

Needless Retesting of Quality-Assured, Commercially Prepared Culture Media

JANICE G. NAGEL AND LAWRENCE J. KUNZ

Francis Blake Bacteriology Laboratories, The Massachusetts General Hospital and the Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02114

Received for publication 12 March 1973

Current revival of interest in quality control of bacteriological culture media prompted this laboratory to develop and implement a system of quality control testing of media purchased from commercial manufacturers. During 8 months we tested more than 900 lots of 46 different media representing 350,000 units of culture media purchased from two major and one minor supplier(s). Only 17 lots were found to be unsatisfactory. This experience raised a question about the real necessity for extensive retesting by users of commercially prepared, quality-assured media. It is suggested that primary responsibility for quality control be placed on the relatively few manufacturer-vendors rather than on the multiple purchaser-users, who may not possess either the expertise or the resources for quality control programs.

Federal and state regulations of clinical laboratories include sections on quality control, occasionally with specific references to testing of bacteriological culture media. For example, the Clinical Laboratories Improvement Act of 1967 stated: "Each batch of medium shall be tested before or concurrently with use with selected organisms to confirm required growth characteristics, selectivity, enrichment and biochemical response" (5).

Publications since then have expressed interest in quality control procedures of various elements of microbiology—the professional society, the commercial supplier, and the clinical laboratory (for examples, see references 1, 2, and 8, respectively).

The development, during the last two decades, of the business of supplying commercially prepared culture media for purchase and use in microbiology laboratories probably has beneficially affected the quality of culture media used in hospital laboratories.

These considerations prompted us to investigate the need and effectiveness of a system of quality control designed to monitor the quality of commercially prepared culture media. The results of this study suggest that requirements for quality assurance of culture media be reassessed by regulating agencies and that the responsibility be assigned to the manufacturers rather than to the consumers.

MATERIALS AND METHODS

Sources and shipment of media. All lots of media included in the study, comprising the majority of types of media used, were purchased from two major and one minor supplier(s) (A, B, and C) over a period of 8 months. The principal media not subjected to quality control studies included: three types of media used for isolation of mycobacteria, two types of broth bottles for blood cultures, SS agar, and certain types of media used in specialty laboratories. Testing of Mueller-Hinton agar used for antibiotic sensitivity testing was performed apart from this study; the results, however, are given in Table 1.

Company A is a manufacturer located on the Eastern seaboard with an international clientele. Media from company A was delivered by air to Boston, then by truck either directly to the hospital or via a local distributor. All media in petri dishes were packaged in moisture-proof plastic wrapping. Tubed media were screw capped. Some plating media were shipped in insulating plastic containers; other plating media and all of the tubed media were shipped in cardboard cartons.

Company B is a Boston area manufacturer with sales and distribution of plated and tubed media restricted to the New England states. Media from company B was delivered by truck directly from the plant to the hospital's receiving platform. Plated media were wrapped in moisture-proof plastic; tubes containing media were stoppered either by screw caps or friction-type plastic closures. Press-on capped tubes and most screw-capped tubes were packaged in plastic racks wrapped in moisture-proof plastic; some screw-capped tubes were not otherwise protected from

