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Quantitative measurement of human anti-HCV Core immunoglobulins on an electrical biochip platform

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ABSTRACT

The importance of early diagnosis devices increased continuously in the last two decades and plays an important role in medical care. Early stage diagnosis of e.g. ovarian cancer, HCV-infection or HIV-infection increased the survival rate of patients significantly. In parallel there is a trend leaving centralized diagnostic laboratories in order to get closer to the patient to perform analysis of even complex parameters in the field. This often saves time, increases the prognosis of the patient significantly and is cheaper in many cases.

In this study we employ a rapid and cost-effective detection system based on electrical biochip technology for decentralized detection of anti-HCV Core immunoglobulins (HCV antibodies). In this system the qualitative and quantitative detection of virus-specific antibodies is done by an ELISA directly on a gold electrode array utilizing HCV Core as capture antigen. The biochip allows antibody detection within 20 min. Signal amplification was done by enzyme labelling and by "Single Electrode Redox Cycling". This method enhances current signals up to 40-fold in comparison to simple oxidation. The sensitivity of this approach is therefore comparable to a standard microtiter plate based ELISA with a 9-fold saving of assay time. This biochip system allows serum or whole blood analysis with no signal loss or increasing background caused by the red blood cells. Fields of application can be hospital emergency units where only single detections have to be conducted in a quick manner or by the general practitioner.

1. Introduction

The Hepatitis C virus (HCV) was first identified in 1989 as transfusion associated non-A, non-B hepatitis (Alter et al., 1989), it is a positive strand RNA virus belonging to the family of *Flaviviridae*, genus *Hepacivirus*. The virus is closely related to the Hepatitis G virus, yellow fever virus, and dengue virus (Robertson et al., 1998). More than 170 million people worldwide are chronically infected with HCV—the prevalence outnumbers the prevalence of HIV by factor 5. Estimated 3.9 million individuals are tested positive for HCV RNA in the United States (Armstrong et al., 2006). Major risk factors for HCV-infection are injection drug usage, blood transfusions before 1992, and sexual exposure. HCV can, together with HBV, also be considered as the leading cause for hepatocellular carcinoma (HCC) which is one of the most frequent forms of cancer worldwide (Bosch et al., 1999). 80% of the HCV patients develop a chronic infection if they are left untreated. Chronic liver cirrhosis can be observed for 20% of chronic HCV carriers. The HCC incidence within the HCV carriers is 1–4% (Lauer and Walker, 2001). There are enormous geographical differences in the prevalence of HCV ranging from 0.2% to 0.5% in central Europe (Wasley and Alter, 2000) and 1.6% in the United States (Armstrong et al., 2006) up to 22% in Egypt. The latter was caused by parenteral antischistosomal therapy (Frank et al., 2000).

HCV-specific antibody assays have been applied since 1992; this caused a drop in the incidence by more than 80% (Schreiber et al., 1996). On average, the interval between infection and seroconversion is between 7 and 8 weeks (Hino et al., 1994; Puoti et al., 1992). Generally an Enzyme-linked immunosorbent assay (ELISA) followed by a recombinant immunoblot assay (RIBA) is used to determine HCV antibodies. For the ELISA a mixture of HCV proteins and peptides is immobilized, HCV antibodies from the patient material are allowed to bind and are later detected by an optical sensor (Pawlotsky, 2002a). RIBA uses similar antigens like the

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ELISA, but here each antigen is analyzed separately. Today RIBA is mostly used in low risk environments like blood banks (EASL, 1999). Positive ELISA or RIBA results have to be confirmed by a second serological test applying a different test principle. HCV RNA is a direct marker for the viral replication and is commonly used for differentiation between active HCV-infections and cured HCV-infections, which cannot be distinguished by serological means, and for therapy monitoring (Neumann et al., 1998). In acute infection the RNA is present whereas specific antibodies appear delayed. In chronic infections both are present at the same time (Pawlotsky, 2002a).

An ELISA with cyclovoltammetric detection of HCV antigen is described to be an alternative to the optical standard methods (Dai et al., 2006). Another convenient alternative to conventional detection of HCV-specific antibodies is our electrical biochip platform which uses "Single Electrode Redox Cycling" as detection method. Here we present a demonstrator device for automated measuring of HCV antibodies in diluted serum or whole blood samples within 20 min emphasizing its point-of-care character. This portable platform could improve diagnostics in small hospitals or in medical practices.

2. Material and methods

2.1. Reagents

Phosphate buffered saline pH 7.4, Tween-20, trehalose, magnesiumchloride, 4-aminophenyl-β-D-galactopyranoside (p-APG) and bovine serum albumine (BSA) were purchased from Sigma–Aldrich GmbH (Taufkirchen, Germany). Human immunoglobulin-G was from Fitzgerald Industries (Concord, Massachusetts, USA) and goat-anti-human-IgG-β-galactosidase-conjugate was purchased from Southern Biotech (Birmingham, Alabama, USA).

