

**Supplementary Table S1.** Solid-phase primers and probes for strip PCR

| Well                                     | Target                                       | Label | Sequence                       | Target region  |
|--|--|-------|--------------------------------|--|
| 1  | GAPDH F                                      | 6FAM  | tgtgctcccactctgatttc           | GAPDH  |
|  | GAPDH Probe 6FAM                             |       | aaaagagctaggaaggacaggcaacttggc |  |
|  | GAPDH R                                      |       | cctagctcccagggttggatt          |  |
|  | TBP F  | HEX   | gcaccactccactgtatecc           | TBP  |
|  | TBP Probe HEX                                |       | acccccateactctgccacgc          |  |
|  | TBP R  |       | cccagaactctccgaagctg           |  |
| 2  | HSV1 F                                       | 6FAM  | cgeatcaagaccacctcctc           | Glycoprotein B (UL27) gene   |
|  | HSV1 Probe 6FAM                              |       | tggcaacgcgcccaac               |  |
|  | HSV1 R                                       |       | gtcagctcgtgRttctg              |  |
|  | VZV F  | HEX   | tactaccagtcatttctatccatctg     | ORF29  |
|  | VZV Probe HEX                                |       | tgtctttcacggaggcaaacacgt       |  |
|  | VZV R  |       | gaaaacccaaaccgttctcgag         |  |
|  | HSV2 F                                       | Cy5   | cgeatcaagaccacctcctc           | Glycoprotein B (UL27) gene   |
|  | HSV2 Probe Cy5                               |       | cggcgtatgccccag                |  |
|  | HSV2 R                                       |       | gtcagctcgtgRttctg              |  |
| 3  | HTLV-1 F                                     | 6FAM  | ggccacctgtccagagca             | Tax protein (tax) gene   |
|  | HTLV-1 Probe 6FAM                            |       | Mtcacctgggacccatcgatgga        |  |
|  | HTLV-1 R                                     |       | actgtagagctgagccgataacg        |  |
|  | HHV8 F                                       | HEX   | cctctgtccccattcattg            | ORF65: small capsid protein on hexon tips; herpesvirus core gene UL35 6FAMily  |
|  | HHV8 Probe HEX                               |       | ccggcgtcagacattctcacaacc       |  |
|  | HHV8 R                                       |       | cgtttccgtcgtggatgag            |  |
|  | HHV6 F                                       | Cy5   | gaagcagcaatcgcaacaca           | DNA polymerase catalytic subunit (U38) gene  |
|  | HHV6 Probe Cy5                               |       | aaccctgtgcccgtctcc             |  |
|  | HHV6 R                                       |       | acaacatgtaactcgggtgtacggt      |  |
| 4  | EBV F  | 6FAM  | tagggccagtcacaagtgtg           | BALF5  |
|  | EBV Probe 6FAM                               |       | cggtcacaatctccacgtg            |  |
|  | EBV R  |       | acctgcgaagacatagag             |  |
|  | HHV7 F                                       | HEX   | cggaaagtcactggagtaatgacaa      | ORF51  |
|  | HHV7 Probe HEX                               |       | ctcgcagattgcttggccatg          |  |
|  | HHV7 R                                       |       | ccaatcctccgaaaccgat            |  |
|  | CMV F  | Cy5   | tcgccccgaagagg                 | UL83: beta gene  |
|  | CMV Probe Cy5                                |       | caccgacgagattccgacaacg         |  |
|  | CMV R  |       | cggccggattgtggatt              |  |
| 5  | <i>C. trachomatis</i> F                      | 6FAM  | gaaaagaacccttgaaggagg          | 16S-23S rRNA intergenic spacer   |
|  | <i>C. trachomatis</i> Probe 6FAM             |       | caaaggcagccgcaac               |  |
|  | <i>C. trachomatis</i> R                      |       | cttaactcctggctcatcatg          |  |
|  | Adenovirus F                                 | HEX   | gacatgactttgaggtgga            | hexon protein gene   |
|  | Adenovirus Probe HEX                         |       | cccatggaYgagccaccct            |  |
|  | Adenovirus R                                 |       | tcgatgacgccgggtg               |  |
