A new anti-HCV EIA based on recombinant antigens derived from different sequence variants of hepatitis C virus

Abstract number: 1134_01_295

Ulanova T., Puzyrev V., Kulikova L., Bochkova G., Golubeva I., Obriadina A., Burkov A.

Objective:

The purpose of this study is the development of a new anti-HCV EIA by using recombinant antigens derived from different sequence variants of different HCV virus genotypes.

Methods:

The 9 sequence variants of recombinant antigens comprising major epitopes from core, NS3, NS4 and NS5 HCV proteins have been selected for the anti-HCV assay development. This new EIA was evaluated using serum specimens (n =511) obtained from patients infected with different HCV genotypes from different parts of the world. The HCV status of these specimens was confirmed using RIBA HCV 3.0 (ORTHO Diagnostic Systems Inc., USA), and Cobas Amplicor HCV Test, v.2.0 (Roche Diagnostics, USA). 376 samples were confirmed as anti-HCV positive, 57 samples were tested anti-HCV 'Indeterminate', and 78 sera were tested anti-HCV negative. Among anti-HCV RIBA negative specimens 3 samples were HCV RNA positive. Additionally, anti-HCV Mixed Titer performance Panel PHV 205, anti-HCV Low Titer Performance Panel PHV 103 (BBI Inc., USA) and 15 anti-HCV seroconversion panels (BioClinical Partners, Inc., USA) were tested. The anti-HCV negative panel was composed of 200 specimens (BBI Inc., USA).

Results:

New EIA detected anti-HCV activity in 100% of specimens tested anti-HCV positive or/and anti-HCV indeterminate by RIBA HCV 3.0 and HCV RNA positive. 92% of RIBA 'indeterminate' samples were immunoreactive with more than one antigen used for the development of the new anti-HCV EIA. Moreover, three anti-HCV negative but HCV RNA positive samples were immunoreactive with NS3 antigens used in this new EIA. Additionally, the EIA was able to detect seroconversion point earlier in 3 anti-HCV seroconversion panels than commercially available anti-HCV EIA. Two anti-NS4 HCV negative but anti-core, anti-NS3 and anti-NS5 positive specimens from PHV 103 and PHV 205 were found anti-NS4 positive by using the NS4 antigen from the new EIA. None of the used anti-HCV negative specimens were tested as positive with new anti-HCV EIA.

Conclusion:

A new highly sensitive and specific anti-HCV detection assay was developed using various sequence variants of HCV antigens. This assay may be used for screening sera from patients infected with different HCV genotypes with almost equal efficiencies.
A new recombinant antigen haemagglutination test for the serological diagnosis of syphilis

Abstract number: 1134_02_25

Chepurchenko N., Ulanova T., Puzyrev V., Loginova L., Burkov A., Obriadina A.

Introduction:

Passive *Treponema pallidum* hemagglutination (HA) – the routine treponemal test has equal sensitivity with the fluorescent treponemal antibody absorption (FTA-ABS) test and with the enzyme immunoassay (EIA) in later stages of syphilis, but some studies have indicated that it is less sensitive in the primary stage of the disease. The use of recombinant *T. pallidum* antigens instead of a poorly defined mixture of antigens from wild-type *T. pallidum* has the potential for improving the sensitivity of hemagglutination test.

Objectives:

The purpose of this study was to evaluate the diagnostic relevance of 4 recombinant proteins that efficiently model the antigenic epitope(s) of the *Treponema pallidum* proteins.

Methods:

Four full-length recombinant proteins (TmpA, 47, 17, 15 kDa) were expressed in *E. coli* and then used individually to develop the hemagglutination test (HT) for the detection of anti-*Treponema pallidum* (anti-TP) activity in serum specimens. Serum samples (N = 267) from patients with clinically proven syphilis in various stages of the disease (primary (N = 36), secondary (N = 70), latent (N = 161)) and from normal blood donors (N = 505) were tested. 24 samples from patients with primary syphilis, 21 samples from patients with secondary and 17 samples from patients with latent stage were tested initially with commercially available hemagglutination test based on mixture of *Treponema pallidum* antigens (HTTP). All specimens were additionally tested for specific antibodies by commercially available enzyme immunoassay (EIA).

Results:

The sensitivity of the HT for the detection of anti-TP activity in human serum specimens varied from 38.9% to 97.2% with primary syphilis sera, from 87.1% to 100% with secondary, and from 96.9% to 98.1% with latent stage sera for each protein. The TmpA was found as the most immunoreactive when serum samples from patients with untreated syphilis were tested. The sensitivity of TmpA HT was identical to those of the HTTP in cases of untreated secondary and latent syphilis and significantly higher with specimens from primary stage of disease. The overall specificity and sensitivity of the TmpA HT were comparable to those of EIA 99% and 99.6%; 98% and 99.6% respectively.

