Evaluation of Three Commercially Available Blood Culture Systems for Cultivation of Helicobacter pylori

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Helicobacter pylori is unable to grow in regular blood culture systems, including the BACTEC (Johnston Laboratories), Septi-Chek (Hoffmann-La Roche), and Bacto (Difco) systems. We tested three blood culture systems used for fastidious organisms: brucella broth with SPS and CO₂ (Becton Dickinson), biphasic brain heart infusion agar or broth (Becton Dickinson), and supplemented peptone broth (Vacutainer). Blood culture bottles were inoculated with H. pylori and human blood and were then incubated by routine diagnostic laboratory procedures. All three blood culture systems were able to sustain the growth of H. pylori, but brucella broth had the highest CFU per milliliter after 72 h. We conclude that a diagnostic laboratory should be able to detect H. pylori bacteremia in a majority of cases by using brucella blood culture bottles.

Helicobacter pylori is a fastidious organism which has been associated with chronic antral gastritis (1, 5, 11), peptic ulceration (2), and gastric carcinoma (6, 7). It is not known if H. pylori can spread and cause disease outside the gastrointestinal tract. There are no reports of bacteremia with H. pylori, despite attempts to isolate it from clinically septic patients (3). This may have to do with the choice of blood culture system. In a previous study (10), we showed that three common blood culture systems, BACTEC (Johnston Laboratories, Inc., Towlon, Md.), Septi-Chek (Hoffmann-La Roche, Inc., Nutley, N.J.), and Bacto (Difco Laboratories, Detroit, Mich.), failed to support the growth of H. pylori. Since the organism can be grown in liquid medium containing brucella broth (9), we wanted to investigate if blood culture systems suitable for growing Brucella and other fastidious organisms could also sustain the growth of H. pylori. Specifically, we wanted to know if H. pylori could be detected in a diagnostic laboratory setting by using commercially available blood culture systems.

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We simulated clinical bacteremia by simultaneously infecting human blood and actively growing H. pylori into three types of blood culture bottles: (i) brucella broth with CO₂ plus SPS (sodium polyanetholsulfonate) (Becton Dickinson Microbiology Systems, Cockeysville, Md.), (ii) biphasic brain heart infusion (BHI) agar or broth (Castaneda bottles; Becton-Dickinson), or (iii) supplemented peptone (SP) broth (Vacutainer Systems; Becton Dickinson). Ten strains of H. pylori from four different geographic regions (Australia, Peru, West Virginia, and Missouri) were used. Control organisms (Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923) were used for all blood culture systems. All isolates had been stored at -70°C and were thawed 3 to 4 days before inoculation. The strains were first plated on fresh H. pylori agars (89% brucella agar, 10% defibrinated calf blood plus 1% IsoVitalex) and were incubated in a microaerobic atmosphere (CampyPak; BBL Microbiology Systems). Control strains were plated onto sheep blood and chocolate agar plates (Remel, Lenexa, Kans.) 1 to 2 days before inoculation. Bacterial inocula were prepared by suspending a few colonies in phosphate-buffered saline to a density of 1.0 McFarland standard. Tenfold dilutions were made, and 500 μl of a 10⁻³ dilution was inoculated into each flask. The size of each inoculum was determined by colony counts, in duplicate, and the colony counts were averaged. Each blood culture bottle was also inoculated with 5 ml of fresh human blood from one of seven healthy volunteers who had been determined to be H. pylori antibody negative. The mean CFU count in each 50-ml bottle immediately after inoculation was 10.0 CFU/ml, corresponding to a bacterial load of 50 CFU/ml of blood if 10 ml is collected from each patient and 100 CFU/ml if 5 ml of blood is drawn. This is slightly above the average CFU count seen in many patients with clinical bacteremia, but it is in the same range as in a previous blood culture study (10) and was intentionally selected so that a direct comparison between the media could be made.