| 6  | <i>Toxocara</i> F                            | 6FAM  | agcacMattgcacgtatgc            | 5.8S rRNA gene and internal transcribed spacer 2   |
|  | <i>Toxocara</i> Probe 6FAM                   |       | tgtggctgRtgcgttggagg           |  |
|  | <i>Toxocara</i> R                            |       | ccttctaactgccagcac             |  |
|  | <i>T. gondii</i> F                           | HEX   | tcccctctgctggcgaaggt           | B1 gene for antigen protein  |
|  | <i>T. gondii</i> Probe HEX                   |       | tctgtgcaacttgggtattcgcag       |  |
|  | <i>T. gondii</i> R                           |       | agcgttcgtggtcaactatcgattg      |  |
|  | <i>Acanthamoeba</i> F1                       | Cy5   | tcaaagcaggcagatYcaatt          | 18S rRNA gene  |
|  | <i>Acanthamoeba</i> F2                       |       | tcaaagcaggcagatttaacca         |  |
|  | <i>Acanthamoeba</i> Probe Cy5                |       | ctGccAccgAatAc                 |  |
|  | <i>Acanthamoeba</i> R                        |       | gtcctattccattatccatgctaa       |  |
|  | <i>Candida</i> F                             | 6FAM  | tggaggagtgattgtctgct           | 18S rRNA gene  |
|  | <i>Candida</i> Probe <i>candida</i> sp. 6FAM |       | ttaacctactaaatagtgtgctagc      |  |
| <i>Candida</i> Probe <i>glabrata</i> HEX | ttaacctactaaatagtgtgctagc                    |       |                                |  |
|  | <i>Candida</i> Probe <i>krusei</i> Cy5       | Cy5   | ttaacctgctaaatagggtcgcgagc     |  |
|  | <i>Candida</i> R                             |       | acagacctgtattgcctcaa           |  |
|  | <i>Fusarium</i> F                            | 6FAM  | gggcccaggtgtaattgt             | 18S rRNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S rRNA gene |
| <i>Fusarium</i> Probe 6FAM               | atagagggtgagagccccgtctggt                    |       |                                |  |
| <i>Fusarium</i> R                        | tctggccggatttagcttt                          |       |                                |  |
|  | <i>Aspergillus</i> F                         | HEX   | acccccctgagccagtcg             | 18S rRNA gene  |
|  | <i>Aspergillus</i> Probe HEX                 |       | acgtccctgccctttgtacacaccg      |  |
|  | <i>Aspergillus</i> R                         |       | gccagcgagtacatcaccttgg         |  |
| 9  | <i>M. tuberculosis</i> F                     | 6FAM  | ggctgtggtagcagacc              | Insertion sequence IS6110 transposase (orfB) gene  |
|  | <i>M. tuberculosis</i> Probe 6FAM            |       | tgtcgacctggcagggttcg           |  |
|  | <i>M. tuberculosis</i> R                     |       | cgggtccagatggcttgc             |  |
|  | <i>P. acnes</i> F                            | HEX   | gcgtgagtgacgtaatgggta          | 16S rRNA gene  |
|  | <i>P. acnes</i> Probe HEX                    |       | agcgttgcggattattggcg           |  |
|  | <i>P. acnes</i> R                            |       | ttccgacgcgatcaacca             |  |
| 10                                       | <i>T. pallidum</i> F                         | 6FAM  | ccaagcgttactaagatg             | 47-kDa antigen gene  |
|  | <i>T. pallidum</i> Probe 6FAM                |       | ttcgaatcttattctcgtgtca         |  |
|  | <i>T. pallidum</i> R                         |       | ctgggacaaactcatic              |  |
| 11                                       | Fungal 28S rRNA F                            | HEX   | gcataatcaataagcggagaaaag       | 28S rRNA gene  |
|  | Fungal 28S rRNA Probe HEX                    |       | cggcgagtgaaagcggsaaragctc      |  |
|  | Fungal 28S rRNA R                            |       | ttagctttagatgRaRttaccacc       |  |
| 12                                       | Bacterial 16S rRNA F                         | 6FAM  | aggcagcagtDRggaat              | 16S rRNA gene  |
|  | Bacterial 16S rRNA Probe 6FAM                |       | tgccagcagccggtaatacRDag        |  |
|  | Bacterial 16S rRNA R                         |       | ggactacYVgggtatctaat           |  |

