

Title Towards standard methods for the detection of *Cryptosporidium parvum* on lettuce and raspberries.
Part 1: Development and optimization of methods

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Abstract

No standard method is available for detecting protozoan parasites on foods such as soft fruit and salad vegetables. We report on optimizing methods for detecting *Cryptosporidium parvum* on lettuce and raspberries. These methods are based on four basic stages: extraction of oocysts from the foodstuffs, concentration of the extract and separation of the oocysts from food materials, staining of the oocysts to allow their visualization, and identification of oocysts by microscopy. The concentration and separation steps are performed by centrifugation, followed by immunomagnetic separation using proprietary kits. Oocyst staining is also performed using proprietary reagents. The performance parameters of the extraction steps were extensively optimized, using artificially contaminated samples. The fully developed methods were tested several times to determine their reliability. The method to detect *C. parvum* on lettuce recovered $59.0 \pm 12.0\%$ ($n = 30$) of artificially contaminated oocysts. The method to detect *C. parvum* on raspberries recovered $41.0 \pm 13.0\%$ ($n = 30$) of artificially contaminated oocysts.