Evaluation of an ELISA Test for Detection of Listeria spp.

B. NORRUNG, M. SOLVE, M. OVESEN, and N. SKOVGAARD*

Institute of Veterinary Microbiology, Royal Veterinary and Agricultural University, 13 Bulowsvej, 1870 Frederiksberg C, Denmark

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ABSTRACT

An ELISA test and two routine culture procedures have been compared in their ability to detect Listeria spp. in food products and swabs of pig tonsils. The culture procedures used were those recommended by the Food and Drug Administration (FDA) and United States Department of Agriculture (USDA). One hundred samples of minced beef, artificially inoculated with Listeria monocytogenes, together with 149 naturally contaminated samples of minced beef, pig tonsil, pig feed and soft, white-molded, blue-veined cheese were tested by the three methods.

The USDA procedure proved to be the most sensitive detection method when samples of artificially contaminated meat containing less than three colony forming units (CFU) of L. monocytogenes were examined. In samples where the L. monocytogenes count was above 3 CFU per gram, the ELISA test and the USDA-detection procedure proved to be equally sensitive. When naturally contaminated samples were examined, the sensitivity of the ELISA test was 92% and the specificity 80%. The detection limit of the ELISA test for demonstration of L. monocytogenes in pure cultures was found to be approximately 10⁶ CFU per ml. The corresponding detection limit of the culture procedure was calculated to be approximately 10⁴ CFU per ml.

Listeria monocytogenes is well established as a foodborne pathogen (3,8). Rapid and reliable tests for detection of L. monocytogenes in food are therefore desirable.

Current isolation and identification methods are laborious and time consuming. These methods include enrichment in liquid selective media followed by plating on selective agar, with further confirmation of suspect colonies by biochemical tests.

A rapid immunological diagnostic test for detection of Listeria spp. in foods has been developed (6). The test uses two monoclonal antibodies specific for Listeria spp. in an enzyme-linked immunosorbent assay (ELISA) format.

In this study two culture procedures [recommended by the USDA (2), and FDA (7)] were compared with the ELISA procedure, with respect to their ability to detect Listeria spp. in food.

MATERIALS AND METHODS

Samples

A total of 249 samples was examined for the presence of Listeria spp. The samples consisted of 100 artificially contaminated samples of minced meat and 149 naturally contaminated samples of minced beef, pig tonsils, pig feed and soft, white-molded, blue-veined cheese.

Artificially contaminated samples of minced beef

One hundred samples, each consisting of 25 g of minced beef, were irradiated with 6 kGy to eliminate the background contamination with Listeria spp. The effectiveness of irradiation was confirmed culturally. After irradiation a competitive microflora was added. This competitive microflora consisted of Pseudomonas spp., Aeromonas hydrophila, and Streptococcus faecium in quantities of 10⁶, 10⁵, and 10⁴, respectively, per gram of meat. These strains had been freshly isolated from nonirradiated minced beef. Ninety-one of the 100 samples were then inoculated with L. monocytogenes in quantities from 0.12 CFU to 67 CFU/g of meat calculated from the addition of the culture to 25 g. Nine samples were not inoculated with L. monocytogenes.

Preparation of cultures used for inoculation

A strain of L. monocytogenes T.31, isolated in our laboratory from a sample of dairy effluent, was used to inoculate the meat samples. The culture was grown overnight at 37°C in brain heart infusion broth, diluted in phosphate buffered saline, and 1 ml of an appropriate dilution was added to each sample of meat. The actual number of L. monocytogenes in the liquid broth was determined by viable counts in plate count agar incubated 24 h at 37°C.

Nonirradiated samples

A total of 149 samples, comprising 61 samples of minced beef, 51 swab samples from pig tonsils, 20 samples of pig feed including both dry feed concentrate and soaked dry feed, and 17 samples of soft, white-molded, blue-veined cheese, was investigated. The tonsil swab samples were collected from pigs on the slaughterlines at an abattoir by using dry cotton swabs which, after swabbing the tonsil cavity, were placed directly into 10 ml enrichment broth. The samples of minced meat, pig feed, and cheese were 25 g each.

Methods of detection

The methods used for isolation of Listeria spp. included an ELISA test and two different culture procedures. These procedures are outlined in Fig. 1 and 2.

Culture procedures

1. USDA method (2). A two-step enrichment procedure in Listeria broths I and II, respectively, followed by streaking on lithium chloride phenylethanol moxalactam agar (LPM agar) with further confirmation of suspect colonies by biochemical tests. Listeria broth I and Listeria broth II consist of nutrients as well as selective components. The difference between them is that Listeria broth I contains half as much of the selective substance acriflavine as Listeria broth II.
Figure 1. Procedure for detection of Listeria in samples of minced beef, pig feed, and pig tonsil swabs.

