In-Use Evaluation of a Commercially Available Set of Quality Control Cultures

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Increasing awareness of the need for a uniform quality control program prompted an evaluation of a commercially available set (Bact-Chek) of eight organisms. A protocol was designed in which this set of control cultures was tested simultaneously in the clinical microbiology laboratory of a 400-bed hospital (the Berkshire Medical Center) and in the reference laboratories of the Bacteriology Section of the Center for Disease Control. The results indicate that the Bact-Chek organisms are essentially as advertised: they constitute a basic set of cultures for a quality control program in clinical microbiology. Ninety percent of the media and reagents (excluding mycobacterial media and reagents) in the clinical laboratory were checked with this set of eight cultures. Additional cultures not in the set were used to check the remaining 10% of the media and reagents.

The increased emphasis on the need for quality control programs in clinical laboratories has prompted the publication of manuals (2, 9) and reports (3, 4, 6, 7, 12-14) on the establishment of such programs in clinical microbiology laboratories. Until now, each of these programs depended upon the maintenance of stock cultures obtained from clinical specimens, purchased from commercial sources, or obtained from another laboratory. Solid media transfer and lyophilization are the two most widely used methods for maintaining stock cultures. Both methods have the inherent disadvantages of mutation or contamination, or both. Aerosolization of viable organisms (10, 11) from lyophilized cultures and the need to use the reconstituted material within one working day are additional disadvantages. Furthermore, lyophilization of cultures requires expensive equipment, which occupies space that can be better used, and the procedure also requires the time of technical personnel which is already at a premium.

A set of eight different bacterial control cultures called Bact-Chek (Roche Diagnostics, Division of Hoffman-La Roche, Inc., Nutley, N.J.) has recently been made commercially available. It promises to alleviate the necessity of maintaining a number of stock cultures of bacteria. Before and immediately after Bact-Chek became commercially available, we had the opportunity to evaluate the set. The cultures are stabilized in the form of dehydrated discs. In use, they represent an inoculum which is inexpensive enough to be discarded after a single day's use, thereby eliminating the hazard of mutation or contamination as well as the inconvenience of subculture and storage of stock strains. A full-range quality control program using these discs was designed and implemented by the clinical laboratories of the Berkshire Medical Center, a 400-bed general hospital in Pittsfield, Mass. The Bacteriology Section, Center for Disease Control (CDC Atlanta, Ga., acted as a reference laboratory and assessed the Bact-Chek organisms for morphological, biochemical, and serological characteristics, as well as purity, stability, and conformity to the manufacturer's brochure.

MATERIALS AND METHODS

The Bact-Chek disc set contains eight different organisms; there are 30 discs of each organism in glass screw-capped vials containing a desiccant packet. Early lots maintained at room temperature showed a loss of viability after approximately 3 months. For this reason, the discs were stored at 2 to 6 C throughout this study. The organisms reported upon in this paper were stored at refrigerator temperature (2 to 6 C) upon receipt from the manufacturer and when not in use. A description of the set of quality control cultures is given in Table 1.
All of the media used at the Berkshire Medical Center were purchased through a single supplier, BBL. Media used by the CDC were prepared in its central media kitchen from commercially obtained dehydrated media. All reagents were laboratory-prepared according to accepted formulas (1, 5, 8).

As the reference laboratory, the CDC examined randomly selected discs for purity, identity, viable organisms, and growth characteristics. In addition, the CDC analyzed each strain for morphological, biochemical, and, where indicated, serological characteristics. Methods and criteria used to establish organism identity and strain characteristics were those recommended by the American Society for Microbiology (5) and the American Public Health Association (8), and those routinely employed by the Center for Disease Control. Several discs of each strain were individually inoculated into tubes containing 2 ml of heart infusion broth (HIB) to determine the viability and stability of the disc. Each tube was shaken for 5 min, and samples of the suspension were transferred to Trypticase soy agar (TSA) plates (5% sheep blood was added for Staphylococcus pyogenes). These were incubated aerobically at 35 C overnight. After this incubation period, colony counts were performed. In addition, discs were placed into 2 ml of HIB and incubated for 2, 4, and 24 hr at 35 C. The resulting broth cultures were plated on TSA for colony counts. Tubes of HIB with discs which yielded no growth at 24 hr were reincubated for 48 and 72 hr before the broth was plated.

