

Review

# Gravimetric Viral Diagnostics: QCM Based Biosensors for Early Detection of Viruses

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**Abstract:** Viruses are pathogenic microorganisms that can inhabit and replicate in human bodies causing a number of widespread infectious diseases such as influenza, gastroenteritis, hepatitis, meningitis, pneumonia, acquired immune deficiency syndrome (AIDS) etc. A majority of these viral diseases are contagious and can spread from infected to healthy human beings. The most important step in the treatment of these contagious diseases and to prevent their unwanted spread is to timely detect the disease-causing viruses. Gravimetric viral diagnostics based on quartz crystal microbalance (QCM) transducers and natural or synthetic receptors are miniaturized sensing platforms that can selectively recognize and quantify harmful virus species. Herein, a review of the label-free QCM virus sensors for clinical diagnostics and point of care (POC) applications is presented with major emphasis on the nature and performance of different receptors ranging from the natural or synthetic antibodies to selective macromolecular materials such as DNA and aptamers. A performance comparison of different receptors is provided and their limitations are discussed.

**Keywords:** antibodies; aptamer; epitope imprinting; molecularly imprinted polymers; quartz crystal microbalance; virus sensor

## 1. Introduction

Early diagnosis of infectious disease-causing agents such as viruses is essential for clinical and point of care (POC) applications [1–3]. Since the viruses have extremely small size and can infect all living beings, i.e., humans, animals, and plants. Therefore, their precise and accurate detection is of significant interest. Viruses usually live in host's living cells, where they replicate. Thus, their detection is a complicated and challenging task due to the complex nature of the medium in which they exist. Since the discovery of first virus, i.e., tobacco mosaic virus (TMV) in 1892, a large number of tests and toolkits have been developed for different types of viruses [4,5]. Especially, modern tools like

enzyme-linked immunosorbent assay (ELISA) and polymerized chain reaction (PCR) amplification are highly sensitive and selective protocols for virus recognition [6,7]. Disposable kits having immobilized enzymes or DNA offer simple and rapid results in minimal cost [8,9]. However, such tests are more suited for qualitative detection of viruses in remote areas.

Regardless of various established techniques for the detection and investigation of infectious viruses, the development of smart biosensors and diagnostic devices for quantitative determination of viruses is mandatory [10–13]. The major impetus in the research and development of such virus sensors is their practical features such as the ease of operation, simple and straightforward device fabrication, possibility to integrate synthetic or natural antibodies, rapid response, high selectivity and cross-sensitivity, portability, and miniaturization capability. It is due to these advantages that biosensors have not only been widely employed for virus recognition [14], but have also been applied for the detection of microorganism such as pathogenic bacteria [15,16] and yeast [17–20], living blood cells [21–23], and different diseases biomarkers [24,25].

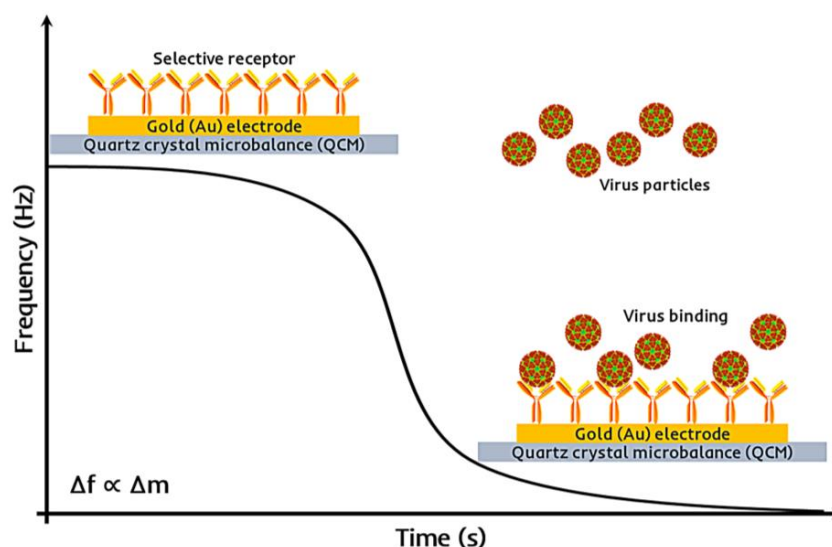
A typical chemical sensor is comprised of natural and-or synthetic receptors coated on a suitable transducer such as optical, electrochemical, magnetic, gravimetric or mass-sensitive etc. [26]. Among many other factors, the selection of a transduction device mainly depends on the nature and physicochemical properties of the sensitive layer material that undergoes changes when exposed to the analyte. For instance, in case of an optical sensor, the optical properties of the selective material (or receptor layer) change due to its interactions with the analyte of interest. However, if the receptor layer is optically inactive, a labeling species or indicator is introduced that translates the receptor-analyte interactions into a recognizable optical signal [27]. Thus, the inclusion of a labeling molecule to the receptor layer introduces the desired sensing feature. Since all receptor surfaces do not necessarily possess the optical, electrochemical, or magnetic characteristics, the addition of a labeling agent causes more complexity in the receptor's binding mechanism and may also affect its sensitivity, which are obvious disadvantages of the labeling techniques.