TABLE 1. Results of tests of quality of plated culture media

Media	Test organisms	Expected results	No. of lots tested	No. of lots approved
Brucella agar with 5% horse blood ^a	<i>Escherichia coli</i>	2 to 3 mm colony	107	106 ^b
	Enterococcus	Gamma reaction 1 to 2 mm colony		
	<i>Streptococcus pyogenes</i>	Beta hemolysis		
MacConkey agar ^a	Alpha streptococcus	Alpha reaction	77	74 ^c
	<i>Hemophilus influenzae</i>	Pinpoint colonies		
	<i>E. coli</i>	Red colonies		
Brain heart infusion agar	<i>Proteus vulgaris</i>	Transparent colonies, no swarming	9	9
	<i>Staphylococcus aureus</i>	No growth		
	<i>Hemophilus influenzae</i>	Appropriate growth with X and V factors; no growth without factors		
Phenylethyl alcohol agar with 5% horse blood	<i>Hemophilus parainfluenzae</i>	Appropriate growth with X and V factors; no growth without factors	20	20
	<i>E. coli</i>	None to pinpoint growth		
	<i>P. vulgaris</i>	None to pinpoint growth, no swarming		
GC agar	<i>Pseudomonas aeruginosa</i>	Pinpoint growth	35	28 ^d
	<i>S. pyogenes</i>	Beta hemolytic colonies		
	<i>Neisseria pharyngitidis</i>	Small yellow colony		
Thayer-Martin medium	<i>Neisseria gonorrhoeae</i>	"Wet" grey colony	37	33 ^e
	<i>N. pharyngitidis</i>	Grey colony		
	<i>S. aureus</i>	Small grey colonies		
Mueller Hinton agar ^f	<i>E. coli</i>	No growth, very light growth	26	26
	<i>S. aureus</i>	No growth		

^a Including 45 lots of blood agar-MacConkey agar biplates.

^b One lot rejected because of pitted surface of agar medium.

^c Three lots received frozen; see also footnotes *d* and *e*.

^d One lot was rejected because of contamination, two lots because of excessive moisture, and four lots because of freezing. Latter included in same shipment as material described in footnote *c*.

^e Three rejected lots were received frozen in the same shipment as the media described in footnotes *c* and *d*; one lot was rejected because of excessive moisture.

Results for 5 of 8 months of study only.

dehydration. Packages of plates and tubes were shipped in cardboard containers.

Company C is a manufacturer located in New England serving a wider area than company B, but having a smaller market than company A. Only plated media from this company were included in the study, and the plates were wrapped in moisture-proof plastic and shipped in cardboard cartons. Delivery from the manufacturer to the hospital was by truck.

In all cases, media were stored in a walk-in refrigerated room in the original packaging, sometimes within the shipping container, but usually without it. In most cases, the original package wrapping was

retained during storage until a working supply was removed for use.

Gross examination of media. Before storage, all media were inspected for general appearance and defects. Especially to be noted were evidence of breakage, spillage, incorrect volume, color, or consistency, tears or breakage of packaging, excess moisture, presence of foreign objects, or microbial contamination in the form of visible colonies or unexpected turbidity. Similar observations were made as lots of media were withdrawn from storage for use.

Tests for contamination. Tests for microbial contamination of media consisted of (i) gross visual

examination through the transparent packaging for macroscopic growth on arrival; and (ii) overnight incubation, and subsequent visual examination for growth, of 2% of plates selected from several packages.

Tests for performance of media. Three types of tests were performed for qualities or properties of culture media in accordance with the specific purpose of each medium: (i) in the case of general purpose media, the ability to support satisfactory growth of certain fastidious organisms and proper growth of eugonic bacteria; (ii) in the case of selective media, the ability to inhibit undesired species and provide for satisfactory growth of selected species; (iii) in the case of differential media, the expected appropriate reactions when challenged with particular selected biotypes. Plating media were not challenged with varying concentrations of organisms as recommended by Stokes (9) and others (8, 10).

Organisms used for challenge. All of the organisms used for testing of media were isolated in these laboratories from clinical specimens and identified by methods employed in our diagnostic bacteriology routine. Cultures were stored in the frozen state on glass beads as previously described (7). The species used in quality control test procedures are listed in the accompanying tables.

RESULTS

More than 900 different lots of 46 different media, representing 350,000 units of culture media, were subjected to the quality control procedures described. The types of media, numbers of lots tested, and numbers of lots failing to pass the quality control tests are listed in Tables 1, 2, 3, and 4. Only 17 lots were not acceptable, and the reasons for rejection of each are provided in the footnotes to the tables. Ten of the 17 rejected lots were unsatisfactory because of evidence of freezing due to mishandling of a single shipment by the local shipper. Three additional lots of media were rejected because excessive moisture accumulated on the interior packaging material, representing wide fluctuations of temperature during shipping. Only 4 of the 17 rejected lots of media were unacceptable because of factors not associated with shipment of media: one lot of blood-agar plates for excessive pitting of media; one lot of GC agar because of contamination; and two lots of carbohydrate test media because of imperfect composition.

