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Towards standard methods for the detection of *Cryptosporidium parvum* on lettuce and raspberries. Part 2: Validation

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Abstract

We report the results of interlaboratory collaborative trials of methods to detect oocysts of the protozoan parasite *Cryptosporidium parvum* on lettuce and raspberries. The trials involved eight expert laboratories in the United Kingdom. Samples comprised 30 g lettuce, and 60 g raspberries. Lettuce samples were artificially contaminated at three levels: low (8.5–14.2 oocysts), medium (53.5–62.6 oocysts), and high (111.3–135.0 oocysts). Non-contaminated lettuce samples were also tested. The method had an overall sensitivity (correct identification of all artificially contaminated lettuce samples) of 89.6%, and a specificity (correct identification of non-contaminated samples) of 85.4%. The total median percentage recovery (from all artificially contaminated samples) produced by the method was 30.4%. The method was just as reproducible between laboratories, as repeatable within a laboratory. Raspberry samples were artificially contaminated at three levels: low (8.5–26.8 oocysts), medium (29.7–65.7 oocysts), and high (53.9–131.3 oocysts). Non-contaminated raspberry samples were also tested. The method had an overall sensitivity (correct identification of all artificially contaminated raspberry samples) of 95.8%, and a specificity (correct identification of non-contaminated samples) of 83.3%. The total median percentage recovery (from all artificially contaminated samples) produced by the method was 44.3%. The method was just as reproducible between laboratories, as repeatable within a laboratory. The results of the collaborative trial indicate that these assays can be used effectively in analytical microbiological laboratories.

Keywords: *Cryptosporidium parvum*; Lettuce; Raspberries; Detection method; Validation

1. Introduction

In Part 1, methods for detecting *Cryptosporidium parvum* on lettuce and raspberries was reported. These methods had been developed by two collaborating laboratories. In these originating laboratories, the methods worked consistently and efficiently. However, the results of methods developed and published by one laboratory can very often be difficult to reproduce in other laboratories. The ability for successful reproduction of results is an absolute prerequisite for adoption of a detection method as a standard diagnostic tool (Lahellec, 1998; Leclerq et al., 2000). Validation of any method should be necessary for its adoption as a standard (Hoorfar and Cook, 2002). This paper describes the validation of the performance characteristics of the methods to detect *C. parvum* on lettuce and raspberries, by collaborative trials involving eight laboratories in the United Kingdom.

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2. Materials and methods

2.1. Parasites

C. parvum oocysts ((Iowa isolate, previously known as *C. parvum* genotype 2) were purchased from Pleasant Hill Farm, Troy, Idaho, USA. Oocysts were less than three months old when used, and their viability, determined by the methods of Campbell et al. (1992, 1993) and Robertson et al. (1993), remained above 85% throughout the course of the trials. Purified oocyst suspensions were prepared in phosphate-buffered saline (PBS, 150 mM; pH 7.2) and enumerated by haemocytometer. Oocyst suspensions contained approximately 100 oocysts in 50 µl PBS. Ten replicate counts were determined for each oocyst suspension, prior to use.

2.2. Samples

Webb's lettuce were obtained from local wholesalers. Each sample consisted of 30 g lettuce leaves. Outer leaves were discarded, and the remaining leaves removed individually and mixed prior to artificial contamination. Fresh raspberries were obtained from local wholesalers, and each sample consisted of 60 g berries.

2.3. The methods

These were as described in the companion paper.

2.4. Design of the collaborative trial

Eight laboratories in the United Kingdom, which had experience of analysis of environmental or clinical samples for the presence of *Cryptosporidium* oocysts participated in the trials. They comprised 3 public health laboratories, 3 drinking water testing laboratories, 1 food research association, and 1 private analytical company. Each participant was sent a detailed trial chronology, standard operating procedures (SOPs), and test reports on which to record the results and return to the trial leader for analysis. The SOPs were based on the methods reported in the companion paper. Monoclonal antibodies and IMS test kits were sent to each participant prior to the commencement of the trial.

Prior to the trials, a pre-collaborative trial was performed between SPDL, CSL and Swansea Public Health Laboratory, Wales to ensure that the SOPs were clear and that the test materials were fit for purpose.

Each collaborative trial comprised three rounds. In each round, 8 blind coded samples were sent to each participant: these were two non-contaminated samples, and two each of samples artificially contaminated at low, medium and high densities with *C. parvum* oocysts. For each round, samples were prepared at CSL and sent out to each participant on Day 0. On Day 0, CSL prepared control slides to verify the artificial contamination level, and also homogeneity samples, which were sent to SPDL for completion of analysis. Each participant received the samples on Day 1, and was asked to analyze them that day and report the results back to CSL within 5 days. Participants were also asked to send their microscope slides from each test to SPDL. Participant results were compared with SPDL results at CSL and deviations were reported by CSL to SPDL. Where results were grossly different from that which was expected (e.g., 0 counts from seeded samples, and positive counts from blank samples) SPDL examined the slides and enumerated (oo)cysts.

2.5. Stability of the test materials

The stability of the test materials was examined in a single experiment performed prior to the collaborative trial. To test the stability of the artificially contaminated samples over 24 h, 10 samples of each food type were artificially contaminated with approx. 100 oocysts on Day 0. Oocysts were extracted from 5 samples on Day 0 and from the remaining 5 samples on Day 1.

2.6. Preparation of food samples for the collaborative trial

Each sample was weighed into a plastic weighing boat prior to artificial contamination. To the non-contaminated samples 5×10 µl PBS were added, to the low level artificially contaminated samples 10 µl of oocyst suspension and 4×10 µl PBS were added, to the medium level artificially contaminated samples 5×5 µl of oocyst suspension and 5×5 µl of PBS were added, and to the high level artificially contaminated samples 5×10 µl of oocyst suspension were added. The samples were left at room temperature for approximately two hours until the liquid was almost dry. Samples were placed into filtered stomacher® bags that were then sealed with autoclave tape. Cool packs were placed in all transport boxes to reduce deterioration of samples during transit.