*Candida* sp., *Candida* species; *Chlamydia trachomatis*, *C. trachomatis*; CMV, cytomegalovirus; EBV, Epstein-Barr virus; F, forward; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HHV, human herpes virus; HTLV, human T-cell lymphotropic virus; HSV, herpes simplex virus; *M. tuberculosis*, *Mycobacterium tuberculosis*; *P. acnes*, *Propionibacterium acnes*; R, reverse; rRNA, ribosomal RNA; TBP, TATA-binding protein; *T. gondii*, *Toxoplasma gondii*; VZV, varicella-zoster virus.

**Supplementary Table S2.** Repeatability tests with a negative control

| Cq values |                        | Day 1   |      | Day 2   |      | Day 3   |      | Day 4   |      | Day 5   |      | All days |      | CV (%) |
|-----------|------------------------|---------|------|---------|------|---------|------|---------|------|---------|------|----------|------|--------|
|           |                        | Average | SD   | Average | SD   | Average | SD   | Average | SD   | Average | SD   | Average  | SD   |        |
| 1         | GAPDH                  | 33.18   | 0.53 | 33.03   | 0.20 | 33.22   | 1.07 | 32.95   | 0.65 | 32.10   | 1.30 | 32.90    | 0.39 | 1.4    |
|           | TBP                    | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
| 2         | HSV1                   | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | HSV2                   | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | VZV                    | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
| 3         | HTLV-1                 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | HHV6                   | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | HHV8                   | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
| 4         | EBV                    | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | CMV                    | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | HHV7                   | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
| 5         | <i>C. trachomatis</i>  | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | Adenovirus             | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
| 6         | <i>Toxocara</i>        | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | <i>T. gondii</i>       | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | <i>Acanthamoeba</i>    | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
| 7         | <i>Candida</i> sp.     | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | <i>C. krusei</i>       | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | <i>C. glabrata</i>     | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
| 8         | <i>Fusarium</i>        | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | <i>Aspergillus</i>     | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
| 9         | <i>M. tuberculosis</i> | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
|           | <i>P. acnes</i>        | 38.76   | 1.68 | 0.00    | 0.00 | 36.32   | 0.36 | 0.00    | 0.00 | 35.02   | -    | 36.70    | 1.90 | 5.2    |
| 10        | <i>T. pallidum</i>     | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
| 11        | Fungal 28S             | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00    | 0.00 | 0.00     | 0.00 | 0.0    |
| 12        | Bacterial 16S          | 39.86   | 3.21 | 40.69   | 3.26 | 39.48   | 1.48 | 37.91   | -    | 38.55   | 2.41 | 69.30    | 1.09 | 2.8    |