To be noted, but not included in the tables, is the fact that all of the quality control defects leading to rejection of the media were easily detectable. To be particularly noted was the fact that the presence of unsatisfactory carbohydrate test media in the laboratory was announced by the quality control technologist simultaneously with recognition of the defective media by the diagnostic laboratory technologists using them.

DISCUSSION

Extended experience in the diagnostic bacteriology laboratory of this institution led to the impression that certain aspects of quality control procedures were redundant because well-trained, experienced bacteriology technologists almost invariably detect deficient culture media simultaneously with formal quality control techniques concurrent with use. The few lots of defective media encountered in the present study thus were noted to be defective by user-technologists at the same time as they were noted by the tester-technologist. The relative rarity of inadequate, substandard, or defective media encountered in the study also confirms our impressions, previously unsupported, of the uniformly high quality of culture media available from certain commercial sources offering quality-assured media for sale.

With the introduction of commercially prepared media for purchase and use in hospital microbiology laboratories there arose new considerations of quality control and quality assurance that have not been generally recognized or expressed. These derive from the probability that the development of the business of manufacturing and selling prepared media has beneficially affected the overall quality of culture media used by microbiology laboratories in hospitals because of: (i) the special experience and expertise of the professional and management staffs of commercial companies in the preparation of media; (ii) uniformity of production methods and use of equipment designed specifically for preparation of media; (iii) production of large batches of media under controlled conditions providing uniformity of quality; (iv) provision of specially conditioned ambient air in media-processing areas; (v) availability and use of packaging materials to minimize dehydration and contamination during shipment and storage; and (vi) systematic quality control testing of all production lots of media. Many of these factors prevail in individual laboratories preparing culture media for in-house use, but it is probable that in most hospital laboratories media are prepared under somewhat less favorable conditions than those provided by commercial manufacturers.

Considerations such as these should influence the requirements of hospital laboratories for quality control, depending on their sources of culture media. Thus, the use of culture media prepared by inexperienced workers under less than ideal conditions, with inadequate supervision, demands rigorous testing for quality prior to use. The use of quality-assured, commercially

TABLE 2. Results of tests of properties of differential media

Media	Test organisms	Expected results ^a	No. of lots tested and approved ^b
Triple sugar-iron-agar	<i>Escherichia coli</i> <i>Edwardsiella tarda</i> ^c <i>Proteus vulgaris</i>	A/A K/A H ₂ S A/A H ₂ S	21
Kligler's iron-agar	<i>E. coli</i> <i>P. vulgaris</i> <i>Pseudomonas aeruginosa</i>	A/A K/A H ₂ S K/K	30
Lysine-iron-agar	<i>E. tarda</i> <i>Proteus rettgeri</i> <i>Citrobacter freundii</i> <i>Proteus morganii</i>	K/K H ₂ S R/A K/A H ₂ S K/A	53
Urea-agar	<i>E. coli</i> <i>P. rettgeri</i> <i>Klebsiella pneumoniae</i>	- + Rapid, strong + Slow, weak	22
Buffered peptone-glucose-broth	<i>E. coli</i> <i>K. pneumoniae</i>	Voges-Proskauer - Voges-Proskauer +	27
Citrate-agar (Simmons)	<i>E. coli</i> <i>K. pneumoniae</i>	- +	37
Tryptophane-broth	<i>E. coli</i> <i>K. pneumoniae</i>	Indole + Indole -	17
Ornithine-semisolid agar	<i>E. coli</i> <i>K. pneumoniae</i>	+ -	26
Semisolid agar	<i>E. coli</i> <i>K. pneumoniae</i> <i>P. aeruginosa</i>	Motile, diffuse Nonmotile Motile, restricted	12
Acetate-agar	<i>E. coli</i> <i>E. tarda</i>	+ -	1
Mucate-broth	<i>E. coli</i> <i>E. tarda</i>	+ -	2
Gelatin	<i>E. coli</i> <i>P. vulgaris</i>	- +	10
Nitrate	<i>E. coli</i> <i>P. aeruginosa</i> <i>Neisseria pharyngitidis</i>	+ + (Gas) -	6
Bile-esculin	<i>Staphylococcus aureus</i> Enterococcus <i>Streptococcus pyogenes</i>	Growth, no blackening Growth, blackening No growth	25
6.5% NaCl in Todd-Hewitt broth	Enterococcus <i>S. pyogenes</i>	Growth No growth	19
10% Lactose (phenol red agar)	<i>Herellea vaginicola</i> <i>P. aeruginosa</i>	+ -	19