In this bargain, acoustic or mass-sensitive devices offer label-free detection of analytes, because mass is a fundamental property of any analyte. Thus, the acoustic wave devices are considered as universal mass-sensitive or gravimetric transducers. These transducers include: (a) the bulk acoustic wave devices such as quartz crystal microbalance (QCM) [28,29]; (b) the surface acoustic wave (SAW) resonators [30,31]; and (c) the shear transverse wave (STW) resonators [32,33], which are frequently studied in a combination with different receptor materials for molecular recognition and biomedical diagnosis. The mass-sensitive biosensors produce direct shift in frequency in the event of receptor-analyte interaction and analyte recognition. Among the aforementioned acoustic devices, QCM based chemical and biosensors have been extensively studied for the detection and quantification of a wide range of analytes from small molecules and ions to biological macromolecules and pathogenic species, e.g., viruses.

Figure 1 shows the principle of a QCM based gravimetric sensor for virus recognition. A QCM transducer, if coated with a suitably selective receptor, is capable of binding virus particles as well as virus proteins. In principle, QCM is highly sensitive to the changes in mass loading and depending upon the nature of analyte (e.g., virus particles) these little changes in mass due to selective virus binding can be detected easily. The selective receptors therefore play a major role in virus recognition and quantification due to their ability to interact and bind with viruses. Hereby, we present a comprehensive review of the gravimetric or mass-sensitive viral diagnostic devices based on QCM and a broad spectrum of synthetic and natural receptors.

The key objective of this work is to deliver an up-to-date literature review and critical analysis of different approaches employed in the design and application of QCM based biosensors and diagnostic devices for the exclusive recognition of different types of viruses. The substance is divided into two sections in which the basic QCM device design and assembly, and different types of receptor materials with special emphasis on the nature, fabrication method, and sensor performance are discussed. In the

end a comparison of different receptor layers is provided along with the benefits and limitations of each receptor. This work also highlights the major achievements and the future research viewpoint.



**Figure 1.** The principle of a QCM-based gravimetric virus sensor. The change in mass in response to virus binding with the selective receptor is detected as a change in frequency of QCM transducer.

## 2. Quartz Crystal Microbalance: Transducer Design and Fabrication

This section presents a brief description of the principle, design, and fabrication of QCM-based gravimetric transducer. During the last few decades, QCM measuring the frequency shift and the electrochemical QCM, i.e., EQCM [34,35] simultaneously computing the electrochemical and frequency changes have been broadly designed for sensing different analytes in the gaseous and liquid phases. The pioneering work of Sauerbrey [36] on calculating the frequency changes as a function of mass adsorbed or deposited on the surface of gravimetric devices has laid the foundation for their applications in chemical and biosensors.

In a typical QCM sensor construction, a thin AT-cut quartz wafer having metal electrodes on opposite sides is connected with oscillator circuit which drives QCM to resonate at characteristic frequency. A bulk transverse wave is generated that propagates in perpendicular direction of quartz surface. The crystal surface is usually covered with a certain receptor material which binds with target analyte and thus leads to increase in the rigid mass of crystal. This results a change in fundamental resonance frequency of crystal and may be used to precisely determine the adsorbed mass on crystal surface therefore, referring it as sensor signal.

QCM, due to its extremely high sensitivity towards minor changes in mass, is considered as one of the best platforms for such applications. If combined with suitable selective material or receptor layer, QCM transforms into an exceptionally smart tiny balance capable of detecting miniscule alterations in surface mass, i.e., usually in the range of ng/cm<sup>2</sup> [37].

