

PATHOGEN DETECTION IN FOOD MICROBIOLOGY LABORATORIES: AN ANALYSIS OF QUALITATIVE PROFICIENCY TEST DATA, 1999–2007

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ABSTRACT

The objective of this study was to assess laboratories' ability to detect or rule out the presence of four common food pathogens: Escherichia coli O157:H7, Salmonella spp., Listeria monocytogenes and Campylobacter spp. To do this, qualitative proficiency test data provided by one proficiency test provider from 1999 to 2007 were examined. The annual and cumulative 9-year percentages of false-negative and false-positive responses were calculated.

The cumulative 9-year false-negative rates were 7.8% for E. coli O157:H7, 5.9% for Salmonella spp., 7.2% for L. monocytogenes and 13.6% for Campylobacter spp. Atypical strains and low concentrations of bacteria were more likely to be missed, and the data showed no trend of improving performance over time. Percentages of false-positive results were below 5.0% for all four pathogens.

PRACTICAL APPLICATIONS

The results imply that food testing laboratories often fail to detect the presence of these four food pathogens in real food specimens. To improve pathogen detection, supervisors should ensure that testing personnel are adequately trained, that recommended procedures are followed correctly, that samples are properly prepared, that proper conditions (temperature, atmosphere and incubation time) are maintained for good bacterial growth and that

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recommended quality control procedures are followed. Supervisors should also always investigate reasons for unsatisfactory proficiency test results and take corrective action. Finally, more research is needed into testing practices and proficiency test performance in food testing laboratories.

INTRODUCTION

Since the first proficiency test event in 1946 (Belk and Sunderman 1947), numerous studies have examined proficiency test performance in clinical laboratories and its impact on the accuracy and reliability of patient test results. Compared with the long history of research in clinical laboratories, however, relatively few studies have examined proficiency test performance in food microbiology testing laboratories; and the research published to date analyzes data from European food testing programs (Peterz 1992; Roberts 1999; Teger 2001; Augustin and Carlier 2002, 2006; Hennekinne *et al.* 2003; Corry *et al.* 2007; Jarvis *et al.* 2007). Consequently, there is still much to be learned about proficiency testing in the food industry, especially in food testing laboratories in the U.S.A.

Here, we present results of a retrospective analysis of qualitative microbiology proficiency test data collected from food testing laboratories in the U.S.A. These laboratories participated in food microbiology proficiency testing programs offered by one proficiency test provider, American Proficiency Institute (API), over the 9-year period 1999–2007. Our objective was to assess whether laboratories could reliably detect or rule out contamination with four common food pathogens: *Escherichia coli* O157:H7, *Salmonella* spp, *Listeria monocytogenes* and *Campylobacter* spp.

MATERIALS AND METHODS

We examined proficiency test results from participants in API's qualitative microbiology programs from 1999 to 2007. During the study period, the number of participating laboratories ranged from 380 to 442. Each year of the study period, participants received two samples in each of three test events (six samples per year) for each program in which they were enrolled.

Proficiency test samples were manufactured for API by Microbiologics, Inc., St. Cloud, Minnesota. Samples to be tested for *E. coli* O157:H7, *Salmonella* spp or *L. monocytogenes* consisted of a vial of 2–5 pellets containing lyophilized bacteria, a 99-mL container of Butterfield's phosphate buffer and an aliquot of nonfat dry milk. Samples to be tested for *Campylobacter* spp. consisted of two lyophilized pellets and a 99-mL container of Butterfield's

phosphate buffer. The samples were assembled in kits at API's headquarters in Traverse City, Michigan, and then shipped to laboratories via 2-day delivery service.

To prepare samples testing for *E. coli* O157:H7, *Salmonella* spp. or *L. monocytogenes*, participants were instructed to rehydrate the pellets in the Butterfield's phosphate buffer and then add 25 mL of this suspension and 25 g of the nonfat dry milk to their own pre-enrichment broth. To prepare samples testing for *Campylobacter* spp., participants were instructed to rehydrate the pellets in the Butterfield's phosphate buffer and test the suspension as if it were a meat carcass rinsate. Participants were instructed to follow their own laboratory procedures to test the samples and then to record the results on the form which was included with the samples.

