

Detection of *Chlamydiaceae* and *Chlamydia*-like organisms on the ocular surface of children and adults from a trachoma-endemic region

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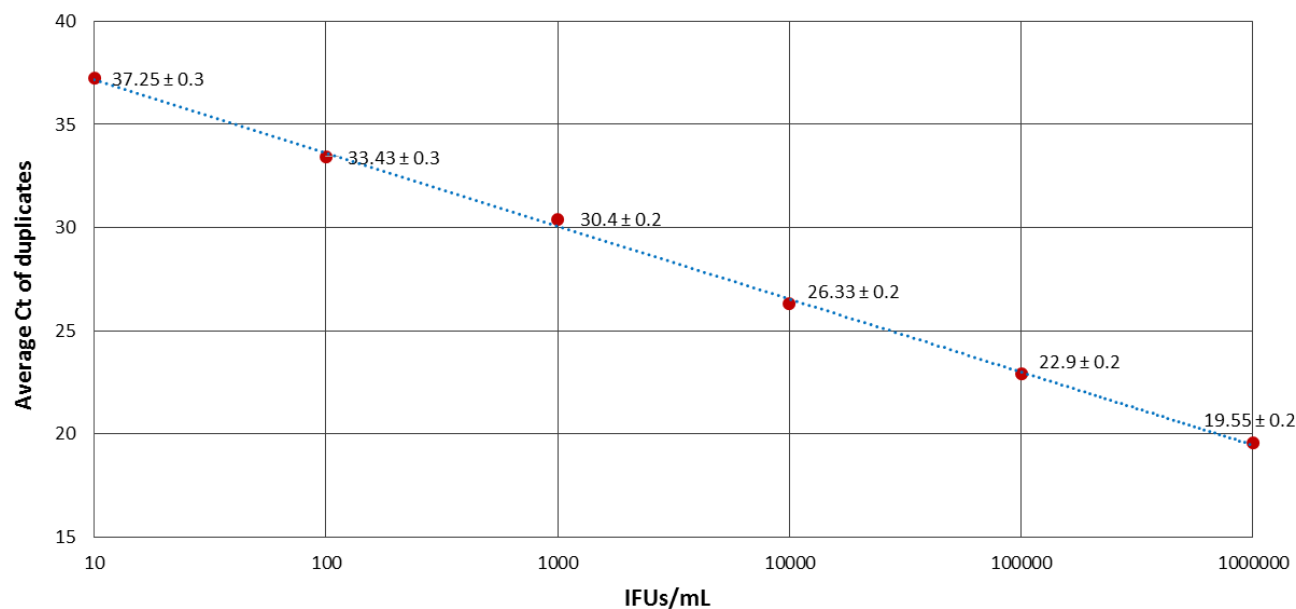


Figure S1. Sensitivity and efficiency of broad-range *Chlamydiae* real-time PCR. A series of 10-fold dilutions of defined numbers of inclusion forming units (IFUs) of *C. trachomatis* (from 1×10^6 to 1×10^1) were prepared for purification of genomic DNA. The prepared dilutions were subjected to each broad-range *Chlamydiae* real-time PCR run in duplicate. Every red dot on the graph indicates the average C_T value for the corresponding dilution factor during the study. The r^2 linearity value from the linear regression is 0.9991. ($y = -3.5474 \times (x) + 40,726$; efficiency = 91.38%).

Table S1. Amplified 16S rRNA sequences and their taxonomic affiliation and closest related sequences in the phylogenetic tree.