The cultures were verified by placing a disc in 2 ml of HIB, incubating for 18 to 24 hr, and then diluting with 3 volumes of HIB; this suspension was inoculated into tubes of various biochemical testing media, which were incubated for a maximum of 7 days. In another set of the various media, a single disc was added directly, as specified in the manufacturer's brochure.

Drug susceptibility tests were performed as described by the manufacturer, as well as by a standardized Kirby-Bauer procedure used routinely at the CDC.

The hospital clinical laboratory (Berkshire Medical Center) evaluated the discs primarily for their applicability in its microbiology quality control program. The microbiologist in charge of quality control or a medical technology student rotating through the quality control program set up the eight Bact-Chek discs and other organisms required to evaluate each new batch of prepared media as it arrived. Alcohol-flamed and cooled forceps were used to pick each disc from its container and transfer it to a tube containing 4 ml of trypticase soy broth. The inoculated tubes were incubated aerobically at 35 C; a loopful of the growth was removed after 2, 4, and 18 hr to be plated onto sheep blood agar as a check for purity, amount of growth, and colony appearance. For the first 2 months of the study, the clinical pathologist, the quality control microbiologist, and a student each examined a set of discs using the same procedure to determine the degree of experience necessary to manipulate the discs. Since the discs stored at 2 to 6 C consistently yielded good growth after 4 hr of broth incubation, the microbiologist used these broth cultures to inoculate both tube and plate media and to control staining reactions and the coagulase tests. Tables 2 and 3 list these tests and the controlling organisms.

### RESULTS

Organisms from the Bact-Chek set gave consistent morphological biochemical, and serological results at both the reference and hospital laboratories. These characteristics are outlined in Tables 4 and 5. All discs in pre-marketed lots yielded pure cultures, and no vials were mislabeled. In the hospital laboratory, there was no evidence of cross-contamination with the alcohol-soaked, flamed forceps technique for handling the discs. The medical technology students learned the technique rapidly. The organisms grew well and consistently. All broths were turbid after 2 hr of incubation; S. pyogenes showed the slowest growth. However, even after 4 to 6 hr of incubation the growth of S. pyogenes was not adequate for susceptibility testing. Three broth cultures of each strain were plated for colony counts monthly. Average counts from each group of three cultures are presented in Table 6. Hospital laboratory results with 4-hr broth incubation of 4-month-old discs were good. The growth from this broth was heavy enough that it always yielded confluent growth in the primary streak when plated out.

### DISCUSSION

Our results indicate that Bact-Chek organisms are essentially as advertised; they provide a good basic set of cultures to use in microbiology quality control programs. At the Berkshire Medical Center, the quality control set provides appropriate characteristics to control the quality of over 90% of the media and reagents used in the laboratory for non-mycobacterial, aerobic bacteriology. Additional cultures not in the set are required to
### Table 2. Media controlled with Bact-Chek organisms

| Medium            | Positive control | Negative control |
|-------------------|------------------|------------------|
| Blood agar, sheep | S. pyogenes      | E. coli          |
| Hektoen Enteric  | Mixture of S.    |                  |
| agar              | pyogenes and S.  |                  |
| MacConkey agar    | E. coli and S.   |                  |
| Trypticase       | epidermidis      |                  |
| Soy agar          |                   |                  |
| Carbohydrates,   |                   |                  |
| phenol red broth  |                   |                  |
| base              |                   |                  |
| Adonitol         | P. aeruginosa    |                  |
| Dulcitol         | E. coli          |                  |
| Inositol         | P. aeruginosa    |                  |
| Maltose          | P. aeruginosa    |                  |
| All other         | E. coli          |                  |
| Citrate, Simmon's| E. coli          |                  |
| Decarboxylases    | P. vulgaris      |                  |
| Enterococcal      | Salmonella       |                  |
| media             | (Klebsiella)     |                  |
| (bile-esculin, SE| E. coli          |                  |
| agar, etc.)       | E. coli          |                  |
| Gelatin           | E. cloacae       |                  |
| KCN broth         | P. pyogenes      |                  |
| Lysine iron agar  | Salmonella/P.    |                  |
| Motility          | vulgaris         |                  |
| MR-VP broth       | E. coli          |                  |
| Nitrate agar or   | E. coli / E.     |                  |
| broth             | cloacae          |                  |
| Phenylalanine     | P. vulgaris      |                  |
| agar              | Salmonella       |                  |
| Selenite broth    | E. coli /        |                  |
| SIM               | Salmonella       |                  |
| Todd Hewitt       | Salmonella       |                  |
| broth             | (Shigella)       |                  |
| Triple sugar iron| S. pyogenes      |                  |
| agar              | Salmonella / E.  |                  |
| Trypticase       | P. aeruginosa    |                  |
| Soy agar          | E. coli          |                  |
| (Christensen)     |                   |                  |