We tested the strip PCR with a negative control in triplicate over 5 days of testing. The intra- and interassay variability indicated that the assay with the negative control was highly reproducible, except for occasional contamination with *P. acnes* and bacterial 16S from the samples or reagents, based on the low standard deviation (<1.30) and low coefficient of variation (CV, 0.0%-1.4%)

CV(%) = standard deviation of population x100 / average

When contamination from *P. acnes* and *bacterial 16S* in the reagents and from the ocular surface was occasionally observed, we calculated the average value and SE. Bacterial 16S, bacterial 16S ribosomal RNA; Cq, quantification cycle; CV, coefficient of variation; fungal 28S, fungal 28S ribosomal RNA; SD, standard deviation.

**Supplementary Table S3.** Repeatability tests using a low concentration of positive controls (each target pathogen DNA of well)

| Cq values | Day 1                  |       | Day 2   |       | Day 3   |       | Day 4   |       | Day 5   |       | All days |       |        |     |
|-----------|------------------------|-------|---------|-------|---------|-------|---------|-------|---------|-------|----------|-------|--------|-----|
|           | Average                | SD    | Average | SD    | Average | SD    | Average | SD    | Average | SD    | Average  | SD    | CV (%) |     |
| 1         | GAPDH                  | 33.33 | 0.42    | 33.27 | 0.66    | 32.79 | 0.34    | 33.06 | 0.23    | 32.13 | 1.25     | 32.81 | 0.43   | 1.3 |
|           | TBP                    | 0.00  | 0.00    | 0.00  | 0.00    | 0.00  | 0.00    | 0.00  | 0.00    | 0.00  | 0.00     | 0.00  | 0.00   | 0.0 |
| 2         | HSV1                   | 32.06 | 0.28    | 32.41 | 0.55    | 31.26 | 1.06    | 32.24 | 0.44    | 32.00 | 0.45     | 31.98 | 0.44   | 1.4 |
|           | HSV2                   | 33.67 | 1.08    | 33.02 | 0.94    | 31.92 | 1.08    | 32.78 | 0.82    | 32.07 | 0.69     | 32.45 | 0.46   | 1.4 |
|           | VZV                    | 33.30 | 0.45    | 33.48 | 1.39    | 31.83 | 1.31    | 32.51 | 0.49    | 32.54 | 0.20     | 32.59 | 0.59   | 1.8 |
| 3         | HTLV-1                 | 33.13 | 0.25    | 33.53 | 1.18    | 31.86 | 1.24    | 32.87 | 0.20    | 32.25 | 0.41     | 32.63 | 0.63   | 1.9 |
|           | HHV6                   | 33.00 | 0.30    | 34.80 | 1.58    | 32.01 | 1.21    | 32.21 | 0.37    | 32.57 | 0.34     | 32.90 | 1.12   | 3.4 |
|           | HHV8                   | 32.67 | 0.48    | 32.69 | 0.55    | 31.91 | 1.05    | 32.04 | 0.66    | 31.90 | 0.22     | 32.14 | 0.33   | 1.0 |
| 4         | EBV                    | 34.28 | 0.38    | 35.62 | 1.81    | 33.19 | 1.27    | 33.85 | 0.42    | 33.72 | 0.86     | 34.09 | 0.92   | 2.7 |
|           | CMV                    | 33.01 | 0.29    | 34.80 | 2.44    | 31.33 | 1.22    | 31.86 | 0.57    | 32.19 | 0.18     | 32.55 | 1.34   | 4.1 |
|           | HHV7                   | 34.44 | 0.78    | 34.45 | 0.25    | 33.71 | 0.36    | 34.01 | 0.26    | 33.92 | 0.59     | 34.02 | 0.27   | 0.8 |
| 5         | <i>C. trachomatis</i>  | 34.69 | 0.26    | 35.14 | 2.26    | 33.68 | 0.87    | 33.91 | 0.12    | 34.67 | 1.26     | 34.35 | 0.59   | 1.7 |
|           | Adenovirus             | 32.31 | 1.71    | 33.49 | 0.72    | 31.91 | 1.74    | 32.11 | 0.38    | 32.33 | 0.63     | 32.46 | 0.61   | 1.9 |
| 6         | <i>Toxocara</i>        | 32.25 | 0.06    | 32.41 | 0.42    | 31.52 | 0.83    | 31.69 | 0.05    | 31.84 | 0.15     | 31.87 | 0.33   | 1.1 |
|           | <i>T. gondii</i>       | 33.04 | 0.13    | 33.67 | 0.77    | 31.30 | 1.61    | 32.55 | 0.41    | 32.08 | 0.44     | 32.40 | 0.86   | 2.7 |
|           | <i>Acanthamoeba</i>    | 32.60 | 0.31    | 32.82 | 0.31    | 31.95 | 0.13    | 31.93 | 0.60    | 31.80 | 0.14     | 32.12 | 0.41   | 1.3 |
| 7         | <i>Candida</i> sp.     | 34.64 | 0.80    | 33.17 | 1.27    | 34.56 | 1.06    | 33.39 | 0.17    | 33.28 | 0.33     | 33.60 | 0.56   | 1.7 |
|           | <i>C. krusei</i>       | 33.79 | 0.59    | 34.97 | 1.37    | 33.08 | 1.67    | 33.52 | 0.16    | 33.50 | 0.30     | 33.77 | 0.71   | 2.1 |
|           | <i>C. glabrata</i>     | 33.58 | 0.46    | 34.34 | 0.53    | 34.19 | 0.30    | 33.12 | 0.40    | 33.09 | 0.32     | 33.69 | 0.58   | 1.7 |
| 8         | <i>Fusarium</i>        | 30.58 | 0.06    | 32.41 | 1.88    | 29.41 | 0.90    | 29.52 | 0.20    | 29.61 | 0.05     | 30.24 | 1.26   | 4.2 |
|           | <i>Aspergillus</i>     | 31.93 | 0.54    | 31.51 | 0.08    | 30.60 | 1.40    | 31.06 | 0.55    | 30.89 | 0.68     | 31.02 | 0.33   | 1.1 |
| 9         | <i>M. tuberculosis</i> | 33.30 | 0.40    | 33.80 | 0.61    | 32.19 | 1.37    | 32.58 | 0.23    | 32.49 | 0.23     | 32.76 | 0.61   | 1.9 |
|           | <i>P. acnes</i>        | 32.11 | 0.55    | 32.14 | 0.88    | 30.50 | 1.19    | 31.56 | 0.92    | 31.25 | 0.53     | 31.36 | 0.59   | 1.9 |
| 10        | <i>T. pallidum</i>     | 32.38 | 1.02    | 31.83 | 0.34    | 31.35 | 0.55    | 31.07 | 0.46    | 31.02 | 0.17     | 31.32 | 0.32   | 1.0 |
| 11        | Fungal 28S             | 34.55 | 0.07    | 34.45 | 0.84    | 33.50 | 0.19    | 34.98 | 1.93    | 32.93 | 0.81     | 33.97 | 0.80   | 2.4 |
| 12        | Bacterial 16S          | 35.49 | 0.57    | 35.81 | 0.98    | 35.48 | 0.77    | 36.27 | 0.68    | 35.18 | 0.29     | 35.68 | 0.40   | 1.1 |