2.2. HCV serum samples

We obtained 111 serum samples from our clinical partner. All samples were previously characterized with the Abbott ARCHITECT system using the ARCHITECT® anti-HCV assay. 20 sera were identified as HCV antibody-negative and 91 as HCV antibody-positive.

2.3. Core antigen

The Core sequence was cloned from a HCV cDNA (subtype 1b) into a pET26b+ vector from Merck Chemicals (Nottingham, United Kingdom) and expressed recombinantly in Escherichia coli (BL21 Rosetta2(DE3)pLysS cells, Merck). Fermentation was performed in synthetic medium using an Applikon fermenter (Schiedam, Netherlands). A glucose feed was installed to prevent T7 promoter dependent leak expression. Lysis was performed with 8 M Urea buffer pH 8.0 over night at 4 °C. Cellular debris was sedimented at 30,000 × *g* and the cleared lysate was purified using an Äkta HPLC system with talon-ultra-flow column from GE Healthcare (Bucks, United Kingdom).

2.4. Microtiter plate ELISA

ELISA was performed at room temperature on a 96-well microtiter high binding plate purchased from Greiner Bio One (Frickenhausen, Germany). The plates were coated with 50 ng/well of recombinant HCV Core protein. The plate was blocked with 1% bovine serum albumin fraction V (BSA) from Carl Roth (Karlsruhe, Germany) diluted in PBS containing 0.05% (v/v) Tween 20 (PBS-T). A serial dilution of sera was prepared in 0.5% BSA PBS-T and applied on the plate. The detection of bound anti-HCV Core immunoglobulins was performed using an anti-human-IgG peroxidase conjugate (Fab specific) from Sigma–Aldrich (1:10,000). 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) was used as substrate for the peroxidase. Optical readout occurred at 405 nm. Between all steps the plate was washed three times with PBS-T. The reaction volume was 50 μl in all steps, 200 μl were used for blocking.

2.5. Biochip fabrication and spotting

The chips were manufactured in the industrial semiconductor production line at the Fraunhofer ISIT on 6 in. silicon wafers as previously described (Elsholz et al., 2006). The chip dimensions were 9 mm × 10 mm. In contrast to the interdigitated electrodes described by Elsholz et al. (2006) our chip carries 16 unstructured gold electrode positions. The dimensions with 500 μm in diameter and 340 μm spacing to each other were the same. The wafers were thermally oxidized to a depth of 650 nm for insulation. The gold electrodes consist of vacuum deposited stacks of 20 nm tantalum as a bonding agent and a final 150 nm upper gold layer. Structuring was carried out with photolithography and lift-off technology. For passivation a combination of 300 nm SiN and 2 μm structured photoresist was used. Here, the conducting paths were coated with an insulator for use in wet samples. Only the electrode area and the contact pads were left open. This combination of the hydrophilic electrode surface and a surrounding hydrophobic passivation allows focusing of aqueous solutions on the electrodes during and after dispensing the capture molecules. A gold counter electrode and an iridium/iridiumoxide reference electrode are also integrated on the chip. Therefore iridium was deposited and structured also with photolithography and lift-off processes. Afterwards it was partially oxidized by oxygen plasma. Its reference potential at a pH of 7.4 was 410 mV against normal hydrogen electrode (NHE).

Application of the antigens at the gold electrodes was performed with a piezodriven microdispensing device from GeSiM GmbH (Großerkmannsdorf, Germany). Therefore human immunoglobulin-G as positive control (50 μg/ml in PBS), HCV Core antigen (10 μg/ml in PBS with 3 M urea), and BSA as negative control (100 μg/ml in PBS) were spotted with 70 droplets on three chip positions each resulting in a total amount of 25 nl per array electrode (Fig. 2C). Positions 4–6 and 10–12 are spotted both with BSA. Positions 10–12 were called "Negative", because they were used for mathematical standardization. Positions 4–6 were unnecessary for this assay but should also be spotted to prevent unspecific binding. The immobilization of these capture molecules was carried out by thiol-gold interaction and hydrophobic adsorption. Spotted chips were incubated for 2.5 h at room temperature and additional 12 h over night at 4 °C in a humidity chamber. After washing with PBS-T chips were blocked with BSA (0.5 mg/ml in PBS) for 90 min, washed with deionized water and dried under vacuum. Spotted chips are stable for more than one year at 4 °C. The spotted chips are disposables and are able to detect only one sample. They will be discarded after one assay and a new chip should be used.