- 25 g sample 225 ml Listeria broth I stomaching for 1 min incubate 24 h/30°C
- 1 cotton swab in 10 ml Listeria broth I stomaching 24 h/30°C
- 0.1 ml from Listeria broth I in 10 ml Listeria broth II incubate 24 h, 30°C

Procedure:
- a) streaking on LPM agar
- b) 1 ml Listeria broth II 4.5 ml KOH for 1 min streaking on LPM agar
- 1 ml for ELISA procedure

Figure 2. Procedure for detection of Listeria in cheese samples (modified FDA procedure).

- 25 g sample 225 ml Listeria enrichment broth (LEB) + MOPS stomaching 2 min incubate 48 h/30°C
- a) streaking on MMA agar
- b) 1 ml LEB in 4.5 ml KOH for 1 min streaking on MMA agar
- 1 ml for ELISA procedure

2. FDA method (1). This method was slightly modified by addition of a buffer system (7) to the enrichment broth. The method includes a one-step enrichment procedure followed by streaking on modified McBride agar and confirmation of suspect colonies by biochemical tests. The FDA has recently substituted Oxford agar for modified McBride.

"Listeria-Tek" ELISA test system from Organon Teknika

The initial enrichment procedures used for the ELISA were performed in the same way as in the culture procedures in order to compare the results. According to the enrichment procedure in the manufacturer's instructions for the "Listeria-Tek" ELISA test system, dairy samples are tested using sample size of 25 g, while only 1 g of sample size is recommended when the samples are nondairy samples. In this study the sample size was 25 g as recommended by the USDA. Following enrichment, the ELISA test system was applied, according to the manufacturers' instructions.

All samples of minced meat, pig feed, and pig tonsil swabs were examined by the USDA method as well as by the ELISA test. Samples of soft cheeses were examined using the ELISA test and the slightly modified FDA method referred to above.

Evaluation of the methods were made by calculation of sensitivity, specificity, efficiency, predictive values, and kappa coefficients (4,5,9). Definitions and calculations of these values are shown in Fig. 3.

Investigation of the detection limits of the ELISA kit

The detection limits of the ELISA kit for demonstration of L. monocytogenes in pure cultures were examined by the use of three different L. monocytogenes cultures. The strains used to prepare the pure cultures were as follows: L. monocytogenes NCTC 7973, L. monocytogenes 7512 isolated from cattle feces, and L. monocytogenes T15 isolated from a soft, white molded, blue-veined cheese. The strains were grown for 24 h in Listeria broth II. After the 24 hours, dilutions in Listeria broth II were made. Each dilution was tested for the presence of L. monocytogenes by the use of the ELISA kit, and the actual number of L. monocytogenes in each dilution was determined in plate count agar incubated 24 h at 37°C.

RESULTS

The results of the USDA method and the ability of the ELISA test to detect Listeria spp. in artificially inoculated samples are shown in Table 1.

The sensitivity, specificity, efficiency, and predictive value are calculated for the ELISA test as well as for the USDA method. The calculation of these parameters is shown in Fig. 3. These calculations are possible since the true Listeria status of the inoculated samples is known, provided inoculation doses and viable counts are correct.

The results are divided into two groups, 1 and 2. Group 1 consists of 56 samples of which 47 were inoculated with L. monocytogenes in quantities from 0.12/g to 2/g, and nine samples free of L. monocytogenes. Group 2 consisted of 44 samples inoculated with L. monocytogenes in quantities from 3/g to 67/g. The results of the findings are presented in Table 1.
TABLE 1. Results from minced beef artificially contaminated with L. monocytogenes.

<table>
<thead>
<tr>
<th>Sample material</th>
<th>Number of samples</th>
<th>Test results</th>
<th>Listeria content in sample</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Efficiency %</th>
<th>Predictive value + test - test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minced beef not inoculated, and inoculated with Listeria at levels 0.12-2 CFU/g.</td>
<td>56</td>
<td>ELISA</td>
<td>+</td>
<td>16</td>
<td>0</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USDA</td>
<td>-</td>
<td>31</td>
<td>9</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minced beef inoculated with Listeria at levels 3-67 CFU/g.</td>
<td>44</td>
<td>ELISA</td>
<td>+</td>
<td>44</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>USDA</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

TABLE 2. Results from naturally contaminated samples.