The sensor response of a typical QCM device, i.e., shift in frequency ( $\Delta f$ ), is directly proportional to the square of its fundamental resonating frequency and the mass deposited [36], as given in the Equation (1) below:

$$\Delta f = -f_o^2 \cdot \Delta m / A \sqrt{\rho_q \mu_q} \quad (1)$$

where  $f_o$  is the fundamental resonant frequency of QCM,  $\Delta m$  is the change in mass,  $A$  is the piezoelectrically active area of QCM in cm<sup>2</sup>,  $\rho_q$  is the density of quartz (i.e., 2.648 g/cm<sup>3</sup>), and  $\mu_q$  is the shear modulus of an AT-cut quartz crystal (i.e.,  $2.947 \times 10^{11}$  g/cm·s<sup>2</sup>).

The Equation (1) is modified for measurements in liquid medium [38,39], i.e., when one face of QCM is in contact with a liquid, the liquid loading effect on frequency change can be summarized as given in Equation (2) below:

$$\Delta f = -f_o^{3/2} \sqrt{\frac{\rho_l \eta_l}{\pi \rho_q \mu_q}} \quad (2)$$

where  $\rho_l$  is the density of the liquid, and  $\eta_l$  is the viscosity of the liquid. It is important to mention here whenever QCM devices are studied in liquid phase, the acoustic properties of liquid medium have to be taken into account for sensor measurements.

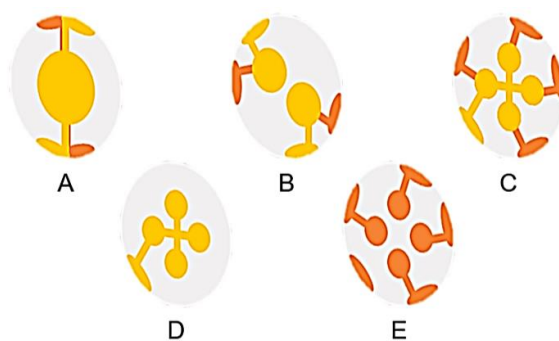
Nonetheless, these equations show that the sensitivity of the QCM device can be improved by increasing its primary frequency, i.e., if the fundamental frequency of a QCM is doubled, its response would be increased by a factor of four. However, this can only be achieved by reducing the thickness of QCM wafer that will result in mechanically fragile devices. Thus, this parameter practically limits the fabrication of devices beyond certain frequency ( $f_o$ ). The other way to increase the sensitivity of QCM devices is to work with overtones, which require special oscillator circuit having band pass filter for attenuation of harmonic resonances. However, this would also lead to an increase in noise level and ultimately making small improvement in signal to noise ratio. Therefore, an increase in the fundamental resonance frequency of crystal is the more appropriate method of tuning sensitivity.

Typically, QCM having frequency in the range of 5–20 MHz are used in liquid phase measurements. Although, a few reports suggest the use of QCM with frequency as high as 110 MHz for liquid phase operation [40]. This is remarkably high frequency and the authors report that substantially high detection limit is achieved in the detection of M13-phages in liquid medium, i.e., the sensitivity improved by a factor of 200 while using 56 MHz QCM as compared to 19 MHz QCM device [40].

Another important aspect in determining the sensitivity of a QCM device is the nature and design of electrodes on QCM wafers [41,42]. If the diameter of the electrode is bigger, the available surface for selective receptor layer integration would be large, which ultimately leads to higher sensor response. For electrode fabrication on QCM wafer, gold or any other noble metal paste can be applied via screen printing. Many of the commercially available QCM have fused metal electrodes that afford better support on QCM surface. Furthermore, the integration of receptor layer with QCM electrode should be firm and stable, i.e., the receptor material should not be deteriorated during the fabrication and testing processes.

Figure 2 shows the design of a single, dual, and tetra-electrode QCM wafer for detecting viruses. A multichannel QCM having more than one electrodes for sensing different analytes is an innovative design [43]. Albeit this unique tetra-electrode, QCM design has not been implemented for simultaneous recognition of different viruses or virus sub-types, but it could be adopted as a single transducer platform for different viruses. However, it is important to mention that there should be a minimal distance between the multiple electrodes to prevent the cross-talk between electrical signals and thereby reducing signal-to-noise ratio for improved sensitivity. Furthermore, in liquid phase measurements, one side of the QCM remains in air to avoid excessive damping problems.

