Label-free detection of H1N1 virus for point of care testing

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Abstract

An assay for the detection of H1N1 virus was established using Reflectometric Interference Spectroscopy (RIfS), a label-free optical detection method. A direct assay format was used to detect the virus by the corresponding antibody immobilized on the chip surface. The used surface chemistry showed low non-specific binding of the virions and gave reproducible signals. The binding of H1N1 virus was monitored in RIfS and verified by atomic force microscopy (AFM) measurements. The proposed assay has the potential to be integrated in portable and miniaturized devices based on RIfS, which can be used in point of care testing at e.g. airports to improve homeland security.

Keywords: biosensor, label-free, point of care testing, influenza, RIfS

1. Introduction

Influenza is an infectious disease of birds and mammals which is caused by RNA viruses of the family of \textit{Orthomyxoviridae}. The pathogen is responsible for 3-5 Mio severe cases of illness and 25,000-500,000 deaths per year all over the world [1]. The influenza virus shows a high variability because of antigen shifts in between different species and antigen drifts arising from mutations of the genome. This leads to new virus strains and increases the potential of influenza epidemics or even pandemics which occur every some years. In times of global travel and commerce the risk of global spread of infectious diseases is highly increasing, as it could be seen with the rapid spread of SARS or avian influenza virus H5N1 [2]. This fact clearly shows the demanding need for fast and reliable detection methods and assays for potentially infectious people. The availability of an easy to use and robust detection method for influenza is a valuable contribution to prevent the expansion of infectious diseases in times of globalization and increases homeland security, as it can be used e.g. at airports without the need of costly laboratory.

In this paper we present an assay for the detection of influenza virus H1N1 based on Reflectometric Interference Spectroscopy (RIfS), a label-free, optical detection method. As the direct optical detection method RIfS is a quantitative method in contrast to commonly used lateral flow assays quantification is possible. Additionally it can be miniaturized and integrated in a portable device [3]; it has the potential to be used in point of care testing. Furthermore, this method admits multiplexing and the development of multianalyte assays [4]. This gives the possibility to develop a whole test panel for different virus strains. The aspect of sample identification is another point where multiparameter assays would be favorable. E.g. at airports where many tests are performed a day, it is...
2. Experimental

2.1. Reflectometric Interference spectroscopy

Reflectometric Interference Spectroscopy is a label-free, optical detection method based on reflection of white light at thin layers. The principle of RIfS is shown in Figure 1. The transducers consist of a multilayer system based on a 1-mm-thick glass substrate coated with 10 nm Ta2O5 and 330 nm SiO2 on top. White light is guided into the multilayer transducer. The light is partially reflected at each of the layers. The reflected light superimposes to an interference spectrum which is detected using a diode array spectrometer.

Alterations in the upper layer change the so called optical thickness of this layer. The optical thickness is the product of the physical thickness and the refractive index. The alterations cause a shift of the interference spectrum which is detected. From this shift a binding curve is calculated and the resulting signal is given as apparent optical thickness (nm) versus time (s).

As the physical thickness and the refractive index are inverse proportional to temperature effects, RIfS is practically temperature insensitive. This means in contrast to SPR, which is another label-free, optical method, RIfS does not need any temperature stabilization. Using RIfS, time-resolved measurements are possible. This is advantageous as not only affinity constants but also kinetic constants can be determined [5]. RIfS has been used in a variety of applications such as DNA-DNA, DNA–protein, protein-cell interaction [6], and receptor-ligand [7]. As RIfS is a label-free detection method it has the advantage that no additional labeling step of the analyte or ligand is necessary, so sample preparation is quite simple.

2.2. Surface chemistry

Label-free detection methods detect every binding event at the transducer’s surface. So it is crucial to prevent non-specific binding. Therefore different types of sophisticated surface chemistries were developed [8]. Either the transducer is modified with two-dimensional biopolymers e.g. PEG or three-dimensional hydrogels like dextran. The glass transducer is functionalized using silan chemistry, upon which the biopolymer layer is applied. This layer reduces non-specific binding sufficiently.

The antibody can then covalently be immobilized using NHS-DIC chemistry. A direct immobilization of the antibody to the glass substrate is possible as well, but may have the disadvantage of high non-specific binding.

Figure 1: Principle of Reflectometric Interference Spectroscopy (RIfS) and the H1N1 assay. a) H1N1 assay is performed in a direct assay format. In RIfS white light is irradiated into a multilayer system and partially reflected. b) Reflected light beams superimpose to an interference spectrum. The minimum of this spectrum shifts upon antigen binding. c) Depending on the shift a binding curve is calculated.
2.3. Measurement procedure

All measurements were carried out in STE-buffer at a pH of 8.0. The binding events were monitored using standard RIfS setup as described in [8]. The sample volume was 1 mL, containing different concentrations of analyte. It was slowly pumped over the sensor surface and the binding of the analyte to the surface was measured.

2.4. Atomic force microscopy (AFM) measurements

For confirmation of virus binding to the immobilized antibody AFM measurements were performed. The Multimode™ SPM atomic force microscope was from Digital Instruments, Santa Barbara, USA, using a J-scanner(97) S/N 3286 JV from Veeco Instruments GmbH, Mannheim, Germany. Images of coated RIfS transducers were done in tapping mode under air using upper SharpSilicon AFM tips, Type: SSS-NCH. AFM can operate in many different modes and media of special interest for biological systems. Because of the possible resolution in nm range, it is possible to confirm virus binding to the antibody coated surface.

3. Results and Discussion

For the binding of bigger particles as cells or large proteins the corresponding binding curves in RIfS show negative signals [9]. As virions are about 80-100 nm and have an approximate weight of $174 \times 10^6$ Da [10] one would expect negative signals for the virion binding. One typical binding curve of 6.5 µg/µL virions to anti-H1N1 is shown in figure 2a. For label-free detection methods it is essential to check for non-specific binding. Therefore non-specific binding was tested using 6.5 µg/ml virions. The sample was pumped over the transducer surface. Non-specific binding caused a negligible signal of 24 pm (Figure 2b). Furthermore AFM measurements were performed to confirm virus binding to the surface (see figure 3).

![Figure 2: a) typical binding curve for influenza H1N1 virions, resulting in negative binding signal due to virus size. b) Test of non-specific binding](image1)

![Figure 3: AFM measurements: a) hydrogel surface as a negative control b) Virions bound to anti-H1N1 antibody immobilized on a hydrogel surface](image2)
Surface structure of the hydrogel without virus is relatively smooth compared to surface with virions. Particles at the size of approximately 100 nm, which is in good correlation of virus size [10], were clearly visible.

Different virus concentrations ranging from 0.65 to 65 µg/mL were measured in triplicates. The assay performance, especially reproducibility and the possibility to quantify are very good, along with a simple test procedure. So a robust assay which needs little sample pretreatment was developed.

Table 1: Measurement data for three different concentrations measured in STE-buffer pH 8.0

| Concentration [µg/mL] | Max. Signal [nm] | Standard deviation |
|-----------------------|------------------|--------------------|
| 0                     | 0.039            | 0.008              |
| 0.65                  | 0.167            | 0.026              |
| 65                    | 1.078            | 0.073              |

4. Conclusion

The presented results demonstrate that it is possible to detect Influenza H1N1 virions with the optical and label-free method RIfS.

Further steps will be: increasing sensitivity to the required clinical range, performing measurements in complex samples as salvia and integration of the developed assay in portable and miniaturized devices based on RIfS. Such devices are developed in the current projects “MoDekt” and “Tierdiagnostik”, where it could be shown that RIfS has the potential to be used in point of care testing.

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