Results were analyzed with API's proprietary software, stratified into peer groups and then evaluated by peer consensus. The consensus result was either "Present" or "Absent" for *E. coli* O157:H7, *Salmonella* spp. and *Campylobacter* spp. Consensus results for samples containing *L. monocytogenes* included response categories for "*Listeria* spp." and identification of species other than *L. monocytogenes* along with the responses "*L. monocytogenes*" and "No *L. monocytogenes*."

During the study period, 12 samples containing pathogens were not graded due to lack of participant consensus (two samples containing *Salmonella* spp., three samples containing *E. coli* O157:H7, three samples containing *L. monocytogenes* and four samples containing *Campylobacter jejuni*). These samples were excluded from our study. Likewise, two samples that were negative for *Salmonella* spp. were not graded due to lack of participant consensus. These also were excluded from our evaluation.

To assess the data, we examined unacceptable responses both to specimens that contained pathogens and to specimens that did not contain pathogens. For each pathogen, we calculated the following:

- (1) The annual percentages of false-negative and false-positive responses;
- (2) The cumulative 9-year percentages of false-negative and false-positive responses;
- (3) The percentages of false-negative responses to samples that contained unusual strains or lower than usual numbers of pathogens.

RESULTS

The percentages of false-negative ("Absent") responses to samples containing pathogens generally persisted above 5.0% throughout the study period for all four pathogens (Table 1). The percentages of false-negative responses

TABLE 1.
PERCENTAGES OF FALSE-NEGATIVE RESULTS FOR SAMPLES CONTAINING
PATHOGENS, 1999–2007

Pathogen	Year									9-year Average
	1999	2000	2001	2002	2003	2004	2005	2006	2007	
<i>Escherichia coli</i> O157:H7	14.0	7.2	8.5	8.2	7.8	8.4	8.8	4.4	6.2	7.8
<i>Salmonella</i> spp	7.2	5.4	5.9	6.2	5.0	2.5	10.6	5.6	4.6	5.9
<i>Listeria</i> <i>monocytogenes</i>	11.0	7.2	7.7	9.1	6.2	8.2	8.2	5.0	5.1	7.2
<i>Campylobacter</i> spp	n/a*	12.5	11.6	17.8	6.1	19.8	14.0	16.7	7.4	13.6

* Proficiency testing for *Campylobacter* spp was not offered in 1999.

TABLE 2.
PERCENTAGES OF FALSE-POSITIVE RESULTS FOR SAMPLES NOT CONTAINING
PATHOGENS, 1999–2007

Pathogen	Year									9-year Average
	1999	2000	2001	2002	2003	2004	2005	2006	2007	
<i>Escherichia coli</i> O157:H7	3.8	5.7	2.8	0.0	1.6	2.0	2.2	1.3	2.6	2.4
<i>Salmonella</i> spp	3.9	4.6	5.6	5.3	5.7	2.3	2.1	5.9	5.0	4.2
<i>Listeria</i> <i>monocytogenes</i>	5.1	2.6	1.4	2.7	1.7	1.0	1.0	1.2	2.1	1.8
<i>Campylobacter</i> spp	n/a*	0.0	0.0	0.0	0.0	4.0	7.0	6.1	2.8	3.3

* Proficiency testing for *Campylobacter* spp. was not offered in 1999.

fell below 5.0% in only three instances: detection of *E. coli* O157:H7 in 2006 and detection of *Salmonella* spp. in 2004 and 2007. Also, the cumulative 9-year percentages of false-negative responses exceeded 5.0% for all four pathogens.

In contrast to the number of false-negative responses, false-positive (“Present”) responses to samples not containing pathogens were generally less than 5.0% (Table 2). During the study period, false-positive responses exceeded 5.0% once for *E. coli* O157:H7, once for *L. monocytogenes* and twice for *Campylobacter* spp. However, false-positive responses for *Salmonella* spp. were 5.0% or more in 5 of 9 years (2001, 2002, 2003, 2006 and 2007). For all four pathogens, the cumulative 9-year percentages of false-positive responses were less than 5.0%.

