**Background** Chlamydia spp. serology is compromised by cross-reactivity of classical antigens. For specific detection of anti- Trachomatosis (Ctt) and anti-C. Pneumoniae (Cpn) antibodies, we developed and validated novel peptide ELISAs.

**Methods** Strongly reactive peptide antigens of 24 Ctt and 48 Cpn-specific B-cell epitopes of multiple immunodominant chlamydial proteins were used in this study. For specific detection of anti-Ctt and anti-Cpn antibodies, 185 human sera were tested in colorimetric ELISAs with mixtures of 12–24 Ctt or Cpn peptide antigens using polyclonal anti-human IgG-HRP conjugates. For comparative evaluation, these sera were tested with 4 Ctt and 4 Cpn commercial IgG ELISAs.

**Results** In commercial ELISAs, Ctt and Cpn individual serum reactivity was 54% biased towards positivity for both species (co-positivity), but unbiased in Ctt and Cpn peptide antibody assays. This finding suggested a severe specificity problem (cross-reactivity) of commercial ELISAs, but not peptide assays. Using hyperimmune mouse sera against each of 11 Chlamydia spp., we confirmed that commercial Ctt and Cpn ELISA antigens are cross-reactive among all Chlamydia spp., but Cpn and Ctt peptide antigens react specifically only with antiserum against the cognate chlamydial species. By comparison at 90% specificity to a Ctt-peptide composite reference standard (CRS) for human anti-Ctt antibody status, the Ctt mixed peptide assays showed 86–83% sensitivity, significantly higher than the 59–34% sensitivity of 4 commercial anti-Ctt ELISAs. Relative to a Cpn-peptide CRS, the Cpn mixed peptide assay showed 86–80% sensitivity at 90% specificity, significantly higher than the 48–25% sensitivity of 4 commercial anti-Cpn ELISAs.

**Conclusion** For detection of anti-Ctt and -Cpn antibodies, commercial ELISAs are not suitable due to cross-reactivity. In contrast, mixed peptide assays are accurate with simultaneous high specificity and sensitivity, and reliably determine anti-Ctt and anti-Cpn antibody prevalence. With convenient use for non-specialized laboratories, these peptide ELISAs will improve Ctt and Cpn serodiagnosis.

**Disclosure** No significant relationships.