

Diagnostic accuracy of clinic-based loop-mediated isothermal amplification and qPCR for fungal and acanthamoeba keratitis

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BACKGROUND

- Delayed diagnosis contributes to poor visual outcomes of fungal keratitis (FK) and acanthamoeba keratitis (AK).
- Current diagnostic approach (i.e. smear and culture) requires experience +/- time.
- qPCR and loop-mediated isothermal amplification (LAMP) can expedite accurate diagnosis.

METHODS

- Settings: Vietnam National Eye Hospital
- Adults presenting with clinical diagnosis of infectious keratitis

Smears +/- cultures

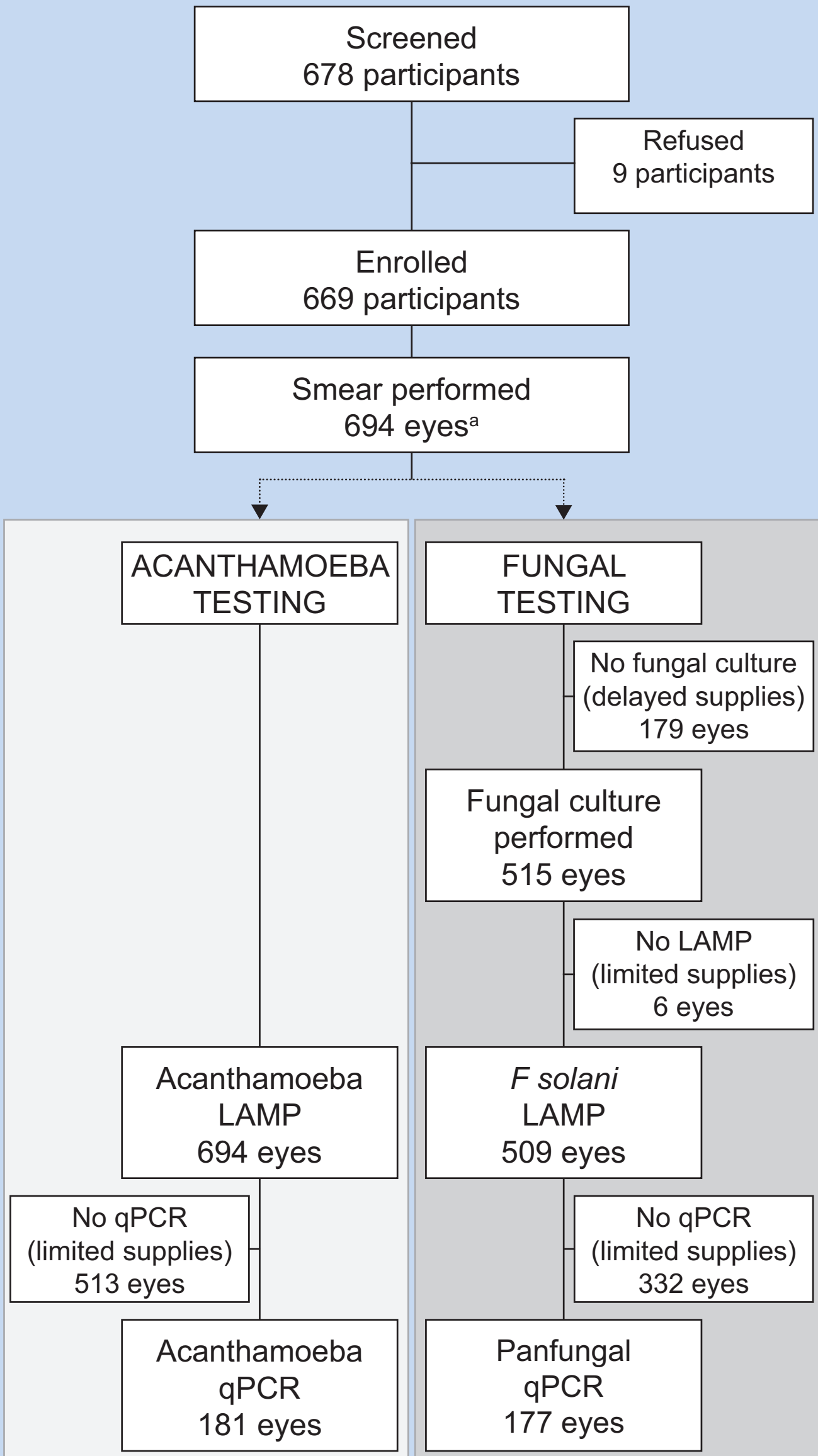
Cornea swabs collected for:
  - qPCR (Acanthamoeba & panfungal)
  - LAMP (Acanthamoeba & *F. solani*)
- Sensitivity and specificity were assessed relative to *smear* for *acanthamoeba qPCR*, *panfungal qPCR*, *acanthamoeba LAMP*, and relative to *culture* for *Fusarium solani* LAMP.