2.6. Biochip platform

The spotted biochips are housed in a polycarbonate chip carrier equipped with a flow through cell as described elsewhere (Elsholz et al., 2006). The automated biochip measuring device consists of a reagent container (Buffer, enzyme conjugate, substrate) and a waste reservoir, 6 pinch solenoid valves, a miniaturized peristaltic pump and a modified chip adapter from AJ eBiochip GmbH (Itzehoe, Germany) (Fig. 2A). Its total size is 10 cm height, 20 cm width and 25 cm depth. The valves together with the pump address the sample and the different reagents sequentially to perform the complete assay. All related electronics like an in house produced 12-channel

parallel multi potentiostat and the control units for temperature, valves and the pump are integrated. This potentiostat is able to perform the below described detection method with a resolution of 5 pA and a range of ±200 nA. The chips carry 16 positions, but we are only able to measure 12 of them with the current device.

2.7. ELISA on-chip

For preparing a 20 minute HCV assay on the electrical biochip platform sera were diluted in steps from 1:800 to 1:102,400. Goat-anti-human-β-galactosidase was diluted 1:400 and the substrate p-APG was provided with 1 mg/ml. In general PBS-T containing magnesiumchloride, trehalose and BSA was used as washing and dilution buffer. The incubation times were 14 min for the serum samples and 3 min for the enzyme conjugate. Additional 3 min were required for washing and detection. Spotted biochips were inserted into the chip adapter and connected to the fluidic, the electrical and the thermal system in parallel. All steps are performed at a chip temperature of 38 °C. The assay run is automatically controlled by proprietary developed software "MCDDE" which controls the action of valves, the pump and chip heating. Data analysis was performed by OriginPro 8 G (OriginLab Corporation, Northampton, Massachusetts, USA).

2.8. Detection method and signal evaluation

The diluted serum samples pass the fluidics to the chip, where the HCV antibodies bind to the HCV Core antigen carrying electrodes. A subsequent enzyme labeling of these bound antibodies and at the human-IgG carrying positions (Positive control) takes place. A position-specific enzymatic generation of the electrochemically active substrate 4-hydroxyaniline (p-AP) out of p-APG allows then the parallel and position-specific readout of the chips using "Single Electrode Redox Cycling" in a stop flow mode (Fig. 2B). The positive control positions always show the strongest signals and the negative control positions the weakest ones independently of the sample allowing therefore standardization.

The method of "Single Electrode Redox Cycling" requires in contrast to redox cycling at interdigital ultramicro electrodes (IDAs) (Liu et al., 2008; Hintsche et al., 2005; Nebling et al., 2004) only unstructured electrode surfaces. This allows less expensive chip fabrication at higher yield because short circuits at several chip positions driven from IDA structures are avoided.

For measuring of the enzyme generated redox active substance 4-hydroxyaniline the gold electrodes on the biochip were switched between +200 mV and −350 mV with a frequency of 1 Hz. Current measurements were taken 120 ms after switching resulting in position-specific increasing oxidation and reduction currents (Fig. 2D). The amounts of these oxidation and reduction currents were summarized to create a current slope of each chip position beginning one second after stop flow mode. Linear regression of a 6 second time period leads to target concentration dependent slopes in the nA/min range (Fig. 2E).

For each triplicate (positive control, HCV, and negative control) at one single chip a mean value was generated. A standardization procedure was carried out by setting the negative control values (chip positions 10–12) to zero while adjusting the positive control and HCV values dependent on this. Then the positive control is set to 1000 nA/min and the HCV signals were normalized relative to it.

3. Results and discussion

3.1. Microtiter plate ELISA

Prior to the biochip-based study we analyzed 111 patient sera in an indirect ELISA on full-length HCV Core protein. 20 sera were previously identified as HCV antibody-negative and 91 as antibody-positive using the ARCHITECT® anti-HCV assay. We analyzed serial dilutions (1:100 to 1:204,800) of each serum sample using HCV Core coated microtiter plates. The dilutions between 1:100 and 1:400 were not taken into consideration as the signal was not linear in this range. All samples reached the level of the negative control in any case at a dilution of 1:102,400. Based on these data we defined the optimal interval for studying the HCV antibody titer between 1:800 and 1:102,400. Fig. 1 shows exemplary the serial dilutions in this range for 8 different patient samples.

This first evaluation of the assay was only based on a graphical evaluation of the serum ELISA. For a reliable determination it was necessary to calculate the exact HCV antibody titer with mathematical methods. The reciprocal value of all titers was plotted against related E_{405} values determined by the ELISA. The resulting graph can be described best as a line that crosses the background level at a certain point, which is defined as HCV antibody titer. The parameters of each line can be evaluated by linear regression. For our analysis it was necessary to define a background level for the ELISA that serves as basis for the titer calculations. We defined it as 2

