Modified Lombard-Dowell Broth as a General Growth Medium

MARIANNE T. JESSEE* AND PATSY J. ROBINSON

Laboratory Medicine Service, Bacteriology Unit, National Naval Medical Center, Bethesda, Maryland 20014

Received for publication 18 April 1977

A new liquid medium (modified Lombard-Dowell broth) was inoculated with stock culture strains of aerobic and anaerobic bacteria and compared with prereduced chopped-meat glucose inoculated with the same anaerobes. Both broths were subcultured at 48 and 72 h to aerobic and anaerobic blood agar plates, and the numbers of colonies were compared after 48-h incubation of the agar plates. This was repeated with mixed cultures of both aerobes and anaerobes. For a period of 11 months all specimens received for anaerobic-aerobic culture were inoculated into prereduced chopped-meat glucose and modified Lombard-Dowell broth plus the appropriate plate medium. Growth from subcultures was compared with primary plate isolates. Chopped-meat glucose and modified Lombard-Dowell broth isolates agreed, with the exception of one, *Fusobacterium necrophorum*, that did not grow in chopped-meat glucose.

In search of a better enrichment broth facilitating growth of a wide range of organisms, aerobic and anaerobic bacteria, we decided to test modified Lombard-Dowell broth (MLDB). We were also interested in the cost of the medium and routine inoculation procedures versus the anaerobic culture system of the Virginia Polytechnic Institute (VPI) (4).

The MLDB was tested for growth and duration of survival of organisms up to 72 h (normal primary-culture holding time for aerobes in our laboratory) and for survival of different aerobic and anaerobic organisms in competition with each other.

After evaluation with stock cultures, we decided to test the MLDB on all patient samples received for anaerobic-aerobic culture against the routine plate media and chopped-meat glucose (CMG).

The organisms used were anaerobic and aerobic stock cultures of the College of American Pathologists, Center for Disease Control, and American Type Culture Collection. The anaerobes consisted of: *Clostridium perfringens*, *C. chauvoei*, *C. sordelli*, *C. bifermentans*, *C. ramosum*, *Bacteroides fragilis* subsp. *fragilis*, *B. melaninogenicus*, *Peptococcus magnus*, *P. asaccharolyticus*, *Peptostreptococcus anaerobius*, *Fusobacterium nucleatum*, *F. necrophorum*, *Actinomyces israelii*, *Bifidobacterium* sp., and *Propionibacterium acnes*. The aerobes consisted of: *Acinetobacter calcoaceticus* (Mima), *Citrobacter freundii*, *Haemophilus influenzae*, *H. parainfluenzae*, *Escherichia coli*, *Neisseria*

gonorrhoeae, *N. lactamica*, *Pasteurella multocida*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens*, *Streptococcus pyogenes* (group A), *S. pneumoniae*, *S. agalactiae* (group B), *Vibrio parahaemolyticus*, and *Yersinia enterocolitica*.

The original formula (8) for Lombard-Dowell broth was given to us by G. Lombard, Anaerobic Section, Center for Disease Control, Atlanta, Ga., and contained the following ingredients: Trypticase (BBL), 5.0 g; yeast extract, 5.0 g; sodium chloride, 2.5 g; sodium sulfite, 0.1 g; L-tryptophan, 0.2 g; vitamin K1 (3-phytylmenadione), 0.01 g; agar, 0.7 g; distilled water, 1,000 ml; L-cystine, 0.4 g; hemin, 0.01 g. The medium was prepared in screw-capped tubes (16 by 125 mm; Granite Diagnostics, Inc., Burlington, N.C.). Since peptone (Difco) was substituted for Trypticase, we are calling the medium MLDB. CMG prereduced broth was purchased from Scott Laboratories, Inc., Fiskeville, R.I.; reducible 5% sheep blood agar plates and kanamycin-vancomycin/colistin-naladixic acid biplates were purchased from C.W. England Laboratories, Beltsville, Md.; and 5% sheep blood agar plates, phenylethyl alcohol blood agar (PEA), mannitol salt agar, MacConkey agar, and enriched chocolate agar plates were purchased from Granite Diagnostics and Bioquest, Cockeysville, Md. All anaerobic plate media were used not later than 1 week after receipt from the manufacturer to assure freshness.

The procedures used were as follows. All stock cultures were streaked onto reducible blood agar

and 5% sheep blood agar plates, respectively, for viability and isolated colonies. This was done at a bench in open room air. Reducible blood agar plates containing palladium chloride as the catalyst were reduced for 24 to 48 h at room temperature in GasPak jars (BBL) before use. Anaerobes were incubated for 48 h in GasPak jars at 35°C. Aerobes were incubated under 5% CO₂ at 35°C for 24 h.

