Extraction of *Acanthamoeba* DNA using Chelex resin.

**Running title: Acanthamoeba Chelex extraction**

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Acanthamoeba keratitis (AK) is a rare corneal infection caused by the free-living amoeba *Acanthamoeba*. \[1\] Diagnosis of AK is based on the culture of corneal scrapings or detection of the organism in pathology specimens. \[1\] Culture positivity and histology may require a long time (3-7 days on average), and are highly dependent on the number of *Acanthamoeba* cysts and trophozoites recovered from the cornea.

Early diagnosis of AK is one of the most important factors determining the final outcome. \[1\] Molecular detection of *Acanthamoeba* using polymerase chain reaction (PCR) provides a rapid and sensitive method for diagnosis. \[1\] However, DNA extraction from *Acanthamoeba* often requires time-consuming and expensive commercial kits, as demonstrated in previous studies. \[2\] *Acanthamoeba* cysts are resistant to physical, mechanical or enzymatic lysis and the presence of inhibitors to *Taq* polymerase can compromise the efficiency of DNA amplification. \[2\]

We report a simple, fast and inexpensive technique for *Acanthamoeba* DNA extraction using Chelex resin (MB Chelex-100 resin®, Bio-Rad Laboratories, Hercules, CA), a commercially available polystyrene-divinylbenzene iminodiacetate material that has been used extensively in DNA extraction for forensic and microbiological purposes. \[3,5-6\]

Our protocol consisted of adding 200 µL of Chelex solution (10% w/v) in 0.1% Triton X-100 and 10 mM Tris buffer, pH 8.0 to cysts suspensions (approx. 10⁴ cysts/ml in balanced salt solution). The material was vortexed for 10 seconds, centrifuged at 10,000 x g for 10 seconds, then heated at 95°C for 20 minutes and finally centrifuged at 10,000 x g for 20 seconds. The supernatant was used as substrate for a PCR reaction (4 µL). The following *Acanthamoeba* genus primers were used: Fwd 5’-
TCTCACAAGCTGCTAGGGCGTCA-3’, Rev 5’-GTCAGAGGTGAAATTCTTGG-3’,
which gave a 250 bp product.[4]

To test the detection limits of our assay, Chelex extraction was performed on serial
dilutions of *A. castellanii* (ATCC, T4 genotype) cysts in saline solution. As shown in
Figure 1, PCR run after Chelex extraction was able to amplify DNA equivalent to a
minimal concentration of 0.1 cysts per PCR reaction.

To assess the potential use of Chelex extraction in the diagnosis of AK, cysts were also
injected into the corneas of eye bank eyes. The corneas of 2 whole eyes obtained from the
local eye bank were injected intrastromally with 0.2 mL of a suspension of $10^4$/mL *A.
castellanii* and *A. lenticulata* (ATCC, T5 genotype) cysts in saline solution, respectively;
a third eye was injected with saline and used as a negative control. The eyes were stored
in Optisol at 35°C for 24-48 hours before the corneas were scraped with a crescent blade.
Chelex solution was added to the corneal scraping and DNA extraction and PCR
performed as described. As shown in Figure 2, The presence of *A. castellanii* (T4) and *A.
lenticulata* (T5) was detectable in the corneal scrapings obtained from both eyes.

Chelex resin DNA extraction appears to be a sensitive and reliable method for
*Acanthamoeba* DNA extraction from culture or corneal scrapings and may represent a
fast and inexpensive alternative to commercial kits for rapid molecular diagnosis of AK
or genotyping of *Acanthamoeba* strains. Further evaluation of Chelex DNA extraction in
a clinical setting are needed to confirm our findings.
References


Figure legends

**Figure 1.** 1% agarose gel showing detection limits of Chelex DNA extraction.

**Figure 2.** 1% agarose gel showing *Acanthamoeba* DNA recovery from *in vitro* infected eyes using Chelex resin. T4 = *Acanthamoeba castellanii*; T5 = *Acanthamoeba lenticulata*; Ctrl = eye injected with saline; + = positive PCR control; - = negative PCR control.