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Tissue Homogenization with Sterile Reinforced Polyethylene Bags for Quantitative Culture of *Candida albicans*

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A simple method of tissue homogenization with sterile reinforced polyethylene bags for quantitative fungal cultures was evaluated with mice infected with *Candida albicans*. This new method correlated well with standard methods ($P \leq 0.01$) for quantifying viable fungus in homogenates of brain, kidney, spleen, liver, and lungs and may be applicable in clinical and experimental mycology laboratories.

Standard methods of tissue homogenization for quantitative fungal cultures include the use of sterile glass tissue grinders (TGs), mechanical shearing devices such as blenders, and the mortar and pestle. The limitations of these systems include equipment expense, cleaning and sterilization time for initial and subsequent uses, potential problems of containment of biologically hazardous samples, and possible damage to the fungus itself by such harsh disruption procedures. We first learned of a novel, alternative technique from S. Shadomy, Division of Infectious Diseases, Medical College of Virginia, Richmond, who uses sterile reinforced polyethylene bags (PBs) in place of glass TGs. Animal tissues are simply homogenized inside the sealed bags, which contain a small amount of diluent. Several studies (1, 2, 4) have used this technique to good advantage; however, the method has not been throughly described or correlated to standards. Therefore, we studied tissue homogenization specifically for quantitative cultures of *Candida albicans* (Robin) Berkhout, comparing the PB technique to the use of glass TGs.

In the PB technique, sterile 150-ml reinforced Whirl-Pak PBs (Nasco, Fort Atkinson, Wis.) were used. Ten Broeck 10-ml glass TGs (A. H. Thomas, Swedesboro, N.J.) were used for TG homogenization. Two isolates of *C. albicans* (UMCC 83-7700 and UMCC 84-3185 from the University of Maryland Culture Collection) from leukemic patients with candidemia were used. The inoculum was grown in DPYE broth consisting of 2% dextrose, 1.0% peptone, and 0.3% yeast extract (BBL Microbiology Systems, Cockeysville, Md.). DPYE broth (50 ml) was inoculated with 1 ml of a 6-h broth starter culture grown at 37°C. After incubation for 12 to 14 h at 37°C with constant agitation, cells were centrifuged at 9,200 × *g* for 15 min at 4°C, washed twice, and suspended in sterile physiological saline. The cell suspensions were adjusted to hemacytometer counts of one of five different 10-fold dilutions (10^4 to 10^8 cells per ml).

Adult outbred CD-1 mice (Baltimore Veterans Administration Medical Center breeding colony) weighing 26 to 30 g received 0.1 ml of the graded concentrations of inoculum via a lateral tail vein. In total, five mice were inoculated with each strain of *C. albicans*. The animals were sacrificed within 5 min of inoculation. The brain, lungs, liver, spleen, and kidneys were promptly removed and sectioned. Approximately half of each organ mass was distributed into aluminum pans, designated for either the PB or TG technique, and then weighed. Sections of the right and left kidneys were combined to obviate any hemodynamic predilection for distribution of *C. albicans* toward one kidney (3). The organs were then placed within the PB or TG, each containing 1 ml of DPYE broth. Homogenization with the PB was performed by rolling a plastic, glass, or wooden rod over the bag until the tissue was thoroughly homogenized (Fig. 1). A thick laboratory marking pen was the most frequently used device for homogenizing the organs, and an outer containing bag was included as a precaution against a ruptured bag (which rarely occurred).

Homogenates from the PB or TG were transferred to sterile tubes for an initial 10-fold dilution in sterile physiological saline by vortex mixing. Samples were removed, and

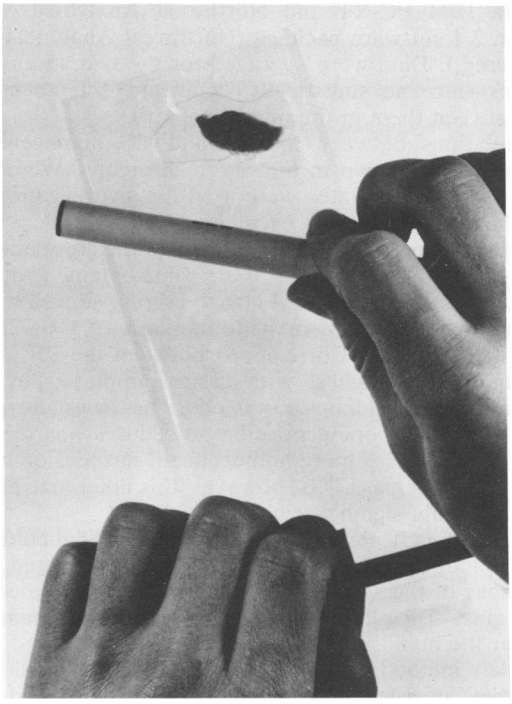

FIG. 1. Method for homogenization of tissue in sterile reinforced PB for quantitative culture of *C. albicans*.