One isolated colony of each anaerobe was picked from the plate media and suspended in the CMG under a stream of 97% CO₂ and 3% hydrogen gas from the VPI anaerobe apparatus. All MLDB was boiled for 10 min and cooled to room temperature before use, and then one colony of each of the anaerobes from the plate media was suspended in the MLDB without use of the VPI apparatus in open room air.

To test the MLDB for competition in mixed cultures, *E. coli* and anaerobes were chosen. One colony of *E. coli* plus one colony of an anaerobe were inoculated into MLDB. One colony of *B. fragilis* subsp. *fragilis* was mixed with one colony of a *Clostridium* species. All tubes were incubated at 35°C for 48 h. The caps on the MLDB were tightened after inoculation to avoid entry of air. CMG was not tested with mixed stock cultures containing aerobes.

All broths were subcultured after 48 and 72 h of incubation to reducible and regular sheep blood agar plates with a calibrated loop delivering 0.01 ml. Visible turbidity in the broth was recorded as 1+, 2+, 3+, or 4+, according to density compared to a Wickerham card (10). Reducible agar plates were incubated for 48 h in a GasPak jar; regular agar plates were incubated at 35°C under 5% CO₂ for 24 and 48 h. Numbers of colonies were estimated as: 5 or less colonies per plate, 1+; 6 to 20 colonies per plate, 2+; 21 to 50 colonies per plate, 3+; more than 50 colonies per plate, 4+.

For a period of 11 months all samples from patients with requests for aerobic and anaerobic culture were inoculated to a reducible sheep blood agar plate, a kanamycin-vancomycin/colistin-nalidixic acid biplate, and pre-reduced CMG for anaerobic culture; 5% sheep blood agar plates, mannitol salt agar plates, MacConkey agar plates, and chocolate agar plates if the specimen source indicated the use of chocolate for aerobic culture; and MLDB for aerobic and anaerobic culture. Anaerobic plate media were incubated in a GasPak jar for 48 h at 35°C; CMG, MLDB, MacConkey, and mannitol salt agar plates were incubated at 35°C without CO₂; and blood agar and chocolate agar plates were incubated under 5% CO₂ at 35°C. All aerobic plates were inspected for growth after overnight incubation. MLDB subcultures

were performed in open room air in the "aerobic" section of the laboratory. The MLDB was subcultured when growth appeared, but not later than after 72 h of incubation, to 5% sheep blood agar plates for aerobic growth (chocolate agar when specimen source indicated a need for it) and PEA agar plates for anaerobic growth, according to standard operating procedures for our laboratory. PEA was chosen to suppress the growth of facultative, anaerobic, gram-negative bacilli.

CMG showing visible growth was subcultured by the "anaerobic" section of our laboratory after 48 h of incubation and up to 5 days of incubation to regular 5% sheep blood agar plates and reducible blood agar plates. For suspected *Actinomyces* sp. the broths were held for 7 days.

All growth from the subcultures was compared with each other and to isolates on the primary plating media. Identification of anaerobes was done according to the VPI method (4). Identification of aerobes was done according to standard laboratory procedures (1-3, 5-7, 9, 11).

Over an 11-month period 277 anaerobes were isolated from 180 patient samples (Table 1). The organisms were recovered equally well from the pre-reduced CMG on reducible blood agar and from the MLDB on PEA. There was one exception: one *F. necrophorum* strain isolated from a pleural fluid did not grow in CMG even after inoculation of the broth with colonies from the blood agar plate. This also had been noted with one of the *Fusobacterium* stock culture strains.

All aerobes present on primary plate media were present in the MLDB subcultures. Many additional aerobic organisms were found that did not appear on primary culture plates. They included: *Bacillus* sp., *Enterobacter agglomerans*, *E. cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, beta-hemolytic streptococci (groups A and B), viridans streptococci, enterococci, and yeast. All aerobic and anaerobic streptococci grew extremely well in the MLDB.

Mixed cultures (Table 2) showed no significant difference between CMG and MLDB. These mixed cultures were repeated numerous times with identical results. Pure cultures from stock strains of peptococcus, peptostreptococcus, *S. pneumoniae*, and *N. lactamica* were viable in MLDB for 3 months. The *H. influenzae* stock strain showed some visible growth in MLDB but did not grow upon subculture to chocolate agar plates. All samples from patients containing *H. influenzae* grew very well when subcultured from the MLDB. *N. gonorrhoeae* did not grow at all in MLDB. All other stock cultures tested gave satisfactory growth (Table 3).