^a A, Acid; K, alkaline; R, red (deaminase positive); numerator, reaction on slant; denominator, reaction in butt. - = negative reaction; + = positive reaction.

^b All lots tested were accepted for use.

^c *E. tarda* was used instead of *Salmonella enteritidis* to avoid unnecessary hazard of infection.

TABLE 3. Results of tests for presence and probable identity of fermentable substances in carbohydrate broths for general use

Substances tested	Results expected with:							No. of lots tested	No. of lots accepted
	<i>E. coli</i>	<i>P. rettgeri</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>	<i>S. marcescens</i>	<i>E. hafniae</i>	<i>C. freundii</i>		
Glucose	+ ^a	+	+	+	+	+		5	5
Maltose	+	-	+	+	+	+		9	9
Lactose	+	-	+	-	-	-		4	4
Sucrose	-	-	+	+	+	-		5	5
Mannitol	+	+	+	-	+	+		17	17
Salicin	-	-	+	-	+	+		5	5
Inositol	-	+ ^b	+	-	+ ^b	-		5	5
Adonitol	-	+	+	-	-	-		6	6
Dulcitol	-	-	-	-	-	-	+	2	2
Raffinose	-	-	+	-	-	-		25	24 ^c
Arabinose	+	-	+	-	-	+		19	19
Rhamnose	+	+	+	+	-	+		22	22
Xylose	+	-	+	+	-	+		7	7
Sorbitol	+	-	+	-	+	-		17	17

^a +, Acid; -, no acid.

^b Weakly acid after 24 h; acid after 48 h.

^c Andrade's indicator not adjusted properly.

prepared media purchased from sources known to be reliable requires only minimal attention to monitoring of quality by purchaser-users.

That only 17 lots of defective media were discovered among more than 900 lots examined during this study is explained by the experience of the quality control laboratory of one of the commercial suppliers included in this study. They report that a significant amount of defective culture media was detected and rejected before shipment. A report from one hospital laboratory in which media is prepared for in-house use indicates a similar experience, in that approximately 5% of media prepared in the laboratory fails to pass quality control testing and is discarded (8).

To be sure, lots of unsatisfactory media do occasionally appear on laboratory benches ready for use for one reason or another—contamination after preparation, failure to undergo quality control, etc. Deterioration during storage appears not to be as serious a problem as is traditionally believed, probably because of modern moisture-proof packaging (6). It is hoped that data relating to the prolonged shelf life of prepared media already accumulated by certain investigators will soon be published for the benefit of practical users of their products.

Indeed, new rules and regulations filed by the Federal Commissioner of Food and Drugs (3) will require that culture media be labeled so as to include "appropriate storage instructions adequate to protect the stability of the product" and "an expiration date based upon the stated

storage instructions." Already one type of culture medium, Transgro, is appearing with expiration dates on package labels and one company, BioQuest, has extended this practice to many other of their prepared culture media. Thus, smaller laboratories which use less media and experience slower turnover of purchased media will thereby have positive temporal guidance for the duration of the reliability of the manufacturer's quality control procedures as they apply to specific lots of media.

