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Claudia Wiese and Sanjeev Galande
Lawrence Berkeley National Laboratory Berkeley, CA, USA

Sterile Preparation of Antibiotic-Selective LB Agar Plates Using a Microwave Oven

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LB agar plates are simple, but powerful tools in molecular biology that allow for the growth of bacteria that can then be used for a wide variety of applications. One of the most common applications for these plates is the selective growth of bacterial cells that have been transformed by a plasmid with an antibiotic-resistant gene linked to it (2). Selection is achieved by matching the antibiotic resistance of the transformed cell to the antibiotic added to the growth media.

Traditional methods for preparing LB agar plates require the media to be autoclaved as a means of sterilization and then enhanced with an antibiotic before pouring the media into plates (1). This method of media preparation can be inconvenient when the plates are needed for immediate use. Recently, a short protocol posted on the Internet (http://www.moorhead.msus.edu/chastain/html/-lbplates.html) suggested that the sterilization of media by autoclave could be replaced with a short round of heating the media in a microwave oven. Furthermore, a commercial vendor offers a product intended for preparation by this method (Immedia; Invitrogen, Carlsbad, CA, USA). To the best of our knowledge, there are no published data that substantiate this method. The following protocol explores the novel method of LB agar and antibiotic plate preparation that uses a microwave oven instead of an autoclave and rapidly produces quality plates without compromising their sterility.

We prepared aliquots of 35 g/L powdered LB agar with powdered antibiotic, either 100 µg/mL carbenicillin or ampicillin, or 50 µg/mL kanamycin (all products purchased from Sigma, St. Louis, MO, USA) in 50-mL sterile conical tubes, sufficient to make 200 mL molten agar solution for eight 100 × 15 mm plates. For each antibiotic condition, seven aliquots were prepared. One set of aliquots was used to prepare

plates immediately. Three sets were dispensed into sterile tubes and stored in a light-protected container at 4°C. The other three sets were also dispensed into sterile tubes and stored in a similar container at room temperature in a thermostatically controlled laboratory (usually 18°C–22°C). Apart from those used immediately, LB agar plates were prepared from these refrigerated and room-temperature aliquots after one, two, or three months of storage; longer storage times were not performed.

At each milestone of storage, eight plates were prepared from one powdered aliquot stored at room temperature and one powdered aliquot stored at 4°C prepared for each of the three antibiotic treatments. Four plates from each condition were immediately plated with bacteria. One plate received host bacteria containing no plasmid. Three plates received host bacteria transformed, by the method recommended by the bacteria supplier, with an antibiotic-resistant plasmid (Stratagene, La Jolla, CA, USA): pBluescript® II KS- (ampicillin and carbenicillin resistant), pBK CMV (kanamycin resistant), or pCAL, into which we cloned a kanamycin-resistant expression insert (ampicillin/carbenicillin/kanamycin resistant). A fifth plate was not plated with bacteria but was used as a control for background contamination. After we spread the bacteria in a standard, sterile manner, the plates were incubated overnight at 37°C. The following day, the plates were scored independently for bacterial growth by two members of the laboratory. The plates that did not receive any bacterial spread were returned to the incubator for an additional night and scored again the next morning. The three remaining plates were wrapped with Parafilm® and stored at 4°C. Over a period of four weeks, we observed the plates periodically for contamination and used some of them for delayed plating experiments.

We found that plates of LB agar and antibiotic prepared in a microwave oven from fresh aliquots of media, and from aliquots stored for one, two, or three months, demonstrated consistent and reliable antibiotic selection (Table 1). This was true for powdered media and antibiotic stored at 4°C and for media and antibiotic stored at room temperature. Under all conditions tested, host cells without an antibiotic-containing plasmid did not grow on any of the plates containing antibiotic, whereas host cells containing an antibiotic-resistant plasmid grew only on the plates containing the appropriate antibiotic. Bacterial colony growth was normal, and there was no evidence of satellite colony formation.

Table 1. Antibiotic Selectivity of Plates

	Host/Vector Plated				
LB agar/ antibiotic	None	DH5-α	pBSII KS- (A/C resistant)	pBK CMV (K resistant)	pCAL + insert (A/C/K resistant)
Ampiicilin	0	0	+	0	+
Carbenicillin	0	0	+	0	+
Kanamycin	0	0	0	+	+

These are the results observed for four different durations of LB agar and antibiotic storage (one, two, and three months) before preparation and at two different storage temperatures (4°C and room temperature for one, two, and three months) of transformed and non-transformed DH5α *E. coli*.
A = ampicillin; C = carbenicillin; K = kanamycin.
"0" indicates no visible growth on 100% of plates after 24 h incubation.
"+" indicates visible growth on 100% of plates after 24 h incubation.

