Utility of Extended Blood Culture Incubation for Isolation of *Haemophilus, Actinobacillus, Cardiobacterium, Eikenella,* and *Kingella* Organisms: a Retrospective Multicenter Evaluation


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The incidence of and average time to detection for *Haemophilus, Actinobacillus, Cardiobacterium, Eikenella,* and *Kingella* (HACEK) bacteria in blood cultures with standard incubation and the utility of extended incubation of blood culture bottles were reviewed at four tertiary care microbiology laboratories. HACEK organisms were isolated from 35 (<0.005%) of 59,203 positive blood cultures. None of 407 blood cultures with extended incubation grew HACEK or other bacteria. Bacteremia from HACEK bacteria is rare, and extended incubation of blood cultures to recover HACEK bacteria is unnecessary.

The *Haemophilus, Actinobacillus, Cardiobacterium, Eikenella,* and *Kingella* (HACEK) group of microorganisms comprises bacteria that commonly colonize the human oropharynx as normal, indigenous flora. Included are *Haemophilus* spp. (except *H. influenzae*), *Actinobacillus actinomycetemcomitans,* *Cardiobacterium hominis,* *Eikenella corrodens,* and *Kingella kingae.* HACEK bacteria infrequently cause bacteremia but, when present, usually are clinically significant and are responsible for approximately 2 to 5% of culture-positive, infective endocarditis cases (6, 20, 21, 29). Traditionally, most laboratories have followed the standard practice of extending the incubation of blood culture bottles for 14 to 21 days to improve the recovery of HACEK bacteria, particularly for patients diagnosed clinically with culture-negative endocarditis. Improvements in blood culture media and implementation of automated blood culture systems that increase recovery of these fastidious organisms were isolated from 35 (<0.005%) of 59,203 positive blood cultures. None of 407 blood cultures with extended incubation grew HACEK or other bacteria. Bacteremia from HACEK bacteria is rare, and extended incubation of blood cultures to recover HACEK bacteria is unnecessary.

A retrospective analysis of blood cultures obtained between 1 January 2003 and 30 April 2004 was performed at four centers: ARUP Laboratories (ARUP), Duke University Medical Center (DUMC), Johns Hopkins Hospital (JHH), and Robert Wood Johnson University Hospital (RWJ). A separate review of blood cultures obtained at DUMC between 1 April 1992 and 30 June 2004 was also performed for comparative, longitudinal analysis. Culturing of blood was performed using a BacT/ALERT (DUMC, JHH) (bioMérieux, Durham, NC) or BACTEC 9240 (ARUP, DUMC, RWJ) (BD Diagnostic Systems, Sparks, MD) automated system. The routine incubation period of bottles varied from 5 days (ARUP, DUMC, RWJ) to 6.5 days (JHH). Requests for extended incubation were recorded by three centers (ARUP, JHH, RWJ). Blood culture bottles that were designated for extended incubation remained in the blood culture instrument for 10 to 14 days. At the end of the extended incubation period, instrument-negative bottles were subcultured to 5% sheep blood, chocolate, and/or anaerobic agar plates, which were incubated for an additional 5 days. A positive blood culture was defined as the recovery of a microorganism(s) from one or more bottles from a blood culture set by either standard or extended incubation protocols. Identification of microorganisms, including HACEK bacteria, was performed by standard laboratory methods (24).

Over the 16-month period, HACEK bacteria were recovered from 16 (13 patients) of 15,826 positive blood cultures (0.1%) from all four centers (Table 1). The mean and median times to detection of HACEK isolates were 3.4 and 3 days, respectively. For the 12-year longitudinal analysis of DUMC data, HACEK bacteria comprised 30 (21 patients) of 48,921 positive blood cultures (<0.1%). The mean and median times to detection of HACEK isolates were 2.6 and 2 days, respectively. Physicians requested extended incubation for 407 blood cultures (182 at ARUP, 30 at DUMC, and 195 at JHH). Beyond the period of routine incubation at each institution, none of these cultures grew HACEK or other bacteria.