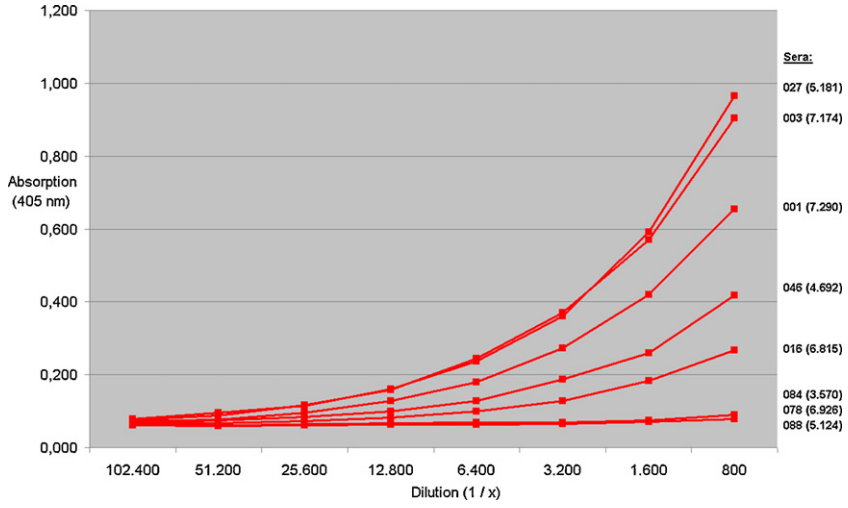

Fig. 1. Microtiter plate ELISA measurements of 8 different patient sera. Bound HCV antibodies were detected with a goat-anti-human-peroxidase conjugate. Visualization occurred with ABTS substrate and optical readout at 405 nm. All steps were carried out for 60 min. Sera 001, 003, 027 showed a high antibody titer, 046 and 016 had medium reactivity and 078, 084, 088 were not reactive.

