Amoebic keratitis (AK) is a potentially blinding ocular infection caused by an Acanthamoeba sp. free-living protozoan parasite that is found ubiquitously throughout the environment worldwide (3). The overwhelming majority of cases of AK occur in immunocompetent contact lens wearers (14), and outbreaks have been linked to contact lens solutions contaminated with acanthamoebae or to those that fail to effectively decontaminate lenses. A recent outbreak in the United States affecting 138 people led to the recall of contact lens solutions and products by both the FDA and Health Canada and has resulted in over 150 lawsuits against the manufacturer (2, 6, 7). Plaintiffs in the lawsuits have been left with impaired vision and, in several cases, have required corneal transplants (7). Although contaminated contact lens solutions or solutions that facilitate growth are usually implicated in large outbreaks of AK, isolated cases occur in individuals who have corneal trauma or who disinfect contact lenses with tap water or other home-based preparations. Swimming and showering while wearing contact lenses are also risk factors for AK. Annual incidences of AK vary by country and are believed to be on the order of 2 to 20 cases per million contact lens wearers, accounting for 10% of the North American population (8, 12, 16, 17, 19, 21).

Clinically, AK can be easily mistaken for herpes simplex virus infection or fungal keratitis, and secondary bacterial infection is common, thus complicating diagnosis (10). Delayed diagnosis has repeatedly been associated with poor visual outcome and more-severe clinical progression (4, 5). Standard laboratory diagnostic procedures include microscopic examination of Giemsa-, periodic acid Schiff-, hematoxylin-and-eosin-, or acridine orange-stained corneal scrapings or contact lens fluids and culture of these specimens on nonnutrient agar overlaid with Escherichia coli or Klebsiella pneumoniae, and all of these procedures are limited by poor sensitivity, the requirement for technical expertise, and, in the case of culture, long turnaround time (4, 5). Due to their excellent sensitivity, molecular methods, including PCR, are increasingly being used to detect acanthamoebae in corneal specimens (9, 10, 18, 20).

MATERIALS AND METHODS

Samples. Consecutive specimens (corneal scrapings, contact lens solutions, and casings) from patients with suspected AK that were sent to the Central Public Health Laboratory for diagnosis between January 2007 and June 2008 were assayed using the standard diagnostic procedures (direct examination and culture) outlined below. Following completion of clinical testing, specimens were deidentified, issued unique study identifiers, aliquoted into cryovials, and stored at −20°C for future qualitative PCR testing. Per the Code of Federal Regulations, Title 45, Part 46, the use of deidentified diagnostic specimens for verifi-
PCR with the Nelson primers were sequenced and analyzed by the Centre for using both the Nelson and the JDP primer sets.

Concentration of organisms in this initial inoculum was calculated using a he-trifuge tube and suspended in RPMI medium (Invitrogen Corp., Carlsbad, CA). The level of significance was set at \( P \) values of \(<0.05\).

**RESULTS**

During the study period, 107 clinical specimens were examined for evidence of *Acanthamoeba* infection. Of these, 81 were corneal scrapings (76%), 17 were contact lens solutions (16%), and 5 were contact lenses (5%). The remaining specimens were one contact lens casing, one eye swab, one corneal biopsy sample, and one tear fluid sample. Of the 107 specimens evaluated, 20 (18.7%) fulfilled the reference standard criteria for a diagnosis of AK (positive results for two of four tests). Twenty-eight (26.2%) specimens were positive by one test, 10 (9.3%) were positive by three tests, and 7 (6.5%) were positive by all four diagnostic tests. Positivity rates varied among the different types of specimens, with 16% (\( n = 13 \)) of corneal scrapings considered positive and 24% (\( n = 4 \)) of contact lens solutions and 50% (\( n = 3 \)) of contact lenses and casings fulfilling the criteria for a diagnosis of AK. *Acanthamoebae* were undetectable in eye swabs, corneal biopsy specimens, and tears.

Results for direct examination of specimens by use of a Giemsa-stained smear were positive for 11 specimens, yielding a sensitivity of 55% (95% CI, 33.2 to 76.8%) and a specificity of 100% (Table 1). Of all methods compared in this study, direct smear analysis had the poorest diagnostic sensitivity (\( P = 0.006 \) for comparison to culture; \( P < 0.001 \) for comparison to Nelson primer PCR). Diagnostic sensitivity of direct smear analysis was greatest for contact lenses and contact lens casings (\( P = 0.034 \) for comparison to corneal scrapings) and poorer for specimens such as contact lens solutions (\( P = 0.09 \).
for comparison to contact lens casings) and corneal scrapings \((P = 0.034)\) (Table 2).