TABLE 1. *Anaerobes isolated from different specimen sources*

Anaerobe	No. of isolates ^a from:			
	Oral surgery	Unspecified wounds	Abdomen	Chest
<i>Acidaminococcus fermentans</i>		1		
<i>Actinomyces</i> sp.	2			
<i>A. israelii</i>	1			
<i>Bacteroides fragilis</i> subsp. <i>fragilis</i>		17	20	3
<i>B. fragilis</i> subsp. <i>thetaiotaomicron</i>		9	4	1
<i>B. fragilis</i> subsp. <i>distasonis</i>		5	9	
<i>B. fragilis</i> subsp. <i>vulgatus</i>		1	3	
<i>B. fragilis</i> subsp. <i>ovatus</i>		1	2	
<i>B. fragilis</i> , other subspecies		8	3	
<i>B. putridinis</i>		1	1	
<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i>	1	6	1	2
<i>B. melaninogenicus</i> subsp. <i>intermedius</i>		6		
<i>B. melaninogenicus</i> subsp. <i>asaccharolyticus</i>		2		1
<i>B. ruminicola</i> subsp. <i>brevis</i>		3		
<i>B. ruminicola</i> subsp. <i>ruminicola</i>		1	1	
<i>B. capillorus</i>			3	
<i>B. corrodens</i>	2			
<i>B. oralis</i>	7			
<i>Bacteroides</i> species		6	1	1
<i>Bifidobacterium</i> sp.	3	3	1	
<i>Clostridium perfringens</i>		13	8	1
<i>C. ramosum</i>			3	1
<i>C. sporogenes</i>		2		
<i>C. cochlearium</i>				1
<i>C. tertium</i>		1	1	
<i>C. subterminale</i>		1		
<i>C. fallax</i>			1	
<i>C. innocuum</i>			1	
<i>C. bifermentans</i>		1		
<i>Clostridium</i> sp.		8	2	
<i>Eubacterium lentum</i>				1
<i>E. alactolyticum</i>				1
<i>Fusobacterium necrophorum</i>				2
<i>F. gonidiaformans</i>			1	
<i>F. nucleatum</i>		1	1	1
<i>Fusobacterium</i> sp.	6			
<i>Lactobacillus plantarum</i>			1	
<i>Lactobacillus</i> sp.		2		
<i>Peptococcus asaccharolyticus</i>		4		
<i>P. constellatus</i>				1
<i>P. magnus</i>		10		
<i>P. prevotii</i>		1		
<i>Peptococcus</i> sp.		6		1
<i>Peptostreptococcus intermedius</i>		4	1	
<i>P. micros</i>				1
<i>P. anaerobius</i>		5		1
<i>Peptostreptococcus</i> sp.		2		1
<i>Propionibacterium acnes</i>		2		
<i>Propionibacterium</i> sp.	2	20	1	2
<i>Veillonella parvula</i>			1	
<i>Veillonella</i> sp.		1	1	

^a Total number of isolates, 277.

Most anaerobic stock cultures (Table 4) had a 1+ difference in broth turbidity between CMG and MLDB. Subculture agar plates showed no significant difference in the number of colonies.

The MLDB functioned surprisingly well as an enrichment broth for aerobes and anaerobes from patient samples. The MLDB has an advantage over the prereduced CMG, because no spe-

TABLE 2. *Mixed-stock cultures: visible growth after 48 h in broth and on plates after subculture*

Organism	CMG		MLDB	
	Broth	Plate	Broth	Plate
<i>Bacteroides fragilis</i> subsp. <i>fragilis</i>	4+	3+	2+	2+
<i>Clostridium perfringens</i>		3+		2+
<i>B. fragilis</i> subsp. <i>fragilis</i>	4+	3+	2+	3+
<i>C. chauvoei</i>		3+		3+
<i>B. fragilis</i> subsp. <i>fragilis</i>	4+	4+	4+	4+
<i>C. bifermentans</i>		3+		3+
<i>B. fragilis</i> subsp. <i>fragilis</i>	4+	4+	4+	4+
<i>C. sordellii</i>		4+		4+
<i>C. ramosum</i>		4+		4+
<i>Peptococcus magnus</i>	4+	4+	4+	4+
<i>Peptostreptococcus anaerobius</i>		2+		4+
<i>C. sordellii</i>	NT ^a	NT		2+
<i>Escherichia coli</i>	NT	NT		2+
<i>P. magnus</i>	NT	NT	2+	2+
<i>E. coli</i>	NT	NT		2+

^a NT, Not tested.TABLE 3. *Aerobe stock cultures: visible growth in broth and on agar plates after subculture*

