Abstract: P631

Epitope specific IgG response to Epstein-Barr virus capsid protein p18 among different age groups

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Objective: Determination of IgG avidity against viral capsid antigen (VCA) frequently have been used for the diagnosis of acute Epstein-Barr virus (EBV) infection and for estimation of seroconversion age in epidemiological studies. Recombinant protein or synthetic peptides with different size often used as diagnostic target. The aim of this study was to compare IgG response to antigenic epitopes: AE1 - located on position 1-119 aa (expressed as GST fusion protein) and AE2 located on position 124-153 (modelled by synthetic peptide) of VCA p18 among different age group.
Materials and methods: A total of 93 serum samples from adult (normal blood donors) and 166 serum samples from children (age range 0-17-years old) were analysed by commercially available anti-EBV-VCA IgG enzyme immunoassay (Diagnostic Systems, Russia). All EBV-VCA IgG positive samples were additionally tested for presence of IgG specific to AE1 and AE2 regions individually. The determination of IgG avidity was performed with 8 M urea as a dissociative agent. Serum samples were divided for 7 groups: less 4 months old, n = 43(1), 4 months to 1 year old, n = 18(2), 1-2 years old, n = 12(3), 3-5 years old, n = 24(4), 5-9 years old, n = 26(5), 10-13 years old, n = 20(6) and 14-17 years old, n = 43(7).
Results: Epitope-specific distribution of anti-VCA-IgG activity was significant different in term of both IgG level (for groups 1, 4-7 and adults) and IgG avidity (for groups 1, 3, 4 and adults) (Table 1).

Table 1. Age distribution of IgG and low-avidity IgG to AE1 and AE2 antigenic domains of VCA p18

Age range	Anti VCA positive samples	Frequency of low-avidity anti VCA	Anti AE1 positive samples	Frequency of low-avidity anti AE1	Anti AE2 positive samples	Frequency of low-avidity anti AE2
less 4m	37/(86%)	9 (24%)	17 (40%)	9 (53%)	35 (81%)	5 (14%)
4 -12 m	8 (44%)	5 (63%)	5 (13%)	3 (60%)	8 (44%)	2 (25%)
1 – 2 yrs	14 (100%)	1 (7%)	10 (71%)	2 (20%)	11 (79%)	0
3 – 5 yrs	22 (92%)	2 (9%)	13 (59%)	3 (23%)	20 (83%)	0
5 – 9 yrs	28 (100%)	3 (11%)	14 (50%)	2 (14%)	25 (89%)	2 (8%)
10 –13 yrs	20 (87%)	4 (20%)	12 (53%)	0	19 (83%)	3 (16%)
14 - 17yrs	40 (93%)	1 (3%)	30 (70%)	3 (10%)	41 (95%)	1 (2.5%)
Adults	89 (96%)	3 (4%)	50 (54%)	13 (26%)	90 (97%)	3 (3.3%)

Conclusion: Specific IgG response to different antigenic epitopes located on VCA p18 of EBV differs in IgG level and IgG avidity among various age groups. Our results strongly indicated that selection of antigenic epitopes (or nature of diagnostic target) for the routine avidity determination of anti VCA IgG may be a critical point in the design of the EIA protocols for both clinical and epidemiological study.

