[P1140] Immunoreactivity studies on synthetic peptides deriving from variable domain IV of *Chlamydia trachomatis* major outer membrane protein

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Objectives: The amino acid sequence of the major outer membrane proteins (MOMPs) from *Chlamydia trachomatis* serovars are predominantly conserved, but have four variable domains (VDs). The major neutralising and serotyping antigenic determinants are located in VD-I, VD-II and VD-IV.The aim of this study was to identify the location of immunodominant regions and to study the relationship between the sequence heterogeneity and immunoreactivity by detecting antigenic reactivity of synthetic peptides derived from VD-I, VD-II and VD-IV of the *C. trachomatis* MOMP. **Methods:** Unique 25-mer long (n = 281) overlapped by 4aa peptides derived from VD-I (aa65–127), VD-II (aa139–202) and VD-IV (aa286–362) sequences corresponding to 9 different *C. trachomatis* serovars (A, C, D, E, F, H, L1, L2, L3) were designed and synthesized. The antigenic reactivity was detected by indirect enzyme immunoassay with known anti-*C. trachomatis* positive (N = 30) and negative (N = 30) sera. **Results:** A strong antigenic region was identified within the MOMP VD IV at amino acids 302–345. The percent amino acid homology between different serovar sequences encompassing this region varied from 43.2% to 95.5%. All except one peptide (serovar C) contained the common motif TTTLNPTIA previously described as the minimal size of the antigenic epitope. However, the range of immunoreactivity with positive serum specimens varied from 50% to 93% for peptides with the common sequence and was 25% for synthetic peptide with substitution of two amino acids in this sequence. The most immunoreactive peptide (QPKSATAIFDTTTLNPTIAGAGDVK) detected 93% of the positive sera with the highest signal to cutoff ratio. This peptide was derived from the *C. trachomatis* serovar E. **Conclusion:** The reactivity of the antigenic epitopes (s) may be differentially affected by neighbouring amino acids. Diagnostic test development requires careful selection of sequence variants, but the length and exact position of diagnostic targets also must be chosen carefully.

[P1172] New enzyme immunoassay "DS-EIA-HIV-AG-SCREEN' for early diagnostics of HIV infection

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Objective: The problem of early diagnostics of HIV infection still remains very important and can be solved by using highly sensitive tests for detection HIV-1 p24 antigen. Improvements in assay sensitivity have been achieved constantly since introduction of the first commercial test. The mostly sensitive current EIA for the detection of HIV p24 have an analytical sensitivity equal 3 pg/mL. Despite the high performance of screening assays, transfusion-associated HIV infection is still reported. The aim of this present study was to develop and evaluate EIA test for p24 detection with analytical sensitivity equal 0.5 pg/mL. **Methods:** New EIA one-step diagnostic test based on biotin-streptavidin amplification. Sensitivity of new test was estimated by testing serial dilutions of the HIV 1 ANTIGEN STANDARD (Bio-Rad Laboratories, Hercules, CA, USA) and serum samples from HIV Antigen Sensitivity Panel No. 801 (Boston Biomedica Inc. (BBI), West Bridgewater, MA, USA). Seroconversion panels No. 62238 and 64578 (ZeptoMetrix Corp. (ZMC), Buffalo, NY, USA), No. 931, 939, 942, and 948 (BBI) have been additionally tested. To challenge the specificity of the new assay, serum samples of healthy blood donors (n = 1,990), pregnant women (n = 400), patients with other infections (n = 256) and patients with noninfectious diseases (n = 305) were investigated. **Results:** Analytical sensitivity of the «DS-EIA-HIV-AG-SCREEN» assay was estimated equal 0.5 pg/mL. All positive samples from commercial panels demonstrated much higher signal to cutoff ratios than with alternative tests. The results obtained during the study of sera samples from seroconversion panels No. 64578 (ZMC, USA), No. 931, 939, 942, and 948 (BBI, USA) showed that the test "DS-EIA-HIV-AG-SCREEN' able to detect p24 antigen either simultaneously or two days later (ZMC, USA, panel No. 62238) than HIV RNA by PCR, but on an average 3 days earlier than lower sensitive tests. General specificity of the new test among different samples cohort was equal 99.95%. **Conclusion:** Development of EIA tests for detection an HIV p24 antigen in concentration lower than 35 pg/mL is not technically insuperable problem. The obtained results showed high diagnostic efficiency of the "DS-EIA-HIV-AG-SCREEN' test which can be used for blood donor screening and examination of patients from risk groups of with the aim of early diagnostics of HIV infection.

[P1182] Differences of immunoreactivity of selected antigenic epitope of tick-borne encephalitis virus envelope protein gE modelled by different variants of recombinant fusion proteins

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Objectives: Tick-borne encephalitis virus (TBEV) is a human pathogenic member of the genus Flavivirus. TBE virus cause severe encephalitis with serious sequelae. Envelope protein (E) of the flaviviruses is a major target of neutralising antibodies and used as a diagnostic reagent for ELISA-format tests. The aim of present study was to evaluate the effect of different N-end tags on immunoreactivity of antigenic epitope(s) of gE protein modeled by recombinant fusion proteins. **Methods:** Artificial gene encoded 296414 aa region of gE TBEV have been synthesized from oligonucleotides by using PCR reaction. Recombinant antigen was expressed in *E. coli* cells as a fusion protein with four different N-end tags variants: 6-histidines (6His), glutathione-S-transferase (GST), GST and 6-histidines (GST+6His) and maltose binding protein (MBP). Recombinant fusion proteins were purified by affinity chromatography and tested in enzyme immunoassay with previously well defined 36 anti-TBEV positive and 56 anti-TBEV negative serum samples. **Results:** Different levels of the protein expression, solubility and immunoreactivity was observed for all four recombinant constructions. GST-N-tagged protein was the most immunoreactive: it has detected IgG anti-TBEV in 97.2% positive serum samples and did not react with any anti-TBEV negative sera. Average signal to cut off (S/C) level with positive samples was equal toa 5.4. Fusion proteins with MBP and 6His also had high enough level of immunoreactivity. IgG anti-TBEV activity was detected in 86.1% and 72.2% of positive sera samples, and average S/C level with positive samples was somewhat higher – 6.3 and 6.1, respectively. But the specificities of these fusion variants were only 96.4% for MBP fusion antigen and 98.2% for 6His fusion protein. The GST+6His N-end tagged antigen demonstrated lower level of immunoreactivity. IgG anti-TBEV activity has been detected only in 5.6% of positive sera samples. Average S/C level with positive samples also was lowest 2.3.**Conclusions:** The achieved results suggest that selection of protein expression system, location and characteristics of fusion tag plays important role in successful modeling of antigenic epitopes by recombinant proteins. Fusion of recombinant antigen comprising TBEV gE regions at 296414 aa position with glutathione-S-transferase at the N-end demonstrated significant diagnostic potential for the development of anti-TBEV ELISA diagnostic assays.