

Evaluation of Mueller-Hinton Agar for Disk Diffusion Susceptibility Tests

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Twelve lots of commercially prepared Mueller-Hinton agar from four medium manufacturers were evaluated for performance with recommended quality control organisms, medium pH, agar depth and uniformity, and characteristics of the zones of inhibition. Only 2 of 12 lots were acceptable for disk diffusion susceptibility tests. Significant problems were observed with the preparation and pouring of the agar media.

One of the most important procedures performed in clinical microbiology laboratories is antimicrobial susceptibility testing. Although a variety of susceptibility testing methods have been developed, the Kirby-Bauer disk diffusion method (1, 8) is used by the majority of clinical laboratories. Jones et al. (4) reported that approximately 82% of laboratories participating in the College of American Pathologists microbiology surveys used the disk diffusion method, with 95% of those laboratories using the Kirby-Bauer method or an acceptable modification. Disk diffusion tests are particularly attractive to small laboratories (4, 6) because the tests are perceived to be technically simple, reproducible, and inexpensive. In fact, when the testing variables are carefully controlled, major discrepancies occur with <1% of the test results (7). However, control of the testing variables is critically important for accurate, reproducible tests.

One variable that has been examined is the quality of Mueller-Hinton agar used for the tests. Variations in the concentration of divalent cations in Mueller-Hinton agar can significantly influence the results of aminoglycoside-*Pseudomonas* tests and all tests with tetracycline (2, 3, 5, 9). Although supplementation of agar media with physiological concentrations of divalent cations has been attempted, the concentration of free cations in the media after autoclaving is unpredictable (5). Thus, the quality of the media should be evaluated by performing tests with established reference organisms.

Each year we routinely request that commercial medium manufacturers submit lots of media to our laboratory for performance testing. We use the results of these tests to select one lot of medium from which all Mueller-Hinton plates for susceptibility tests must be prepared. Al-

though we have seen a gradual improvement in the performance of the media with quality control organisms, we have not seen a similar improvement in other parameters by which we evaluate the media. This report is a summary of a recent evaluation of 12 lots of media received from four large, nationally known commercial manufacturers (BBL Microbiology Systems, Cockeysville, Md.; GIBCO Diagnostics, Madison, Wis.; Remel, Lenexa, Kans.; and Scott Laboratories, Fiskville, R.I.).

At the time of the evaluation, we contacted the companies and requested that they send us samples of different lots of prepared Mueller-Hinton agar plates. We informed them that the results of this evaluation would be used to select a lot of medium for all susceptibility tests performed at Barnes Hospital during the next year (ca. 17,000 tests). We also told the companies that the following parameters would be examined: performance with recommended quality control organisms, medium pH, agar depth and uniformity (e.g., whether or not the 150-mm plates were poured evenly), and characteristics of the zones of inhibition. Quality control tests for each lot of medium were performed in triplicate on successive days. We performed these disk diffusion susceptibility tests precisely in accordance with recommended procedures (8). The test inocula were standardized by comparison with a McFarland 0.5 standard and quantitated to ensure testing accuracy. The following organisms and antimicrobial agents were tested: *Staphylococcus aureus* (ATCC 25923) against ampicillin, cephalothin, chloramphenicol, clindamycin, erythromycin, gentamicin, tobramycin, oxacillin, penicillin G, tetracycline, and vancomycin; *Escherichia coli* (ATCC 25922) against ampicillin, carbenicillin, cephalothin, cefamandole, cefoxitin, chloramphenicol, genta-

micin, tobramycin, amikacin, nitrofurantoin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole; and *Pseudomonas aeruginosa* (ATCC 27853) against carbenicillin, gentamicin, tobramycin, amikacin, cefoperazone, cefotaxime, mezlocillin, and piperacillin. The National Committee for Clinical Laboratory Standards quality control guidelines (8) were used to determine if the tests were within acceptable limits. Depth of the agar medium was measured with a millimeter ruler. Medium pH was measured with an Orion 601A meter after macerating the medium in neutral distilled water. The sharpness of the zones of inhibited growth was recorded after the inoculated plates were incubated for 18 to 24 h at 35°C.

The results of the medium evaluation are summarized in Table 1. Only four lots of media were within established quality control ranges for all drugs tested against the three recommended quality control strains. Of the remaining eight lots of media with tests not within established quality control ranges, only 1 to 3 quality control tests (from a total of 32) were outside acceptable limits for four lots. However, one lot of medium failed to support the growth of the *S. aureus* quality control strain. The tests that were most commonly not within established quality control ranges were *S. aureus* with chloramphenicol (six lots), clindamycin (five lots), and oxacillin (five lots), *E. coli* with chloramphenicol (six lots), and *P. aeruginosa* with cefotaxime (two lots).