DISCUSSION

- A clinic-based qPCR was highly sensitive, specific, and reliable for diagnosis of Acanthamoeba keratitis but had poorer diagnostic performance for fungal keratitis.
- The LAMP protocol used in this study requires further optimizations to improve sensitivity and reliability.
- Although qPCR was promising for detecting Acanthamoeba keratitis in the clinic, future studies with a larger sample size are warranted.

RESULTS

Figure 1. Flow diagram.



<sup>a</sup>5 participants with bilateral disease, 20 had repeat tests

Table 1. Demographic and clinical characteristics.

| Patient characteristics                    | N = 669 patients (%)<br>(694 eyes <sup>a</sup> ) |
|--------------------------------------------|--------------------------------------------------|
| Age (median, IQR)                          | 55 (42-64)                                       |
| Female                                     | 318 (48%)                                        |
| Estimated ulcer size                       |                                                  |
| Small (<3mm)                               | 270/681 (40%)                                    |
| Medium (3-6mm)                             | 233/681 (34%)                                    |
| Large (>6mm)                               | 178/681 (26%)                                    |
| Time-to-presentation in days (median, IQR) | 10 (7-30)                                        |
| Contact lens wear                          | 13 (2%)                                          |
| Agricultural trauma                        | 127 (19%)                                        |
| Prior eye drops/medication                 | 649 (97%)                                        |

<sup>a</sup>5 patients with bilateral disease, 20 patients had repeat tests

Table 2. Diagnostic accuracy of qPCR and LAMP.

|                          | Smear (+) | Smear (-) | Sensitivity and specificity<br>(%, 95%CI) |
|--------------------------|-----------|-----------|-------------------------------------------|
| <b>Acanthamoeba qPCR</b> |           |           |                                           |
| Positive                 | 5         | 2         | Sens = 100% (57-100%)                     |
| Negative                 | 0         | 174       | Spec = 99% (96-100%)                      |
| <b>LAMP</b>              |           |           |                                           |
| Positive                 | 2         | 0         | Sens = 40% (12-77%)                       |
| Negative                 | 3         | 176       | Spec = 100% (98-100%)                     |
| <b>Fungus qPCR</b>       |           |           |                                           |
| Positive                 | 51        | 33        | Sens = 84% (72-91%)                       |
| Negative                 | 10        | 83        | Spec = 72% (63-79%)                       |
| <b>F. solani LAMP</b>    |           |           |                                           |
| Positive                 | 5         | 25        | Sens = 10% (4-21%)                        |
| Negative                 | 46        | 433       | Spec = 95% (92-96%)                       |

Figure 2. Repeatability and reproducibility of qPCR and LAMP.

REPEATABILITY

Two aliquots from same swab

Acanthamoeba  
qPCR

+

-

+

6

0

-

0

7

$\kappa = 1.00$

Acanthamoeba  
LAMP

+

-

±

+

1

0

1

-

0

8

1

±

0

0

2

$\kappa = 0.70 \ (0.36-1.00)$

Panfungal  
qPCR

+

-

+

4

2

-

3

4

$\kappa = 0.24 \ (-0.29-0.76)$

*Fusarium solanii*  
LAMP\*

+

-

±

+

4

10

0

-

0

19

2

±

1

1

1

$\kappa = 0.28 \ (0.03-0.52)$

REPRODUCIBILITY

Aliquots from 2 different swabs

Acanthamoeba  
qPCR

|   |   |    |
|---|---|----|
|   | + | -  |
| + | 1 | 0  |
| - | 0 | 41 |

$\kappa = 1.00$

Acanthamoeba  
LAMP

|       |   |    |       |
|-------|---|----|-------|
|       | + | -  | $\pm$ |
| +     | 0 | 3  | 0     |
| -     | 0 | 39 | 0     |
| $\pm$ | 0 | 0  | 0     |

$\kappa = 0.00$

Panfungal  
qPCR

|   |    |    |
|---|----|----|
|   | +  | -  |
| + | 12 | 13 |
| - | 5  | 12 |

$\kappa = 0.17 \ (-0.10-0.45)$

*Fusarium solanii*  
LAMP

|       |   |    |       |
|-------|---|----|-------|
|       | + | -  | $\pm$ |
| +     | 0 | 2  | 0     |
| -     | 2 | 38 | 0     |
| $\pm$ | 0 | 0  | 0     |

$\kappa = -0.05 \ (-0.10-0.00)$

\*The original sample of 13 participants were all negative twice, so we selected additional samples for repeatability analysis.



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