*Media not listed, such as chocolate agar, were controlled by organisms not in the Bact-Chek set.

Organisms in parentheses were not in Bact-Chek set.

Zero = negative control not indicated.

check the quality of the remaining 10% of media and reagents. These additional cultures include the recommended ATCC strains of *Staphylococcus aureus* and *Escherichia coli* for antimicrobial susceptibility testing and a strain each of *Streptococcus viridans*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *N. meningitidis*, *Streptococcus pneumoniae*, and *S. faecalis*.

The disc form of preserved cultures offers a number of advantages over lyophilized material. The discs are much easier to manipulate, are less hazardous to use, and take less time to reconstitute. There is no risk of cross-contamination when reasonable care is taken. Bact-Chek discs are conveniently stored in their vials at refrigerator temperature, and retrieval is very simple.

The characteristics of each organism remained stable during the 6-month shelf life recommended by the manufacturer, and those characteristics used in the quality control program were typical for each strain. There may be some objection to the lack of motility of the *Proteus vulgaris* and *Pseudomonas aeruginosa*, but, because the motility of neither organism is being used as a control characteristic, it is not a problem in these instances. At the CDC, several biochemical reactions differed from those listed by the manufacturer. *S. pyogenes* showed a positive celllobiose fermentation in 2 days; *Salmonella typhimurium* failed to ferment glycerol in 7 days but did utilize gluconate in 2 days. *E. coli* did not show arginine dihydrolase activity after 7 days. *Enterobacter cloacae* gave a positive test for gluconate utilization in 2 days, and the *S. aureus* gave a weakly positive reaction for sodium hippurate hydrolysis. These discrepancies do not detract from the intended use of these control cultures;

### Table 3. Reagents and stains controlled with Bact-Chek organisms

| Reagent* | Positive control | Negative control |
|----------|------------------|------------------|
| Bacitracin discs | *S. pyogenes* | (Alpha - hemolytic streptococcus)* |
| Coagulate plasma | *S. aureus* | |
| Ferric chloride, 10% | *P. vulgaris* | |
| Hydrogen peroxide, 3% | *S. aureus* | |
| Kovac's | *E. coli* | *S. pyogenes* |
| Nitrates reagents | *E. coli* | *E. cloacae* |
| Oxidase reagent | *P. aeruginosa* | *S. pyogenes* |
| Gram stain | *S. aureus* | *E. coli* |

*Media not listed, such as chocolate agar, were controlled by organisms not in the Bact-Chek set.

Organisms in parentheses were not in Bact-Chek set.

Zero = negative control not indicated.

*Reagents not listed were controlled by organisms not in the Bact-Chek set.

*Not in Bact-Chek set.
however, if these discs are used for purposes other than those advocated, proper care and controls must be used for each new application.

From our experience with premarketed and commercially available lots of Bact-Chek, we recommend (i) that the discs be stored at 4 to 8°C in the original vials over the drying agent, (ii) that the discs be incubated at least 4 hr in broth before being used to inoculate any further media or for other test procedures, and (iii) that each new vial of discs be checked for strain identity and purity before being incorporated into the quality control program. Contamination of these discs either by the manufacturer or by repeated entry by the user can occur, and the user must always exercise caution and good judgement with these or any other quality control cultures.

The CDC laboratory checked the antimicrobial susceptibility pattern of each Bact-Chek strain but does not recommend using these organisms for quality control of antimicrobial susceptibility tests because of the recent National Committee for Clinical Laboratory Standards designation of E. coli ATCC 25922 and S. aureus ATCC 25923 as “standard” control strains for these procedures.

