A multiplexed protein microarray for the simultaneous serodiagnosis of human immunodeficiency virus/hepatitis C virus infection and typing of whole blood

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A multiplexed protein microarray for the simultaneous serodiagnosis of human immunodeficiency virus/hepatitis C virus infection and typing of whole blood**

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Abbreviations used:
SNBTS, Scottish National Blood Transfusion Service; ELISA, enzyme-linked immunosorbent assay; HCV, hepatitis C virus; HBV, hepatitis B virus; HIV, human immunodeficiency virus; EIA, enzyme immunoassay; PBS, phosphate buffered saline; PBS-T, phosphate buffered saline +0.05% Tween 20; BSA, bovine serum albumin; IgG, immunoglobulin G; IgM, immunoglobulin M; MAbs, monoclonal antibodies; PMT, photomultiplier tube; ROC, receiver operator characteristic; NIBSC, National Institute for Biological Standards and Control; RDI, Research Diagnostics Inc USA; Ag, antigen; Ab, antibody; RSD, relative standard deviation.

Keywords: protein microarray; HIV; HCV; blood typing; serodiagnosis

Abstract

All donor blood samples must be tested pre-transfusion to determine the donor blood type. Standard testing protocols also require that assays be performed for important blood borne pathogens such as hepatitis C, syphilis, hepatitis B and human immunodeficiency virus. We have developed a protein microarray which can type whole blood and detect antibodies to significant pathogens simultaneously from the same donor blood sample. The data shown demonstrate the ability of the array to accurately type blood samples whilst also detecting the presence of antibodies against both HIV and HCV. In conclusion we have successfully developed a platform capable of typing human whole blood samples, whilst at the same time testing for the presence of antibodies specific for HCV/HIV. The major benefits of this system are its amenability to expansion with additional assays, e.g. rhesus typing; syphilis and/or HBV detection; and also the adaptability of the assay for higher throughput analysis, currently 16 individual samples per slide, but readily expandable to a 96-well format.

1. Introduction

All donor blood samples must be blood typed prior to transfusion. This ensures the correct assignment of blood donations and avoids 'transfusion reactions' that are caused by agglutination or antibody-dependent complement-mediated lysis of erythrocytes in the recipient when blood is transfused to an individual of a different blood group[1,2]. Current methods of blood testing include hemagglutination, enzyme-linked immunosorbent assays (ELISA) and nucleic acid testing; standard testing protocols also require that serological assays for important blood borne pathogens such as hepatitis C (HCV), syphilis, hepatitis B (HBV) and human immunodeficiency virus (HIV) be performed to ensure donation safety[3,4,5,6] (and also see http://www.blood.co.uk/pdfdocs/tests_on.pdf). Although these tests are well characterised and widely used, they are performed separately and require that a number of centrifugation and/or washing steps be performed on the whole blood sample prior to analysis.

Current screening for HIV in blood donations involves the detection of HIV-specific host antibodies in an Enzyme Immunoassay (EIA)/ELISA format. Samples with a non-reactive result from the EIA/ELISA are considered to be HIV negative, whilst those with a reactive result are re-tested in duplicate. If the re-test is also reactive, then the sample is tested further with an alternative method to ensure the correct diagnosis is given[7]. A similar principle is also applied to HCV testing[6].

An improved testing system for transfusion medicine would involve a robust test, capable of minimising sample and reagent use, whilst performing multiple tests on a single platform[[8,9]. Previous studies from our group have demonstrated the development and evaluation of a protein microarray platform capable of accurately phenotyping human erythrocytes[10,11,12]. However, the true value of a microarray platform in transfusion medicine is realised when multiple tests are combined

on the same array, since the cost of running a single microarray remains fairly stable regardless of how many individual components are incorporated into it[8,9]. Here we demonstrate that a protein microarray can be used to simultaneously type whole blood and perform serodiagnosis of pathogen infection. To achieve this, viral antigens were used for serodiagnosis of HIV and HCV, whereas antibodies were printed for the purpose of blood typing.

2. Materials & Methods

Selection of antigens for the serodiagnosis of HIV and HCV

The suitability of a range of HIV/HCV antigens was initially tested by preparation of microarrays which were incubated with HIV antibody positive serum (Human HIV CAL+, Abbott, UK), HCV antibody positive serum (Human HCV CAL+, Abbott, UK) or negative control sera (Human HIV CAL- and HCV CAL-, Abbott, UK). A range of serum dilutions (1/20 – 1/1000) was tested and the optimum serum dilution was selected (1/30 in phosphate buffered saline (PBS) + 0.05% Tween 20 (PBS-T) with 1 % bovine serum albumin (BSA)). The most promising antigens in terms of the greatest level of background corrected signal and the least amount of cross reactivity were chosen for use on the array and are listed in Table 1.

