Efficacy of brain heart infusion-egg albumen agar, yeast extract phosphate agar and peptone glucose agar media for isolation of *Blastomyces dermatitidis* from sputum


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**Abstract**

The efficacy of brain heart infusion (BHI)-egg albumen agar, yeast extract phosphate agar and several modified peptone glucose agar media was evaluated for isolation of *Blastomyces dermatitidis* from sputum concomitantly seeded with the yeast form of the pathogen and *Candida albicans*. Based upon high percent culture positivity of sputum, improved recovery (CFU/ml) of the seeded inoculum, faster growth rate of *B. dermatitidis* and low level of contamination, BHI-egg albumen agar, followed by yeast extract phosphate agar are recommended as the media of choice for the isolation of *B. dermatitidis* from contaminated clinical specimens.

**Introduction**

Blastomycosis, a pulmonary and systemic mycotic disease was only recently recognized in India [6, 12]. The most important criterion for its definitive diagnosis is the culture of the etiologic agent, *Blastomyces dermatitidis*, from clinical specimens. Lung being the principle focus of primary infection, sputum is generally the commonest clinical material available for laboratory diagnostic investigations. A prolonged incubation of up to 4 weeks has been recommended for isolation of *B. dermatitidis* from clinical specimens [2, 3, 11]. In addition to this long incubation period, a common difficulty in isolating the fungus from clinical specimens is the concomitant occurrence of saprobic or commensal microbiota which may interfere with its growth. Sabouraud glucose agar, a widely used primary isolation medium in mycological diagnostic work, has the serious drawback of frequent overgrowth by *Candida albicans*, a commensal of the human respiratory tract. In fact, *C. albicans* is known to be an *in vitro* inhibitor of *Histoplasma capsulatum*, a related dimorphic fungal pathogen [9]. Smith & Goodman [14] reported that yeast extract phosphate agar supplemented with ammonium hydroxide yielded higher isolations of *H. capsulatum* and *B. dermatitidis* from contaminated clinical specimens than did BHI-blood agar and Sabouraud glucose agar. As far as we are aware, these findings have not been corroborated although yeast extract phosphate agar has been listed in several medical mycology text books for the selective isolation of *H. capsulatum* and *B. dermatitidis* [5, 11, 13]. More recently, Kane et al. [8] observed that brain heart infusion (BHI)-egg albumen agar yielded more isolations
of *B. dermatitidis* from sputum than did BHI-blood agar and Sabouraud glucose agar. This paper embues the results of an investigation of the comparative efficacy of yeast extract phosphate agar, BHI-egg albumen agar and several modified peptone glucose media for the isolation of *B. dermatitidis* from experimentally seeded sputum specimens.

**Materials and methods**

**Media**

The culture media used in this study were as follows: Brain heart infusion (BHI)-egg albumen agar, yeast extract phosphate ammonium hydroxide agar, yeast extract phosphate cycloheximide agar, peptone glucose cycloheximide agar, peptone glucose ammonium hydroxide agar, buffered peptone glucose cycloheximide agar and buffered peptone glucose ammonium hydroxide agar.

Brain heart infusion-egg albumen agar was prepared as described by Kane *et al.* [8]. The composition of the medium was BHI powder (Difco), 37 g; fresh egg albumen, 30 ml; sheep blood, 50 ml; cycloheximide, 0.5 g; chloramphenicol, 0.05 g; gentamicin, 0.04 g; agar, 15 g and distilled water to make 1000 ml. The egg albumen was prepared by carefully draining it into a sterile McCartney bottle and homogenizing it on a vortex mixer after addition of glass beads. All the ingredients were combined except albumen, blood and gentamicin and sterilized at 121 °C for 15 minutes. The remaining ingredients were added after the medium was cooled to 50 °C.

Yeast extract phosphate agar was prepared as described by Smith & Goodman [14]. It contained yeast extract (Difco), 1 g; phosphate buffer (0.6 M), 2 ml; chloramphenicol, 0.05 g; agar, 15 g and distilled water to make 1000 ml. The medium was sterilized at 121 °C for 15 minutes and poured into Petri plates. The stock solution of 0.61 M phosphate buffer was prepared as follows: Four grams of disodium hydrogen phosphate (Na₂HPO₄) were dissolved in 30 ml of distilled water, followed by addition of 6 g of potassium dihydrogen orthophosphate (KH₂PO₄) and the pH was adjusted to 6 with either 1 N HCl or 1 N NaOH. The buffer was made up to 40 ml with distilled water and stored at 4 °C. Approximately, 0.05 ml of ammonium hydroxide was dropped on each medium plate after inoculation.

The composition of the basal peptone glucose agar was as follows: peptone, 10 g; glucose, 10 g; agar, 15 g and distilled water to make 1000 ml. The medium was fortified with cycloheximide, 0.5 g; chloramphenicol, 0.05 g and distilled water at 121 °C for 15 minutes. The buffered peptone glucose agar was prepared in the same manner as described above except that 2 ml of phosphate buffer was incorporated.

**Inoculum**

Three isolates of *B. dermatitidis*, B-1145, IP-973 and VPCI-S70, originating from U.S.A., Tunisia and India, respectively, and a solitary isolate of *C. albicans*, ATCC 10259, served as the test fungi. The inoculum was prepared from 3 days old growth of the yeast form of *B. dermatitidis* cultured on Pharmamedia glucose agar at 37 °C. A loopful of the growth was suspended in sterile physiological saline, and the cell density was so adjusted with haemocytometer that 0.1 ml of the suspension would contain about 10 or 100 yeast cells of *B. dermatitidis*. To test the inoculum viability, a 0.1 ml aliquot was cultured on peptone glucose agar plates. Inoculum of *C. albicans* was also prepared in the same manner and it was adjusted with haemocytometer so that 0.1 ml would contain about 10⁶ yeast cells of *C. albicans*.

**Collection and processing of sputum**

Early morning sputum specimens were collected in sterile McCartney bottles from patients with miscellaneous respiratory disorders attending the Clinical Research Centre of V.P. Chest Institute, Delhi. They were homogenized by shaking with sterile glass beads on a vortex mixer. Before seeding with *B. dermatitidis*, each sputum was cul-