We added positive controls (each target pathogen DNA of well) to each well in triplicate over 5 days of testing. The intra- and interassay variability indicated that the assay was highly reproducible based on the low standard deviation (<2.44) and low coefficient of variation (CV, 0.0%-4.2%).

**Supplementary Table S4.** Repeatability tests using a low concentration of the positive control (all target pathogen DNA of strip)

| Cq values | Day 1                  |       | Day 2   |       | Day 3   |       | Day 4   |       | Day 5   |       | All days |       |        |     |
|-----------|------------------------|-------|---------|-------|---------|-------|---------|-------|---------|-------|----------|-------|--------|-----|
|           | Average                | SD    | Average | SD    | Average | SD    | Average | SD    | Average | SD    | Average  | SD    | CV (%) |     |
| 1         | GAPDH                  | 32.92 | 0.65    | 32.65 | 0.28    | 32.41 | 1.34    | 33.02 | 0.49    | 33.60 | 0.80     | 32.92 | 0.45   | 1.4 |
|           | TBP                    | 0.00  | 0.00    | 0.00  | 0.00    | 0.00  | 0.00    | 0.00  | 0.00    | 0.00  | 0.00     | 0.00  | 0.00   | 0.0 |
| 2         | HSV1                   | 30.58 | 0.73    | 31.10 | 0.40    | 31.18 | 0.24    | 31.33 | 0.39    | 32.01 | 0.34     | 31.24 | 0.51   | 1.6 |
|           | HSV2                   | 31.47 | 0.45    | 31.83 | 0.03    | 32.07 | 0.09    | 32.38 | 0.49    | 33.37 | 1.07     | 32.22 | 0.72   | 2.2 |
|           | VZV                    | 31.43 | 0.80    | 31.90 | 0.76    | 31.95 | 0.37    | 31.95 | 0.40    | 32.73 | 0.53     | 31.99 | 0.47   | 1.5 |
| 3         | HTLV-1                 | 31.67 | 0.81    | 32.54 | 0.31    | 32.46 | 0.30    | 32.56 | 0.65    | 32.85 | 0.44     | 32.42 | 0.44   | 1.4 |
|           | HHV6                   | 31.75 | 0.75    | 32.63 | 0.05    | 32.57 | 0.10    | 32.32 | 0.30    | 33.19 | 0.54     | 32.49 | 0.52   | 1.6 |
|           | HHV8                   | 31.44 | 0.70    | 31.48 | 0.36    | 31.71 | 0.34    | 31.62 | 0.58    | 32.34 | 0.12     | 31.72 | 0.36   | 1.1 |
| 4         | EBV                    | 32.60 | 0.61    | 32.54 | 0.17    | 32.70 | 0.20    | 32.80 | 0.46    | 33.26 | 0.40     | 32.78 | 0.28   | 0.9 |
|           | CMV                    | 30.64 | 0.51    | 31.19 | 0.41    | 31.28 | 0.47    | 31.41 | 0.24    | 32.81 | 1.23     | 31.47 | 0.81   | 2.6 |
|           | HHV7                   | 33.77 | 0.14    | 33.73 | 0.35    | 33.74 | 0.37    | 34.07 | 0.53    | 34.62 | 0.66     | 33.99 | 0.38   | 1.1 |
| 5         | <i>C. trachomatis</i>  | 33.48 | 0.47    | 34.23 | 0.57    | 34.73 | 0.99    | 34.30 | 0.35    | 34.73 | 0.39     | 34.30 | 0.51   | 1.5 |
|           | Adenovirus             | 31.27 | 1.05    | 32.51 | 0.60    | 33.15 | 0.14    | 32.53 | 0.75    | 34.04 | 0.41     | 32.70 | 1.01   | 3.1 |
| 6         | <i>Toxocara</i>        | 29.96 | 0.33    | 29.98 | 0.26    | 30.26 | 0.51    | 29.95 | 0.35    | 30.92 | 0.43     | 30.21 | 0.42   | 1.4 |
|           | <i>T. gondii</i>       | 30.38 | 0.39    | 31.39 | 0.95    | 31.09 | 0.19    | 30.84 | 0.73    | 33.22 | 1.81     | 31.38 | 1.09   | 3.5 |
|           | <i>Acanthamoeba</i>    | 32.04 | 0.48    | 32.04 | 0.41    | 32.14 | 0.41    | 31.89 | 0.34    | 32.69 | 0.10     | 32.16 | 0.31   | 1.0 |
| 7         | <i>Candida</i> sp.     | 33.12 | 0.71    | 32.60 | 0.44    | 33.13 | 1.57    | 32.90 | 0.31    | 34.02 | 1.82     | 33.15 | 0.53   | 1.6 |
|           | <i>C. krusei</i>       | 31.77 | 1.09    | 31.90 | 0.16    | 32.20 | 0.58    | 32.09 | 0.22    | 33.47 | 1.18     | 32.28 | 0.68   | 2.1 |
|           | <i>C. glabrata</i>     | 32.68 | 0.53    | 32.16 | 0.13    | 32.36 | 0.66    | 32.46 | 0.22    | 33.64 | 1.16     | 32.66 | 0.58   | 1.8 |
| 8         | <i>Fusarium</i>        | 29.39 | 0.49    | 30.37 | 1.31    | 29.69 | 0.51    | 29.83 | 0.23    | 30.90 | 0.95     | 30.04 | 0.60   | 2.0 |
|           | <i>Aspergillus</i>     | 29.07 | 0.27    | 28.96 | 1.37    | 29.22 | 0.63    | 29.28 | 0.48    | 31.36 | 1.36     | 29.58 | 1.00   | 3.4 |
| 9         | <i>M. tuberculosis</i> | 31.63 | 0.99    | 32.10 | 0.49    | 32.05 | 0.55    | 32.15 | 0.11    | 32.49 | 0.11     | 32.08 | 0.30   | 1.0 |
|           | <i>P. acnes</i>        | 30.53 | 1.83    | 31.45 | 0.58    | 31.62 | 0.16    | 31.59 | 0.47    | 32.37 | 0.73     | 31.51 | 0.66   | 2.1 |
| 10        | <i>T. pallidum</i>     | 31.38 | 0.44    | 31.22 | 0.28    | 31.58 | 0.44    | 31.67 | 0.09    | 32.86 | 1.39     | 31.74 | 0.65   | 2.0 |
| 11        | Fungal 28S             | 33.37 | 0.47    | 33.13 | 0.59    | 33.04 | 0.59    | 32.73 | 0.66    | 34.39 | 1.26     | 33.33 | 0.64   | 1.9 |
| 12        | Bacterial 16S          | 35.81 | 0.76    | 35.71 | 1.15    | 35.72 | 0.54    | 35.96 | 0.75    | 35.89 | 1.51     | 35.82 | 0.11   | 0.3 |

We added positive controls (all target pathogen DNA of strip) to each well in triplicate over 5 days of testing. The intra- and interassay variability indicated high reproducibility based on the low standard deviation (<1.83) and low coefficient of variation (CV, 0.0%-3.5%).