Microarray preparation

In order to allow multiple samples to be processed on the same slide, the Whatman Fast Frame™ system was utilised in conjunction with 16-well incubation chambers (Whatman Schleicher & Schuell, UK). This system allows up to four microarray slides to be held firmly together in a single frame. Microarrays were prepared by printing probes onto gold coated slides (25 x 75 x 1 mm, Ssens BV, NL) using a MicroGrid II contact printer (Genomic Solutions, UK) with 200 µm solid pins. Multiple spotted replicates of the following capture, blood typing antibodies LA2, n = 3 (A-blood type specific, 0.82 mg/ml)) and LB2, n = 3 (B-blood type specific, 0.484 mg/ml) were printed onto the arrays. In addition, the range of HCV and HIV antigens (as selected above) were printed in triplicate (Table 1) along with PBS, n = 9 (pH 7.0) and PBS-T, n = 9 (pH 7.0) blank control spots and mouse IgG, n = 3 (0.31 mg/ml) as a secondary antibody positive control. Each slide was printed with 16 separate arrays, allowing the simultaneous processing of up to 16 samples on the same slide. Each array consisted of a grid of 7x9 printed spots. Arrays were checked by microscopy after printing to ensure that probes were correctly printed and when not in use the arrays were stored under N_2 at 4 °C. Prior to use arrays were submerged 10 times in PBS-T pH 7.0 + 1 % BSA before being placed in a fresh container of PBS pH 7.0 containing 1 % BSA for 1 hr at room temperature, with constant

agitation. Arrays were then submerged 10 times in a fresh container of PBS and centrifuged to dryness in an Eppendorf 5810R centrifuge at 1000 rpm for 1 min.

Sample preparation

Due to the difficulties involved in the safe handling of HIV/HCV infected blood samples in a research laboratory, and also in obtaining ethical approval for their use, a mock antibody-positive plasma sample was prepared utilising non-infected plasma diluted 1/30 in PBS-T (pH 7.0) containing 1% BSA. A range of monoclonal antibodies (MAbs) specific for HIV/HCV antigens was used to produce a mock antibody-positive plasma sample with a range of different antibody concentrations (6×10^{-8} M, 6×10^{-9} M, 6×10^{-10} M, 2.4×10^{-10} M, 1.2×10^{-10} M, 6×10^{-11} M) see Table 2. These samples were then used for testing the HIV/HCV assays. For blood typing analysis, erythrocytes of A and O blood types (Alba Biosciences, UK) were prepared by centrifuging for 5 minutes at 1,000 rpm prior to washing twice in ice cold PBS. In order to produce a mock whole blood sample the washed erythrocytes were re-suspended in plasma producing a final erythrocyte concentration equivalent to that of a normal human haematocrit level, i.e. 40%. For combined blood typing and serodiagnosis of pathogen infection a mock antibody-positive whole blood sample was created by adding erythrocytes (equivalent of a 40% haematocrit level) to a mock antibody-positive plasma sample.

Sample incubation

Samples (prepared as above) were contained on the array using 16-well incubation chambers (capacity = 120 µl) for 1 hr at room temperature with agitation. After incubation the samples were aspirated from the wells and the wells were washed 3 times with 100 µl PBS-T. The incubation chambers and Fast Frame supports were removed and the slides were then washed for 20 minutes in PBS-T and for 2 x 10 minutes in PBS. The slides were then centrifuged to dryness at 1000 rpm for 1 min. Three separate arrays were used for each different condition.

Blood typing data collection

In order to assess blood-typing results, the microarray slides were scanned with a ScanArray 5000 confocal microarray scanner (Packard Biochip Technologies) at 488 nm excitation and measuring emission at 520 nm to detect bound erythrocytes via their auto-fluorescence.

Serodiagnosis of pathogen infection data collection

Following the collection of the blood typing data the microarray slides were placed back into the Fast Frame support along with fresh 16-well incubation chambers. In order to detect the bound anti-HIV/HCV antibodies, 80 µl of Cy3 labelled anti-mouse IgG secondary antibody (1/1000 dilution, Sigma, UK) was added to each well and incubated, with agitation, for 1 hour at room temperature in the dark. A range of Cy3 labelled secondary antibodies was tested over a range of dilutions and a goat anti-mouse IgG (M412, Sigma, UK) was found to give the optimum result at a dilution of 1/1000 in PBS-T + 1 % BSA. The secondary antibody was then aspirated and each well rinsed with 3 x 100 µl PBS-T. The Fast Frame supports and incubation chambers were carefully removed and the slides were washed for 20 minutes in PBS-T and 2 x 10 minutes in PBS. The slides were centrifuged to dryness (1,000 rpm for 1 min) and scanned using a ScanArray 5000 confocal microarray scanner (Packard Biochip Technologies) with excitation at 550 nm and measuring emission at 570 nm to detect the Cy3 fluorescence signal.