Results for specimen culture using four different types of NM were positive for 14 specimens, yielding a sensitivity and specificity of 73.7\% \((95\% \text{ CI, 54.4 to 93.0\%})\) and 100\%, respectively (Table 1). Performance of culture was greatest for specimens such as contact lenses and contact lens casings, where the burden of organisms is presumably higher (Table 2). There was a trend toward poorer performance of culture for contact lens solutions than for corneal scrapings (Table 2). Only 18 of 26 specimens fulfilled the reference standard criteria for a diagnosis of AK, thus, eight specimens were considered to be false positive. By specimen type, Nelson primer PCR appeared to have the greatest diagnostic sensitivity for contact lens solutions, casings, and the lenses themselves, with lesser performance for specimens such as corneal scrapings, though these differences did not achieve statistical significance, possibly due to low specimen numbers (Table 2).

PCR using the JDP primer set revealed poorer diagnostic and analytic sensitivity than that using the Nelson primer set \((P < 0.001)\), though the former was more specific (Table 1). The sensitivity of JDP primer PCR was 65.0\% \((95\% \text{ CI, 44.1 to 85.9\%})\), while the specificity was 100\%. The performance of JDP primer PCR varied by specimen type, with the best diagnostic performance observed for contact lens solutions \((P < 0.001)\) for comparison to corneal scrapings) and casings and actual lenses \((P = 0.034)\) for comparison to corneal scrapings), with poorer performance for corneal scraping specimens (Table 2).

Serial dilutions of whole acanthamoebae were made to a concentration of <1 organism per \(\mu\)l and then subjected to both Nelson and JDP primer PCRs as described in Materials and Methods. PCR product was detectable at a concentration of 0.05 organisms per \(\mu\)l, or roughly 1 or 2 organisms per 25-\(\mu\)l aliquot, with the Nelson primer set, suggesting a single-organism level of analytic sensitivity. Analytic sensitivity was lower with JDP primer PCR, which detected down to 1.56 organisms per \(\mu\)l, or roughly 40 organisms per 25-\(\mu\)l aliquot.

Most organisms were not identified to the species level by sequencing, having greatest homology with the Acanthamoeba sp., though some shared 100\% sequence similarity with Acanthamoeba castellanii \((n = 4)\); GenBank accession numbers AY690455.1 and AF260724.1), Acanthamoeba polyphaga \((n = 4);\) GenBank accession numbers AF132135.1 and AF026243.1), or Acanthamoeba culbertsoni \((n = 2);\) GenBank accession number AY690459.1).

**DISCUSSION**

We have demonstrated that amplification of Acanthamoeba DNA by use of PCR with the Nelson primers \((11)\) is a sensitive means by which to diagnose AK in a clinical laboratory setting. PCR in general had a particular performance advantage with specimens such as contact lens solutions, where a dilutional effect may be observed. While traditional direct smear analysis and culture of specimens are highly specific diagnostic methods, they are limited by high false-negativity rates, the requirement for significant technical expertise, and, in the case of culture, a very long turnaround time \((4, 10, 18)\). The yield of culture using NM reported herein is almost identical to that reported by others using buffered charcoal yeast agar, non-nutrient agar with E. coli, and Trypticase soy agar with sheep or horse blood \((15)\). Our results are also consistent with others in that culture has previously been shown to outperform JDP primer PCR in the diagnosis of AK from clinical specimens \((18)\).

That Nelson primer PCR had a high false-positivity rate in this verification may simply reflect the outperformance of this highly sensitive molecular technique compared to that of the comparator methods. It is possible that Acanthamoeba DNA was detectable by the primer set at a concentration below the limit of detection of whole organisms or parasite DNA for the other assays. This represents an inherent limitation of any

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**TABLE 1. Comparison of four diagnostic methods used for evaluation of 107 clinical specimens from patients suspected to have AK**

| Assay          | No. positive | No. negative | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---------------|-------------|-------------|----------------|----------------|---------|---------|
| Direct microscopy | 11          | 96          | 55.0           | 100            | 100     | 90.6    |
| Culture\(^a\) | 14          | 91          | 73.7           | 100            | 100     | 94.6    |
| PCR            | 26          | 81          | 90.0           | 90.8           | 69.2    | 97.5    |
| Nelson primers | 13          | 94          | 65.0           | 100            | 100     | 91.6    |

\(^a\) PPV, positive predictive value; NPV, negative predictive value.