Abstract: P632

Antigenic properties of new recombinant polypeptide from TBEV IgE protein

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Objectives: Tick-borne encephalitis virus (TBEV) is a pathogenic human flavivirus endemic in some parts of Europe and Asia. Detection of specific antibody is a base for diagnosis of TBEV-infection. Recent studies have shown the necessity of further improvement of existing TBEV-tests regarding both sensitivity and specificity. Majority manufactures used cultural antigens (virion or purified protein) producing from Central European type of TBEV (Naidorff strain). The common problems of this approach are: (1) insufficient purity of antigen; (2) problem with recognition of other TBEV-types (Sibirean and Far-East); (3) possible cross reactivity with another flaviviruses. In this study we investigated antigenic properties of new recombinant protein corresponding to domain III of TBEV IgE protein. Domain III is highly antigenic, consists primarily of linear epitopes and have high degree of homology among all types of TBEV.
Methods: Recombinant polypeptide comprising 296-414 aa region of TBEV IgE-protein was produced in *E. coli* as GST-fusion protein. Assay conditions for the detection of anti-TBEV-IgG were optimised to reduce the possibility of false positive and false negative results. Two groups of sera have been used to evaluate sensitivity and specificity of the test: serum samples from TBEV-infected individuals collected in European part of Russia (n = 78) and sera from normal blood donors (n = 109). All specimens were previously tested for IgG anti-TBEV activity by commercially available EIA.
Results: All sera from TBEV-infected patients were positive in EIA with new recombinant protein. The average of signal to cutoff ratio was 24, 5.108 out 109 samples from normal blood donors sera were negative. Assay sensitivity was calculated at 100%, assay specificity - 99.1%.
Conclusions: The recombinant protein derived from C-term of IgE TBEV used in this study demonstrated significant potential as diagnostic reagent in EIA for the detection of specific IgG to TBEV in serum specimens.

Abstract: P633

Development a new enzyme immunoassay for detection anti-EBNA1 antibody to Epstein-Barr virus based on new p72 mosaic protein

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Background: The Epstein-Barr virus (EBV) is a human herpes virus 4 (HHV4) that infects and establishes latency in B-lymphocytes. Primary infection leads to a life-long past infection which is normally asymptomatic. EBV expresses a number of genes, however, Epstein-Barr nuclear antigen 1 (EBNA1) p72 is the only viral protein which characterizes EBV past-infection.
Objective: The aim of this study was to evaluate diagnostic relevance of new artificial protein composed of 2 antigenic epitopes of the EBV EBNA1 and to develop and evaluate a screening enzyme immunoassay (EIA) for the detection of anti-EBNA1 IgG activity to EBV in serum specimens.
Materials and methods: Two potential antigenic epitopes of EBNA1 protein have been predicted by bioinformatics analysis. Mosaic of two antigenic domains from the protein p72 (1-98 aa) and (408-498 aa) of HHV4 was expressed in *E. coli* as hybrid proteins with Glutathione S-transferase to develop an assay for the detection anti-EBNA1 antibodies. Assay conditions were optimised to reduce the possibility of false positive and false negative results. The new IgG-EIA was evaluated using serum specimens obtained from EBV PCR positive patients (n = 51), HIV-infected individuals (n = 72) and from normal blood donors (BD) (n = 504). All PCR positive specimens were additionally tested for IgG anti-EBNA1 activity by commercially available EIA based on full-length EBV nuclear antigen. The specificity was estimated on the EBV negative samples (n = 23).
Results: The EBV past-infection for PCR positive patients was confirmed by the detection of high avidity IgG to EBV Viral Capsid Antigen (VCA). All of the 51 EBV PCR positive patients had IgG antibodies to EBNA1 on the novel EIA. Concordance with commercially available EIA was 98.03%. The frequency of IgG antibodies to EBNA1 in all investigated groups were as follows: 92.21% for health blood donors, 95.39% for HIV-infected individuals and 90.23% for children. Specificity of the assay was around 95.63%.
Conclusion: Recombinant protein comprising theoretically predicted antigenic epitopes of EBNA1 protein demonstrated a significant potential as diagnostic reagent. The new EIA is highly specific diagnostic assay for the detection of anti-EBNA1 IgG HHV4 activity in serum specimens and in combination with VCA IgG and IgM may be useful tool for routine diagnosis of acute EBV infections or EBV immune status.

