Microbiological Culture Versus Polymerase Chain Reaction (PCR) for Diagnosing Corneal Infection: the Nottingham Infectious Keratitis Study

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Background

- *Infectious keratitis* is the leading cause of corneal blindness globally (1,2).
- Preventing poor IK outcomes relies on a timely and accurate diagnosis, which guides treatment (3).
- Diagnosis currently relies on *microbiological culture*, the drawbacks of which include a variably low yield and slow turnaround (2-14 days) (3).

Aim

- To compare the diagnostic performance of culture methods and polymerase chain reaction (PCR) in infectious keratitis within a clinical environment.

Methods

- A retrospective, comparative diagnostic test clinical study.
- Inclusion criteria: consecutive cases of presumed fungal/bacterial keratitis that underwent corneal sampling for conventional culture (direct or indirect or both), bacterial (16S) and fungal (18S) ribosomal ribonucleic acid (rRNA) PCR at Queens Medical Centre between June 2021 and September 2022.
- Corneal sampling, microbiological and molecular tests were carried out according to departmental guidelines.
- The parameters for diagnostic performance compared in this study were:

1. Test yield – compared using Cochran's Q test.
2. Pair-wise agreement between tests – using (a) percent agreement and (b) Cohen's kappa (κ).
3. Accuracy (sensitivity and specificity) in comparison to (a) the direct culture method and (b) a composite reference standard.

Results

- Sample size: n=81 patients (86 samples)
- All cases were bacterial keratitis cases.
- PCR-tested = 86 samples
- Direct culture-tested = 66 samples
- Indirect culture-tested = 85 samples

1.Yield

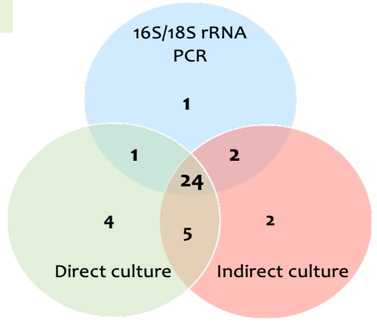


Figure 1: A diagram showing the number of positive results yielded by each test in patients who had undergone all three tests (n=65).

☆***No significant difference in yield*** was found between tests (52.3% vs 50.8% vs 43.1%; p=0.13).

2. Agreement

☆The strength of PCR's agreement with culture methods ranged from ***moderate to substantial,*** with a ***stronger agreement with indirect culture*** and on the ***patient level.***

Table 1: A summary of 16S/18S rRNA PCR agreement with direct and indirect culture on a patient and an organism genus level.

Tests being compared to 16S/18S rRNA PCR	Percent agreement (%)	Cohen's kappa (κ)		
		κ ± standard error (95% confidence interval)	Strength of agreement	P-value
Patient level (agreement on positive/negative results)				
Direct culture	81.8	0.67±0.09 (0.49-0.84)	Substantial	P<.001
Indirect culture	85.9	0.69±0.08 (0.54-0.84)	Substantial	P<.001
Organism genus level (agreement on aetiologic organism down to the genus level)				
Direct culture	88.5	0.55±0.10 (0.36-0.74)	Moderate	P<.001
Indirect culture	90.6	0.62±0.08 (0.46-0.79)	Substantial	P<.001

3. Accuracy

☆Compared to the composite reference standard, ***PCR had the lowest sensitivity.*** Compared to direct culture, ***PCR had a high specificity.***

Figure 2: The sensitivity of all three tests*.

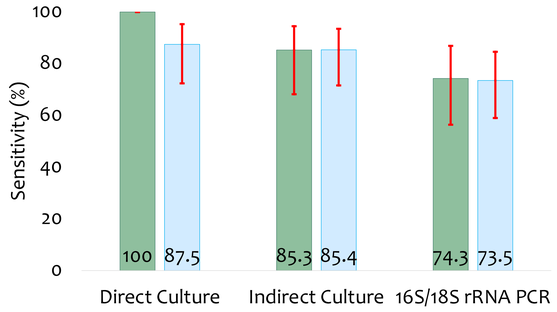
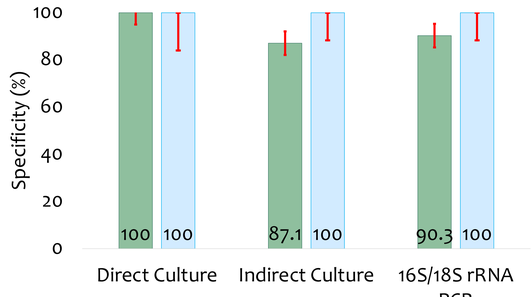


Figure 3: The specificity of all three tests*.

*The error bars represent the 95% confidence intervals



Legend: Direct culture as the reference standard; Composite reference standard

Discussion

1. PCR's diagnostic performance is comparable to the current culture method. PCR testing identified the causative organisms in some culture-negative cases, improving the overall diagnostic yield in IK (refer to figure 1).
2. PCR agreed well with the culture methods, although this is difficult to define. More disagreement on the organism genus level may have stemmed from contamination on either test.
3. PCR had a low sensitivity overall and a relatively high specificity when compared to the direct culture method. However, estimating accuracy is difficult due to the lack of a "perfect" gold standard (4).

Conclusion

- This is the first study evaluating the diagnostic performance of direct culture, indirect culture and PCR for IK.
- Despite PCR's comparable performance to culture methods in IK diagnosis, its lower sensitivity suggests that it cannot supplant culture methods. Instead, PCR can serve as an adjunctive modality to maximise the overall yield and reap both methods' unique benefits.
- Suggestions for further study:
  - ☆ A larger, prospective study including bacterial, fungal, and Acanthamoeba keratitis cases.
  - ☆ Analysis of the impact of culture/PCR-positivity on disease outcomes.

References

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