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NOTES

Comparison of the Plating Efficiencies and Shelf Lives of Three Different Commercial Buffered Charcoal Yeast Extract Media Supplemented with α-Ketoglutaric Acid[▽]

Paul H. Edelstein* and Martha A. C. Edelstein

University of Pennsylvania School of Medicine and Department of Pathology and Laboratory Medicine, Philadelphia, Pennsylvania 19104

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The plating efficiencies and shelf lives of locally made buffered charcoal yeast extract medium supplemented with α-ketoglutaric acid (BCYEα) were compared to those of media made by BD, Hardy, and Remel. Lung homogenates from guinea pigs infected with *Legionella pneumophila* were plated monthly onto different medium lots. All media performed equally well and had shelf lives of at least 12 months.

The performance of commercially made buffered charcoal yeast extract media supplemented with α-ketoglutaric acid (BCYEα) is not known with certainty, as the last published evaluation of the media was in 1993 and did not include media made by Hardy Diagnostics, more than one lot of any medium type, or use of a non-medium-passaged test strain (9). BCYEα medium is a nonselective growth medium designed to cultivate *Legionella pneumophila* (3) and is distinguished from selective media such as PAC, BMPA, and others by the absence of antimicrobial agents. These media can be difficult to make properly, as minor differences in pHs, cation contents, and agar compositions can have a major impact on growth rates, plating efficiencies, and colony sizes (4, 6–8). Virulent *L. pneumophila* is known to be especially salt sensitive, and spontaneous mutations in stock strains may result in salt resistance (1, 10), so testing media with stock strains of bacteria may not be valid. The current CLSI guidelines for the quality control of this medium use a crude index of medium performance, using a medium-adapted stock strain (2). We have detected two lots of commercial media that passed manufacturer or CLSI quality control guidelines over a 10-year period yet failed more rigorous quality control testing, growing <1% of the bacteria in the test inoculum (P. Edelstein, unpublished observation). Despite these problems in quality control standards, our recent informal observations have been that commercial BCYEα media are generally of high quality, with much longer shelf lives than the usual 2- to 3-month expiration dates specified by the manufacturers. In this study, we formally tested and compared commercially made media and a laboratory-prepared BCYEα medium, using a virulent non-medium-passaged *L. pneumophila* strain.

Plated BCYEα media were obtained from three manufacturers, BD BBL (Franklin Lakes, NJ), Hardy (Hardy Diagnostics, Santa Maria, CA), and Remel (Lenexa, KS). In addition, two different batches of locally made BCYEα media were prepared and used as the "gold standard" media throughout the 1-year testing of the media (3). The commercial media were obtained at study onset in sufficient quantities to be tested over a 1-year period. Additional lots of the commercial media were obtained at two monthly intervals throughout the study, to allow comparison of the performance of freshly made media with that of older media. The culture media were stored in the dark in a cold room (1 to 3°C) in their original plastic bags.

Media were inoculated with *L. pneumophila* serogroup 1 strain F889, a virulent clinical isolate strain that infects guinea pigs, amoebae, and a variety of cell types, including human monocyte-derived macrophages (5). Guinea pigs were inoculated by the intratracheal route with F889 to produce pneumonia, after which the lungs were harvested, ground in Mueller-Hinton broth, and then frozen in *N*-(2-acetamido)-2-aminoethanesulfonic acid (ACES) buffered glycerol at −70°C. The frozen lung suspensions were diluted in Mueller-Hinton broth so that the inoculum contained 100 to 300 CFU (median, 165 CFU; mean, 185 CFU). Each test of the media used a newly thawed vial, which was used to inoculate all media being tested that day with an identical inoculum. Media were tested in triplicate to quadruplicate, except for one test day when testing in duplicate was performed. Inoculated plates were incubated at 35°C for 4 days before counting colonies or determining colony sizes. Bacterial colony sizes were determined once, for one plate each of two different lots of media from each manufacturer, and for one plate each of three different lots of locally made media, including the two lots used as the reference standard. Colony diameters of 20 randomly selected colonies were measured for each plate, using a dissecting microscope equipped with a calibrated optical micrometer. The identity of the bacteria growing on the media as *L. pneumophila* was based on their typical colony morphology when observed with a dissecting microscope and on their typical colony texture and odor.

Comparisons of the recovery efficiencies for each medium batch were performed using colony counts and standard deviations normalized to the average colony counts of the two different

* Corresponding author. Present address: Clinical Microbiology Laboratory, 4 Gates, Hospital of the University of Pennsylvania, 3400 Spruce St., Philadelphia, PA 19104-4283. Phone: (215) 662-6651. Fax: (215) 662-6655. E-mail: phe@mail.med.upenn.edu.