These are some general but imperative design and fabrication parameters that regulate the ultimate QCM device sensitivity. The following study emphasizes a variety of synthetic receptors based on imprinted polymers, natural antibodies, DNA and aptamers for the detection of different viruses in combination with QCM devices.



**Figure 2.** The design of quartz crystal microbalance (QCM) transducers: (A) A working single-electrode QCM for detecting a specific virus; (B) A dual-electrode QCM with working and reference electrodes for detecting a specific virus; and (C) A tetra-electrode QCM for simultaneous detection of multiple viruses. The front and back sides of the multichannel tetra-electrode QCM are shown in D and E, respectively. For sensor measurements, QCM is assembled into a flow cell so that its front side (D) is exposed to the liquid phase containing the target analyte (e.g., viruses), and its back side (E) is exposed to air to prevent damping of the sensor signal.

### 3. Receptors for QCM Virus Sensors

A number of academic research articles and review papers have been published previously that emphasize the QCM's working principle and study their performance in a wide range of complex biochemical processes taking place at the receptor-analyte interface [44–47]. These investigations highlight the surface chemistry and mechanistic events during the attachment or binding of various target analytes. This work is primarily focused on different types of synthetic and natural receptors that directly influence QCM device sensitivity and selectivity for improved recognition of viruses. In case of virus sensing, the recognition characteristics are governed by nature of receptor/selective layer. In the following sections, we shall describe the virus recognition principle, mechanism, and performance including sensitivity and selectivity of various receptors. The potential receptors for selective sensing of different viruses are divided into four classes based on their nature and virus-binding mechanism: (a) Synthetic antibodies based on molecularly imprinted polymers; (b) natural antibodies; (c) DNA; (d) aptamers; and (e) other biomacromolecular receptors such as proteins.

#### 3.1. Synthetic Antibodies: Molecularly Imprinted Polymers for QCM Virus Sensors

Artificially designed polymeric receptors such as molecularly imprinted polymers (MIPs) are often referred as synthetic or artificial antibodies, because they can mimic the molecular recognition characteristics of naturally occurring receptors or antibodies [48,49]. Among several receptors, synthetic antibodies based on MIPs have placed themselves as highly efficient recognition elements for a wide range of bio-analytes [50–52]. Molecular imprinting is a straightforward way to introduce the target analyte or a closely related molecule as the template in polymer matrix to develop highly specific affinity centers [53]. These interaction centers are usually tailored to template size, structure, and functionality; thus, offering complementary geometrical and chemical fits for the target analyte. The interactions between such affinity centers and analyte molecules are mainly driven by non-covalent forces such as hydrogen bonding,  $\pi$ - $\pi$  stacking, van der Waals forces etc., which allow reversible re-inclusion [54–57].

The ease of synthesis, profound sensitivity, unmatched selectivity, resistance against chemical and thermal degradation, storage stability under ambient conditions are the key features of MIPs, which make them highly competitive in sensing applications. According to some researchers, the selectivity of synthetic antibodies (MIPs) toward specific target molecules is found to be comparable to natural antibodies [58]. The regeneration of these synthetic antibodies after every sensing cycle is fairly simple that makes them viable for several rounds of assays without any significant loss in sensitivity and-or



selectivity. Furthermore, the cost of MIPs for a typical analyte is in the range of 0.1–0.5 \$/mg, whereas the natural antibodies depending upon the target molecules are offered in 100–1000 \$/mg [59].

The foremost advantage of MIPs in chemical and/biosensing applications is the possibility to easily integrate MIPs with different transducer devices such as QCM [60,61]. Due to their exceptional characteristics, MIPs are considered as potential candidate for virus sensing applications and often exhibit high recognition proficiency and selectivity [62]. There are a variety of imprinting strategies for microorganisms' detection [63]; however, in the following sub-sections, we shall focus on the selected approaches that have been typically employed in QCM based viral diagnostics. Table 1 shows the selected examples of virus-MIP based QCM biosensors and their detection limits.

**Table 1.** The selected examples of the molecularly imprinted polymers (MIP) as synthetic receptors for QCM-based viral diagnostics.