Conclusions:

The results of this study indicate that the TmpA HT may be a reasonable alternative to the routine hemagglutination test and the ELISA as a confirmatory test for syphilis.
Enzyme immunoassay for the detection of anti-Kaposi's sarcoma-associated herpes virus IgG antibodies based on new mosaic protein

Abstract number: 1134_03_316

Kornienko N., Gladysheva M., Ulanova T., Puzyrev V., Burkov A., Loginova L., Obriadina A.

Background:
Kaposi's sarcoma-associated herpes virus (or human herpes virus type 8 HHV-8) is a recently discovered gamma-herpesvirus associated with 4 clinical and epidemiological variants of Kaposi sarcoma (classic, endemic, iatrogenic, and acquired immunodeficiency virus-associated), primary effusion lymphoma and multicentric Castleman's disease.

Objective:
The aim of this study was to learn the antigenic properties of new recombinant mosaic protein and to develop and evaluate a screening enzyme immunoassay (EIA) for the detection of anti HHV-8 IgG activity in serum specimens.

Materials and methods:
Mosaic of 2 antigenic domains from the proteins encoded by open reading frames 65 (140–170 aa) and K8.1 (32–62 aa) of HHV-8 was produced as GST fusion protein to develop an assay for the detection of anti HHV-8 antibodies.

Assay conditions were optimized to reduce the possibility of false positive and false negative results. To validate the specificity and sensitivity of new EIA sera from HIV-infected individuals (n = 163), children (0–15 years) (n = 170), patients with sexual transmitted diseases (STD) (n = 136), and from European normal blood donors (n = 1349) were tested. Serum samples from KS patients (n = 30) were initially tested as positive by immunofluorescence assay with LANA protein. All specimens were additionally tested for IgG anti-HHV-8 activity by commercially available EIA (Vecto HHV-8-IgG-strip,Russia).

Results:
27 out of 30 HHV-8-positive samples were positive on the novel EIA. Assay sensitivity was calculated at 90%. Coincidence with commercially available EIA was 98.21%. The percentages of positive reactivity in all investigated groups were as follows: 119 for health blood donors, 1.76 for children, 2.21 for STD-patients and 3.38 for HIV-infected. Specificity of the assay was around 98.2%–98.8% and there were no significant differences between health donors/children and groups at highest risk of acquiring HHV8 infection.

Conclusion:
The artificial mosaic protein used in this study demonstrated significant potential as diagnostic reagent. The new EIA is highly specific diagnostic assay for the detection of anti-HHV-8 activity in serum specimens and may be useful tool for studies of HHV-8 epidemiology.

KSHV seroprevalence in the Russian European population is low both among health donors and HIV-infected people.
Immunoreactivity of predicted antigenic determinants of *Toxoplasma gondii* ROP 4 protein

Abstract number: 1134_01_190

Ulanova T., Puzyrev V., Savel’eva N., Volkova T., Loginova L., Burkov A., Obriadina A.

**Objectives:**

The purpose of this study was to determinate and evaluate diagnostic relevance of antigenic epitopes encoded by open reading frame for *Toxoplasma gondii* ROP 4 protein (Serine/Threonine protein kinases, catalytic domain).

**Methods:**

Four potential antigenic epitopes of *Toxoplasma gondii* ROP 4 protein have been predicted by bioinformatics analysis. Recombinant genes encoded selected amino acids sequences have been constructed from synthetic oligonucleotides by using PCR reaction. Proteins were expressed in *E. coli* as hybrid protein with Glutathione S-transferase. To study antigenic property of new proteins 25 well defined positive (N = 15) and negative (N = 10) serum samples were tested. All serum samples were previously tested by three commercially available assays for the detection of IgG anti *Toxoplasma gondii*. In absence of known IgM positive samples and high discordance between commercially available assays for the IgM detection the 3rd International Standard for ANTI-TOXOPLASMA SERUM, HUMAN (TOXM, NIBSC, UK) was used. Also 200 serum specimens from normal blood donors were tested.

**Results:**

Predicted antigenic determinants were located at position 30–159 aa, 447–467 aa, 491–505 aa and 548–558 aa. Gene encoded amino acid sequences of ROP 4 protein at position 30–145 aa (RH2) and artificial mosaic gene encoded amino acid sequences at position 447–467 aa, 491–505 aa, 548–558 (RH1) aa were synthesized. The pure samples of two recombinant proteins were obtained. All proteins were weak immunoreactive with IgG antibody of anti *Toxoplasma gondii* positive serum samples. When TOXM was tested RH1 and RH2 were able to detect 1.6 preliminary units of IgM anti-toxoplasma antibodies. None of specimens from normal blood donors were tested as IgM positive with new recombinant proteins. Antigenic epitopes located at 30–159 aa was found as most immunoreactive for the specific IgM detection.

**Conclusion:**

The results indicated that 4 antigenic epitopes have been predicted of the ROP 4 protein of *Toxoplasma gondii*. All proteins used in this study demonstrated a significant diagnostic potential as candidates for the development of diagnostic assays for the detection of anti-*Toxoplasma gondii* IgM activity in serum specimens.
Prediction of potential antigenic domains of HIV-1 Pol protein and evaluation their immunoreactivity

Abstract number: 1135_248

Puzyrev V., Savel'eva N., Baranova E., Van'kova O., Pimenov V., Obriadina A., Burkov A., Ulanova T.