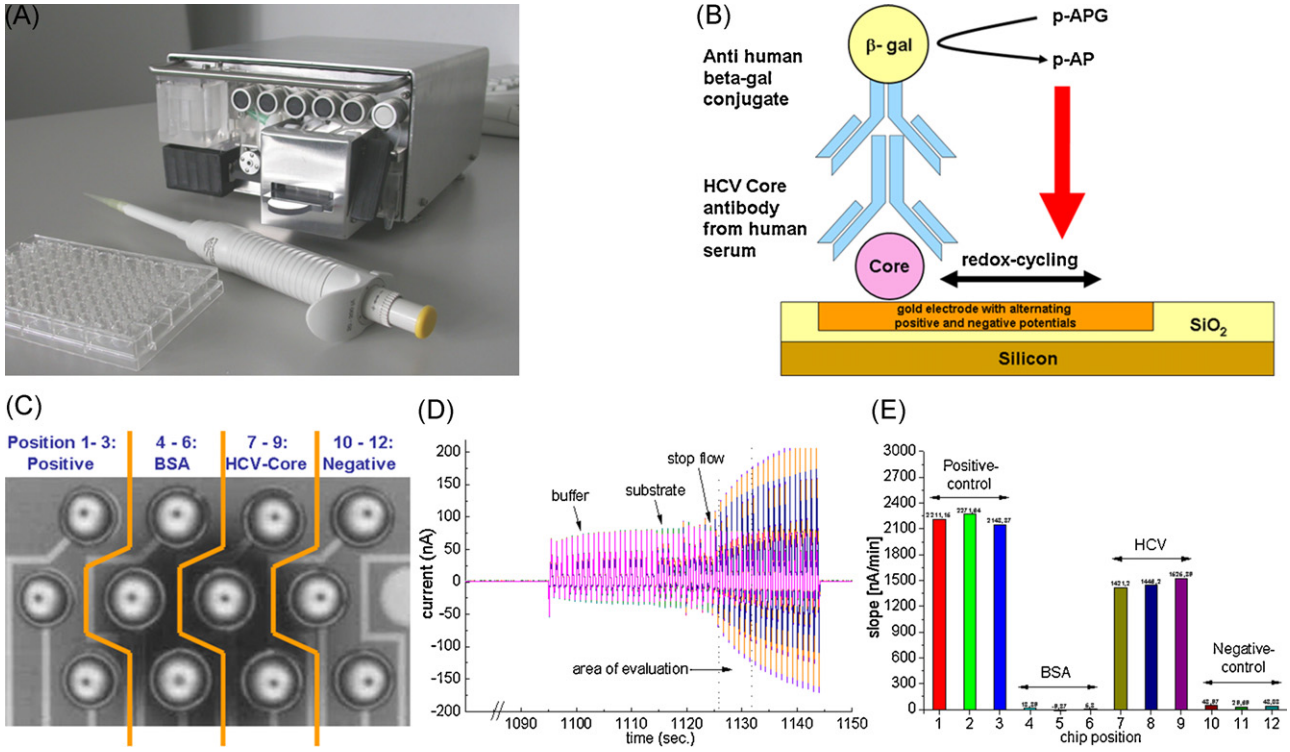

Fig. 2. Biochip System for HCV antibody detection. (A) Photograph of the biochip reader system: the system has quite small dimensions (10 cm height, 20 cm width, 25 cm depth) and can be easily carried to the point-of-care. (B) Schematic depiction of the on-chip ELISA: the gold electrode uses alternating positive and negative potentials for signal detection. (C) Photograph of the spotted biochip: positions 1–3: positive control (human IgG), positions 4–6: BSA, positions 7–9: recombinant HCV Core protein and positions 10–12: BSA for negative control. (D) Reading process of the chip: after addition of the substrate the flow is stopped and the electrochemical measurement is performed. Whole assay time: 20 min. (E) Exemplary analysis of the biochip raw data: all parameters are measured in triplicate. The median is than calculated from each of the 4 groups. The positive control is an internal standard for the evaluation. It is usually set to 1000 nA/min and the HCV signals are normalized relative to it.

times the value of the median negative control. We used OriginPro 8G SR2 for the linear regression. The obtained line slope and intercept can be used together with the predefined background level to calculate the titer:

$$\frac{1}{[(\text{Background-Intercept})/\text{Slope}]}$$

All titers matched nicely with the titer obtained from the graphical evaluation. We defined a titer below 1:500 as HCV negative. With these parameters our assay succeeded in the correct classification of all 20 HCV antibody-negative and of 88 from 91 HCV antibody-positive samples resulting in a sensitivity of 96.7% and a specificity of 100%. A share of false negative results was expected, as it was reported that some antibody-positive HCV patients lack Core protein specific antibodies.

For the subsequent comparison of the biochip assay with the conventional ELISA we focused on 8 sera that represent the complete spectrum of HCV antibody titers that we observed in the first screening. Samples 001, 003, 016, 027 and 046 were previously identified as HCV antibody-positive and 078, 084, 088 sera as HCV antibody-negative using a commercial system. This classification was confirmed by our assay antibody-positive samples showed a significant activity in our assay.

The calculated HCV antibody titers for sera 001, 003, 016, 027, 046 are: 9406; 21,113; 2330; 19,286 and 4464. Sera 078, 084 and 088 were not considered as the ELISA reading was below the defined background. The mathematical evaluation also confirms the determination of HCV positive and negative samples by the commercial system.

3.2. ELISA on-chip

The ELISA data presented in Fig. 1 served as a reference for the biochip reading. Fig. 2 gives a short impression of the assays setup: Fig. 2A shows the automated electrical biochip reader system equipped with reservoirs and fluidics. Fig. 2B shows a schematic depiction of the on-chip ELISA for HCV antibody detection. The chip position is coated with recombinant HCV Core protein that captures relevant antibodies from a serum sample. Bound antibodies are labelled by anti-human-β-galactosidase conjugate that facilitates the converse from p-APG to electroactive 4-hydroxyaniline (p-AP). The gold electrode uses alternating positive and negative potentials for signal detection. The received electric currents can be measured and correlate directly to the amount of antibody in the sample. Fig. 2C displays the spotted gold electrodes of one chip as described above in “Biochip fabrication and spotting”. The human immunoglobulin, the HCV Core antigen and the BSA are immobilized through thiol gold interactions. For automated assay procedure all incubation and washing steps similar to the microtiterplate ELISA were performed by the systems internal microfluidic. The whole process takes 20 min starting with a diluted serum sample. The electrochemical detection of a 1:800 diluted HCV positive serum sample is shown in Fig. 2D. Partially increasing alternating oxidation and reduction currents of all 12 positions are obtained in parallel. The needed measuring interval for receiving relevant data points is just 6 s (Fig. 2D). Fig. 2E shows the raw data of slope calculation for these 12 positions of the same measurement. Slopes are obtained for the positive control positions (1–3) and the HCV positions (7–9) while the negative positions 4–6 and 10–12 showed nearly no slopes. Human IgG is constantly detected as positive control and as internal standard

