Research Note

Pooling of Noncollaborative Multilaboratory Data for Evaluation of the Use of DNA Probe Test Kits in Identifying Listeria monocytogenes Strains

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ABSTRACT

The Accuprobe test kit, a DNA probe culture confirmation test kit for identifying bacterial isolates as Listeria monocytogenes was evaluated with 148 food-borne Listeria isolates. The identities of the isolates were confirmed by conventional tests. A subset of the Listeria isolates was also used to evaluate the Gene-Trak Listeria monocytogenes isolation kit. The data obtained with each kit were pooled with data for that kit from independent published studies. Interlaboratory performances were then estimated statistically, to achieve the main features of orthodox interlaboratory collaborative studies. The large number of strains required in this noncollaborative evaluation method advantageously provided a more representative sampling of the potential isolates that may be found in practice. Exact comparability between the two kit studies was not possible nor is it a feature of orthodox collaborative identification kit studies. However, the kits apparently performed equivalently given the different statistical confidences dictated by the study designs.

Key words: Listeria ID test kits

U.S. Food and Drug Administration (FDA) microbiological analysts use the methods published in their manual (9) for regulatory analyses. When feasible, the FDA prefers to use methods that have had their performance verified by methods that have had their performance verified by a common set of samples.

For the food-borne pathogen Listeria monocytogenes a variety of commercial test kits enable putative Listeria isolates to be identified more rapidly or easily than with conventional microbiological tests. The performances of these kits have been validated to different extents. For example, two biochemical identification kits have been collaboratively validated (11, 13). Two DNA probe kits have been collaboratively validated for detection use with different matrices (5, 14) with only in-house validation of their intrinsic identification performances. Two other DNA probe test kits, which can identify isolates as L. monocytogenes in less than 4 h, the AccuProbe® Listeria monocytogenes Culture Confirmation Test (1) and the Gene-Trak Listeria monocytogenes Assay DNA Hybridization Test (10) have not been collaboratively validated. However, the AccuProbe test kit has been extensively studied noncollaboratively (1,2,6,7,12,15,16). The availability of all these data makes using them for validation an attractive proposition, especially since they cover a more comprehensive array of bacterial strains than the 50 to 100 strains used in a conventional multilaboratory collaborative study.

The aim of this study was to gather more performance data, especially for the Gene-Trak test kit, about a substantial set of regulatory food-borne Listeria isolates and to pool this new data with published data in order to show that data from noncollaborative multilaboratory studies generates results effectively equivalent to those obtained by collaborative laboratory study of a small common sample set of isolates.

MATERIALS AND METHODS

Microorganisms

One hundred and forty-eight food isolates of Listeria monocytogenes, L. seeligeri, L. welshimeri, L. ivanovii, L. innocua, L. grayi, and L. murrayi were obtained (see Acknowledgments). They were maintained on tryptose phosphate agar slants at 4°C. Isolate purity was verified by streaking on Trypticase soy agar with 0.6% (wt/vol) yeast extract. Strains were inoculated into Trypticase soy broth with 0.6% (wt/vol) yeast extract and incubated at 30°C for 18 to 24 h for confirmation of their identities by conventional tests (9). Hybridization assays were performed with samples of cultures grown in tryptose phosphate broth at 30°C for 18 to 24 h. Powdered media were obtained from Difco Laboratories, Detroit, MI.
Test kits

The AccuProbe Listeria monocytogenes culture confirmation test (Gen-Probe Inc., San Diego, CA) (1,2) was used to determine whether isolates were or were not L. monocytogenes. The kit’s proprietary nonisotopically tagged DNA probe is specific for a target in the 16S r-RNA (12,15,17). The kit was used according to the manufacturer’s instructions for broth cultures.

The Gene-Trak Listeria monocytogenes Assay DNA Hybridization Test kit (Gene-Trak Systems, Framingham, MA) (10) was also used to determine whether isolates were or were not L. monocytogenes. The kit’s probe is specific for a target in r-RNA, presumably the 16S r-RNA (10). Broth culture samples were tested according to the manufacturer’s instructions.

Experimental design

The test kit identities of the isolates were compared with their conventionally determined and confirmed identities. The study data for a test kit were pooled with data published by other laboratories. The data pool for the test kit represents a noncollaborative multilaboratory study of different isolate sets. The number of cases of particular isolates being common to two or more laboratory studies was negligible, so a correction was not needed.

From the observed false-result rates, the maximum probable combined number of false-positive and -negative results expected if every laboratory had tested all the isolates in the pool was computed at the $P = 0.05$ significance level, using sampling statistics. In effect, this treated the pooled data as a sample of a collaborative interlaboratory study involving a potential, but unrealized, number of observations equal to the number of laboratories multiplied by the total number of pooled isolates. Separate probable false-positive and -negative rates were calculated from the appropriate isolate subpools representing inclusivity and exclusivity isolate panels relevant to the test kits.

