Contamination of Cultures Processed with the Isolator Lysis-Centrifugation Blood Culture Tube

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Overall contamination (on- plus off-streak) of the Isolator (Du Pont Co.) blood culture tube (23%) was greater than that of a conventional broth blood culture bottle (0.6%) or that of a biphasic blood culture bottle (1.3%). To determine the source of this contamination, Isolator cultures of blood from 59 healthy volunteers and of sterile broth from 60 vials were made. A total of 37% of the blood cultures and 22% of the broth cultures were contaminated (P = 0.06). Staphylococcus epidermidis-contaminated cultures represented 31 and 10% of the blood and broth cultures, respectively (P = 0.06). Contamination of plates processed on a bench top, in front of horizontal laminar flow, and in a biological safety cabinet with vertical laminar flow were compared. Processing plates in a biological safety cabinet resulted in a significant reduction in the number of contaminated plates (P < 0.05). The contamination rate for 7,574 Isolator blood vials processed in the biological safety cabinet was significantly decreased to 6.7% on-streak (9.3% on-plus off-streak). Contamination of Isolator-processed blood cultures originated from the laboratory and the patient. The former can be reduced by inoculating plates in a vertical laminar flow biological safety cabinet and by maintaining adequate quality control of media. The latter may be unavoidable.

The primary goal of blood cultures is to provide early detection and rapid identification of pathogenic microorganisms. Contamination of blood cultures with Staphylococcus epidermidis, Corynebacterium spp., Propionibacterium spp., and Bacillus spp. not only presents problems in interpretation but also consumes a significant amount of time spent in identification (6). Conventional broth systems have contamination rates ranging from 0.6% for an unvented tryptic broth bottle to 1.3% for a vented biphasic brain heart infusion medium (4). In general, rates of contamination in excess of 3% are considered unacceptable (5).

The Isolator lysis-centrifugation blood culture tube (Du Pont Co.) is an innovation in blood culture methodology. In a Mayo Clinic evaluation of 6,010 blood cultures, the Isolator offered a significant improvement over an unvented broth bottle or a vented biphasic bottle for the detection of septicemia due to certain microorganisms (4). Since the greater sensitivity of the Isolator tube for the detection of bacteria and fungi yielded an unacceptably high isolation rate for contaminants (4), this study was designed to investigate the source(s) of contamination and to determine means of contamination control during the collection and processing of Isolator blood cultures.

**MATERIALS AND METHODS**

Isolator cultures were made from blood from 59 healthy volunteers. After skin antisepsis of the venipuncture site with povidone-iodine (5), blood was collected in 7.5-ml Isolator blood culture tubes with the needle-collar assembly for vacuum tubes. Isolator tubes were processed according to the instructions of the manufacturer on an open bench top which had been cleaned with 5% phenol. Equal amounts of the concentrate were inoculated onto a sheep blood agar (SBA) plate, a chocolate blood agar (CBA) plate, a brucella blood agar (BBA) plate, and a brain heart infusion agar (BHIA) plate. The SBA and CBA plates were incubated in CO₂ at 35°C. The BBA plate was incubated at 35°C in a GasPak anaerobe jar (BBL Microbiology Systems), and the BHIA plate was incubated at 30°C in room air. Within 12 to 24 h, each BHIA plate was sealed in a polyethylene bag to prevent fungal contamination. The BHIA plate was used for the isolation of yeasts and fungi. SBA, CBA, and BBA plates were inverted after 24 h; all plates were inspected daily for growth and discarded on day 4. The SBA, CBA, and BBA plates were purchased from GIBCO Laboratories. The BHIA plates were purchased from Diagnostics Inc.

Isolator cultures were made from sterile brain heart infusion broth from 60 25-ml vials. After disinfection of the rubber stopper with povidone-iodine, broth was collected in 7.5-ml Isolator tubes and processed as described above.

Laboratory contamination of Isolator-processed plates was evaluated by “handling” SBA plates on an open bench top (320 plates), in a horizontal laminar flow cabinet (200 plates; Clean Air Systems Corp.), and in a biological safety cabinet (BBL Microbiology Systems with vertical laminar flow (200 plates). Handling was defined as (i) removing the lid of the culture plate and simulating inoculation of the concentrate with an empty syringe, (ii) streaking the plate with a sterile plastic loop, and (iii) examining the plate on day 4 and discarding it. The control group was 100 unopened SBA plates that were incubated along with the other SBA plates. The technologists processing the Isolator cultures washed their hands and disinfected the bench top on which the culturing was performed and the inside surface of both hoods. The disinfectant used was 5% phenol.

For the purpose of this study, contamination was defined as any colony of S. epidermidis spp., Corynebacterium spp., Propionibacterium spp., or Bacillus spp.; a gram-negative non-fermentative bacillus (other than Pseudomonas aeruginosa); or filamentous fungi detected on or off the streaked portion of the plate. A contaminated plate contained at least one

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colony. A contaminated culture of blood from a volunteer or of brain heart infusion broth from a vial contained at least one colony on one of the four plates. Statistical analysis was performed by Fisher's exact test or chi-square test (1).