Household ID	Taxonomy in phylogenetic tree	Closest related sequence in tree	% Similarity	Other sequences with same similarity
Children Case				
cTF-006	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-008	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-012	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	99.5	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-013	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	99.5	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-017	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	99.5	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-018	<i>Neochlamydia</i>	AF098330, Endosymbiont of <i>Acanthamoeba</i> sp. TUME1	96	
cTF-020	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-021	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-025	<i>Parachlamydia</i>	AF478463, Uncultured <i>Parachlamydiaceae</i> bacterium cvC7	95.1	
cTF-029	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-031	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-037	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-038	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-044	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-048	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-051	<i>Criblamydiaceae</i>	JN538005, Uncultured organism	96.1	
cTF-057	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-059	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	98.9	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-060	<i>Chlamydia</i>	AP006861, <i>Chlamydia felis</i> Fe/C-56	99.5	AE015925, <i>Chlamydia caviae</i> GPIC
cTF-068	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-069	<i>Chlamydia</i>	GQ398031, <i>Chlamydia gallinacea</i> 08-1274	99	
cTF-074	<i>Parachlamydiaceae</i>	FR872580, <i>Parachlamydia acanthamoebae</i> UV-7	96.6	
cTF-088	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
cTF-092	Unclassified <i>Chlamydiales</i>	JN616113, Uncultured bacterium	96.2	
Children Control				
cC-015	<i>Chlamydia</i>	AP006861, <i>Chlamydia felis</i> Fe/C-56	99.5	AE015925, <i>Chlamydia caviae</i> GPIC
cC-042	<i>Chlamydia</i>	AP006861, <i>Chlamydia felis</i> Fe/C-56	100	AE015925, <i>Chlamydia caviae</i> GPIC
cC-099	<i>Chlamydia</i>	AP006861, <i>Chlamydia felis</i> Fe/C-56	97.1	AE015925, <i>Chlamydia caviae</i> GPIC
Adults Case				
aTT-200	Unclassified <i>Chlamydiales</i>	AY114316, Uncultured <i>Chlamydia</i> sp.	94.6	
aTT-201	Unclassified <i>Chlamydiales</i>	JN606074, <i>Chlamydiales</i> bacterium NS11	95.1	
aTT-203	<i>Chlamydia</i>	AP006861, <i>Chlamydia felis</i> Fe/C-56	100	AE015925, <i>Chlamydia caviae</i> GPIC
aTT-207	Unclassified <i>Chlamydiales</i>	JN701140, Uncultured bacterium	90.2	
aTT-209	<i>Chlamydia</i>	AP006861, <i>Chlamydia felis</i> Fe/C-56	100	AE015925, <i>Chlamydia caviae</i> GPIC
aTT-211	Unclassified <i>Chlamydiales</i>	EU363464, <i>Chlamydiales</i> bacterium CRIB 32	98	
aTT-214	<i>Chlamydia</i>	AP006861, <i>Chlamydia felis</i> Fe/C-56	96.6	AE015925, <i>Chlamydia caviae</i> GPIC
aTT-216	Unclassified <i>Chlamydiales</i>	JN536584, Uncultured organism	93.6	
aTT-217	Unclassified <i>Chlamydiales</i>	JN606074, <i>Chlamydiales</i> bacterium NS11	98	
aTT-225	Unclassified <i>Chlamydiales</i>	JN701140, Uncultured bacterium	99	
aTT-226	Unclassified <i>Chlamydiales</i>	JX279901, Uncultured bacterium	91.2	
aTT-228	Unclassified <i>Chlamydiales</i>	HM063023, Uncultured bacterium	98	
aTT-237	<i>Chlamydia</i>	AP006861, <i>Chlamydia felis</i> Fe/C-56	99.5	AE015925, <i>Chlamydia caviae</i> GPIC
Adults Control				
aC-250	Unclassified <i>Chlamydiales</i>	HM063023, Uncultured bacterium	97.5	
aC-253	<i>Parachlamydia</i>	AM412760, <i>Parachlamydia acanthamoebae</i> OEW1	98.5	
aC-257	Unclassified <i>Chlamydiales</i>	AY114316, Uncultured <i>Chlamydia</i> sp.	91.2	
aC-259	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	96.8	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
aC-260	Unclassified <i>Chlamydiales</i>	JX279901, Uncultured bacterium	97.5	
aC-261	<i>Chlamydia</i>	AP006861, <i>Chlamydia felis</i> Fe/C-56	98	AE015925, <i>Chlamydia caviae</i> GPIC
aC-262	Unclassified <i>Chlamydiales</i>	JN701140, Uncultured bacterium	95.8	

aC-263	Unclassified <i>Chlamydiales</i>	JN606074, <i>Chlamydiales</i> bacterium NS11	95.1	
aC-265	Unclassified <i>Chlamydiales</i>	AF364563, Uncultured <i>Chlamydiales</i> bacterium	95.5	
aC-268	Unclassified <i>Chlamydiales</i>	HM063023, Uncultured bacterium	98.5	
aC-279	Unclassified <i>Chlamydiales</i>	EU090709, Uncultured <i>Candidatus</i> <i>Rhabdochlamydia</i> sp.	87.7	
aC-282	Unclassified <i>Parachlamydiaceae</i>	AF364563, Uncultured <i>Chlamydiales</i> bacterium	96.1	
aC-284	<i>Chlamydia</i>	CP000051, <i>Chlamydia trachomatis</i> A/HAR-13	100	AE001273, <i>Chlamydia trachomatis</i> D/UW-3/CX
aC-285	Unclassified <i>Parachlamydiaceae</i>	LN995859.1 <i>Neochlamydia</i> sp. Trut23-12-2015	94.5	
aC-290	Unclassified <i>Chlamydiales</i>	JN536584, Uncultured organism	92.6	

Table S2. BLAST-n analysis of *Chlamydiae* 16S rRNA sequences detected in the ocular swabs of children and adults. Results are categorized based on the first and second BLAST-hit identification.

