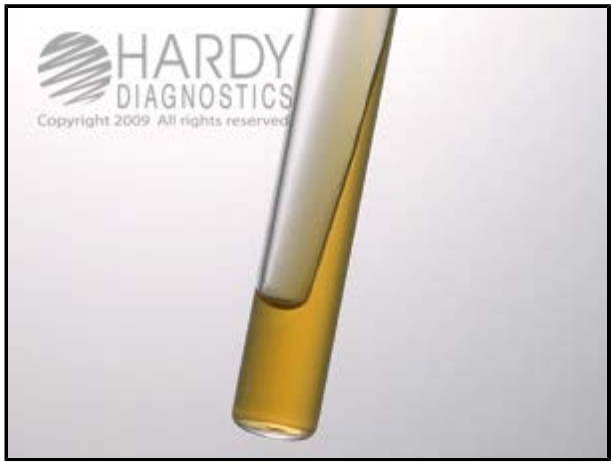
PHYSICAL APPEARANCE

- Modified ALP Media with Carbohydrate should appear slightly opalescent, and pink-red in color.
- Modified ALP Media with Organic Salts or Amides should appear slightly opalescent, and yellow-orange in color.
- Motility Nitrate Media should appear slightly opalescent, and whitish in color.
- FID Media should appear slightly opalescent, and whitish in color.

Pseudomonas aeruginosa (ATCC® 27853) growing on Modified ALP with Urea (Cat. no. Y70). Incubated aerobically for 24 hours at 35ºC. The pink-red color development was indicative of a positive reaction.



Burkholderia cepacia (ATCC® 25416) growing on Modified ALP with Urea (Cat. no. Y70). Incubated aerobically for 24 hours at 35ºC. No pink-red color development was indicative of a negative reaction.



Uninoculated tube of Modified ALP with Urea (Cat. no. Y70).

BIOCHEMICAL PROFILES OF COMMONLY ENCOUNTERED NFB

The ALP Identification System is designed to identify the following non-fermenters:

- *Acinetobacter anitratus*
- *Flavimonas oryzihabitans*
- *Acinetobacter lwoffi*
- *Pseudomonas aeruginosa*
- *Bordetella bronchiseptica*
- *Pseudomonas putida*
- *Burkholderia cepacia* complex
- *Pseudomonas stutzeri*
- *Ralstonia pickettii*
- *Chryseobacterium* spp.
- *Stenotrophomonas maltophila*
- *Delftia acidovorans*
- *Sphingomonas paucimobilis*

Oxidase-Positive:									

	P. aeruginosa	*Chryseo-bacterium* spp.	*P. putida*	*P. stutzeri*	*Ralstonia pickettii*	*Delftia acidovorans*	*B. bronchiseptica*	*S. paucimobilis*	*B. cepacia* complex
Motility Nitrate (Y23)									
Motility	+	-	+	+	+	+	+	-	+
Nitrate	+	-	-	(-)	(-)	-	-	-	-
FID(Y21)									
Fluorescence	+	-	+ or -	-	-	-	-	-	-
Indole	-	+	-	-	-	orange indole	-	-	-
Denitrification	+	-	-	-	-	NA	NA	NA	NA

Oxidase-Negative:					
	A. anitratus	*S. maltophilia*	*A. lwoffi*	*C. luteola*	*F. oryzihabitans*
Motility Nitrate (Y23)					
Motility	-	+	-	+	+
Nitrate	-	-	-	-	-
Fluorescence	-	-	-	-	-
Indole	-	-	-	-	-
Lactose	+	-	-	-	-
Denitrification	-	-	-	NA	NA
Modified ALP					
Sucrose (Y69)	-	+ or -	-	(-)	(-)
Arabinose (Y62)	+	-	-	+	+
Urea (Y70)	+ or -	-	-	(-)	(-)

NA	=	Results not available	-	=	10% or less of the strains are positive
+	=	90% or more of the strains are positive	(-)	=	11-50% of the strains are positive
(+)	=	51-89% of the strains are positive			

FID is incubated at room temperature (15-30ºC.).

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. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.

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

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

5. Gilardi, G.L., ed. 1985. Non-Fermentative Gram-Negative Rods, Vol. 16. Marcel Dekker, Inc., New York, NY.

ATCC is a registered trademark of the American Type Culture Collection.

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