We were initially concerned that plates prepared in this manner could have a shortened shelf life because of premature microorganism contamination. Therefore, in a separate experiment with the microwave method, we tested media prepared under four conditions, using two different glass containers (autoclaved or non-autoclaved) and two different sources of water (double-distilled or distilled). These plates were compared to plates prepared with the traditional autoclave method. All plates contained 100 µg/mL carbenicillin. The plates were then wrapped in Parafilm, stored at 4°C, and assessed daily for visible signs of contamination. After 14 days, almost all of the plates displayed microorganism contamination, regardless of the method of preparation or the quality of the component. Other plates prepared with a microwave oven and stored for the short-term (1–10 days at 4°C) did not show contamination and demonstrated reliable antibiotic selection (data not shown).

These data demonstrate that antibiotic-selective media prepared in the microwave oven perform reliably, at least for three commonly used antibiotics. Our data suggest that the efficacy of the antibiotic present in the media was not compromised by the microwave-heating process. This is true for powdered media and antibiotics prepared directly from their original containers, as well as for the combinations that were stored for up to three months. The media and antibiotics stored at room temperature demonstrated efficacy equal to those refrigerated. With respect to microorganism contamination, plates prepared with the microwave protocol (Table 2) appear to have the same shelf life as those prepared by the traditional autoclave method. Using variations of glassware and water with the microwave technique, we could not identify a method of media preparation that conferred a better storage life than media prepared in the autoclave.

The environmental conditions of the laboratory could influence the results from this type of preparation. The climate of our laboratory is a moderate temperature with low-to-moderate humidity, conditions that are favorable for limiting microorganism contamination and growth. Our laboratory is generally clean, with a low volume of personnel and outside traffic. Therefore, glassware, environmental surfaces, and the microwave oven we used for media preparation have a low degree of contamination. Similar conditions are likely to be available in many laboratories.

The microwave oven-prepared media, when processed from readily avail-

Table 2. Protocol

1. Pre-mix the powdered LB agar and antibiotic in the correct proportion, according to the manufacturer's suggestions. If preparing for future use, store it in a tightly capped container protected from light and moisture.
2. When ready to prepare plates, combine distilled water and the LB agar and antibiotic powder mixture in an oversized clean flask. Slowly add the powder to the water, while gently swirling the flask to prevent the formation of clumps.
3. Cover the flask with plastic wrap and poke a hole in the top to allow steam to vent.
4. Heat on high in a microwave oven until all of the powder clumps are gone. Beware of the heat of the flask and the agar. Wearing a heat-protective glove, periodically swirl the contents of the flask until the solution is homogenous. If the contents of the flask begin to boil vigorously, take out the flask and allow the contents to settle.
5. Once in solution, place the flask into a 55°C water bath to cool the agar until the flask can be easily handled with bare hands.
6. When the temperature of the flask has equilibrated, dispense the agar into sterile culture plates. Leave the lids cocked until condensate evaporates from the lid, at which time the plates can either be used or wrapped with Parafilm and stored at 4°C.

able reagents such as LB agar and antibiotics, costs about one-fifth the amount of a comparable commercially available product. This cost savings comes at no expense to the quality of the media, which performed reliably in all of our tests.

This simple method is important because it provides an alternative for preparing antibiotic-selective plates that is less time-consuming and easier than preparing media by autoclaving, tempering the media, and then adding antibiotic. It is not necessary to prepare and store concentrated stocks of dissolved antibiotics. We do not recommend long-term storage (beyond two weeks) of plates poured from media prepared in a microwave oven. However, there is little reason to store such plates because the convenience of microwave-prepared media obviates the customary reason to keep reservoirs of plates stored for future experiments. On the other hand, aliquots of powdered media containing agar and antibiotics may be prepared ahead of time and stored for at least three months at room temperature before preparing them in the microwave, thereby providing additional convenience for this technique. This technique may find widespread application in both the controlled laboratory environment and the field. Obviously, we do not recommend this technique for use in a clinical laboratory because our method may not meet the rigorous specifications for detecting and testing the growth of pathogenic microorganisms.

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Address correspondence to Dr. Stanley A. Rubin, Cardiology (111E), 11301 Wilshire Blvd., Los Angeles, CA 90073, USA. e-mail: sarubin@ucla.edu

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Michael P. Iacoviello and Stanley A. Rubin
UCLA School of Medicine
Los Angeles, CA, USA