Receptor	Template/Target	Polymer	Detection Limit	Reference
Soft-lithographically (surface) imprinted MIP	TMV	Polyurethane	8 ng/mL	[64]
	PPOV	Polyurethane	$5 \times 10^5$ virus particles/mL	[65]
	HRV 1A, HRV 2, HRV 14	Polyurethane	100 µg/mL	[66]
	H5N1, H5N3, H1N1, H1N3, H6N1	Poly(acrylamide-co-methacrylic acid-co-methylmethacrylate-co-N-vinylpyrrolidone) with N,N'-(1,2-dihydroxyethylene)bisacrylamide	$10^5$ particles/mL	[67]
Epitope imprinted MIP	Dengue virus NS1 protein	Poly(acrylic acid-co-acrylamide) with ethyleneglycol dimethylacrylate	1 µg/L	[68]
	HIV-1 GP41	Polydopamine	2 ng/mL	[69]
Plastic antibody replica	HRV 14	Poly(vinylpyrrolidone-co-methacrylic acid) with N,N'-(1,2-dihydroxyethylene)bisacrylamide	$2.5 \times 10^{12}$ virus particles/mL	[70]

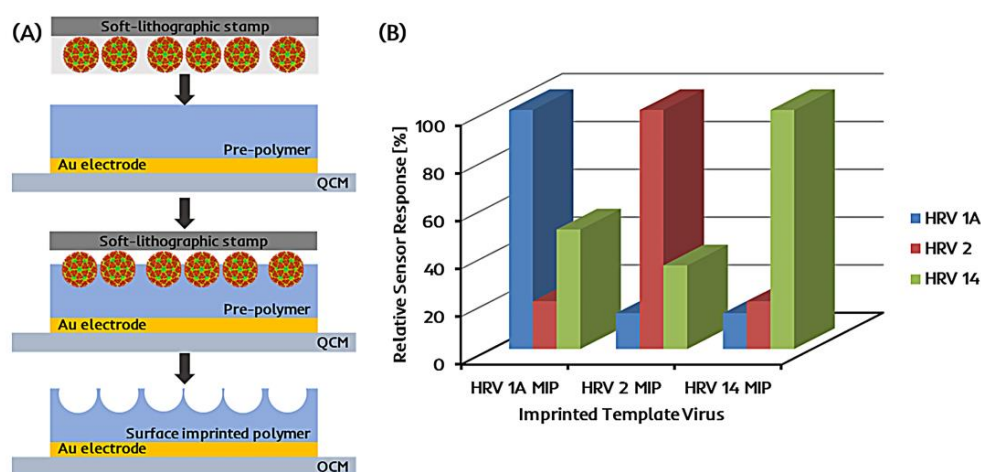
### 3.1.1. Soft-Lithography

Owing to the delicate nature of microorganisms, soft-lithography is an appropriate stamping technique for patterning polymeric materials, thus generating selective biomimetic surfaces [71,72]. It is a surface imprinting technique that has been extensively applied for macromolecular imprinting in the last two decades [73,74]. In this case, a suitable monolayer of targeted microorganism or biomacromolecule is deposited as template on an inert substrate to make the template-stamp, which is subsequently pressed on the surface of a pre-polymer. The stamp-pressed pre-polymer layer is allowed to undergo polymerization under moderate conditions, and then washed with mild solvents to remove template from the polymer surface [75]. A schematic of the soft-lithography based surface imprinting procedure is shown in Figure 3A. This method allows transfer of precise structural details of bioanalytes onto synthetic polymer surface. Consequently, this biomimetic interface is capable of selectively recognizing target analytes through non-covalent chemical interactions.

Soft-lithography allows faster mass transfer of bioanalytes with enhanced reversibility. Furthermore, the pre-polymer can be coated on QCM electrodes before soft-lithographic stamping procedure for simple and easy integration of the selective layer with the transducer. Soft-lithographic imprinting has been extensively studied in combination with QCM devices for different types of viruses. For instance, Dickert et al. [76,77] first patterned a pre-polymer coated on QCM electrode surface with tobacco mosaic viruses (TMV) using soft-lithography, and the resulting sensor was reported to be highly sensitivity towards TMV. The device also exhibited reversible sensor signal indicating the complete removal of adsorbed species and perfect layer regeneration for further analyses. In a subsequent work [64], the sensitivity of QCM devices coated with surface imprinted polyurethane monolayer was substantially improved and the selectivity was investigated revealing high affinity of stamped polymers for the targeted virus. The detection of parapox ovis virus (PPOV) is another

example of the sensing platform using a combination of QCM and soft-lithographically patterned polymer surfaces [65].