**Supplementary Table S5.** Linearity tests of strip PCR assay

|    |                        | Serial dilution of standard DNA (copies/mL, Cq values) |                 |                 |       |   | Linear dilution graph |           | Amplification efficiency | Correlation coefficient |
|----|------------------------|--|-----------------|-----------------|-------|---|-----------------------|-----------|--------------------------|-------------------------|
|    |                        | 10 <sup>6</sup>  | 10 <sup>4</sup> | 10 <sup>2</sup> | 50    | 0 | Slope                 | Intercept |                          |                         |
| 1  | GAPDH                  | 18.78  | 25.60           | 32.36           | 33.92 | 0 | -3.47                 | 39.56     | 0.94                     | 1.00                    |
|    | HSV1                   | 18.10  | 24.77           | 31.57           | 31.98 | 0 | -3.28                 | 37.85     | 1.02                     | 1.00                    |
| 2  | HSV2                   | 18.50  | 25.22           | 31.64           | 32.70 | 0 | -3.29                 | 38.28     | 1.01                     | 1.00                    |
|    | VZV                    | 18.83  | 25.87           | 32.46           | 33.41 | 0 | -3.38                 | 39.24     | 0.97                     | 1.00                    |
|    | HTLV-1                 | 20.22  | 26.76           | 33.48           | 34.10 | 0 | -3.26                 | 39.82     | 1.02                     | 1.00                    |
| 3  | HHV6                   | 20.13  | 26.94           | 33.93           | 34.01 | 0 | -3.31                 | 40.11     | 1.00                     | 1.00                    |
|    | HHV8                   | 18.55  | 25.36           | 32.09           | 33.20 | 0 | -3.40                 | 38.93     | 0.97                     | 1.00                    |
|    | EBV                    | 19.80  | 26.52           | 32.88           | 33.87 | 0 | -3.26                 | 39.45     | 1.02                     | 1.00                    |
| 4  | CMV                    | 18.01  | 24.80           | 31.39           | 33.21 | 0 | -3.46                 | 38.69     | 0.95                     | 1.00                    |
|    | HHV7                   | 18.58  | 25.02           | 31.82           | 34.28 | 0 | -3.53                 | 39.51     | 0.92                     | 1.00                    |
| 5  | <i>C. trachomatis</i>  | 18.74  | 25.62           | 32.87           | 33.91 | 0 | -3.54                 | 39.90     | 0.92                     | 1.00                    |
|    | Adenovirus             | 18.83  | 25.82           | 31.75           | 33.48 | 0 | -3.31                 | 38.82     | 1.00                     | 1.00                    |
|    | <i>Toxocara</i>        | 17.28  | 24.36           | 31.01           | 29.32 | 0 | -3.03                 | 35.87     | 1.14                     | 1.00                    |
| 6  | <i>T. gondii</i>       | 17.54  | 24.78           | 31.69           | 34.40 | 0 | -3.77                 | 40.00     | 0.84                     | 1.00                    |
|    | <i>Acanthamoeba</i>    | 17.97  | 24.86           | 31.83           | 33.70 | 0 | -3.59                 | 39.37     | 0.90                     | 1.00                    |
|    | <i>Candida</i> sp.     | 18.98  | 25.8            | 32.53           | 33.64 | 0 | -3.40                 | 39.38     | 0.97                     | 1.00                    |
| 7  | <i>C. krusei</i>       | 18.88  | 25.74           | 32.69           | 32.78 | 0 | -3.32                 | 38.88     | 1.00                     | 1.00                    |
|    | <i>C. glabrata</i>     | 18.91  | 25.67           | 32.11           | 32.89 | 0 | -3.26                 | 38.56     | 1.03                     | 1.00                    |
| 8  | <i>Fusarium</i>        | 19.08  | 25.63           | 30.63           | 31.44 | 0 | -2.84                 | 36.43     | 1.25                     | 1.00                    |
|    | <i>Aspergillus</i>     | 17.74  | 24.39           | 30.70           | 31.76 | 0 | -3.24                 | 37.26     | 1.03                     | 1.00                    |
| 9  | <i>M. tuberculosis</i> | 18.51  | 25.13           | 31.71           | 32.32 | 0 | -3.24                 | 38.03     | 1.03                     | 1.00                    |
|    | <i>P. acnes</i>        | 18.13  | 25.38           | 32.19           | 32.36 | 0 | -3.38                 | 38.58     | 0.98                     | 1.00                    |
| 10 | <i>T. pallidum</i>     | 16.79  | 23.58           | 30.43           | 33.94 | 0 | -3.77                 | 39.09     | 0.84                     | 1.00                    |
| 11 | Fungal 28S             | 18.21  | 24.84           | 31.26           | 33.88 | 0 | -3.49                 | 39.01     | 0.93                     | 1.00                    |
| 12 | Bacterial 16S          | 20.36  | 26.84           | 34.30           | 36.20 | 0 | -3.63                 | 41.86     | 0.89                     | 1.00                    |

We performed linearity tests with DNA from each pathogen at various dilutions (10<sup>6</sup> copies, 10<sup>4</sup> copies, 10<sup>2</sup> copies, 50 copies). We set a limited measurable goal of pathogen concentration for strip PCR as 50 copies. These experiments showed a linear relationship with a good correlation coefficient (1.00) between the Cq value in the range of 50 copies to 10<sup>6</sup> copies of DNA. We believe that the strip PCR assay will make semi-quantitative analysis possible in this range.