<table>
<thead>
<tr>
<th>Sample material</th>
<th>Number of samples</th>
<th>ELISA result</th>
<th>Culture procedure</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Efficiency %</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minced beef</td>
<td>61</td>
<td>+</td>
<td>51</td>
<td>3</td>
<td>98</td>
<td>67</td>
<td>93</td>
</tr>
<tr>
<td>Tonsil swabs</td>
<td>51</td>
<td>+</td>
<td>15</td>
<td>6</td>
<td>83</td>
<td>81</td>
<td>82</td>
</tr>
<tr>
<td>Pig feed</td>
<td>20</td>
<td>+</td>
<td>15</td>
<td>0</td>
<td>79</td>
<td>(100)</td>
<td>80</td>
</tr>
<tr>
<td>Cheese</td>
<td>17</td>
<td>-</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
<td>94</td>
</tr>
</tbody>
</table>

Total sample material | 149 | + | 90 | 10 | 92 | 80 | 88 | 0.7 |

by the culture procedures and the ELISA test of Listeria spp. in natural samples are shown in Table 2. The sensitivity, specificity, and efficiency of the ELISA kit, and its predictive value of a positive and a negative test, respectively, are all shown in Table 2. This method of calculation assumes that the culture procedure is the true value. Furthermore, the two tests are compared by calculation of the kappa coefficient (Table 2).

The detection limit of the ELISA kit for detection of Listeria spp. in pure cultures was found to be approximately 10^6 CFU/ml (Table 3).

**DISCUSSION**

Artificially contaminated samples of minced beef inoculated with L. monocytogenes in quantities from 0.12-2 bacteria per gram.

Using the USDA method, 45 out of 47 inoculated samples were recognized as positive giving a sensitivity of 96%. This difference could be due to the selective principle of the USDA method or insufficient mixing of cultures used for inoculation, resulting in an inoculated sample free of L. monocytogenes. The sensitivity of the ELISA test was 34%.

The specificity of both the USDA-method and the ELISA-test was found to be 100%. It is known that the specificity of an ELISA-test might be influenced by the presence of potential cross-reacting microorganisms resulting in false-positive reactions. The competitive microflora (Pseudomonas spp., Aeromonas hydrophila, and Streptococcus faecium) used in this study did not produce false-positive reactions and did not influence the specificity of the ELISA test.

The efficiencies of the ELISA test and the USDA
TABLE 3. Examination of the detection limit of the ELISA kit.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>ELISA absorbance</th>
<th>Viable count</th>
</tr>
</thead>
<tbody>
<tr>
<td>neg. control</td>
<td>0.083</td>
<td>8 x 10⁶/ml</td>
</tr>
<tr>
<td>pos. control</td>
<td>2.351</td>
<td>8 x 10⁶/ml</td>
</tr>
<tr>
<td>cut-off value</td>
<td>0.252</td>
<td></td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCTC 7973</td>
<td>2.031</td>
<td>8 x 10⁶/ml</td>
</tr>
<tr>
<td>dil. 1:10</td>
<td>2.080</td>
<td>8 x 10⁶/ml</td>
</tr>
<tr>
<td>dil. 1:100</td>
<td>0.470</td>
<td></td>
</tr>
<tr>
<td>dil. 1:1,000</td>
<td>0.176</td>
<td>8 x 10⁶/ml</td>
</tr>
<tr>
<td>dil. 1:10,000</td>
<td>0.106</td>
<td>8 x 10⁶/ml</td>
</tr>
<tr>
<td>dil. 1:100,000</td>
<td>0.097</td>
<td>8 x 10⁶/ml</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75.1.2.</td>
<td>1.896</td>
<td>8 x 10⁹/ml</td>
</tr>
<tr>
<td>dil. 1:10</td>
<td>1.494</td>
<td>8 x 10⁹/ml</td>
</tr>
<tr>
<td>dil. 1:100</td>
<td>0.282</td>
<td>8 x 10⁹/ml</td>
</tr>
<tr>
<td>dil. 1:1,000</td>
<td>0.103</td>
<td>8 x 10⁹/ml</td>
</tr>
<tr>
<td>dil. 1:10,000</td>
<td>0.104</td>
<td>8 x 10⁹/ml</td>
</tr>
<tr>
<td>dil. 1:100,000</td>
<td>0.091</td>
<td>8 x 10⁹/ml</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.15</td>
<td>1.860</td>
<td>6 x 10⁹/ml</td>
</tr>
<tr>
<td>dil. 1:10</td>
<td>2.361</td>
<td>6 x 10⁹/ml</td>
</tr>
<tr>
<td>dil. 1:100</td>
<td>1.023</td>
<td>6 x 10⁹/ml</td>
</tr>
<tr>
<td>dil. 1:1,000</td>
<td>0.201</td>
<td>6 x 10⁹/ml</td>
</tr>
<tr>
<td>dil. 1:10,000</td>
<td>0.111</td>
<td>6 x 10⁹/ml</td>
</tr>
<tr>
<td>dil. 1:100,000</td>
<td>0.099</td>
<td>6 x 10⁹/ml</td>
</tr>
</tbody>
</table>