Organism	MLDB	Plate
<i>Acinetobacter calcoaceticus</i> (Mima)	3+	3+
<i>Citrobacter freundii</i>	3+	3+
<i>Escherichia coli</i>	3+	4+
<i>Haemophilus influenzae</i>	±	NG ^a
<i>H. parainfluenzae</i>	2+	2+
<i>Neisseria gonorrhoeae</i>	NG	
<i>N. lactamica</i>	2+	3+
<i>Pasteurella multocida</i>	2+	3+
<i>Pseudomonas aeruginosa</i>	3+	3+
<i>Proteus vulgaris</i>	3+	4+
<i>Salmonella typhimurium</i>	3+	4+
<i>Serratia marcescens</i>	2+	3+
<i>Streptococcus pyogenes</i> (group A)	3+	3+
<i>S. agalactiae</i> (group B)	3+	4+
<i>S. pneumoniae</i>	2+	3+
<i>Vibrio parahaemolyticus</i>	2+	3+
<i>Yersinia enterocolitica</i>	3+	3+

^a NG, No growth.

cial apparatus is needed for its inoculation and its purchase price is lower.

MLDB with the addition of 1% dextrose was also tested with several stock cultures but proved unsatisfactory when mixed populations

TABLE 4. *Anaerobe stock cultures: visible growth after 48-h incubation in broth and on plates after subcultures*

Organism	CMG		MLDB	
	Broth	Plate	Broth	Plate
<i>Actinomyces israelii</i>	NG ^a		2+	2+
<i>Bifidobacterium</i> sp.	2+	2+	1+	2+
<i>Bacteroides fragilis</i> subsp. <i>fragilis</i>	4+	4+	3+	4+
<i>B. melaninogenicus</i>	3+	3+	2+	2+
<i>Clostridium bifermentans</i>	3+	3+	2+	4+
<i>C. chauvoei</i>	3+	3+	2+	2+
<i>C. perfringens</i>	4+	4+	3+	3+
<i>C. ramosum</i>	3+	4+	2+	4+
<i>C. sordellii</i>	3+	3+	2+	4+
<i>Fusobacterium necrophorum</i>	NG		2+	2+
<i>F. nucleatum</i>	1+	2+	2+	2+
<i>Peptococcus asaccharolyticus</i>	3+	3+	2+	2+
<i>P. magnus</i>	4+	4+	2+	2+
<i>Peptostreptococcus anaerobius</i>	2+	2+	2+	2+
<i>Propionibacterium acnes</i>	2+	3+	2+	3+

^a NG, No growth.

were added. Clostridia or bacteroides overgrew the other organisms present. Studies with smaller amounts of dextrose were not performed.

PEA plates became inhibitory to anaerobes, especially fusobacteria, as the media aged, approximately 1 week after receipt from the manufacturer.

We thank G. L. Lombard for supplying us with the formula of the Lombard-Dowell broth, and Lisa Tennison for secretarial assistance in the preparation of the manuscript.

LITERATURE CITED

1. Austrian, R. 1974. *Streptococcus pneumoniae* (pneumococcus), p. 109-115. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
2. Catlin, W. B. 1974. *Neisseria meningitidis* (meningococcus), p. 116-123. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Facklam, R. R. 1974. Streptococci, p. 96-108. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
4. Holdeman, L. V., and W. E. C. Moore (ed.). 1975. *Anaerobe laboratory manual*, 3rd ed. Virginia Polytechnic Institute and State University, Blacksburg.
5. Ivler, D. 1974. *Staphylococcus*, p. 91-95. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
6. Kellogg, D. S., Jr. 1974. *Neisseria gonorrhoeae* (gonococcus), p. 124-129. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.

- ton, D.C.
7. Smith, P. B., K. M. Tomfohrde, D. L. Rhoden, and A. Balows. 1972. API system: a multitube micro-method for identification of *Enterobacteriaceae*. Appl. Microbiol. 24:449-452.
 8. Stargel, M. D., F. S. Thompson, S. E. Phillips, G. L. Lombard, and V. R. Dowell, Jr. 1976. Modification of the Minitek miniaturized differentiation system for characterization of anaerobic bacteria. J. Clin. Microbiol. 3:291-301.
 9. Tatum, H. W., W. H. Ewing, and R. E. Weaver. 1974. Miscellaneous gram-negative bacteria, p. 270-294. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
 10. Wickerham, L. H., and K. A. Burton. 1948. Carbon assimilation tests for the classification of yeasts. J. Bacteriol. 56:363-371.
 11. Young, V. M. 1974. *Haemophilus*, p. 302-307. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.