We also evaluated the preparation of the media. The medium pH should be between 7.2 and 7.4, and the medium should be poured into petri dishes so as to give a uniform depth of between 3 and 5 mm. Only five lots of media were between pH 7.2 and 7.4. Eight lots of media were poured to the right depth; however, the plates from six lots were poured so that the depth of agar in the plates was not uniform. Only six lots of media (from companies 1 and 4) were poured properly. Finally, the zones of inhibition were difficult to interpret with six lots of media from two companies (companies 1 and 2). This was particularly a problem with *S. aureus* tests, for which a definite point separating growth and no growth could not be defined. We were initially concerned that this effect was due to the size of the testing inoculum. However, the inoculum was quantitated to ensure that it was properly controlled. The mean inoculum sizes were 1.6×10^8 CFU/ml for *S. aureus*, 2.9×10^8 CFU/ml for *E. coli*, and 2.3×10^8 CFU/ml for *P. aeruginosa*. In addition, the same inoculum preparation was used to inoculate each lot of medium. Thus, the indistinct zones were due to the media rather than to the testing conditions.

During the last few years, we have seen a steady improvement in the results of quality

TABLE 1. Summary of medium evaluation

Co.	Medium lot	No. of tests in control ^a	Agar			Zone characteristics
			pH	Depth (mm)	Uniformity	
1	A	31	7.1	5	Level	Indistinct
	B	29	7.2	5	Level	Indistinct
	C	29	7.2	5	Level	Indistinct
	D	27	7.1	4-5	Level	Distinct
2	E	30	7.1	4-6	Uneven	Indistinct
	F	27	7.1	5-7	Uneven	Indistinct
	G	26	7.1	4-6	Uneven	Indistinct
3	H	32	7.0	3-5	Uneven	Distinct
	I	20 ^b	7.0	3-5	Uneven	Distinct
	J	32	7.3	2-5	Uneven	Distinct
4	K	32	7.2	4	Level	Distinct
	L	32	7.2	4	Level	Distinct

^a A total of 32 tests were performed for each lot of medium.

^b *S. aureus* failed to grow on this medium.

control tests with Mueller-Hinton agar. The results of all quality control tests were acceptable with only four lots of media in this study. However, that was an improvement over our experience in previous years. When we initially started our medium evaluations 5 years ago, as many as 50% of all test results were unacceptable. Although the National Committee for Clinical Laboratory Standards has made many revisions in quality control guidelines, we believe that the changes that have occurred are due to an overall improvement in medium quality. For example, the quality control guidelines for tests with tetracycline and gentamicin have not been changed. Tests with these two antibiotics were commonly outside the control limits in our initial evaluations. However, in the present evaluation no aminoglycoside or tetracycline tests were outside the control limits. We believe that this reflects the medium manufacturers' increased awareness of problems associated with variations in divalent cation concentration. Furthermore, efforts by the National Committee for Clinical Laboratory Standards to standardize all lots of Mueller-Hinton agar by performance testing are currently under way (A. L. Barry, personal communication). This should further improve the quality control results with Mueller-Hinton agar and theoretically eliminate the difficulties we encountered with indistinct zones of inhibition.

A problem that has not been described previously is the actual preparation and pouring of Mueller-Hinton agar plates. In our experience, we found that only two companies (1 and 4) were able to prepare media within the proper pH range and to pour plates at the appropriate depth

and uniformity. Although seven lots of media were outside the acceptable pH range (pH 7.2 to 7.4), the small variations in pH did not affect the results of the quality control tests. However, the reproducibility of the test results was affected by the uniformity of the agar in the plates. The performance test results were less reproducible with the incorrectly poured agar plates made by companies 2 and 3 than with the uniformly poured agar plates made by companies 1 and 4. The zones of inhibited growth measured on the former group of plates varied by as much as 4 mm, compared with an average variation of <1 mm on the latter group of plates. The variations observed with the incorrectly poured media were significantly greater than what we have previously reported with well-controlled tests and could cause clinically significant changes in the interpretation of some test results (7).

In summary, only 2 of 12 lots of Mueller-Hinton agar were found to be acceptable for disk diffusion susceptibility tests. Although improvements have been made in the media, as determined by performance tests, the manufacturers must devote more attention to the preparation and pouring of the agar media.

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