Data processing and analysis

Slides were scanned as described above; for each array five separate scans were taken using a constant photomultiplier tube (PMT) setting and incrementally increasing laser power settings. Microarray images were analysed with Quantarray software (Perkin Elmer, USA) using the fixed circle method and spot-specific background was corrected by subtracting measured spot background from spot signal. From the five scans of each array, the optimal scan in terms of linear range was selected on the basis of comparative scatterplot analysis[13].

Blood typing

We have previously described the development and evaluation of a protein-based microarray capable of successfully phenotyping human whole blood samples[12]. We developed a novel diagnostic algorithm which allowed the accurate determination of the ABO blood type of randomly assigned blood samples. Herein we utilised the same algorithm to phenotype erythrocytes incubated on the microarrays for combined testing. The algorithm utilises Receiver Operator Characteristic (ROC) curve analysis to produce a series of threshold values within which a sample can be assigned a particular blood phenotype. The threshold values are based upon an index score (ratio) of the A and B blood type antibody responses (LA2/LB2) and are defined in Table 3.

The background corrected signals were used to determine the index scores, with background noise being calculated from the median signal of the 18 PBS/PBS-T control spots printed on each array. Representative mean signal for a probe x_i (probe x on array i) was calculated as the median fluorescence across all replicates of a probe on an array (LA2, n = 3, LB2, n = 3). The relative signal (index score) between the anti-A (LA2) and anti-B (LB2) probes was then defined as the ratio $LA2_i/LB2_i$. The threshold values defined in Table 3 were then applied to the blood typing data collected in this study to determine whether the combined pathogen and blood phenotyping array was capable of accurately determining the blood type of the erythrocytes contained in the mock blood samples. A sample of blood type A would be expected to give a strong response to the type A specific antibody and no response to the type B specific antibody, whilst a sample of blood type O would be expected to show no response to both of these probes. The reason for this is that type O erythrocytes carry neither, type A or B antigens as such a double negative response would be the expected outcome for this blood type.

Serodiagnosis of pathogen infection analysis

The median signals obtained from the triplicate printed HIV/HCV antigen spots were background corrected by dividing them by the median fluorescence signal from the 18 PBS/PBS-T printed control spots. The mean signal/background value for each sample was used to calculate the relative signal value for each antigen by dividing it by the mean signal/background value obtained with a plasma only reference sample. The mean relative signal values for each antigen were then used to assess the performance of each of the pathogen antigens across a range of plasma and blood samples.

3. Results

Antigen cross-reactivity analysis

To ensure specificity, the individual HIV/HCV antigens (See Table 1) were tested for cross-reactivity with HIV/HCV antibody positive sera. In this experiment a range of secondary antibody dilutions was also tested (1/400, 1/1000, 1/2000). Encouragingly, the data demonstrated very little cross reactivity between the HCV antigens and the HIV antibody positive serum with relative signal values of between 0.28 – 1.16, whilst the HIV antigens showed far higher relative signal values ranging from 1.15 - 6.27 (at the optimum secondary antibody dilution of 1/1000) (See Supplementary Figure 1). In addition the HIV antigens showed very little cross reactivity with the HCV antibody positive serum (relative signal value range = 0.65 – 1.74) whilst the HCV antigens showed higher relative signal values of 2.53 – 8.61 (See Supplementary Figure 1).

Performance of mock antibody-positive plasma against selected HIV/HCV antigens

The best performing antigens from initial trials, Virostat HCV NS3 Fusion, Fitzgerald HCV NS3, Virogen HIV-2 env gp36 and RDI HIV-2 env gp36 were tested using a range of antibody concentrations. Figure 1 shows a dose response curve for the HIV/HCV mock antibody-positive plasma against the four best performing HIV/HCV antigens. In order to aid visualisation of this data it was plotted on a log scale of the monoclonal antibody concentrations (calculated based upon the molecular mass of IgG as 1.5×10^6). As can be seen, the best sensitivity for detection of all four antibodies in the mock antibody-positive plasma falls between 6×10^{-10} M and 2.4×10^{-10} M where the dose response curve is at its steepest point within the linear range. We chose three concentrations that spanned the linear phase for the response to these antigens (6×10^{-10} M, 4×10^{-10} M and 2.4×10^{-10} M) for use in further evaluation of the array for combined testing.