\(^b\) Two specimens were not set up for culture.

**TABLE 2. Performance characteristics of four methods for diagnosis of AK used for evaluation of 107 clinical specimens by specimen type**

| Assay          | Corneal scraping \((n = 81)\) | Contact lens solution \((n = 17)\) | Contact lenses and casings \((n = 6)\) |
|---------------|------------------------------|----------------------------------|-----------------------------------|
|               | Sensitivity | Specificity | Sensitivity | Specificity | Sensitivity | Specificity |
| Direct microscopy | 46.2       | 100.0       | 50.0        | 100.0       | 100.0       | 100.0       |
| Culture\(^a\) | 75.0        | 100.0       | 50.0        | 100.0       | 100.0       | 100.0       |
| PCR            | 84.6        | 91.2        | 100.0       | 92.3        | 100.0       | 66.7        |
| Nelson primers | 46.2        | 100.0       | 100.0       | 100.0       | 100.0       | 100.0       |
| JDP primers    | 46.2        | 100.0       | 100.0       | 100.0       | 100.0       | 100.0       |

\(^a\) Two specimens were not set up for culture.
diagnostic evaluation in the absence of a well-performing reference standard (1).

Corneal specimens for the diagnosis of AK are notoriously difficult to obtain, and few patients tolerate corneal scraping well (5,10). Obtaining a sufficient volume of clinical specimen to facilitate decent smear or culture yields is challenging (5,10). Similarly, large-volume specimens such as contact lens solutions have a demonstrable dilutional effect and are therefore subject to poor smear and culture results, as demonstrated by our specimen-based performance analysis. Thus, diagnostic methods that detect very few organisms in a clinical specimen are clearly advantageous. We have demonstrated an analytic sensitivity for Nelson primer PCR to the single-organism level. Such analytic sensitivity has implications not only for routine diagnosis of AK but also for a test of cure, where one would expect the burden of acanthamoebae to be extremely low in clinical specimens. In addition, Nelson primer PCR could prove to be a rapid, sensitive tool for screening batches of contact lens solutions in outbreak situations.

In the clinical laboratory setting, care must be taken to balance maximization of Acanthamoeba culture yield through prolonged incubation and production of a timely and clinically useful result. In the case of AK, prompt initiation of appropriate therapy is necessary to limit ocular morbidity and optimize visual outcome (4,5). Thus, employment of a rapid, sensitive screening tool, such as Nelson primer PCR, followed by a rapid, specific confirmatory test, such as JDP primer PCR, may offer benefits beyond those achieved through direct specimen microscopy and culture alone. Strategies which simplify the procedure for laboratory investigation of AK are likely worthwhile and cost-effective (4).

Of particular interest to clinicians is that the kind of specimen most frequently submitted to the laboratory, the corneal scraping, statistically had the lowest rates of detectable whole organisms and Acanthamoeba DNA by all tests but Nelson primer PCR. While this may simply reflect that patients with actual AK in our sample were more likely to be contact lens wearers and thus have contact lens-related specimens to submit, it may also reflect that the burden of organisms in contact lenses, cases, and fluids is greater than what is seen in a corneal scraping. This hypothesis is supported by our specimen-based performance analysis of the individual diagnostic assays, though these results should be interpreted cautiously given the low numbers of positive samples by each specimen type evaluated. While detection of acanthamoebae from contact lenses, fluids, or casings does not strictly confirm the diagnosis, it is virtually diagnostic in the setting of a compatible clinical history (10,13). Thus, submission and processing of these types of atypical specimens may be as important as those for corneal scrapings. Other potential explanations for the noted assay performance disparities by specimen type involve the presence of PCR inhibitors in the corneal tissue itself and the low volume of clinical material obtained by corneal scrapings. Future evaluation of the disparity in yield by specimen type where the potential bias of contact lens use can be controlled is warranted.

The evaluation herein highlights the limitations of commonly employed diagnostic assays for AK and supports the potential utility of at least one primer set for molecular detection of acanthamoebae in clinical specimens. PCR had a clear diagnostic advantage over conventional techniques for large-volume specimens, such as contact lens solutions, where a dilutional effect would be expected. PCR is less labor-intensive than culture, requires fewer specialized technical skills, is more sensitive, and offers a much more rapid turnaround time, all of which culminate in the ability of the clinical laboratory to produce a meaningful, clinically relevant result. We would encourage clinicians to consider submission to the laboratory of contact lens-associated materials in addition to corneal scrapings from any patient in whom AK is suspected.

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We have no conflicts of interest to declare.

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