In this study involving four major tertiary care medical centers, bacteremia from HACEK bacteria was rare, comprising approximately 0.1% of positive blood cultures over the 16-month period. The 12-year longitudinal comparative analysis
from a single study site corroborated these findings. Recommendations for the extended incubation of blood cultures to increase the recovery of HACEK bacteria (2, 25) are based on studies that were performed before improvements in blood culture media and implementation of automated blood culture instruments (9, 12, 13, 30). Even before the widespread use of automated systems, data have suggested that extended incubation may be unnecessary. For example, a 1996 survey of microbiology laboratories using Septi-Chek blood culture bottles concluded that fastidious bacteria, including HACEK, were recovered from blood cultures usually within 7 days of incubation (8). A Mayo Clinic study of 45 cases of endocarditis caused by HACEK bacteria diagnosed from 1970 to 1992 found that the mean time to detection of growth in blood cultures was 3.4 days (6). In a comprehensive review that documented the time to detection of 55 endocarditis cases due to HACEK bacteria, the overall mean time to detection for positive blood cultures was 7.1 days. However, 52 of these cases were published between 1964 and 1993; the mean time to detection for A. actinomycetemcomitans was 2.0 days in the 3 cases published after 1993 (26). More recent case reports of endocarditis caused by HACEK bacteria consistently demonstrate isolation of HACEK microorganisms within 5 days of incubation (3–5, 10, 16, 17, 19). Yet, physicians routinely request extended incubation for the HACEK bacteria and, moreover, often mistakenly associate “culture-negative,” infective endocarditis and/or bacteremia with the HACEK group. Although other fastidious pathogens (e.g., Bartonella spp., Legionella spp.) associated with endocarditis may require special incubation requirements (7), HACEK bacteria, as we have shown, do not require extended incubation or special media to improve their recovery.

We recognize that given the rarity of HACEK bacteria causing endocarditis, the numbers of blood cultures that were held for extended incubation in our study may not have sufficient power to detect a significant benefit. However, an excess of 20,000 blood cultures with extended incubation would be required to detect a statistically significant difference, making such a study prohibitive. In further support of our findings, we reviewed published reports that have studied the roles of nucleic acid amplification and sequencing analysis using 16S rRNA genes for the diagnosis of bacteremia and endocarditis. To date, molecular detection of HACEK bacteria from blood or excised heart valves has not been demonstrated reliably for patients with “culture-negative” endocarditis or for cases in which blood cultures were obtained before the initiation of antibacterial therapy (1, 11, 14, 18, 22, 27, 28). Molecular methods may prove useful for diagnosis, however, for patients already receiving antimicrobial therapy (1, 18) or for diseases caused by difficult-to-culture or unculturable pathogens (e.g., Bartonella spp., Coxiella burnetii, Tropheryma whipplei) (2, 15, 23).

We conclude that bacteremia from HACEK bacteria is rare and that extended incubation of blood cultures to recover HACEK bacteria is unnecessary. None of the hundreds of special requests for extended incubation yielded HACEK bacteria or other fastidious organisms in our extensive, multi-center experience. For suspected cases of culture-negative endocarditis owing to more unusual microorganisms, dialogue with the microbiology laboratory is essential to optimize the use of nonculture-based detection methods (e.g., serology or nucleic acid amplification) or special media to improve the detection of select pathogens (e.g., Legionella spp., Bartonella spp.) from blood cultures (2). HACEK bacteria can be recovered well from currently available automated blood culture systems and media within the standard incubation period of 5 days.

We thank the staff of the Clinical Microbiology Laboratories at ARUP Laboratories, Duke University Medical Center, Johns Hopkins Hospital, and Robert Wood Johnson University Hospital.

REFERENCES


### Table 1. HACEK bacteria isolated from routine blood culture by study site

<table>
<thead>
<tr>
<th>Study site(s)</th>
<th>Total no. of positive blood cultures</th>
<th>HACEK bacteria isolated (no. of cultures)</th>
<th>Time to detection in days (no. of cultures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARUP</td>
<td>2,301</td>
<td>Haemophilus parainfluenzae (1)</td>
<td>4</td>
</tr>
<tr>
<td>DUMC</td>
<td>48,921</td>
<td>Eikenella corrodens (3)</td>
<td>1, 3 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kingella kingae (1)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemophilus spp. (5)</td>
<td>2 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemophilus aphrophilus (1)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemophilus haemolyticus (1)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemophilus parainfluenzae (15)</td>
<td>2 (7), 3 (6), 7 (2)</td>
</tr>
<tr>
<td>JHH</td>
<td>6,519</td>
<td>Actinobacillus actinomycetemcomitans (1)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eikenella corrodens (1)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cardiobacterium hominis (2)</td>
<td>3 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemophilus parainfluenzae (3)</td>
<td>3, 4, 5</td>
</tr>
<tr>
<td>RWJ</td>
<td>1,462</td>
<td>Haemophilus parainfluenzae (1)</td>
<td>4</td>
</tr>
<tr>
<td>All</td>
<td>59,203</td>
<td>All (35)</td>
<td>3</td>
</tr>
</tbody>
</table>

* Includes January 2003 to December 2003 only.
* Value represents median and mean.