Blood typing

Human erythrocytes of either A or O blood type were added to the mock antibody-positive plasma to create mock antibody-positive 'whole blood' samples. These samples were blood typed using the protein microarray platform (Fig. 2) and the data analysed against the blood typing thresholds defined in a previous study by our group[12].

The protein microarray method was able to successfully discriminate between the A and O blood type erythrocytes used in the mock antibody-positive human 'whole blood' samples (Fig. 2). A relative signal value of between 5-7 was seen with the A blood type whilst a signal of approximately 1 was seen with the O blood type for the LA2 antibody (Type A specific). In addition, the LB2 (Type B specific) antibody showed relative signals of approximately 1 for both the A and O blood types. The relative signal values were also very low (less than 2 for all but one of the antigens) for the HIV/HCV antigens, indicating that there was very little cross reactivity between the erythrocytes and the pathogen specific antigens. However it should be noted that the Virostat HCV core antigen showed some cross reactivity with the type O erythrocyte sample (Fig. 3), as such this antigen was removed from the final analysis. The results from the blood-typing analysis were analysed using the previously defined blood typing thresholds described in Table 3, the results showed that the protein microarray method successfully typed the A (Index Score = 3.63 – 3.93) and O (Index Score = 1.31 – 1.56) blood type samples both in the presence and absence of the HIV/HCV specific monoclonal antibodies.

Serodiagnosis of pathogen infection

Relative signal responses to the HIV/HCV antigens were assessed with the mock antibody-positive human 'whole blood' samples. During the blood typing analysis the Virostat HCV core antigen showed cross-reactivity with the O erythrocyte sample, and was removed from the pathogen detection analysis, thus leaving two HCV antigens (Virostat HCV NS3 Fusion and RDI HCV NS4) and four HIV antigens (NIBSC HIV-1 gp41, NIBSC HIV-1 p24 His, RDI HIV-2 env gp36 and Virogen HIV-2 env gp36). Positive control spots of mouse IgG (reacts with the anti-mouse IgG secondary antibody) were spotted onto the arrays and in all cases these spots, as expected, gave relative signal values close to one, indicating similar responses for plasma only and mock antibody-positive 'whole blood' samples.

With the monoclonal antibodies at a concentration of 6 $x10^{-10}$ M, the most promising HCV antigens were the RDI HCV NS4 antigen (relative signal values of 42 (O type blood) and 40 (A type blood), indicating that the response with the mock antibody-positive plasma was approximately 40 times greater than that with the plasma sample) and Virostat HCV NS3 antigen (relative signal values of 8 (O type blood) and 7 (A type blood)) (Fig. 3). The most promising HIV antigens were the RDI HIV-2 env gp36 antigen (relative signal values of 9 (O type blood) and 7 (A type blood)) and Virogen HIV-2 env gp36 (relative signal values of 4 (O type blood) and 3 (A type blood)). The two HIV-1 antigens from NIBSC (HIV-1 gp41 and HIV-1 p24 His) did not perform as well with relative signal values between 0.42 and 1.36 (Data not shown).

It was noted that the relative signal responses between the A and O blood type samples and the sample with no erythrocytes correlated very well, indicating the robustness of the assay to differences in blood composition and different ABO blood types. By far the best performing antigens were the RDI HCV NS4 and the RDI HIV-2 env gp36. The best sensitivity for monoclonal antibody detection used in the mock antibody-positive plasma differed slightly for the best performing antigens but generally they fell in the sub-nanomolar range between 6 x 10^{-10} and 2.4 $x10^{-10}$ M as predicted by the dose response curve (Fig. 1). In addition all antibodies were detected at 6 x 10^{-10} M regardless of the presence of erythrocytes (Fig. 3).

4. Discussion

Previous results from our group have demonstrated the feasibility of using a protein based microarray platform to type donor blood samples with minimal sample treatment[10,11,12]. This study has built further on these results by demonstrating that the technique can be successfully combined with the serodiagnosis of multiple pathogen infections on the same microarray platform. While other publications have shown impressive multiplexed virus detection,[14, 15] we believe that this is the first

example of integration of such tests with blood typing and is a critical step in the development of integrated pre-transfusion tests.

In order to make the assay more amenable to multiple probe testing and to allow the optimisation steps required of a multiple ELISA type assay we miniaturised the array footprint to allow the printing of multiple arrays per slide, currently 16. This layout enabled us to make use of 16 well incubation chambers and Fast Frame™ format (Whatman, Schleicher & Schuell, UK) meaning that we could run multiple samples (such as different antibody concentrations) on the same slide with no leakage between samples. This allowed us to minimise the variation associated with sample handling and processing and also allowed accurate comparisons to be made between different conditions. In previous studies we have demonstrated greater signal-noise responses when blood typing was performed on gold coated slides[11]. We acknowledge that gold is not a well-recognised surface for performing immunoassays and while we also printed and analysed on epoxy silane coated glass slides (data not shown) we found that the best fluorescent signals were observed on the gold surface and as such we have only shown these results here.