Abstract: P638

Development of sensitive and specific real-time PCR assay for simultaneous diagnostics and genotyping of cytomegalovirus

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Background: Human cytomegalovirus (HCMV) is an important pathogen capable of establishing lifelong persistent infections, which normally remain asymptomatic. Previous studies indicated that sequence variation among CMV strains frequently occurs even in highly conserved genes' regions. Genetic variation of functionally important genes may complicate CMV diagnostics.
Objective: The purpose of this work was designing primers and probes for simultaneously Real-Time PCR diagnostics and genotyping of CMV.
Materials and methods: In this study 527 serum samples obtained from pregnant women and 3 samples from children with CMV congenital infection were used. Virus DNA was extracted by using the MagNA Pure DNA purification kit (Roche, Indianapolis, IN, USA). 4 sets of primers directed to IE2, gN, gO and gB CMV genes and 10 sets of probes for Real-Time detection with LidhtCycler instrument (Roche, Indianapolis, IN, USA) have been designed and evaluated. Genotyping was being carried out by sequencing analysis on ABI 3100 Avant Genetic Analyzer instrument (ABI, Foster City, CA, USA). Phylogenetic analysis of the nucleotide sequences was conducted with Clastal X, the Tamura-Nei substitution model, Grow tree based on Neighbour-Joining tree building method, and Maximum Parsimony method implemented in MEGA 3.0 package.
Results: The Real-Time PCR analysis with IE2 gene detected CMV activity in 72 isolates among 527 analyzed samples The PCR tests with previously described primers to gN, gO and gB genes revealed 7, 1 and 2 positive samples accordingly. The PCR test sensitivity was defined with quantitative CMV control (ABI, Foster City, CA, USA). The sensitivity of PCR test on IE2 gene was 20 copies per 50 μl. The phylogenetic analysis of IE2 region sequences demonstrated that this region could be successfully used for virus genotyping. The Real-Time FRET test divided all analyzed samples into two groups, those that had a melting peak like laboratory strain Davies and those that had a melting peak like Towne and AD169 laboratory adapted strain. Two pairs of specific hybridisation probe covering two mutations inside IE2 gene's region were used to confirm it.
Conclusion: Nested PCR with primers to IE2 gene incorporated with Real-Time FRET analyse described here, provides a sensitive and specific assay for detecting CMV in clinical isolates. The IE2 gene can serve as a target for simultaneously detecting and genotyping of CMV using the Real-Time PCR opportunities.

Abstract: P994

Potential antigenic determinants of *Chlamydia trachomatis* major outer membrane protein modeled by overlapped recombinant proteins

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Background: Major outer membrane protein (MOMP) is a primary target of specific anti-*Chlamydia trachomatis* antibodies. This protein performs important structural and immunity-related roles and contains 4 variable segments (VS I, II, III, IV). Previously it has been shown that immunoreactive antigenic epitopes are located within VS I, II and IV.
Objective: The purpose of this study was determination of potential antigenic epitopes encoded by open reading frame for major outer membrane protein of *Chlamydia trachomatis* and modelling them by using overlapped recombinant proteins about 100 amino acids (aa).
Methods: Several antigenic determinants of *Chlamydia trachomatis* MOMP have been predicted by bioinformatics analysis. Six pairs of primers were designed to produce six overlapping DNA fragments from genomic DNA *Chlamydia trachomatis* by using PCR reaction. Recombinant genes encoding selected amino acid sequences about 100 aa with overlapping 30 aa have been synthesized. Proteins were expressed in *Escherichia coli* as hybrid proteins with Glutathione S-transferase and 6-Histidine tag. To study antigenic properties of new proteins 64 well defined positive (n = 39) and negative (n = 25) serum samples were tested. All serum samples were previously characterised by three commercially available assays for the detection of IgG anti-*Chlamydia trachomatis*.
Results: Six clusters of potential antigenic determinants have been predicted within major outer membrane protein of *Chlamydia trachomatis*. Recombinant genes encoding predicted amino acid sequences of MOMP at positions 1-116 aa, 66-165 aa, 128-216 aa, 191-286 aa, 252-354 aa and 317-398 aa were synthesised. The pure samples of 6 proteins were obtained by affinity chromatography. All proteins were immunoreactive and demonstrated different specific activity with anti-*Chlamydia trachomatis* antibodies in serum samples. The 191-286 aa protein showed the highest level of immunoreactivity. This antigenic epitope(s) comprise VS III.
Conclusion: The predicted antigenic epitope(s) located within 191-286 aa demonstrated a significant diagnostic potential as candidates for the development of diagnostic assays for the detection of anti-*Chlamydia trachomatis* IgG activity in serum specimens.