Performance of Five Agar Media for Recovery of Fungi from Isolator Blood Cultures

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We studied the recovery of 1,270 fungal isolates from 176,144 Isolator blood cultures (0.72% positive) on bacterial and fungal media, under routine and differing incubation conditions. Except with Histoplasma capsulatum, chocolate agar incubated for only 3 days proved to be an excellent medium for the recovery of fungi from the Isolator system.

Fungemia is common in many patients with serious fungal disease and may be detected by a variety of blood culture systems (2–4, 6, 7, 9, 10, 12–16, 18, 23–33, 35, 36). The Isolator lysis-centrifugation system (Wampole Laboratories, Cranbury, N.J.) has been shown to be an accurate and effective method for detecting fungemia and fungal bloodstream infections (3, 7, 8, 10, 12–18, 20, 21, 24, 25, 32, 33, 36, 37). However, the use of excessive media with the Isolator system may be cost-prohibitive and may not reveal additional diagnostic information.

To determine if significant differences exist in the frequencies of recovery of fungi among five agar media used for routine blood culture, we retrospectively examined the recovery of 1,270 fungal isolates from 176,144 Isolator blood cultures performed at the Mayo Clinic during 1991 to 1995. No attempt was made to identify or remove repetitive cultures. For each culture, blood was collected in an Isolator tube and processed according to the manufacturer’s instructions. The sediment was inoculated onto five agar media. Sheep blood agar (SBA) (Trypticase soy agar with 5% sheep blood, TSAII formulation; Becton Dickinson Microbiology Systems, Sparks, Md.) and chocolate agar (CA) (Becton Dickinson) were inoculated primarily for the recovery of bacteria and were incubated at 35°C with 5 to 7% CO2. These plates were examined for 72 h and then discarded. Inhibitory Mold agar (IMA) (Remel Inc., Lenexa, Kans.), brain-heart infusion agar (BHI) (Remel), and Sabouraud dextrose agar (SAB) (Remel) were inoculated for the recovery of fungi. Fungal cultures were incubated at 30°C with 10% humidity. They were examined daily for 6 days and then discarded. Inhibitory Mold agar (IMA) (Remel Inc., Lenexa, Kans.), brain-heart infusion agar (BHI) (Remel), and Sabouraud dextrose agar (SAB) (Remel) were inoculated for the recovery of fungi. Fungal cultures were incubated at 30°C with 10% humidity. They were examined daily for 6 days and then twice a week for a total incubation time of 21 days. Fungi that were recovered on any media were completely identified by standard methods in a mycology laboratory.

Only organisms (genus and species) that were recovered on ≥6 separate occasions were deemed suitable for statistical analysis. The isolation frequency for fungal isolates was determined for each agar medium (Table 1). Cochran’s Q test (6) was used to assess if any significant differences existed in frequencies of recovery of each organism among the five media (Table 2). Pairwise sign tests were used to assess differences between the various media for each organism (Table 2). When a significant difference was found in the pairwise analysis, the data in Table 1 were used to determine which of the two media isolated the organism more frequently. Some statisticians promote an adjustment when multiple comparisons are made, such as a Bonferroni adjustment (22). The P values reported in Table 2 are the unadjusted P values, with significance assumed to be ≤0.05. Bonferroni’s adjustment requires a significance level of ≤0.005. Data both with and without the Bonferroni adjustment are discussed.

Four genera and eight species from 1,248 isolates met the inclusion criteria. The frequency of isolation of these organisms for each medium is presented in Table 1. The most frequently isolated fungus was Candida albicans. Cochran’s Q analysis revealed that there were no significant differences detected among the five media for the recovery Candida lusitaniae (P = 0.1309), Candida tropicalis (P = 0.6726), Candida parapsilosis (P = 0.8168), or Coccidioides immitis (P = 0.8198). Significant Cochran’s Q P values (P ≤ 0.05), without adjustments for multiple comparisons, were found for C. albicans (P = 0.0158), Candida glabrata (P = 0.0147), Candida neoformans (P = 0.0028), and Histoplasma capsulatum (P < 0.0001). Pairwise comparisons revealed that C. albicans was recovered significantly more frequently on CA than on IMA or BHI (P = 0.0392 and P = 0.0019, respectively). Although C. albicans was recovered more frequently on CA than SAB (455 versus 429 isolates), the difference was not significant (P = 0.0963). Similarly, although C. albicans was recovered more frequently on SBA than any of the fungal agars, the differences were not significant. The pairwise comparisons for both C. glabrata and C. neoformans demonstrated that these fungi