Objectives:

The aim of this study is prediction and evaluation diagnostic relevance of potential antigenic epitopes of HIV-1 polymerase protein (protease; reverse transcriptase; integrase).

Methods:

Five potential antigenic epitopes of HIV-1 pol protein have been predicted by bioinformatics analysis. Recombinant genes encoded selected amino acids sequences have been assembled by PCR from synthetic oligonucleotides and expressed in E. coli as hybrid proteins with Glutathione S-transferase. Proteins were tested by enzyme immunoassay against a panel of human serum specimens positive for anti HIV (n = 228) and specimens from normal blood donors (n = 200). Status of samples has been confirmed by ‘New Lav Blot’ (Bio-Rad, USA).

Results:

Potential antigenic determinants were predicted at position 1–51 aa, 198–347 aa, 242–347aa, 352–411 aa and 922–1002 aa pol protein. Only protein comprising 922–1002 aa of pol protein (integrase) was immunoreactive and detected IgG antibody in 87% anti-HIV positive serum samples. Average of signal to cutoff ratio is 8.5. None of specimens from normal blood donors were tested as positive with this recombinant protein. Other theoretically selected amino acids sequences demonstrated no immunoreactivity. This may be connected with the conformational nature of other predicted epitopes.

Conclusion:

The results indicated that recombinant protein comprising one of the theoretically predicted antigenic epitope(s) of HIV-1 pol protein demonstrated a significant diagnostic potential and can be used for the development EIA for the detection of anti-HIV activity in serum specimens.
IgG response to herpes simplex virus type 2 gG and gD recombinant proteins among adults and children

Abstract number: 1135_273

Klimashevskaia S., Puzyrev V., Ulanova T., Volkova T., Burkov A., Obriadina A.

Objectives: Recent studies have shown that both HSV-2 glycoprotein D (gD2) and glycoprotein G (gG2) have high potential as diagnostic reagents for the reliable detection of the type-specific IgG. The purpose of this study was to evaluate specific IgG response to HSV-2 gG2 and gD2 among child and adult populations and to investigate the protein-specific pattern of low avidity IgG reactivity.

Methods: A total of 160 serum samples from adults (normal blood donors) and 55 serum samples from children (age range 2–3 years-old) were analysed by type-specific HSV2 IgG enzyme immunoassay (DS-EIA-ANTI-HSV2-IgG, Russia), based on recombinant HSV 2 gG2 (525–578aa) and gD2 (266–394 aa) proteins. All HSV-2 IgG positive samples were additionally tested for presence of IgG to gD2 (anti gD2) and gG2 (anti gG2) individually. The determination of IgG avidity was performed with 8M urea as a dissociative agent.

Results: 73 out of 160 (45.6%) serum samples from normal blood donors (adults) and 14 out of 55 (25.5%) serum samples from children were positive for HSV2 antibodies by EIA. Protein-specific distribution of IgG activity was significantly different in terms of both IgG level and IgG avidity for two investigated groups (Table 1, Table 2).

Table 1. Frequency of IgG antibodies to gD2 and gG2

<table>
<thead>
<tr>
<th>HSV2-positive samples</th>
<th>Frequency of IgG antibodies to gD2 only</th>
<th>Frequency of IgG antibodies to gG2 only</th>
<th>Frequency of IgG antibodies to gG2 and gD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults (n = 160)</td>
<td>73</td>
<td>50 (68.5%)</td>
<td>5 (6.8%)</td>
</tr>
<tr>
<td>Children 2–3 years (n = 55)</td>
<td>14</td>
<td>9 (64.3%)</td>
<td>3 (21.4%)</td>
</tr>
</tbody>
</table>

Table 2. Frequency of low-activity antibodies to gD2 and gG2

<table>
<thead>
<tr>
<th>HSV2-positive samples</th>
<th>Frequency of low avidity antibodies</th>
<th>Frequency of low avidity antibodies to gD2 only</th>
<th>Frequency of low avidity antibodies to gG2 only</th>
<th>Frequency of low avidity antibodies to gG2 and gD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults (n = 160)</td>
<td>73</td>
<td>12 (16.4%)</td>
<td>11 (15%)</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>Children 2–3 years (n = 55)</td>
<td>14</td>
<td>6 (42.9%)</td>
<td>2 (14.3%)</td>
<td>3 (21.4%)</td>
</tr>
</tbody>
</table>

Conclusion: Specific IgG response to HSV 2 gG2 and to gD2 differs among various age groups. Frequency of low-avidity antibodies in children significantly exceeds frequency of low-avidity antibodies in adults. Furthermore, low-avidity anti gG2 was found predominant in children, and low-avidity anti gD2 in adults. Serologic assay based on the only protein (gG2 or gD2) has a potential for false negative result. It may be especially important for primary HSV2 infection detection.