

Recombinant ELISA test

For the ELISA assay, Costar® high-affinity 96-well plates (Corning Inc., ME, USA) were used. The plate was sensitized with 100 µL of recombinant major outer membrane protein (rMOMP) from *Chlamydia trachomatis* or *Chlamydia abortus* at a concentration of 200 pg/mL in carbonate-bicarbonate buffer (0.1 M, pH 9.5). The plate was incubated at 4 °C for 24 h, followed by one hour at 37 °C. After which, the solution was removed, and the wells were blocked with 200 µL of 1% bovine serum albumin (BSA) in phosphate-buffered saline with 0.05% Tween 20 (PBS-T). The plate was incubated for one hour at 37°C. Afterward, one wash was done with 200 µL of PBS-T per well and with gentle shaking for 1 min; the solution was removed, and 100 µL of the human serum to be analyzed was added to each well (diluted 1:256 in PBS-T). Each serum was studied in duplicate. The microplate was incubated for one hour at 37 °C and washed three times with PBS-T, as described above. After washing, 100 µL of goat anti-total human Ig serum peroxidase-conjugated (Cappel Organon Teknika Corp. USA) was added. The microplate was incubated for one hour at 37 °C and washed five times with PBS-T, as described above. Subsequently, 100 µL of substrate solution (4 mg of orthophenylenediamine in sodium citrate-citric acid buffer, 0.1 M, pH 5.5, and 8 µL of H₂O₂ at 30%) was added to each well, and the plates were incubated for 30 min at room temperature. Following incubation, the enzymatic reaction was stopped by adding 50 µL of 2.5 M sulfuric acid solution to each well. The optical density (OD) was determined using a 490-nm filter (Elx808, Biotek, Winooski, VT, USA). For the analysis of the results, the following equation was used:

$$S/Co \text{ ratio} = \frac{\text{Abs sample} - \text{Abs negative control}}{\text{Abs positive control} - \text{Abs negative control}}.$$

The signal-to-cutoff (S/Co) is calculated by subtracting the absorbance value of the samples from the absorbance value of the negative control and then subtracting the absorbance value of the positive control from the absorbance value of the negative control. Samples with absorbance values greater than or equal to the cutoff were considered positive.