The manufacturer states that liquid media
may be inoculated directly with a Bact-Chek disc; it soon became obvious to us that this would be a very expensive procedure. For this reason, we elected to inoculate numerous media from a 4-hr broth culture (Berkshire Medical Center laboratory) which had been inoculated with only one disc. When the latter approach was used, one set of Bact-Chek discs provided the Berkshire Medical Center laboratory with an adequate supply of quality control cultures for the shelf life of the set.

**TABLE 5. Bacteriological test results of gram-positive organisms in Bact-Chek set**

| Bacteriological test | *Staphylococcus aureus* | *Staphylococcus epidermidis* | *Streptococcus pyogenes* |
|----------------------|-------------------------|-----------------------------|-------------------------|
| Hemolysis (sheep blood) | B                      | B                           | B                       |
| Bacitracin disc       | -                      | -                           | +                       |
| Sodium hippurate      | +                      | +                           | -                       |
| Growth, 6.5% NaCl broth | ND                   | ND                         | ND                      |
| Growth at 45 °C       | ND                     | ND                          | ND                      |
| Coagulase             | +                      | +                           | -                       |
| Catalase              | -                      | -                           | -                       |
| Deoxyribonuclease     | +                      | +                           | +                       |
| Glucose (anaerobic)   | +                      | +                           | +                       |
| Mannitol (anaerobic)  | (+)2                    | -                           | -                       |
| Glucose oxidation/fermentation | F            | F                           | F                       |
| Gas from glucose      | -                      | -                           | -                       |
| Malonate              | -                      | -                           | -                       |
| Adonitol              | -                      | -                           | -                       |
| Arabinose             | -                      | -                           | -                       |
| Dulcitol              | -                      | -                           | -                       |
| Maltose               | +                      | +                           | +                       |
| Raffinose             | -                      | -                           | -                       |
| Salicin               | -                      | -                           | (+)4                    |
| Sorbitol              | -                      | -                           | -                       |
| Sucrose               | +                      | (+)4                        | -                       |
| Lactose               | (+)4                    | (+)4                        | (+)4                    |
| Rhamnose              | -                      | -                           | -                       |
| Trehalose             | +                      | +                           | (+)4                    |
| Xylose                | -                      | -                           | -                       |
| Cellobiose            | -                      | -                           | (+)12                   |
| Nitrate               | +                      | +                           | +                       |

* B, beta; +, positive; -, negative; W+, weak positive; (+) delayed positive with days of incubation; ND, not done.

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**Table 6. Colony counts of Bact-Chek discs after 2 hr of broth incubation to demonstrate 6-month stability**

| Organism     | January | February | March | April | May  | June |
|--------------|---------|----------|-------|-------|------|------|
| *E. cloacae*  | 66 × 10^4 | 110 × 10^4 | 18 × 10^4 | 21 × 10^4 | 70 × 10^4 | 26 × 10^4 |
| *E. coli*     | 4 × 10^4  | 4 × 10^4  | 3 × 10^4  | 4 × 10^4  | 10 × 10^4 | 2 × 10^4  |
| *P. vulgaris* | 58 × 10^4 | 53 × 10^4 | 43 × 10^4 | 11 × 10^4 | 74 × 10^4 | 9 × 10^4  |
| *P. aeruginosa* | 24 × 10^4 | 40 × 10^4 | 34 × 10^4 | 28 × 10^4 | 27 × 10^4 | 6 × 10^4  |
| *S. typhimurium* | 153 × 10^4 | 138 × 10^4 | 105 × 10^4 | 42 × 10^4 | 40 × 10^4 | 45 × 10^4 |
| *S. aureus*   | 6 × 10^4  | 4 × 10^4  | 3 × 10^4  | 4 × 10^4  | 10 × 10^4 | 2 × 10^4  |
| *S. epidermidis* | 5 × 10^4  | 9 × 10^4  | 8 × 10^4  | 5 × 10^4  | 7 × 10^4  | 2 × 10^4  |
| *S. pyogenes* | 2 × 10^4  | 2 × 10^4  | 8.6 × 10^4 | 31.3 × 10^4 | 2 × 10^4  | 2 × 10^4  |

* Represents a dilution of 10⁴; no growth obtained on three plates at 10⁴.