A Research Note

Performance of a DNA Probe-Based *Salmonella* Test in the AACC Check Sample Program

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ABSTRACT

The performance of the GENE-TRAK® *Salmonella* assay in food as been evaluated with samples of flour or flour based bakery mix provided by the American Association of Cereal Chemists (AACC) check sample program. Six pairs of coded samples were tested from November 1986 through September 1987. The test correctly identified all six positive and six negative samples. This included a lactose positive *Salmonella* strain that was missed by a large majority of the other laboratories that participated in the survey. These results indicate that DNA probe technology can provide not only more timely results than traditional methods, but in some cases can actually provide more accurate information.

Food microbiology continues to play a critical role in the prevention of human disease by foodborne pathogens. Large outbreaks of food associated human clinical illness (1,10) have also resulted in well publicized economic losses. Classical techniques have served the food industry well over the last decade and will continue to do so for many years to come. Recently, however, DNA probes have been applied to problems in food microbiology (2,3,4,5). These probe assays offer a number of advantages including rapid availability of results, quantitative criteria for assessment of results, and greater test specificity.

We recently concluded a year-long study of the performance of our GENE-TRAK DNA probe *Salmonella* assay with samples provided by the American Association of Cereal Chemists (AACC) check sample program (AACC Check Sample Service, 3340 Pilot Knob Road, St. Paul, MN 55121). Two coded samples are sent to participants in this service every 60 days for analysis of several microbial pathogens. The results from these samples are reported to the service. About 45 days later the final results are reported to all participants. To ensure confidentiality, these results are reported by individual code number. Thus, the participant can review results from his or her laboratory in complete privacy against those obtained by other anonymous participants and make improvements as necessary.

METHODS AND MATERIALS

A detailed description of the GENE-TRAK DNA probe method has been published elsewhere (6), but briefly, the sample is initially culturally enriched by the conventional BAM procedure for *Salmonella* (7). This includes an overnight pre-enrichment in lactose broth, selective enrichments in selenite cystine and tetrathionate brilliant green broths and a post enrichment in GN broth for a total of 44 h. At this point 1-mL samples of each post enrichment broth are combined in 25-mm filter cups supplied as part of the GENE-TRAK *Salmonella* test kit. These cups are mounted on a vacuum filter manifold that is connected to a vacuum source of 8-10 inches of Hg. After filtration, the filters are treated sequentially with three solutions which are also supplied as part of the kit. The first solution disrupts the bacterial cell wall and renders the target DNA single stranded. The second solution neutralizes the first solution and the third solution fixes the DNA to the filters. The filter cups are then snapped apart and up to 28 filters placed in a 50 mL screw-capped centrifuge tube. A prehybridization solution is added to the filters to block any unoccupied sites on the filters. After this prehybridization solution is decanted, the P-32 labelled probe is added and incubated at 65°C in a water bath for 2 h. During this incubation, the probe molecules form hybrids or double stranded helices of with *Salmonella* DNA, if present. DNA from other bacteria, even very closely related species such as *Citrobacter* and other Enterobacteriaceae, do not react with these probes at 65°C. After the incubation with probe, the filters are washed in buffer six times to remove any probe that did not form a stable hybrid. These washes are also done at 65°C to increase the stringency of hybridization, which also decreases reactivity from closely related organisms. After decanting the wash solution, the filters are air dried briefly and the radioactivity quantitated either in a scintillation counter or in a compact beta counter, also supplied by GENE-TRAK Systems.

Controls are also supplied as part of the kit. The positive control is a suspension of heat killed *Salmonella typhimurium* and the negative control is a suspension of heat killed *Escher-
TABLE 1. DNA probe Salmonella test data summary.

<table>
<thead>
<tr>
<th>Assay date</th>
<th>Result</th>
<th>Sample A Identification</th>
<th>Result</th>
<th>Sample B Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/6/86</td>
<td>Positive</td>
<td>K:z4</td>
<td>Negative</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>1/15/87</td>
<td>Negative</td>
<td>Uninoculated</td>
<td>Positive</td>
<td>l:d</td>
</tr>
<tr>
<td>3/12/87</td>
<td>Positive</td>
<td>Lactose +, C1:z9</td>
<td>Positive</td>
<td>N:G complex</td>
</tr>
<tr>
<td>5/19/87</td>
<td>Positive</td>
<td>B:1 complex</td>
<td>Negative</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>7/6/87</td>
<td>Negative</td>
<td>Uninoculated</td>
<td>Positive</td>
<td>E:1 complex</td>
</tr>
<tr>
<td>9/10/87</td>
<td>Positive</td>
<td>E:Factor 19</td>
<td>Negative</td>
<td>Uninoculated</td>
</tr>
</tbody>
</table>

Note: The atypical Salmonella in Sample A assayed on 3/12/87 was missed by a majority of the participants in the AACC Check Sample service.

Three negative and one positive control are run with each manifold. Results are calculated with reference to the negative controls. The three negatives are averaged and a constant of 500 cpm is added to yield a cutoff value. Any sample having a count that is higher than the cutoff is considered positive for Salmonella and any sample which has a count lower than the cutoff is considered negative. The positive control typically gives counts that are between 5000 cpm and 45000 cpm and is used to verify that the procedure was performed correctly. A typical operator can comfortably do 144 samples per day with this procedure.

RESULTS

The data from our study appears as Table 1. They clearly indicate that the method can detect a wide variety of Salmonella species including an atypical lactose-positive strain that was missed by the majority of the participants in the check sample service. No false-positive results were obtained in this study, although conventional microbiology testing indicated the presence of large numbers of competitor organisms in the uninoculated samples. Results were obtained in less than three days after the start of the assay, thus saving several days in comparison to conventional methods.

The results of this study indicate the value of two recent innovations in food microbiology: quality control reference systems such as the AACC check sample service, and the utility of DNA probes in testing for foodborne pathogens. We have recently introduced an assay for Listeria (8,9) which follows a procedure that is almost identical to that described for the Salmonella test. Others are under development. We hope that other professional societies will follow the lead of the American Association of Cereal Chemists in providing blinded test samples of interest to their members so that they can assess performance in a controlled and confidential environment. Such information can be invaluable in identifying deficiencies in analytical procedures before they cause serious problems in actual samples.

REFERENCES