In a later study [66], this strategy was extended to imprint different strains of human rhinovirus (HRV) as three serotypes, i.e., HRV 1A, HRV 2, and HRV 14, were used as the templates to produce the respective biomimetic selective layers on QCM. The sensing measurements revealed that each type of imprinted surface offered the highest sensor response to its templated strain virus as shown in Figure 3B, thus proving excellent selectivity. Similarly, the surface imprinted polymers designed by soft-lithography procedure are successfully employed in QCM-based viral diagnostics for influenza A virus sub-types, i.e., H5N1, H5N3, H1N1, H1N3, and H6N1 [67,78]. The fabricated sensors demonstrated considerable selectivity for screening of influenza A virus sub-types as each sub-type was best recognized by its own imprinted surface. The reported detection limit was  $10^5$  virus particles/mL [67]. In view of these examples, it is obvious that soft-lithography approach to design surface MIPs makes them capable of selectively distinguishing different serotypes of the same virus, which may be termed as intra-group selectivity.



**Figure 3.** (A) A schematic representation of soft-lithography: A stamp of assembled virus particles is pressed onto the pre-polymer coated on a QCM electrode. After polymerization, the stamp is removed and the template is washed away to obtain the surface imprinted synthetic antibodies; (B) Relative sensor response of different human rhinovirus (HRV) serotype-imprinted polymers towards different strains of HRV. It is evident that each sub-type of HRV is preferentially identified by the respective MIP surface. Figure 3B is reproduced with permission from Jenik et al. [66]. Copyright by the American Chemical Society, 2009.

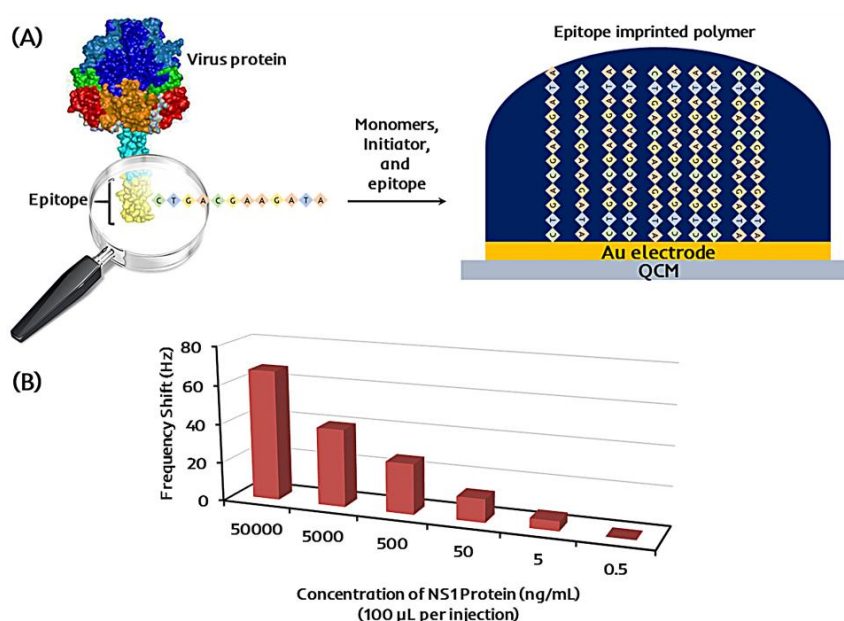
### 3.1.2. Epitope Imprinting

The living cells, viruses and other microorganisms are sometimes difficult to fit and imprint as templates due to their large size and the lack of binding site accessibility [79]. To overcome such challenges in biomacromolecular imprinting, epitope imprinting is introduced as an efficient macromolecular imprinting method, especially for proteins [80,81]. Epitope imprinting is also a method of choice for imprinting and recognition of viral proteins and different species of viruses. This technique, unlike whole-cell imprinting or soft-lithographic patterning of viruses on polymer surfaces, introduces a small peptide fragment as the template for imprinting polymers. The resultant imprinted material interacts with target protein through its epitope, i.e., a small part of the antigen. A schematic of the epitope imprinting process is shown in Figure 4A.