Four samples (two in 2002 and two in 2005) contained *Salmonella choleraesuis* subsp. *choleraesuis* (ATCC# 10708), which does not produce the

24-h H₂S reaction that is typical of many *Salmonella* species. Twenty-three of 96 (24.0%) and seven of 48 (14.6%) respondents in 2002 failed to detect this atypical *Salmonella*, as did 45 of 148 (30.4%) and 13 of 47 (27.7%) respondents in 2005. Also, one sample in 2006 contained *Campylobacter coli* instead of the usual *C. jejuni*; six of 25 (24.0%) respondents failed to detect this pathogen.

Six samples contained low concentrations of pathogens. One sample in 1999 and one sample in 2001 contained low numbers of *E. coli* O157:H7. Three of three (100%) respondents in 1999 and five of 57 (8.8%) respondents in 2001 failed to detect the *E. coli* O157:H7 in these samples. One sample in 2000 and two samples in 2001 contained low concentrations of *Salmonella*. Two of 72 (2.8%) respondents in 2000 failed to detect this pathogen, as did nine of 70 (12.8%) and one of 59 (1.7%) respondents in 2001. Finally, in 2000, three of 35 (8.6%) respondents failed to detect a low concentration of *L. monocytogenes*.

DISCUSSION

The false-negative results suggest that food testing laboratories consistently fail to detect pathogens more than 5.0% of the time. In our data, *Campylobacter* spp. were most likely to be missed (13.6% 9-year average), followed by *E. coli* O157:H7 (7.8% 9-year average), *L. monocytogenes* (7.2% 9-year average) and *Salmonella* spp. (5.9% 9-year average).

Atypical strains of bacteria were more likely to be missed than common strains. False-negative responses for detecting an atypical *Salmonella* strain ranged from 14.6 to 30.4%, compared with an overall false-negative response rate of 5.9%. Also, 24.0% of respondents failed to detect *C. coli*, compared with an overall false-negative rate of 13.6% for *Campylobacter* spp.

These results are similar to results reported by other researchers. A study of data from laboratories participating in the French food proficiency testing program (Réseau d'Analyses et d'Échanges en Microbiologie des Ailiments) reported that the number of laboratories providing false-negative responses to samples containing *Salmonella* spp. exceeded 5.0% in 22 of 27 test events (Augustin and Carlier 2002). Another study of proficiency test performance in laboratories enrolled in the Nordic food microbiology proficiency testing program reported a combined false-negative and false-positive rate in two test events of 6.7 and 10.0% (Peterz 1992).

Our finding that higher false-negative rates were associated with atypical strains of bacteria and, to a lesser extent, lower concentrations of bacteria also is consistent with results reported by other researchers. Augustin and Carlier (2002) attributed a highly variable rate of false-negative responses to the level

of inoculum; specifically, samples with low levels of *Salmonella* yielded significantly more false-negative responses. In a study of data from the Nordic food testing program, participants had problems isolating *Salmonella dublin* (Peterz 1992).

Our data suggest not only that laboratories often fail to detect pathogens but also that performance has not improved during the past 9 years. Although the rates of false-negative results fluctuate, they show no clear upward or downward trend. This is consistent with results of a study of data from the French food microbiology testing program which noted a variable rate of false-negative results for detection of *Salmonella* but no improvement over time (Augustin and Carlier 2002).

Whether performance in individual laboratories improved during the study period is uncertain, because we analyzed only the aggregate performance of a changing population of laboratories as participants enrolled in and dropped out of API's proficiency testing programs. Thus, it is possible that high rates of unacceptable responses from newly enrolled participants obscured improving performance by longtime participants. However, results of one study found that longtime participants in a proficiency testing program performed better than recent enrollees (Peterz 1992).

A comparison of the data in Tables 1 and 2 shows that, for all four pathogens, the 9-year cumulative false-negative rates exceeded the 9-year cumulative false-positive rates. For three pathogens (*E. coli* O157:H7, *L. monocytogenes* and *Campylobacter* spp.), the disparity was more than three-fold. This disparity between rates of false-positive and false-negative results suggests that one reason why laboratories fail to detect pathogens may be that the bacteria fail to grow. This in turn may indicate problems with media or incubation conditions such as improper temperature, improper atmosphere or insufficient incubation time. In clinical microbiology laboratories, poor performance has been linked to failure to follow recommended practices for culturing and identifying pathogens (Boyce *et al.* 1995; Kiehlbauch *et al.* 2000; Novak 2002; Voetsch *et al.* 2004; Edson *et al.* 2005; Edson and Massey 2007) and to inadequate staffing and insufficient training of personnel (Belk and Sunderman 1947; Hurst *et al.* 1998; Jenny and Jackson-Tarantino 2000; Kiehlbauch *et al.* 2000; St. John *et al.* 2000). Research into reasons for poor performance in food testing laboratories is less extensive, but investigators have mentioned issues such as insufficient incubation time and incorrect incubation temperature (Augustin and Carlier 2006) and improper plating of media (Corry *et al.* 2007).