To mimic the handling of the blood culture bottles in a diagnostic microbiology laboratory, the blood culture bottles were ventilated according to the manufacturers' instructions and were incubated under aerobic conditions at 37°C, high humidity, and 6% CO₂. After inoculation, each bottle was routinely subcultured on days 1, 2, 3, 7, and 14. One hundred microliters of broth was plated onto commercially available chocolate and 5% sheep blood agars (Remel). Growth was recorded as positive if at least one colony of H. pylori was detected on any of the agars. Simultaneous plating on our own H. pylori agar served as a control. H. pylori strains were identified by morphology, and the identities were confirmed by positive urease, catalase, and oxidase tests (9); control strains were identified by colony morphology, and the identities were confirmed by Gram staining, showing either gram-positive cocci in clusters or gram-negative rods.

To determine the actual growth in each blood culture bottle, we also performed daily colony counts for the first 72 h. These were done on our own H. pylori plates (described above), which were incubated in CampyPak jars, and the results are expressed as CFU per milliliter of broth. Geometric means for each time point were calculated by using Statistgraphics 3.0 software (Graphics Software Systems, Inc., Rockville, Md.).
We found that all three blood culture systems were able to sustain the growth of *H. pylori*. On the first day after inoculation, the growth curves were very similar. By day 2, growth was more rapid in the brucella and SP broth bottles compared with that in the BHI agar or broth bottles (Fig. 1). On the third day, the brucella bottles outperformed both the SP broth and BHI agar or broth bottles. Control organisms showed good growth in all culture systems. The growth of only control organisms was visible on the agar slant in the biphasic BHI agar or broth bottles.

Most of the subcultures onto commercially available agars showed some growth. During the first 3 days after inoculation, the blood agar plates had positive growth more often than the chocolate agars. By day 7, more than 70% of the plates showed growth regardless of the type of medium used (Table 1). Four blood cultures were likely to have been misdiagnosed as negative if this had been a true clinical situation. One each grown in SP broth and BHI broth still remained negative by day 14, while growth was simultaneously verified on our own *H. pylori* medium. One brucella blood culture was positive only once (two colonies by day 2 on the blood agar), and a fourth culture grown in a BHI bottle remained negative on all plates until day 14.

The experiment described here showed that *H. pylori* can grow in commercially available blood culture systems for fastidious organisms: brucella broth with CO₂ and SPS, biphasic BHI agar or broth, and SP broth. Of these systems, brucella broth seemed to be superior to the others. Comparing the growth of *H. pylori* when subcultured onto blood agar plates and chocolate agar plates, growth was usually seen earlier on the blood agar plates. It is possible that this was a selection induced by growing the organism in the presence of blood in the broth. Another possible explanation is that the *Helicobacter* strains used in the present experiment were better adapted to growth on blood agar plates since they had originally been isolated on blood-containing media. In the biphasic BHI agar or broth bottles, growth was primarily found in the liquid phase. No visible colonies were seen on the agar slant, which is consistent with the poor growth that *H. pylori* exhibits on BHI agar without any additives.

We conclude that bacteremia with *H. pylori* could be detected in a diagnostic microbiology laboratory by using brucella blood culture bottles. Those clinical situations in which it may be appropriate to test for this include culture-negative endocarditis, nosocomial pneumonia or sepsis in patients on ventilators given acid-reducing ulcer prophylaxis, and unexplained fever following gastroduodenoscopy with biopsies, recurrent peptic ulcer disease, or gastric cancer. Anecdotal reports of *H. pylori* in patients with purulent peritonitis (8) and in respiratory secretions following aspiration pneumonia (4) suggest that it could act as a pathogen outside the stomach under certain circumstances. Whether *H. pylori* can cause bacteremia is not known at present, but the judicious use of brucella blood culture bottles in the proper clinical setting may answer this question in the future.

REFERENCES


TABLE 1. Percentage of plates showing growth of *H. pylori* when cultivated in brucella bottles, biphasic BHI bottles, or SP broth and subcultured onto 5% sheep blood agar and chocolate agar

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<th>Day</th>
<th>Brucella</th>
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<th>SP</th>
<th>SBA</th>
<th>CA</th>
<th>SBA</th>
<th>CA</th>
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</table>

* For brucella bottles, n = 10; for biphasic BHI bottles, n = 10; for SP broth, n = 10. SBA, sheep blood agar; CA, chocolate agar.

b One plate was discarded because of fungal overgrowth (n = 9).