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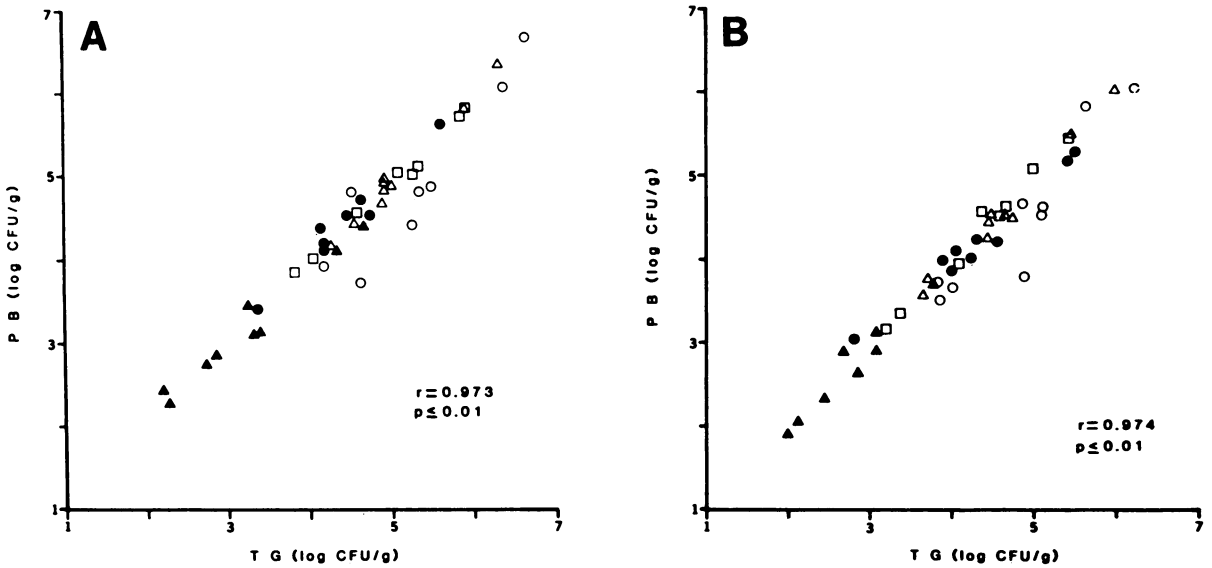

FIG. 2. Correlation between CFU derived from PB and glass TG techniques for homogenization of mouse liver (●), spleen (□), kidney (△), lung (○), and brain (▲) infected with graded concentrations of *C. albicans*. (A) CFU determined by using serial dilutions and pour plates; (B) CFU determined by using the SIS.

C. albicans CFU were quantified by using Sabouraud dextrose agar (BBL) dilution pour plates and a spiral inoculator system (SIS) (Spiral System Instruments, Inc., Bethesda, Md.). This SIS, which deposits microliter volumes in an archimedes spiral (spiral plates) on ordinary plates, has been described in detail elsewhere (5). Pearson linear coefficients and linear regression curves were determined for comparison of the PB and TG techniques by the SIS and pour plate method for all organs, for each organ, and for all organs except lungs. Similar analyses were performed to compare the new PB and SIS techniques with the conventional TG and pour plate method systems. Analysis was performed with an IBM PC-XT and Northwest Analytical Statpak Version 2.1 software package (Northwest Analytical, Portland, Oreg.). Data were averaged for the two strains of *C. albicans* since no statistically significant differences were seen between them in the number of CFU.

Correlations between the PB and TG homogenization techniques are shown in Fig. 2 for all organs. When serial dilutions and pour plates were used for quantification (Fig. 2A), the correlation was excellent ($r = 0.973$; $P \leq 0.01$). When the newer SIS was used (Fig. 2B), the correlation was equally good ($r = 0.974$; $P \leq 0.01$). Data obtained with both new systems, PBs and spiral plates, correlated well with the conventional TG and pour plate methods ($r = 0.972$; $P \leq 0.01$) for all organs. Correlations between the PB and TG techniques were highest with tissue samples from brain, liver, spleen, and kidneys ($r = 0.995$). The lowest correlation between the two homogenization procedures was seen with lungs ($r = 0.953$). A more homogeneous suspension may be achievable with glass TGs; however, this difference may not be a practically significant one.

This study indicated that quantitative candidal cultures of tissue homogenized by the PB technique correlated highly with those of the same tissue homogenized by the conventional glass TG method. The correlation was maintained whether the homogenate was quantitatively cultured by the pour plate method or by the SIS. We found this "Shadomy technique" of tissue homogenization with reinforced sterile plastic bags for quantitative fungal cultures superior with respect to increased productivity and efficiency when compared with glass TGs. Although this study examined only *C. albicans*, we suspect that the results would be similar for other yeastlike organisms. However, further investigation is necessary for the filamentous and the dimorphic fungi.

We suggest that this simple method for tissue homogenization may be applicable for quantitative fungal cultures in studies of pathogenesis, in in vivo antifungal investigations, and in evaluation of the concentration of fungi within clinical tissue specimens. Moreover, we have found the new method clearly superior to conventional methods for collecting qualitative data.

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