The natural antibody-antigen binding inspired epitope imprinting approach is useful in producing artificial receptors for bioanalytes recognition. Albeit it has been extensively studied for protein recognition [59,82], some researchers also investigated its applications in diagnosis of viruses and viral proteins. The selection of peptide fragment as template and its sequence are important to achieve

improved recognition as the surface groups on the epitope yield specific structural and functional memory in polymers. These surface groups are suited for epitope imprinting due to their accessibility and functionality that leads to the selective recognition of targeted species.

Tai and coworkers [68,83] adopted the epitope imprinting approach for QCM-based serological assay of dengue virus, i.e., mosquito-borne virus, infections. They used acrylic polymer system for epitope imprinting of 15-mer peptide that is known as the linear epitope of dengue virus NS1 protein [68]. The resulting epitope-imprinted polymer (EIP) QCM sensor achieved the direct and quantitative detection of the NS1 protein, as shown in Figure 4B. Furthermore, the EIP-QCM sensors exhibited a good correlation with enzyme-linked immunosorbent assay (ELISA) indicating reliable detection of dengue virus. The sensor also correctly identified samples for the presence or absence of virus species. The EIP sensor coatings remained stable for a period of one month and could be regenerated five times. The analysis time of one sample was 20–30 min with the detection limit of 1–10  $\mu\text{g/L}$ . Such devices are useful in clinical diagnosis of dengue virus infections as they prevent the use of monoclonal antibodies [84,85].



**Figure 4.** (A) A schematic representation of the epitope imprinting procedure; (B) The frequency shifts as a function of the epitope-imprinted polymer's (EIP) complexation with dengue virus NS1 protein. The EIP-coated QCM chip is capable to bind NS1 protein in the range of 5 ng/mL to 50  $\mu\text{g/mL}$  with 100  $\mu\text{L}$  injection of the unpurified NS1 protein solution. Figure 4B is reproduced with permission from Tai et al. [68]. Copyright by the American Chemical Society, 2005.

Chen and coworkers [69] used a synthetic peptide, i.e., 35 amino acid residue as template to developed epitope-imprinted polydopamine coated on QCM for human immunodeficiency virus (HIV) type-1 detection. The selection of epitope was made by similar amino acid residues of HIV-1 glycoprotein 41, i.e., amino acid numbers 579–613. The device exhibited high sensitivity and selectivity for HIV-1 glycoprotein (GP41) with the estimated detection limit of 2 ng/mL. The highpoint of this approach is its applicability to real samples, where the sensor exhibits recovery values of 86.5%–94.1% of HIV-1 GP41 in spiked human urine. These findings are interesting in view of their potential for clinical tests of target viruses and-or viral proteins without using labeling indicator.

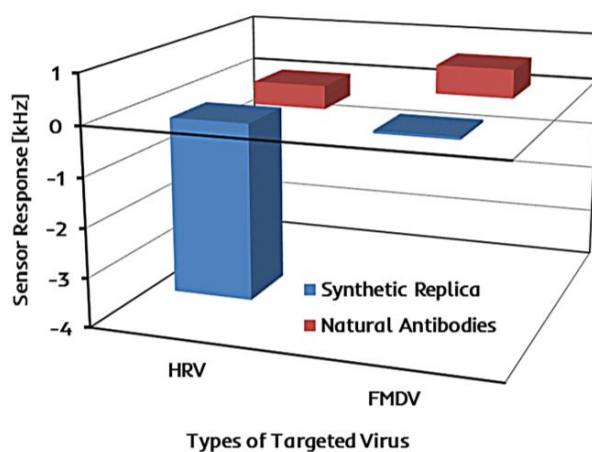
### 3.1.3. Plastic Antibody Replica

Dickert and coworkers [70,86] employed an innovative strategy to produce plastic antibody replica for virus sensing via two-step soft-lithography technique. Firstly, they used natural antibodies



for a specific target as the template in synthetic polymer system, which could be precipitated in appropriate solvent to produce nanoparticles. Then, natural antibody-imprinted nanoparticles were used as the master stamp on the surface of pre-polymer already coated on QCM electrode. Thus, two-step soft-lithography leads to the formation of precisely patterned polymer interface that perfectly mimics natural antibodies used as template.