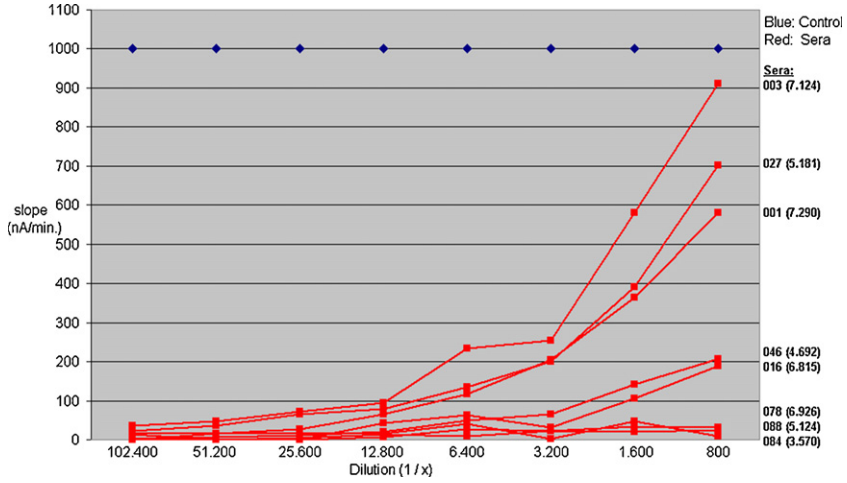

Fig. 3. Biochip ELISA measurements of 8 different patient sera. Sera 001, 003 and 027 showed a high antibody titer, 046 and 016 had medium reactivity and 078, 084 and 088 were not reactive at all. The signals were normalized to the negative control which was set to 0.0 nA/min. Than the positive control was set to 1000 nA/min and the HCV signals were calculated appropriate to this. Each data point was measured in triplicate. The median values are indicated in this figure. Overall the biochip assay results are comparable to the microtiter plate ELISA.

resulting in the highest slope. It is always used to normalize the HCV antibody measurement and allows together with the negative control positions to obtain standardized data that is not influenced by external factors like temperature or enzyme activity (Fig. 2E).

Fig. 3 shows serial dilutions of the five HCV antibody-positive sera and the three HCV antibody-negative sera mentioned above measured on the biochip. Analogous to the ELISA experiments dilutions were prepared in the range between 1:800 and 1:102,400. In the biochip assay sera 001, 003 and 027 showed a strong reactivity on the recombinant HCV Core protein. Sera 046 and 016 were medium reactive furthermore the signal of sera 078, 084 and 088 did not rise over the background level of 50 nA/min. The raw signals were standardized in the way described in Section 2. The titration curves were comparable with the curves of the microtiter plate ELISA shown in Fig. 1. Only the slope values of serum 003 and serum 016 at a dilution of 1:3200 were a little bit lower than expected. The median value of the negative controls was approximately 50 nA/min. Overall the reactivity was comparable to the previously shown ELISA data.

The calculated HCV antibody titers measured in the biochip assay for the 5 HCV antibody-positive sera are: 001: 14,602; 003: 65,090; 016: 4088; 027: 36,148 and 046: 4263. Calculation has not been performed for sera 078, 084 and 088 because they were all below the background level in all dilutions. The calculated HCV antibody titers on the biochip are comparable to the values obtained from ELISA. There is a trend observable that the titers obtained from the biochip are approximately factor 2 higher than in ELISA.

Fig. 4 shows a biochip measurement of patient serum 027 at a dilution of 1:800 which was previously identified as HCV antibody-positive. The sample was measured on 28 biochips according to the same conditions to evaluate the standard deviation that has to be expected within a series of measurements. The mean value for the positive control raw signals is 1911.8 ± 205.4 nA/min ($\pm 10.7\%$) and for the raw HCV data 1397.3 ± 128.9 nA/min ($\pm 9.2\%$) (data not shown). Then the raw data is standardized using the negative control signals and the internal positive control standard (Fig. 4). The normalized HCV mean value for this experiment is 734.0 ± 51.4 nA/min ($\pm 7.0\%$).

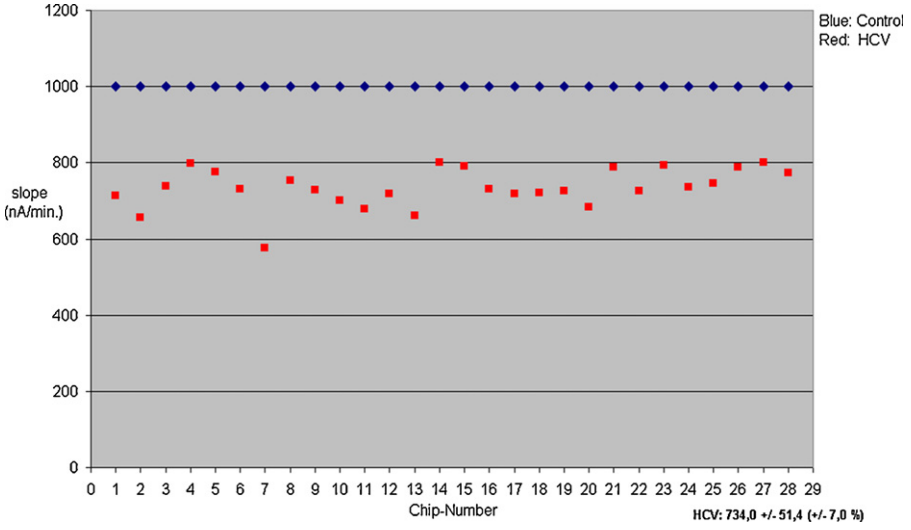

Fig. 4. Biochip ELISA measurements for repeatability tests. The HCV positive serum 027 was diluted 1:800 and analyzed in our electrical biochip system for HCV antibody reactivity. Standardized HCV signals are shown. Each data point was measured in triplicate. The median values are indicated in this figure. The sample was measured on 28 biochips according to the same conditions to see if the experiments were repeatable. HCV signal: 734.0 ± 51.4 ($\pm 7.0\%$).