method were 45% and 96%, respectively. The predictive value of a positive test result was 100% for both methods. The predictive value of a negative test result was 22% for the ELISA test, while it was 82% for the USDA method (Table 1). As efficiency and predictive values are dependent on the prevalence of positive and negative samples, respectively, these parameters should only be used as estimates for the probability of a given result being correct. The evaluation of tests should be based on their sensitivities and specificities.

Artificially contaminated samples of minced meat inoculated with L. monocytogenes in quantities from 3-67 bacteria per gram

As this sample material consisted of true-positive samples only, the sensitivity is the only parameter of interest. The sensitivity was found to be 100% for the ELISA test as well as for the USDA method.

It is concluded that the culture procedure and the ELISA test are equally sensitive when the L. monocytogenes content in samples is above 3 CFU/g of meat while the culture procedure is the more sensitive when the quantities of L. monocytogenes are less than 3 CFU/g. When Listeria spp. are naturally present in food products, they will often be sublethally injured and the sensitivity of both methods correspondingly lower. The higher sensitivity of the culture procedure is not in accordance with the investigations of Mattingly et al. (6) who found the ELISA test to be more sensitive than the culture procedure when testing 136 naturally contaminated food samples. Mattingly et al., however, did not describe the culture procedure or the kind of food samples used in their study, and it is therefore difficult to explain the difference in the results. Our results concerning the sensitivity of the ELISA test and the culture procedure agree with our findings on the detection limit of the ELISA test for demonstration of L. monocytogenes in pure cultures. The detection limit was found to be approximately 10⁶ CFU/ml (Table 3). The corresponding detection limit of the culture procedure has been calculated to be approximately 10⁸ CFU/ml. The sensitivity of the ELISA test might therefore be markedly improved by only a short prolongation of the enrichment period.

Naturally contaminated samples

The overall sensitivity of the ELISA test was found to be 92% and the specificity to be 80% (Table 2). As the true Listeria spp. status of naturally contaminated samples is not known, one has to assume the result from the most sensitive and specific test to be the true value.

The sensitivity and specificity of the ELISA test at 92% and 80%, respectively, reflect that the ELISA test tested 10 samples as false positive and 8 samples as false negative, among a total of 149 samples. The kappa coefficient was calculated to be 0.73. No agreement beyond chance would give a kappa value of 0 and a kappa value of 1 would indicate perfect agreement. The value of the kappa coefficient is, like efficiency, dependent on the prevalence of positive and negative samples. It is a useful parameter when comparing a new test with a standard test if no information on the sensitivity and specificity of the standard test is available (5). From Table 2 it can also be seen that the sensitivity, specificity, efficiency, and kappa coefficient differ within the different types of samples. These differences, however, are not significant and may be explained by the rather low number of samples as well as by differences in the prevalence of positive samples within the different types of samples. An evaluation of the ability of the ELISA test to detect Listeria spp. in different food products has to be based on investigations made on the food product in question.

In conclusion the present study shows that the ELISA test is limited in its detection of Listeria spp. in raw food products where the number of Listeria is often low and the competitive microflora at a high level. The ELISA test could, however, with great advantage be used as a screening test in the end of a production line to establish whether a possible propagation of Listeria has taken place.

ACKNOWLEDGMENT

The “Listeria-Tek” ELISA test system was kindly provided by Novo Nordisk Food Diagnostics A/S, Hilleroedgade 31, 2200 Copenhagen N, Denmark.

REFERENCES


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the recovery of survivors from sealed, totally immersed TDT tubes indicates that L. monocytogenes Scott A, if present at high levels, may survive in small numbers if heated to 160°F with no dwell time. Further study of these questions is needed.

The heat resistance data obtained for the Scott A strain of L. monocytogenes should be expanded to include other strains to determine if presently used cooking processes for meat and poultry products result in adequate destruction of populations of L. monocytogenes normally encountered in these products.

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REFERENCES


Skovagaard, et. al., cont. from p. 755