**Supplementary Table S6.** Analytical specificity measurements

|    | Cq value               | HSV1   | HSV2   | VZV    | HTLV-1 | HHV6  | HHV8   | EBV    | CMV    | HHV7   | <i>C. trachomatis</i> | Adenovirus | <i>Toxocara</i> | <i>T. gondii</i> | <i>Acanthamoeba</i> | <i>Candida</i> sp. | <i>C. krusei</i> | <i>C. glabrata</i> | <i>Fusarium</i> | <i>Aspergillus</i> | <i>P. acnes</i> | <i>T. pallidum</i> | Bacterial 16S |
|----|------------------------|--------|--------|--------|--------|-------|--------|--------|--------|--------|-----------------------|------------|-----------------|------------------|---------------------|--------------------|------------------|--------------------|-----------------|--------------------|-----------------|--------------------|---------------|
|    |                        |        |        |        |        |       |        |        |        |        |                       |            |                 |                  |                     | Fungal 28S         | Fungal 28S       | Fungal 28S         | Fungal 28S      | Fungal 28S         |                 |                    |               |
| 1  | GAPDH                  | 30.02  | 32.92  | 31.02  | 26.01  | 30.58 | 24.02  | 33.50  | 33.77  | 22.25  | 24.35                 | 27.69      | 32.28           | 31.91            | 26.15               | 31.99              | 32.64            | 32.50              | 32.54           | 32.54              | 32.10           | 31.23              | 33.83         |
|    | TBP                    | 30.23  | 34.73  | 31.37  | 24.01  | 31.02 | 23.73  | 0.00   | 0.00   | 21.39  | 24.41                 | 27.98      | 0.00            | 0.00             | 23.91               | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 31.19              | 0.00          |
| 2  | HSV1                   | 20.79  | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | HSV2                   | 0.00   | 26.81  | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | VZV                    | 0.00   | 0.00   | 20.01  | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
| 3  | HTLV-1                 | 0.00   | 0.00   | 0.00   | 23.57  | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | HHV6                   | 0.00   | 0.00   | 0.00   | 0.00   | 28.51 | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | HHV8                   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 20.92  | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
| 4  | EBV                    | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 29.52  | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | CMV                    | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 23.36  | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | HHV7                   | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 13.60  | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
| 5  | <i>C. trachomatis</i>  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 30.56                 | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | Adenovirus             | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 14.14      | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | <i>Toxocara</i>        | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 18.24           | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
| 6  | <i>T. gondii</i>       | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 22.54            | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | <i>Acanthamoeba</i>    | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 22.86               | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | <i>Candida</i> sp.     | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 21.47              | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
| 7  | <i>C. krusei</i>       | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 19.04              | 19.38            | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | <i>C. glabrata</i>     | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 20.92              | 0.00             | 19.54              | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
|    | <i>Fusarium</i>        | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 21.81              | 0.00            | 0.00               | 0.00          |
| 8  | <i>Aspergillus</i>     | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 21.16           | 0.00               | 0.00          |
|    | <i>M. tuberculosis</i> | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 0.00               | 0.00          |
| 9  | <i>P. acnes</i>        | 35.05  | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 35.24* | 0.00   | 34.43* | 33.30*                | 37.08*     | 0.00            | 35.75*           | 33.39*              | 36.57*             | 35.68*           | 34.94*             | 33.19*          | 0.00               | 17.99           | 0.00               | 0.00          |
| 10 | <i>T. pallidum</i>     | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 0.00   | 0.00   | 0.00   | 0.00   | 0.00                  | 0.00       | 0.00            | 0.00             | 0.00                | 0.00               | 0.00             | 0.00               | 0.00            | 0.00               | 0.00            | 34.38              | 0.00          |
| 11 | Fungal 28S             | 0.00   | 0.00   | 0.00   | 0.00   | 0.00  | 37.20* | 0.00   | 0.00   | 38.22* | 0.00                  | 0.00       | 36.21*          | 0.00             | 35.55*              | 22.57              | 20.75            | 21.53              | 44.00*          | 22.84              | 0.00            | 34.27*             | 39.55*        |
| 12 | Bacterial 16S          | 40.15* | 41.98* | 43.82* | 0.00   | 0.00  | 42.57* | 0.00   | 42.44* | 35.83* | 0.00                  | 0.00       | 40.07*          | 40.40*           | 36.25*              | 42.58*             | 39.03*           | 40.23*             | 42.39*          | 36.18*             | 35.10*          | 36.64*             | 19.96         |

We performed analytical specificity measurements with high concentrations of non-targeted pathogens in each well to truly assess specificity. None of the non-targeted pathogens showed positive signal excepting *P. acnes*, bacterial 16S and fungal 28S contaminated from reagents and ocular surface.

\* Cut-off Cq levels: bacterial 16S and fungal 28S, < 35.00; *P. acnes*, < 33.00; others, < 40.00.

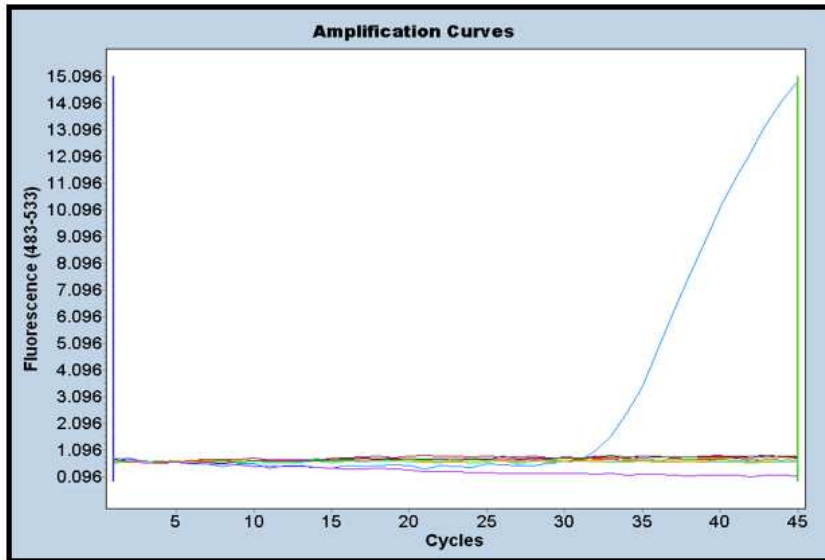
**Supplementary Table S7.** Strip PCR test results in control samples