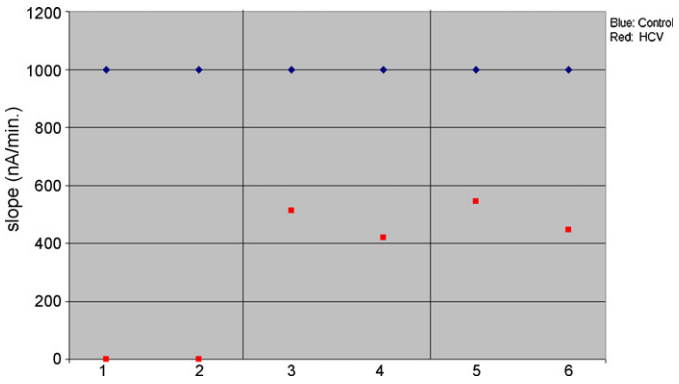

Fig. 5. Biochip ELISA measurements on 6 chips under the same conditions with different samples: (1) HCV negative serum 084 diluted 1:500. (2) HCV negative whole blood diluted 1:500. (3, 4) HCV positive serum 027 diluted 1:1600. (5, 6) HCV positive serum 027 diluted 1:1600 with whole blood prediluted 1:500. Standardized HCV signals are shown. Each data point was measured in triplicate.

For point-of-care diagnostics detection of HCV antibodies in diluted whole blood samples is urgent. For evaluation of validity of the anti-HCV biochip for the use of whole blood we spiked whole blood with the HCV antibody-positive serum 027 by performing a 1:1600 dilution of this serum with 1:500 prediluted whole blood. HCV antibody-negative serum (1:500), HCV antibody-negative whole blood (1:500), and serum 027 (1:1600) were used as control samples. The results achieved with the electrical biochip platform are presented in Fig. 5. The determination of the HCV antibody-negative serum 084 and a HCV antibody-negative whole blood sample show standardized HCV signals near to zero comparable to the measurements shown above. The determination of HCV antibody in serum 027 and in whole blood spiked with serum 027 was done in duplicate. All four standardized results were in between 430 and 550 nA/min with no loss of signal intensity in the diluted whole blood sample in comparison to the diluted serum sample. This emphasizes the electrical biochip platform to be suitable for detection of HCV antibodies in whole blood.

For elucidation of the diagnostic potential of the "Single Electrode Redox Cycling" principle applied by our biochip we compared the measurement of a serum sample using "Single Electrode Redox Cycling" with the measurement of the identical sample using simple oxidation of the generated p-AP at +200 mV. For this we diluted 2 μl of whole blood in 1 ml dilution buffer. One aliquot was measured as described above. A second aliquot was measured with a new chip using simple oxidation of the generated p-AP at +200 mV (Fig. 6). A more than 40-fold decrease of the positive control signals was obtained with this second setting. HCV- and negative control signals were much below zero which could lead to a loss of weak HCV signals and therefore of sensitivity. This indicates that "Single Electrode Redox Cycling" is an outstanding method for electrochemical detection.

3.3. Advantage of the biochip-based ELISA and the detection method

The detailed HCV antibody data analyses revealed that the values obtained from the biochip system showed an up to 2-fold increase of signal-to-noise ratio compared to the microtiter plate based reference ELISA. This means that the on-chip HCV antibody assay was at least as sensitive as the reference assay, but could be performed at a fraction of time. The standard deviation of the HCV antibody measurements was about ±10% and could be improved to ±7.0% after normalization. Therefore standardization will increase the reliability of the system significantly.

The biochip system features an amplifying electrical detection method. This "Single Electrode Redox Cycling" highlights electrochemical detection in that way, that the obtained signals are more than 40-fold higher than only measuring simple oxidation. Further described redox cycling on interdigitated electrodes (Hintsche et al., 2005) enhances current signals too, but the electrodes structure is susceptible to short circuits. In contrast to the static anodes and cathodes in the interdigitated electrode system we switched one electrode to act as anode and cathode. Therefore the alternating potential oxidizes and reduces electro active species nearly independent of diffusion. This method allows us to use a simple electrode layout on the biochip which enables further miniaturization and offers bulk production and cost reduction cycles.