We utilised HIV and HCV antibody positive sera to determine the level of cross reactivity between the selected HIV/HCV antigens. The data showed very little cross reactivity between the HIV positive sera and the selected HCV antigens and vice versa, indicating good specificity with the antigens selected. This result showed that it was possible to use multiple antigens to simultaneously detect host antibodies specific for multiple pathogens in serum with very little cross reactivity, strong relative signals and minimal sample fractionation and purification.

While we assayed for antigen cross reactivity using positive sera, the limited availability of this resource, meant that it was more practical to use mock antibody-positive plasma for dose response and further experiments. The HIV/HCV mock antibody-positive plasma proved to be an excellent model analyte and the array was able to detect the presence of HIV/HCV specific monoclonal antibodies in the sample with strong responses being observed with all four antigens. The best sensitivity for monoclonal antibody detection in the mock antibody-positive plasma was chosen as the region of the dose-response curve with the highest gradient (http://goldbook.iupac.org/S05606.html).

Mock antibody-positive human ‘whole blood’ samples were simultaneously blood typed and analysed for the presence of HIV/HCV specific antibodies using the microarray platform. The mean index scores for the mock antibody-positive human ‘whole blood’ samples were calculated and the threshold values defined in Table 3 were then used to assign the samples with a particular ABO blood type. The combined use of the protein microarray and the threshold values allowed us to accurately predict the blood types for the A and O type mock antibody-positive human ‘whole blood’ samples. In addition, by testing in the presence and absence of the monoclonal antibodies we were able to conclude that the mock antibody-positive plasma had no detrimental affect on the array’s ability to

accurately predict the blood type of the erythrocytes. In order to successfully phenotype type O erythrocytes our microarray test depends on the presence of two negative responses (LA2 and LB2), however one way of improving the assay and ensuring that type O samples are correctly predicted would be to utilise a positive control for type O blood such as an additional probe for H-antigen, which is the common precursor of the A and B antigens[16].

Once the slides had been scanned for ABO blood typing the secondary (goat anti-mouse) antibody was added to each well to measure the responses to the pathogen specific antigens. Slides were then scanned a second time (for Cy3) and the relative signal responses to the HIV/HCV antigens were determined. The detection of the antibodies by printed antigens in the mock antibody-positive plasma is measured by the relative signal response, calculated by dividing the signal/background value for each antigen by the signal/background value for that antigen in the plasma only sample. The results of the pathogen detection demonstrated clear positive responses for two of the HCV and two of the HIV antigens. The data showed that for the monoclonal antibodies the best sensitivity was in the region of 6×10^{-10} M. By far the most promising HCV antigen was the RDI HCV NS4 which showed a 40-fold increase in relative signal responses for the mock antibody-positive plasma compared to the plasma only control. The response for the Virostat HCV NS3 fusion antigen was also easily detectable with relative signal values of up to 10. The HIV antigens also showed very encouraging results, with the most promising antigen being the RDI HIV-2 gp36 with relative signal responses up to 13.

This study has described the development of a combined assay for simultaneous blood typing and serodiagnosis of pathogen infection on a single protein microarray platform. The assay as described utilises an anti-mouse secondary antibody (Cy3 labelled) to detect the binding of mouse anti-HIV/HCV monoclonal antibodies from the mock antibody-positive plasma. As the blood typing antibodies used (LA2 and LB2) are mouse antibodies, the anti-mouse secondary antibody can cross react with these probes giving false signals. In the development of the assay we overcame this problem by first incubating the mock antibody-positive human 'whole blood' samples on to the arrays and scanning for the erythrocyte specific signal and then adding the secondary antibody and scanning for the Cy3 pathogen specific signal. In a real diagnostic assay, the antibodies detected would be human anti-HIV/HCV, therefore the secondary antibody used could also be human specific and would not then be expected to react with the LA2 and LB2 antibodies printed on the array. This would allow us to scan the slide on a single occasion, obtaining both the erythrocyte auto-fluorescence signal and Cy3 pathogen-specific signal without any pre-processing or further incubations between scans, giving a faster result and a less complicated assay.

Further improvements to the assay could be gained by printing additional features such as antibodies specific for HIV/HCV antigens thus allowing the detection of pathogen specific antigens in the blood[8]. This would add further power to the assay by allowing the simultaneous detection of host

antibodies and pathogen specific antigens. In addition, further HIV/HCV antigens could be added to the assay to provide a more in depth coverage of viral strains. Our group have also investigated the use of this assay to detect other blood borne pathogens and in particular further tests for syphilis and HBV have shown great promise.