Household ID	Best BLAST match for 16S rRNA gene*	% Similarity	Second best BLAST match for 16S rRNA gene*	% Similarity
Children Case				
cTF-006	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-008	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-012	<i>Chlamydia trachomatis</i>	99	<i>Chlamydia trachomatis</i>	99
cTF-013	<i>Chlamydia trachomatis</i>	99	<i>Chlamydia trachomatis</i>	99
cTF-017	<i>Chlamydia trachomatis</i>	99	<i>Chlamydia trachomatis</i>	99
cTF-018	<i>Neochlamydia</i> SP.	95	<i>Neochlamydia endosymbiont of Acanthamoeba</i> sp.	95
cTF-020	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-021	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-025	<i>Chlamydiales</i> bacterium CRIB 32	95	<i>Parachlamydia acanthamoebae</i>	94
cTF-029	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-031	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-037	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-038	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-044	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-048	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-051	<i>Criblamydia sequanensis</i>	95	<i>Parachlamydia acanthamoebae</i>	93
cTF-057	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	100
cTF-059	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	98
cTF-060	<i>Chlamydia caviae/felis</i>	99	<i>Chlamydia felis</i>	99
cTF-068	100% <i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-069	<i>Chlamydia gallinacea</i>	99	<i>Chlamydia psittaci</i>	99
cTF-074	<i>Parachlamydia acanthamoebae</i>	97	<i>Parachlamydia acanthamoebae</i>	96
cTF-088	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
cTF-092	<i>Chlamydiales</i> bacterium CRIB33	92	<i>Criblamydiaceae</i> bacterium cvE70	90
Children Control				
cC-015	<i>Chlamydia caviae/felis</i>	99	<i>Chlamydia felis</i>	99
cC-042	<i>Chlamydia caviae/felis</i>	100	<i>Chlamydia felis</i>	100
cC-099	<i>Chlamydia</i> sp. 2742-308	98	<i>Chlamydia caviae</i>	97
Adults Case				
aTT-200	<i>Chlamydiales</i> bacterium NS11	95	<i>Chlamydiales</i> bacterium CRIB 32	94
aTT-201	<i>Chlamydiales</i> bacterium NS11	95	<i>Chlamydiales</i> bacterium CRIB 32	94
aTT-203	<i>Chlamydia caviae/felis</i>	100	<i>Chlamydia felis</i>	100
aTT-207	<i>Neochlamydia hartmannellae</i>	92	<i>Chlamydiales</i> bacterium NS13	92
aTT-209	<i>Chlamydia caviae/felis</i>	100	<i>Chlamydia felis</i>	100
aTT-211	<i>Chlamydiales</i> bacterium CRIB 32	98	<i>Chlamydiales</i> bacterium NS11	96
aTT-214	<i>Chlamydia</i> sp./ <i>Chlamydia pecorum</i>	97	<i>Chlamydia pecorum</i>	97
aTT-216	<i>Chlamydiales</i> bacterium CRIB 32	97	<i>Chlamydiales</i> bacterium NS11	94
aTT-217	<i>Chlamydiales</i> bacterium NS11	98	<i>Chlamydiales</i> bacterium CRIB 32	97
aTT-225	<i>Chlamydiales</i> bacterium NS11	95	<i>Chlamydiales</i> bacterium cvE18	94
aTT-226	<i>Chlamydiales</i> bacterium CRIB 32/ NS11	95	<i>Chlamydiales</i> bacterium CRIB 32	95
aTT-228	<i>Chlamydiales</i> bacterium NS11	96	<i>Chlamydiales</i> bacterium CRIB 32	95
aTT-237	<i>Chlamydia caviae/ felis</i>	99	<i>Chlamydia felis</i>	99
Adults Control				
aC-250	<i>Chlamydiales</i> bacterium CRIB 32	97	<i>Chlamydiales</i> bacterium NS11	95
aC-253	<i>Parachlamydia acanthamoebae</i>	98	<i>Parachlamydia acanthamoebae</i>	98
aC-257	<i>Simkania negevensis</i>	94	<i>Chlamydiales</i> bacterium NS11	93
aC-259	<i>Chlamydia trachomatis</i>	96	<i>Chlamydia trachomatis</i>	96
aC-260	<i>Chlamydiales</i> bacterium CRIB 32	97	<i>Chlamydiales</i> bacterium NS11	96
aC-261	<i>Chlamydia caviae/felis</i>	98	<i>Chlamydia felis</i>	98
aC-262	<i>Chlamydiales</i> bacterium CRIB 32	93	<i>Chlamydiales</i> bacterium NS11	92
aC-263	<i>Chlamydiales</i> bacterium NS11	97	<i>Chlamydiales</i> bacterium cvE18	94

aC-265	<i>Neochlamydia</i> sp.	95	<i>Candidatus Protochlamydia</i> sp. cvE14	94
aC-268	<i>Chlamydiales</i> bacterium CRIB 32	98	<i>Chlamydiales</i> bacterium NS11	96
aC-279	<i>Neochlamydia hartmannellae</i>	90	<i>Candidatus Clavichlamydia salmonicola</i>	89
aC-282	<i>Neochlamydia</i> sp.	94	<i>Candidatus Protochlamydia amoebophila</i>	94
aC-284	<i>Chlamydia trachomatis</i>	100	<i>Chlamydia trachomatis</i>	99
aC-285	<i>Protochlamydia naegleriophila</i>	96	<i>Protochlamydia naegleriophila</i>	95
aC-290	<i>Chlamydiales</i> bacterium CRIB 32	95	<i>Chlamydiales</i> bacterium NS11	94

* Uncultured bacteria were excluded from final BLAST hits by choosing the “Exclude” option in BLAST-n page for “Uncultured/environmental sample sequences”.