4. Conclusion

Although we performed our analysis of HCV antibodies in microliter scale directly on the biochip's gold electrodes we reached at least the sensitivity of a standard ELISA. We obtained these results on a mobile diagnostic platform that allows the quantification of HCV antibodies in only 20 min. That allows a 9-fold reduction of assay time and enormous savings of assay components. Especially expensive antigen that has to be produced under GMP conditions can be reduced by coating with only 30 nl on one chip position in contrast to 50 μl in a regular ELISA. We also succeeded in the quantification of HCV antibodies from whole blood samples. We measured the same sensitivity that we observed in serum samples. No interference with the red blood cells occurred. This simplifies

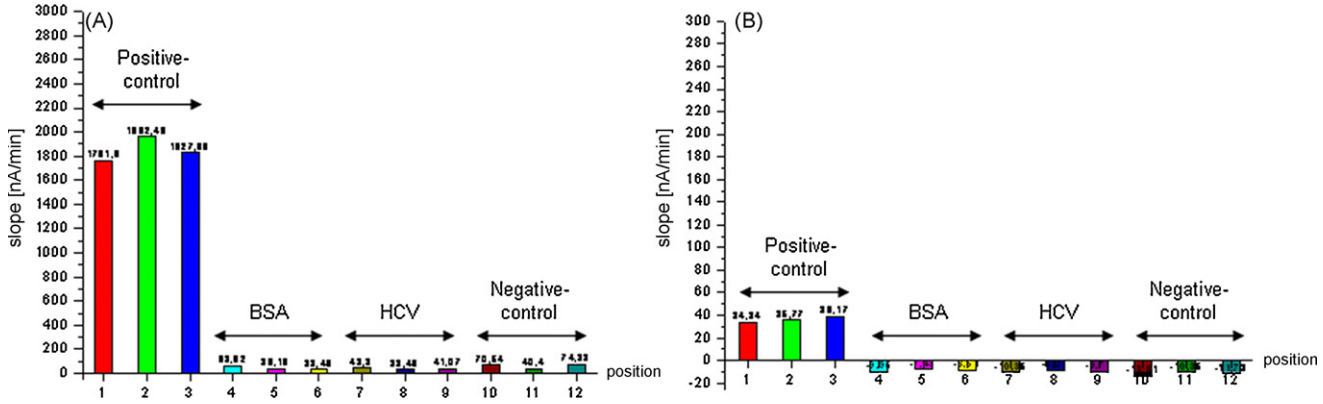

Fig. 6. Biochip ELISA measurements of a 1:500 diluted whole blood sample. (A) Slope calculation of current signals resulting from "Single Electrode Redox Cycling" as detection method. (B) Slope calculation of current signals resulting only from p-AP oxidation at +200 mV of an identical second test with the same sample at a new biochip. The scale is only a tenth of (A).

enormously the sample preparation protocol, as serum does not have to be prepared from every blood sample. So it is not longer necessary to obtain a whole vacutainer with 20 ml of blood which have to be centrifugated. A sample volume of 2 μl whole blood required by the biochip facilitates numerous tests even from limited amounts of patient sample. Especially samples collected from infants do usually not exceed 500 μl. Common detection systems often require 200 μl or more for the determination of one single blood parameter. Our system can be operated by inexperienced users. Blood can be taken with a lancet out of the finger tip analogous to blood sugar tests. The assay reached a standard deviation of (+/− 7.0%) which is with respect to the small volume handling a promising result but should be improved to reach medical standards.

Most of the commercial systems use a truncated HCV Core protein. Only amino acids 1–150 are usually considered. In contrast to this we could express recombinantly a full-length protein with 191 amino acids, which binds specifically HCV antibodies in serum samples. It is reported that HCV infected individuals do not always develop detectable antibody amounts against the core protein of the virus (Lauer and Walker, 2001). To reach a sensitivity of 99% or more it might be helpful to integrate other recombinantly expressed HCV proteins like NS3, NS4, and NS5 (Colin et al., 2001) into the biochip system. Commercially available tests systems use recombinantly expressed and pooled HCV proteins and peptides, mainly the transmembrane domain-free parts of core and parts of NS3, NS4 or NS5, to avoid false negative results at the cost of inter-assay comparability of the obtained HCV-score values (Pawlotsky, 2002b). The HCV-scores allow qualitative decisions on the HCV serostatus but are unsuitable for monitoring antibody titer trends. A quantification of the HCV antibody titer is not promising in the commercial systems as there is always a mixture of antigens being analyzed.

In further experiments the biochip system described here could easily analyze multiple parameters from one serum sample and record exact titers for each measured HCV antigen separately. We will exceed the limit from 12 positions now to 16 chip positions and spot the mentioned antigens additional to the core antigen. If a patient would be analyzed repeatedly with such a system it would be possible to add quantitative long-term trends to his anamnesis. After the spontaneous HCV clearance antibody titers usually decrease slowly and can clear completely within decades (Takaki et al., 2000). For these kinds of measurements exact long-term quantifications would be helpful.

In the future the system will allow the combination of different parameters on one chip which will enable the parallel testing of all relevant data associated with the differential diagnoses of a given spectrum of symptoms (e.g. respiratory disease) in hospitals or emergency units. The whole platform could be easily adapted for non-infectious targets like cardiac or tumor markers which are also detectable via an ELISA on-chip.

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