The extent to which performance with proficiency test samples reflects performance with real food specimens is unclear for three reasons. First, testing personnel may test proficiency test samples more carefully than they test actual food samples. If so, the rate of false-negative results from real food

samples may be higher than the proficiency test data imply. Second, as proficiency testing assesses only the analytical phase of the testing process, it may not detect pre-analytical errors in collecting, transporting and processing specimens, or post-analytical errors in reporting results that could occur with real food samples. Again, this suggests that the rate of erroneous results in real food specimens may be higher than indicated by the proficiency test results. Finally, the artificial nature of proficiency test samples (freeze-dried bacteria and nonfat dry milk) can introduce bias into the testing process even though results are evaluated by peer group consensus. These matrix effects could increase the number of erroneous results in proficiency test samples. However, as Corry and colleagues (2007) have asserted, as proficiency test samples are more homogenous than real food samples and contain laboratory-attenuated strains of bacteria, performance with these samples is likely to be better than performance with real food samples.

Even if the false-negative rates with real food samples correlate with the 5.9–13.6% false-negative rates which we found with proficiency test samples, it is cause for concern. However, for the reasons discussed above, it seems likely that the rate of false-negative results with real food samples is even greater. If so, this is clearly a serious public health issue that demands resolution.

Recommendations

Laboratory supervisors can improve detection of pathogens by ensuring that policies and procedures deemed essential for good performance are followed. In the microbiology laboratory, the following good laboratory practices will optimize performance (Corry *et al.* 2007; Edson *et al.* 2007):

- (1) Ensure that the staff are trained and proficient.
- (2) Follow consensus guidelines issued by panels of scientific experts.
- (3) Ensure that equipment is properly maintained and used to promote good bacterial growth.
- (4) Ensure that diluents, reagents and media are correctly prepared and properly stored.
- (5) Use proper technique to prepare primary and analytical test samples.
- (6) Perform quality control on reagents, media and equipment as recommended by the manufacturer.
- (7) Always investigate unsatisfactory proficiency test results.

Finally, further research into testing practices and proficiency test performance in food microbiology laboratories is needed to clarify the reasons why food testing laboratories so often fail to detect pathogens. Whether factors that have been linked to poor performance in clinical laboratories also contribute to

poor performance in food testing laboratories or whether other issues are involved needs to be determined, so that steps can be taken to improve pathogen detection in the food supply.

CONCLUSION

Food testing laboratories in the U.S.A. could learn from clinical laboratories' experience not only with proficiency testing but also with government regulation. For many years, only clinical laboratories involved in interstate commerce were federally regulated; thousands of clinic and physician's office laboratories were exempt. Then, in 1987, an article in the *Wall Street Journal* about widespread errors in Pap smear testing alarmed Congress and the public (Bogdanich 1987). Congressional hearings and subsequent news reports in the mainstream media revealed that careless laboratory practices involved much more than just Pap smear testing. Concern for the public health and a lack of confidence that clinical laboratories could properly police themselves prompted Congress in 1988 to pass legislation that tightened standards for all laboratories engaged in testing patient specimens, including thousands of previously unregulated laboratories.

Today, events in the food laboratory industry in the U.S.A. parallel those that unfolded in the clinical laboratory industry 20 years ago. Although accrediting agencies set standards that laboratories must meet, accreditation itself is voluntary. As a result, the public must rely on food testing laboratories to regulate themselves, just as they had to trust clinical laboratories to regulate themselves before 1988. Reminiscent of the news stories about laboratory errors in the 1980s, recent high-profile media coverage of food recalls prompted by illnesses and deaths caused by tainted food have once again alarmed the public and the Congress. It may well be that, if the food industry cannot retain the public's trust, Congress will impose regulations on food testing laboratories as it did for clinical laboratories in 1988.

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