An additional consideration in the reevaluation of quality control of bacteriological culture media is that the design of a methodology for testing culture media for quality probably requires a level of bacteriological sophistication greater than that needed for actual employment of the media in clinical situations. For example, Table 3 indicates the complexity of the pattern of biochemical reactions of selected organisms necessary to assure to a high degree of certainty that the carbohydrates present in the broths are as labeled. Only the presence of a metabolizable substance is assured if only two organisms are used for testing, one that will produce acid from all of the substrates and another that will produce acid from none of them (4). Even the procedures listed in this report lack sophistication, for example, the failure to challenge plating media with differing concentrations of the various test organisms.

Purposive, intelligently performed implementation of the quality control program entails little less expertise. The use of poorly designed

TABLE 4. Results of tests for presence and probable identity of carbohydrates in special-purpose differential media

Media ^a	Test organisms	Expected results	No. of lots tested	No. of lots passed
CTA-dextrose	<i>Neisseria pharyngitidis</i>	+ ^b	18	18
	<i>Neisseria gonorrhoeae</i>	+		
	<i>Neisseria meningitidis</i>	+		
CTA-maltose	<i>N. pharyngitidis</i>	+	18	17 ^c
	<i>N. gonorrhoeae</i>	-		
	<i>N. meningitidis</i>	+		
CTA-sucrose	<i>N. pharyngitidis</i>	+	22	22
	<i>N. gonorrhoeae</i>	-		
	<i>N. meningitidis</i>	-		
OF-dextrose	<i>Proteus vulgaris</i>	+	26	26
	<i>Serratia marcescens</i>	+		
	<i>Enterobacter hafniae</i>	+		
	<i>Herellea vaginicola</i>	+		
OF-lactose	<i>P. vulgaris</i>	-	4	4
	<i>S. marcescens</i>	-		
	<i>E. hafniae</i>	-		
	<i>E. coli</i>	+		
OF-maltose	<i>P. vulgaris</i>	+	22	22
	<i>S. marcescens</i>	+		
	<i>E. hafniae</i>	+		
	<i>H. vaginicola</i>	-		
OF-mannitol	<i>P. vulgaris</i>	-	3	3
	<i>S. marcescens</i>	+		
	<i>E. hafniae</i>	+		
OF-sucrose	<i>P. vulgaris</i>	+	5	5
	<i>S. marcescens</i>	+		
	<i>E. hafniae</i>	-		
OF-xylose	<i>P. vulgaris</i>	+	3	3
	<i>S. marcescens</i>	-		
	<i>E. hafniae</i>	+		

^a Prefix CTA denotes cystine trypticase basal medium; prefix OF denotes oxidation-fermentation basal medium of Hugh and Leifson.

^b +, Acid reaction; -, no acid reaction.

^c CTA-lactose tubes were mislabeled as CTA-maltose.

or understood quality control procedures may add little or nothing to assurance of good performance of test procedures and may, moreover, promote a false sense of security. Under certain circumstances, the employment of quality control methodology may even be detrimental to good practice by unnecessarily diverting limited resources of the laboratory to the testing of media previously certified by a responsible manufacturer.

The expense of quality control procedures ultimately must be considered, especially by those agencies professedly dedicated to mini-

mizing the costs of medical care. Bartlett et al. (1) estimate that the quality control program in their hospital laboratory represents an expenditure of 4% of technologists' time alone—a not inconsiderable expense—although it includes procedures other than those employed in control of culture media. That certain companies, at least, through rigorous quality control techniques, can deliver media of uniformly high quality that make unnecessary multiply replicated quality control programs by purchaser-users of the media, suggests that federal, state, and other regulatory agencies reassess their

requirements for quality control of culture media and reassign that responsibility to the relatively few manufacturer-vendors whose products can easily and less expensively be monitored for quality.

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