One major advantage of this system is the fact that we can simultaneously process up to 16 samples on a single microarray slide. This helps to reduce the level of variation due to handling/operator issues as the samples are processed and scanned together and it also helps to reduce the overall costs of the assay as up to 16 test arrays can be printed onto a single slide. A further development to this system would be to move to a 96-well plate format, whereby single arrays are printed into individual wells of a 96-well plate. This would allow 96 individual samples to be processed simultaneously, greatly reducing the overall cost per assay. This would also bring the assay closer in line with the industry standard tests and would be more amenable to automation[8,9].

Our results demonstrate proof of concept for the combination of multiplexed serodiagnosis of viral infection and blood-typing assays. However, blood typing requires identification of a larger range of surface antigens (such as the Rhesus group) and extensive pathogen testing also includes assays for syphilis and HBV. Further work will include the integration of these assays into the current microarray format[11].

In conclusion, we have successfully developed a proof of concept platform capable of blood typing human whole blood samples based on the ABO blood system, whilst at the same time testing the sample for the presence of antibodies specific for HIV/HCV. The major benefits of this system are its amenability to expansion with additional assays, i.e. rhesus typing or syphilis, HBV detection and also the ability to simultaneously test a number of samples on the same test slide, currently 16 but readily expandable to a 96-well format.

Tables

Antigen (Ag) / antibody (Ab)	Molecular Weight (Ag) / Class (Ab)	Origin (Ag) /Target (Ab)	Source
HIV-1 gp41 (ARP 680) (Ag)	16×10^3-28×10^3	HIV	NIBSC
HIV-1 p24 His (EVA 673) (Ag)	26×10^3	HIV	NIBSC
HIV-2 env gp 36 (Ag)	34×10^3 (+114×10^3 β-Gal Fusion)	HIV	RDI
HIV-2 env gp 36 (Ag)	32×10^3 (+114×10^3 β-Gal Fusion)	HIV	Virogen
HCV Core (Ag)	36×10^3	HCV	Virostat
HCV NS3 Fusion (Ag)	1×10^6	HCV	Virostat
HCV NS4 (Ag)	19×10^3 (+114×10^3 β-Gal Fusion)	HCV	RDI
LA2 (Ab)	IgM	A antigen	Alba
LB2 (Ab)	IgM	B antigen	Alba

Table 1. HIV/HCV pathogen specific antigens and blood typing antibodies utilised on the protein microarray.

Antibody Target	Subtype	Source
anti-HCV Core	Mouse IgG1	Virogen
anti-HCV NS3	Mouse IgG1	Virogen
anti-HCV NS4	Mouse IgG2a	RDI
anti-HIV-1 p24	Mouse IgG1	Virogen
anti-HIV-1 gp41	Mouse IgG1	RDI
anti-HIV-2 gp36	Mouse IgG2a	Virogen

Table 2. Monoclonal antibodies used in the preparation of the mock antibody-positive plasma samples (HIV/HCV).

ABO Blood Type	Threshold Value of Index Score
A	≥ 2.585
B	≤ 0.612
O	0.929 – 2.58

Table 3. Thresholds derived from ROC curve analysis within which a blood sample can be defined as belonging to a particular ABO blood type group[12]. Threshold values are based upon an index score (ratio) of the A and B blood type antibody responses (LA2/LB2).