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**TABLE 1. Isolation frequencies by medium type**

<table>
<thead>
<tr>
<th>Species</th>
<th>% (no.) of isolates recovered on:</th>
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<tbody>
<tr>
<td></td>
<td>SBA</td>
</tr>
<tr>
<td>C. albicans</td>
<td>64.7 (435)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>51.9 (63)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>73 (116)</td>
</tr>
<tr>
<td>H. capsulatum</td>
<td>0 (0)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>67.9 (57)</td>
</tr>
<tr>
<td>C. neoformans</td>
<td>54.8 (23)</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>57.1 (12)</td>
</tr>
<tr>
<td>Coccidioides immitis</td>
<td>77.8 (7)</td>
</tr>
<tr>
<td>Total</td>
<td>58.7 (733)</td>
</tr>
</tbody>
</table>
were recovered significantly more frequently on IMA and SAB than on SBA. Although these organisms were recovered slightly more frequently on the fungal agars than on CA, the differences were not statistically significant. After the Bonferroni adjustment for multiple comparisons, no statistically significant differences were detected for C. albicans, C. glabrata, or C. neoformans. Only the recovery of H. capsulatum retained statistical significance after the Bonferroni adjustment.

Both with and without adjustments for multiple comparisons, H. capsulatum demonstrated significant P values by Cochran’s Q analysis (P = <0.0001). Pairwise comparisons showed that the difference was due to its recovery on the fungal agars, rather than that on the SBA or CA. There was no significant difference detected in frequencies of recovery of H. capsulatum among the fungal agar media. Although H. capsulatum was not recovered on SBA or CA, this was an expected finding since the incubation time for the CA and SBA plates, which were inoculated primarily for the recovery of bacteria, was only 72 h. Geha and Roberts analyzed four years of blood culture data using the Isolator lytic centrifugation system and found that the mean recovery time for H. capsulatum was 11.5 days, with a range of 6 to 21 days (11). Therefore, we feel confident that these results represent a selection bias against H. capsulatum and that on the fungal agars. In this assessment, CA performed as well as or better than the fungal agars, even though cultures were incubated for a shorter period of time.

In the current climate of stringent medical economics, studies that provide insights into cost-effective means for providing effective laboratory diagnosis should be explored. The clinical laboratorian must allocate resources judiciously and establish diagnoses in the most cost-effective manner, without compromising the quality of the tests performed. The Isolator system is a proven method of diagnosing fungemia, but its cost-effectiveness has been questioned (19). Our study suggests that, with the exception of H. capsulatum, clinically relevant fungi that cause bloodstream infections will be adequately recovered on CA within 3 days of incubation using the Isolator system. Although isolates other than H. capsulatum were occasionally recovered after incubation times greater than 3 days, the number of these isolates was not enough to provide significant differences in favor of the fungal media. This study also suggests that the 5 to 7% CO₂ atmospheric condition employed for the recovery of bacteria is, at least, not inhibitory to fungi.

Additional studies, which examine extended incubation of CA and SBA, are needed to determine the suitability of these media for the recovery of H. capsulatum. Until such a study has been performed, it would be prudent to continue the use of at least one of the proven fungal media. Among the fungal media, H. capsulatum was recovered most frequently on SAB compared with recoveries on IMA and BHI, but the differences in detection were not significant. The recovery of H. capsulatum on SBA and CA held for extended incubation times should be studied further. This may necessitate tapping, bagging, or using 20 ml, rather than 15 ml, of SBA or CA to prevent desiccation.

If a three-plate system (SBA, CA, and a fungal medium) is used, we recommend using all the Isolator sediment and extending incubation for the optimal recovery of fungi. The use of one, rather than three, fungal media with the Isolator system should substantially reduce laboratory costs without compromising the detection of fungemia.

**REFERENCES**