| No. | Patient | Diagnosis     | Sample | GAPDH (Cq) | TBP (Cq) | Bacterial 16S (Cq) | Fungal 28S (Cq) | <i>P. acnes</i> (Cq) | Other pathogens |
|-----|---------|---------------|--------|------------|----------|--------------------|-----------------|----------------------|-----------------|
| 1   | 74M     | Cataract      | Aqh    | 33.58      | 34.75    | 40.00              | 40.00           | 37.93                | N.D.            |
| 2   | 79F     | Cataract, ERM | VF     | 33.57      | 33.57    | 40.00              | N.D.            | N.D.                 | N.D.            |
| 3   | 77M     | Cataract      | Aqh    | 33.58      | 33.75    | 40.00              | 40.00           | N.D.                 | N.D.            |
| 4   | 65M     | RD            | VF     | 32.82      | 32.32    | 40.00              | N.D.            | 38.82                | N.D.            |
| 5   | 67F     | POAG          | Aqh    | 32.28      | 32.28    | N.D.               | 41.66           | N.D.                 | N.D.            |
| 6   | 60F     | POAG          | Aqh    | 33.24      | 33.24    | N.D.               | N.D.            | 37.76                | N.D.            |
| 7   | 73F     | NTG           | Aqh    | 34.79      | 35.87    | N.D.               | N.D.            | 35.27                | N.D.            |
| 8   | 68M     | XFG           | Aqh    | 34.67      | 36.85    | 38.85              | N.D.            | 35.74                | N.D.            |
| 9   | 64F     | MH            | VF     | 33.75      | 33.75    | N.D.               | N.D.            | N.D.                 | N.D.            |
| 10  | 72M     | MH            | VF     | 33.92      | 33.92    | N.D.               | N.D.            | N.D.                 | N.D.            |

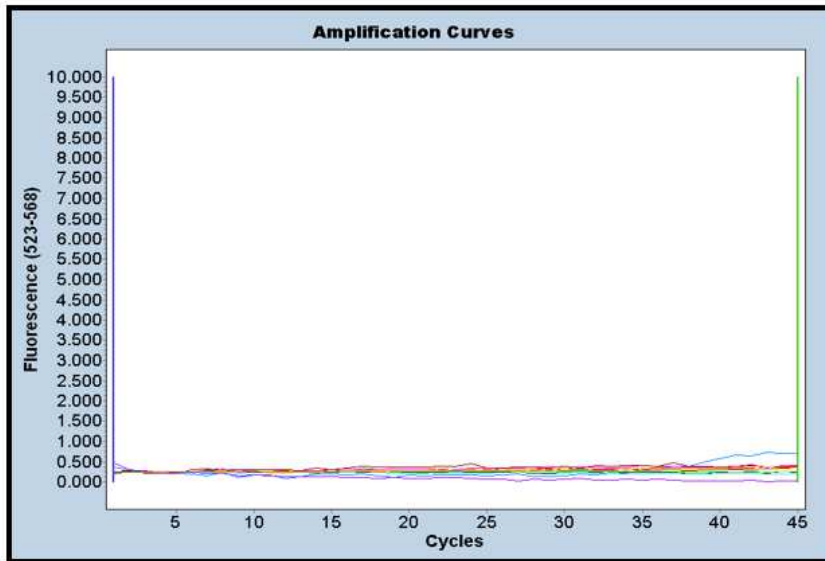
Some control samples (n = 10) were positive for bacterial 16S rRNA (5/10 samples,  $39.77 \pm 0.51$  cycles) and fungal 28S rRNA (3/10 samples,  $40.55 \pm 0.96$  cycles) present in the PCR amplification enzyme as well as *P. acnes* (5/10 samples,  $37.10 \pm 1.52$  cycles). Cut-off Cq values were set based on data from control samples to avoid false-positive results, as follows: 35.00 for bacterial 16S and fungal 28S rDNA; and 33.00 for *P. acnes*.

Aqh, aqueous humor; ERM, epiretinal membrane; MH, macular hole; N.D., not detected; NTG, normal tension glaucoma; POAG, primary open angle glaucoma; RD, retinal detachment; rRNA, ribosomal RNA; VF, vitreous fluid; XFG, exfoliative glaucoma.

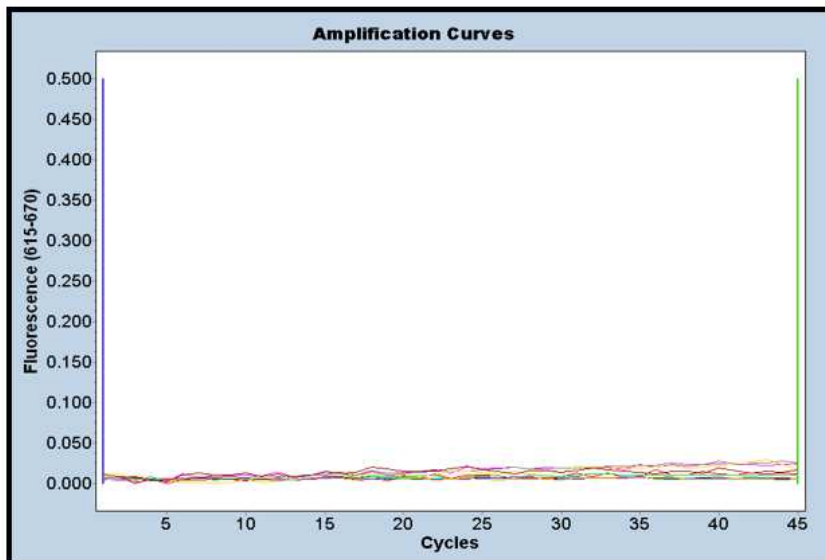
6FAM



HEX



Cy5



### Supplementary Figure S1.

Strip PCR results with negative DNA control (DW) were negative for all items except GAPDH.