Comparison of the two test kits’ performances was not an intended goal of this study. In any case, different numbers and types of strains were tested with them in this and the other studies considered. Nevertheless, in this study, the Listeria strains used with the least examined kit were a substantial sample of those used with the most examined kit. Thus, the former kit’s performance was assessed relative to the latter’s by using sampling statistics.

RESULTS

This study considered 148 Listeria isolates, mainly from foods regulated by the Food and Drug Administration. The AccuProbe test kit (1) is intended for identification only. The Gene-Trak kit is intended for detection in selective enrichment cultures (10), but in this study it was used to identify pure cultures in nonselective broth.

All isolate cultures were confirmed as pure and their nominal identities were generally confirmable. A few discrepancies were strains of non-L. monocytogenes species which were identified as species of Listeria different from the nominal identities. These remained discrepant upon reidentification but were never identified as L. monocytogenes. Thus, the confirmed identities of strains in this study’s Listeria collection consisted of 76 strains of L. monocytogenes and 72 strains of other species of Listeria.

The observed AccuProbe identities of the Listeria coincided with the conventional identities at the classification level of L. monocytogenes or not L. monocytogenes, as shown in Table 1. Thus, the observed intragenus specificity and sensitivity rates were both 100%, there being no observed false results.

Of the 148 isolates, 112 were tested and were all correctly identified by the Gene-Trak test kit as L. monocytogenes (46 of 46) or not L. monocytogenes (66 of 66) (Table 1). The observed intragenus sensitivity and specificity rates were both 100%. At the 95% confidence level, the Gene-Trak kit’s probable false-positive (f+) rate was ≤2.8% and the false negative (f−) rate was ≤3.9%, relative to the AccuProbe kit. The relative combined false positive and negative (f±) rate was ≤1.4%. Due to the unequal numbers of strains tested with each kit, the apparent slight nonequivalence of the kits is probably not significant as is also implied by published data considered later.

The overall performance results from this study confirm those of other laboratories for the AccuProbe (1,2,6–8,12,14–16) and the Gene-Trak (10) test kits. A cumulative total of 1,556 strains of Listeria and other genera have been examined with the AccuProbe test kit by 10 laboratories including the present study. A total of 732 strains were L. monocytogenes. The remaining 828 strains were other species, including 143 non-Listeria strains. For the pool of strains, excluding one f+ result, unconfirmed by retesting (2), and two false results associated with a critical technique variation (7), the estimated f− rate was ≤0.38% and the estimated f+ rate was ≤0.34%. The estimated combined f± rate was ≤0.18%. The estimated specificity and sensitivity rates were 99.66 and 99.62%, respectively.

In a previous study of the GeneTrak L. monocytogenes test kit (10), 403 strains were tested. Pooling these data with those of the present study for a two-laboratory noncollaborative study of 515 strains (including 189 L. monocytogenes strains) yielded estimated f+, f−, and f± rates of ≤0.61, ≤1.06 and ≤0.39%, respectively. The estimated specificity and sensitivity rates were 99.39 and 99.94%, respectively.

That so many diverse strains, both Listeria and non-Listeria (including strains of 81 species from 52 non- 

<p>| TABLE 1. Comparison of the numbers of strains of Listeria species classified by conventional tests with the numbers reacting positive and negative by the AccuProbe and Gene-Trak Listeria monocytogenes tests |
|---------------------------------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Listeria species</th>
<th>AccuProbe test</th>
<th>Gene-Trak test</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. monocytogenes</td>
<td>76</td>
<td>46</td>
</tr>
<tr>
<td>L. seeligeri</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L. welshimeri</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>L. ivanovii</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>L. innocua</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>L. grayb</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>All species</td>
<td>76</td>
<td>46</td>
</tr>
</tbody>
</table>

* Only 112 of the set of 148 strains were examined with both the AccuProbe and Gene-Trak test kits.

" Identification to subspecies was not needed for this study.
Listeria genera), were correctly identified with the Accuprobe test kit by 10 laboratories, including the present study, would indicate that it is a reliable, rapid kit for identifying isolates as being *L. monocytogenes*. These cumulative study data were obtained by laboratories independently examining their own choices of conventionally identified isolates. The Gene-Trak DNA probe kit also performed well according to the pooled results of two laboratories that independently tested a combined total of 515 strains.

The design of the noncollaborative multilaboratory study method for identification kits described here could be formalized by making it prospective rather than retrospective and by choosing parameters to achieve consensus-established performance levels with optimally standardized numbers and varieties of strains. An appropriately minimal number of laboratories could agree to test a small panel of relevant strains, possibly furnished by a central source (4). This panel would control any bias due to a laboratory somehow producing unusual numbers of anomalous data. Each laboratory would also independently test a suitable larger number of strains of its own choosing. While only maximal estimates of interlaboratory reproducibility would be generated, there would be the advantage of their being based on a wider spectrum of isolates than that used in orthodox interlaboratory studies.

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**REFERENCES**