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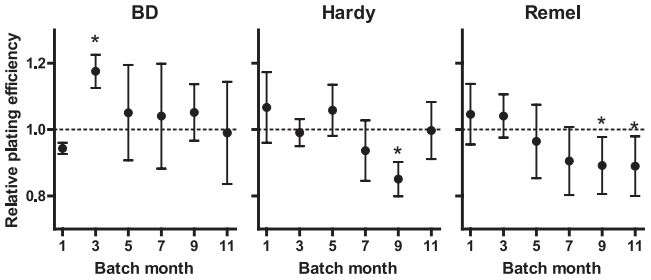

FIG. 1. Normalized plating efficiencies of BCYEα medium for the growth of *L. pneumophila*, using six different lots of media from BD BBL, Hardy Diagnostics, and Remel. A value of 1.0 indicates that the plating efficiency was equivalent to that of locally made media that served as the reference standard. $n = 4$. Dots represent mean values, and error bars represent 95% confidence intervals. *, $P < 0.05$ for comparison with reference standard by one-way ANOVA.

batches of the reference locally made media. Plating efficiency that was not significantly different from that of the gold standard would have a mean of close to 1.00 and a 95% confidence interval that encompassed 1.00. For comparisons between media or between different lots of the same media, including colony size comparisons, one-way analysis of variance (ANOVA) was used unless stated otherwise (GraphPad Prism version 5.02; San Diego, CA). Comparisons of month 1 to month 12 plating efficiencies were made by an unpaired two-tailed *t* test, with Welch's correction (GraphPad Instat, version 3.06). To calculate 95% confidence intervals of differences between ratios (e.g., normalized lot A versus normalized lot B), a nonpaired *t* test was used, which derived the 95% confidence intervals of the differences between ratios (GraphPad Prism).

All freshly prepared media, which were tested within days after receipt from manufacturer, or within a month of manufacture if locally made, had similar performances for plating efficiency (between manufacturer comparison, $P = 0.19$) (Fig. 1). For each manufacturer, one or more batches had performances that were significantly different than that of the reference medium, although the absolute differences were at most 20% different from the reference media, a nonsignificant difference in practical terms (within batch comparison, $P < 0.001$).

The shelf lives of the Hardy and Remel media tested were in excess of 1 year, as plating efficiencies of the same lots did not decrease over a 12-month period, in comparison with month 1 and month 12 plating efficiencies (data not shown) ($P > 0.05$, nonpaired *t* test with Welch's correction). The plating efficiency of the year-old BD plates was 11% better than that of the same lot plated a year previously, a statistically significant but practically insignificant difference ($P = 0.01$, nonpaired *t* test). To exclude the possibility that the reference standard medium performance degraded with time, these reference standard lots were compared with freshly made local media at 8 and 12 months after the reference media were made; no significant differences of plating efficiencies were observed when comparing the reference medium lots to the freshly made media (data not shown) ($P > 0.05$).

Colony sizes were significantly greater for bacteria plated onto BD media, which had a mean diameter of 3.99 mm, in comparison to mean diameters of 2.95, 2.71, and 2.51 mm for the Hardy, Remel, and locally made media, respectively (Fig. 2) ($P < 0.0001$). Medium age had no uniform effect on colony sizes, as 3- to 5-month-old media from Remel, BD, and locally

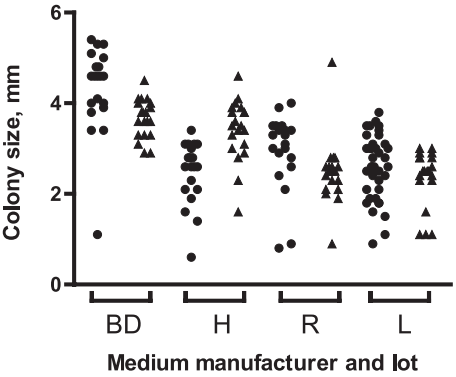

FIG. 2. *L. pneumophila* colony diameters for freshly prepared and 3- to 5-month-old lots of BCYEα media. $n = 20$ for each lot, except for the old lot of locally made media, for which $n = 40$. BD, BD BBL; H, Hardy Diagnostics; R, Remel; L, locally made; circles, old lot; triangles, newer lot.

made media had larger colony sizes than did month-old media, whereas 5-month-old media from Hardy had smaller colony sizes than did month-old media ($P > 0.05$ for old versus new for Remel and locally made media, and $P < 0.01$ for the same comparisons for Hardy and BD media, all by nonpaired *t* tests).

Quality of BCYEα media from all manufacturers tested was high, using a stringent test of plating efficiency. In addition, the media maintained their quality for at least a year under proper storage conditions, despite much shorter manufacturer's designated expiration dates. The larger colony sizes of *L. pneumophila* grown on BD media do not necessarily mean that the medium is superior for the clinical laboratory detection of the bacteria in clinical specimens, as the standard laboratory procedure is plate examination using a dissecting microscope, which can accurately detect *L. pneumophila* colonies as small as 0.3 mm.

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