

Supplementary Table 3: Sensitivity and specificity values with 95% confidence intervals (brackets) of culture, polymerase chain reaction and *in vivo* confocal microscopy compared to different reference standards for *Acanthamoeba* and filamentary fungus, expanding on the data presented in Table 2. The sensitivity/specificity values are shown on the column to the right and the number of positive and negative test results, for the organism in question, are shown on the left.

Diagnostic Modality	Culture Reference Standard		Totals	Indices	Value (% CI)
	Positive	Negative			
Acanthamoeba (n=50 detected including both mono and polymicrobial)					
PCR					
Positive	11	16	27	Sensitivity %	73.3 (44.9-92.2)
Negative	4	118	144	Specificity %	88.1 (81.3-93.0)
Total	15	134	149		
IVCM					
Positive	10	24	34	Sensitivity %	71.4 (41.9-91.6)
Negative	4	90	94	Specificity %	78.9 (70.3-86.0)
Total	14	114	128		
Fungus (n=14 detected including both mono and polymicrobial)					
PCR					
Positive	1	3	4	Sensitivity %	20.0 (0.50-71.6)
Negative	4	148	152	Specificity %	98.0 (94.3-99.6)
Total	5	151	156		
IVCM					
Positive	3	5	8	Sensitivity %	60.0 (14.7-94.7)
Negative	2	118	120	Specificity %	95.9 (90.8-98.7)
Total	5	123	128		
Diagnostic Modality	PCR Reference Standard		Totals	Indices	Value (% CI)
	Positive	Negative			
Acanthamoeba (n=50 detected including both mono and polymicrobial)					
Microbiology					
Positive	11	4	15	Sensitivity %	40.7 (22.4-61.2)
Negative	16	118	134	Specificity %	96.7 (91.8-99.1)
Total	27	122	149		
IVCM					
Positive	20	16	36	Sensitivity %	69.0 (49.2-84.7)
Negative	9	81	90	Specificity %	83.5 (74.6-90.3)
Total	29	97	126		
Fungus (n=14 detected including both mono and polymicrobial)					
Microbiology					
Positive	1	4	5	Sensitivity %	25.0 (0.63-80.6)
Negative	3	148	151	Specificity %	97.4 (93.4-99.3)
Total	4	152	156		
IVCM					
Positive	3	7	10	Sensitivity %	100 (29.2-100)
Negative	0	99	99	Specificity %	93.4 (86.9-97.3)
Total	3	106	109		

Diagnostic Modality	IVCM Reference Standard		Totals	Indices	Value (% CI)
	Positive	Negative			
Acanthamoeba (n=50 detected including both mono and polymicrobial)					
Microbiology					
Positive	10	4	14	Sensitivity %	29.4 (15.1-47.5)
Negative	24	90	114	Specificity %	95.7 (89.5-98.8)
Total	34	94	128		
PCR					
Positive	20	16	36	Sensitivity %	69.0 (49.2-84.7)
Negative	9	81	90	Specificity %	83.5 (74.6-90.3)
Total	29	97	126		
Fungus (n=14 detected including both mono and polymicrobial)					
Microbiology					
Positive	3	2	5	Sensitivity %	37.5 (8.52-75.5)
Negative	5	118	123	Specificity %	98.3 (94.1-99.8)
Total	8	120	128		
PCR					
Positive	3	0	3	Sensitivity %	30.0 (6.67-65.2)
Negative	7	99	106	Specificity %	100 (96.3-100)
Total	10	99	109		

PCR = Polymerase chain reaction; IVCM = In vivo confocal microscopy; CI = Confidence interval.

*IVCM was not performed for cases of bacterial keratitis;

†Composite diagnosis reference standard is defined as a positive result for at least 1 of the following: culture, PCR or IVCM.