Figures

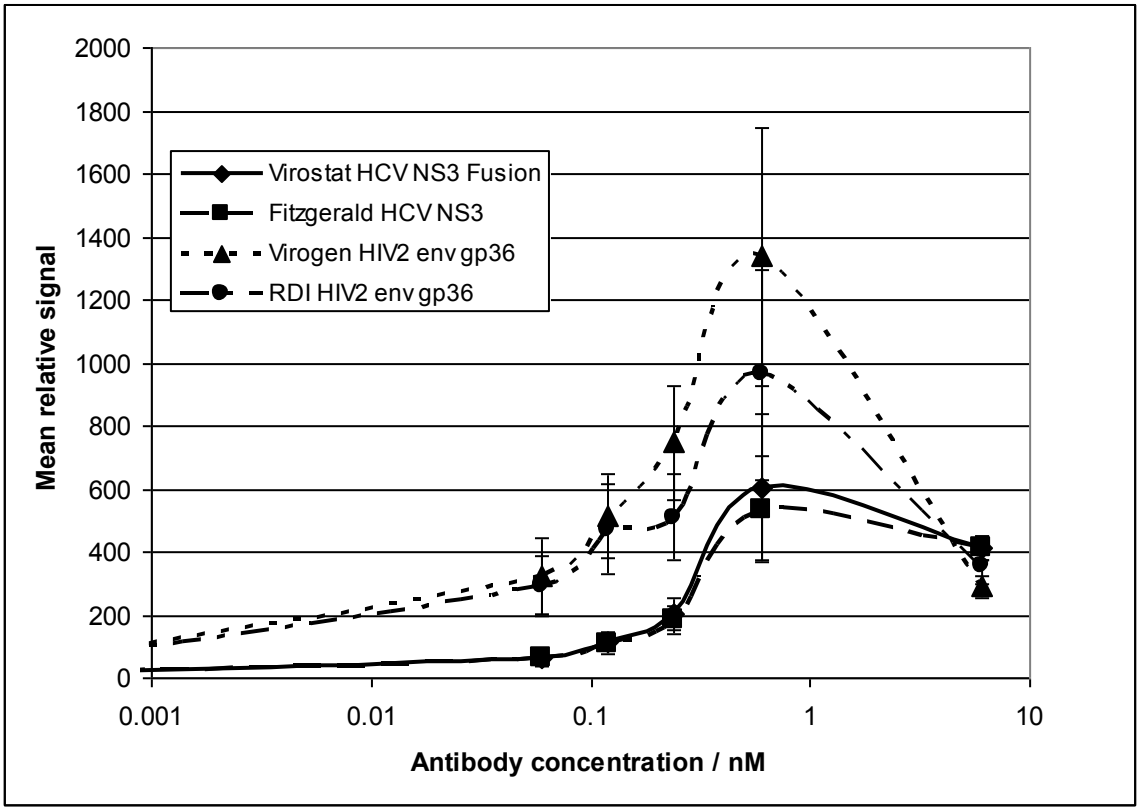

Figure 1. Dose response curve demonstrating optimum conditions for mock antibody-positive plasma antibodies with the four best performing HIV/HCV antigens. HIV/HCV combined mock infected plasma at a 1/30 dilution.