RESULTS

Isolator cultures of blood from healthy volunteers and of sterile broth from vials had contamination rates of 37 and 22%, respectively, if on-streak and off-streak colonies were counted (Table 1). S. epidermidis was the most common contaminant, appearing in 31% of the blood cultures and 10% of the broth cultures. Although statistical significance was approached, neither the overall nor the S. epidermidis contamination rate showed a significant difference between the blood and broth cultures. Although the overall on-streak contamination rates in Isolator cultures of blood and broth were similar (Table 2), there was a difference (P = 0.054) in the on-streak contamination due solely to S. epidermidis.

The relationship between the location in the laboratory where Isolator cultures were processed and the contamination rate is shown in Table 3. Plates processed in a biological safety cabinet with vertical laminar flow were significantly less likely to be contaminated than those processed on an open bench top. The use of horizontal laminar flow did not significantly reduce the incidence of contamination.

DISCUSSION

Henry et al. (4) reported an on-streak contamination rate of 9.6% in an evaluation of over 6,000 Isolator blood cultures. The on- plus off-streak contamination rate was 23% (unpublished data). Although Dorn et al. (3) suggested that colony counts of <1 CFU/ml for common contaminants were not indicative of septicemia, colony counts of clinically significant organisms can fall within this range (2, 4). Since single colonies detected on streaked areas of the Isolator plates are of potential clinical significance, subculture and species identification is required.

This study investigated reasons for the high number of Isolator cultures which become contaminated. Isolator cultures of blood from healthy volunteers were compared with those of sterile broth from vials to determine whether contaminants were inoculated into the Isolator tube from percutaneous venipuncture. Although more contaminants, particularly S. epidermidis, originated from blood than from broth cultures, these differences were not statistically significant. On the basis of our data (Tables 1 and 2) and those of Zierdt (7), who reported S. epidermidis in 11.6% of blood cultures processed by a lysis-filtration technique, we suggest that a component of the increased Isolator contamination originates from the patient and is primarily S. epidermidis. Whether the staphylococci originate from the skin or represent a transient bacteremia was not resolved by our study. If this contaminant arises from the skin during venipuncture, elimination may be difficult.

Blood agar plates processed in a manner identical to that used for Isolator cultures demonstrated that a component of the Isolator contamination originates from laboratory handling. Comparing plates processed on an open bench top with those processed in a biological safety cabinet with vertical laminar flow demonstrated significantly few contaminant colonies on plates processed by the latter method. Control plates which were never opened were contaminated 1% of the time. If the routine processing of Isolator cultures requires four agar plates per culture and if random contamination of agar plate media is 1%, then a 4% contamination rate of cultures may be observed regardless of the care taken during processing.

The Isolator, in combination with a broth blood culture bottle and a biphasic medium blood culture bottle, has been used routinely for patient blood cultures since November 1982. After this study we began processing all Isolator blood cultures in a vertical laminar flow biological safety cabinet (Belco Glass, Inc.). Comparison of the contamination rates of Isolator blood cultures obtained from patients and processed before and after installation of the hood substantiates the effectiveness of the biological safety cabinet. The contamination rate for 2,679 blood cultures processed on an open bench top was 9.5% on-streak (15.1% on- plus off-streak). The contamination rate for 3,530 Isolator blood cultures processed similarly by the same technicians but inside the biological safety cabinet was significantly decreased (P < 0.05) to 7.8% on-streak (11% on- plus off-streak). After processing 7,874 Isolator blood cultures from patients in the biological safety cabinet, contamination rates continued to decrease to 6.7 and 9.3% for on-streak and on- plus off-streak, respectively.

Contamination of Isolator blood cultures appears to originate from multiple sources: patients, laboratory environ-

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TABLE 1. On- plus off-streak contamination of Isolator-processed cultures

<table>
<thead>
<tr>
<th>Cultures</th>
<th>No. (%) of cultures*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
</tr>
<tr>
<td>Normal volunteers</td>
<td>59</td>
</tr>
<tr>
<td>BHI broth in vials</td>
<td>60</td>
</tr>
</tbody>
</table>

a Cultures were processed on an open bench top.
b P = 0.061; P > 0.05 is not significant (chi-square test).
c P = 0.060; P > 0.05 is not significant (Fisher's exact test).

TABLE 2. On-streak contamination of Isolator-processed cultures

<table>
<thead>
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</table>

a Cultures were processed on an open bench top.
b P = 0.58; P > 0.05 is not significant (chi-square test).
c P = 0.054; P > 0.05 is not significant (Fisher’s exact test).

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TABLE 3. Influence of location on the contamination of Isolator-processed plates

<table>
<thead>
<tr>
<th>Location</th>
<th>No. (%) of plates</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open bench top</td>
<td>320</td>
<td>0.012</td>
</tr>
<tr>
<td>Horizontal laminar flow</td>
<td>200</td>
<td>0.001</td>
</tr>
<tr>
<td>Biological safety cabinet with vertical laminar flow</td>
<td>200</td>
<td>0.001</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>ND</td>
</tr>
</tbody>
</table>

a P values for horizontal laminar flow and biological safety cabinet were calculated by comparing each with that for the open bench top (chi-square test). NS, Not significant; ND, not determined.
CONTAMINATION OF ISOLATOR BLOOD CULTURES

ments, and commercially prepared agar plate media. Contamination from the laboratory environment can be significantly decreased by processing cultures in a biological safety cabinet with vertical laminar flow. Contamination of media can be minimized by an active quality control program. Contamination, primarily S. epidermidis, from the patient may be unavoidable.

LITERATURE CITED