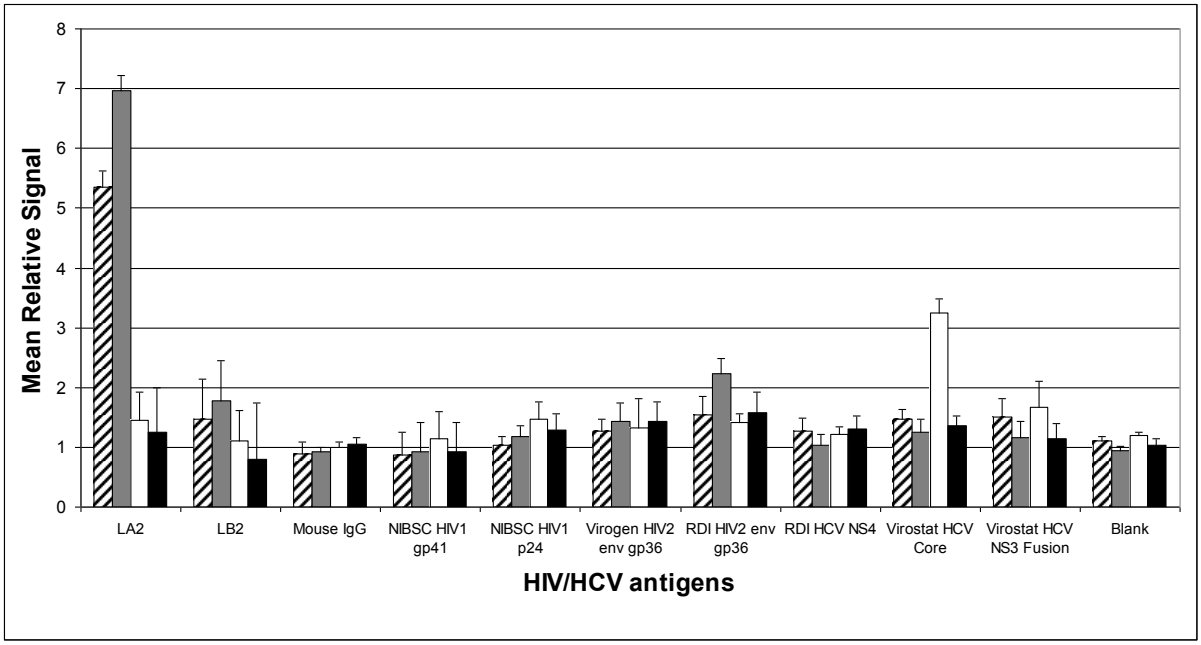

Figure 2. Blood typing results for human mock antibody-positive 'whole blood' samples. HIV/HCV mock antibody-positive plasma at a 1/30 dilution with erythrocytes of type A or O blood type both in the presence and absence of monoclonal antibodies (MAbs) against HIV/HCV antigens. Mean relative signal value was calculated by dividing the mean signal/background value for each sample by the mean signal/background value obtained with a plasma only reference sample. Blank sample value is calculated based on the median response from 14 blank, non-spotted array locations. Error bars display standard error based on the ratio of the relative standard deviation (RSD) of the mock antibody-positive signal (a) and of the plasma only signal (b) respectively, i.e. $\sqrt{((RSDa)^2 + (RSDb)^2)}$. = Type A erythrocytes only, = Type A erythrocytes + MAbs, = Type O erythrocytes only, = Type O erythrocytes + MAbs**.**

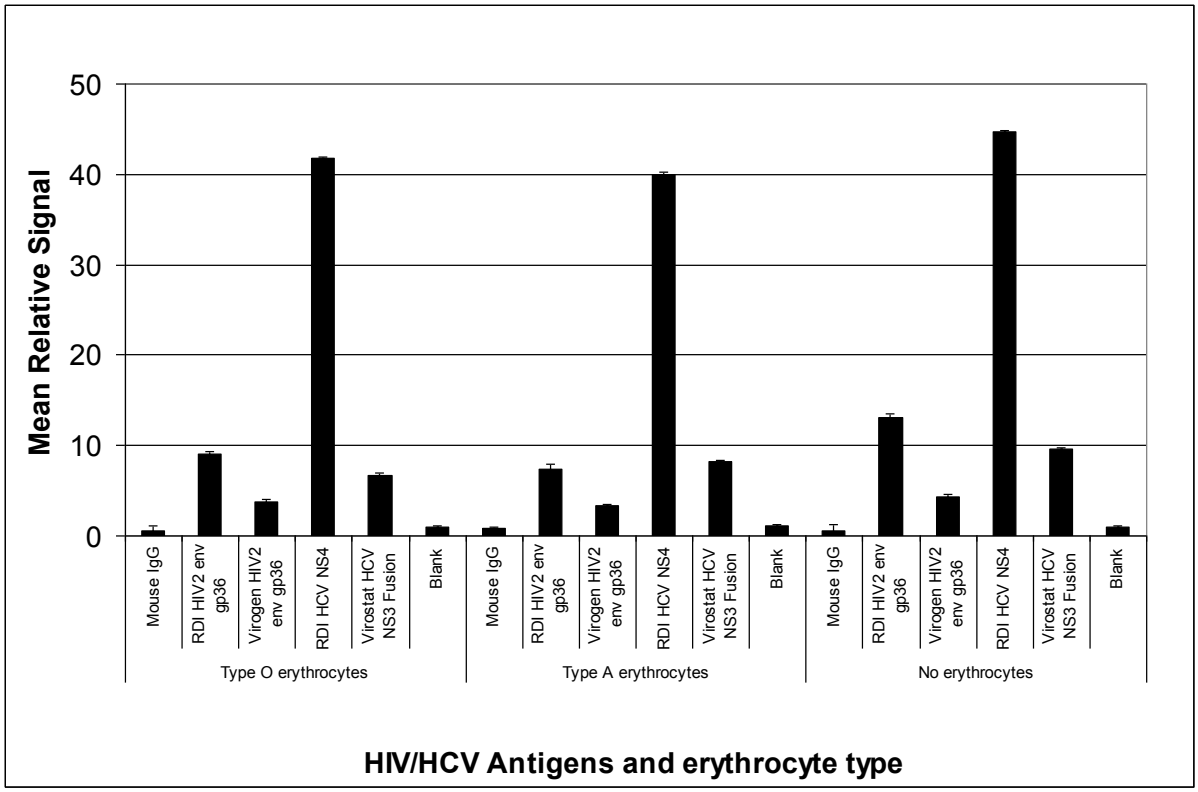

Figure 3. Serodiagnosis of pathogen infection analysis from mock antibody-positive human 'whole blood' samples. HIV/HCV mock antibody-positive plasma at a 1/30 dilution in the presence of monoclonal antibodies ((MAbs) at a concentration of 6 x 10^{-10} M) against HIV/HCV antigens. The mean relative signal value was calculated by dividing the mean signal/background value for each sample by the mean signal/background value obtained with a plasma only reference sample. The blank sample value is calculated based on the median response from 14 blank, non-spotted array locations. Mouse IgG antibody serves as a positive control. Error bars display the standard error based on the ratio of the relative standard deviation (RSD) of the mock antibody-positive signal (a) and of the plasma only signal (b) respectively, i.e. $\sqrt{((RSDa)^2 + (RSDb)^2)}$.

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