The authors compared QCM sensor results of natural antibodies and their plastic antibody replica for HRV (the template and target analyte) and foot-and-mouth disease virus (FMDV; the interferent), as shown in Figure 5. Astonishingly, plastic antibody replica exhibited higher response, i.e., six times higher, as compared to natural receptors. It was suggested that high sensitivity of plastic antibody replica might be attributed to the higher surface area of MIP-nanoparticles. Furthermore, the sensor response of both natural and synthetic receptors towards FMDV was negligible showing very low cross-sensitivity and excellent selectivity. This approach provides an alternative and innovative way to precisely pattern synthetic receptors that are plastic copies of natural antibodies and that can be used in microorganisms' detection and bioassay.



**Figure 5.** A comparison of the sensor response of natural antibodies and their synthetic replica prepared via imprinting for human rhinovirus (HRV) and foot-and-mouth disease virus (FMDV) recognition. HRV is the template as well as the target analyte, while FMDV is used for cross-sensitivity measurements. Reproduced with permission from Schirhagl et al. [70]. Copyright by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2009.

### 3.2. Natural Antibodies for QCM Virus Sensors

This section reviews strategies for fast and reliable detection of viruses with the help of natural antibody (NAb)-based QCM biosensors. NAb-QCM biosensors for viral recognition have versatile advantageous features compared to other sensing devices. They are eminently suitable for the rapid diagnosis of viruses with high sensitivity and specificity. Natural antibodies are proteins produced by the immune system to protect the body by identifying and neutralizing pathogens [87]. Natural antibodies function as the selective receptors in gravimetric viral diagnostic devices and offer site-specific affinity with either viral shell proteins or with the proteins released by the viruses when they enter the host body. In fact, the antigenic amino acids occur as patches and function as protein binding sites on the virus coat.

The accumulation of bioanalyte (the targeted virus) on the surface of QCM biosensor coated with NAb results in the resonant frequency shift that is used as the sensor signals. The sensitivity of the NAb-QCM biosensor depends on the type of antibody used and its orientation on the electrode surface. Although considerable data has been documented on NAb-based methods for the detection of pathogenic viruses by conventional techniques [88,89], relatively little work has been done on specifically utilizing NAb-QCM biosensors as viral diagnostics. Nonetheless, NAb-QCM based

detection systems have been developed to detect harmful virus species causing fatal diseases to human beings, plants, and animals in the last two decades [90–92]. Table 2 shows the selected examples of NAb-QCM biosensors for viruses.

**Table 2.** The selected examples of the natural antibodies (NAb) as receptors for QCM-based viral diagnostics.

Receptor	Target	Fabrication/Immobilization Method	Detection Limit	Reference
Anti-H5 NAb	AIV H5N1	Anti-H5 attached to nanobeads immobilized on 16-mercaptohexadecanoic acid monolayer	0.128 HAU	[93]
Anti-MCMV NAb	MCMV	3-mercaptopropanoic acid and 11-mercaptoundecanoic acid (10:1 ratio) crosslinked with anti-MCMV	250 ng/mL	[94]
Monoclonal anti-CIV NP antibody	CIV H3N2	ProLinker™ B immobilized anti-CIV monoclonal antibody	14 nM	[95]
Secondary antibodies	HepBV	Secondary antibodies linked through carboxylated hyper-branched polymer	2 ng/mL	[96]

For instance, Li et al. [93] fabricated a QCM immunosensor using magnetic nanobeads and polyclonal anti-H5 antibodies for the detection of avian influenza virus (AIV) H5N1 in agricultural, food, environmental, and clinical samples. The surface antigen hemagglutinin (HA) was deposited on QCM through self-assembled monolayer of 16-mercaptohexadecanoic acid. The target AI H5N1 viruses were then captured by the immobilized anti-H5 antibodies attached to magnetic nanobeads. The addition of magnetic nanobeads coated with anti-H5 results in amplification of binding reaction between antibody and virus antigens. This anti-H5 coated NAb-QCM immunosensor exhibited good sensitivity (limit of detection: 0.128 HAU [97]) due to nanobeads amplification. It was also noticed that signal amplification was more significant at lower virus concentration that could be favorable for early stage screening of H5N1 virus.

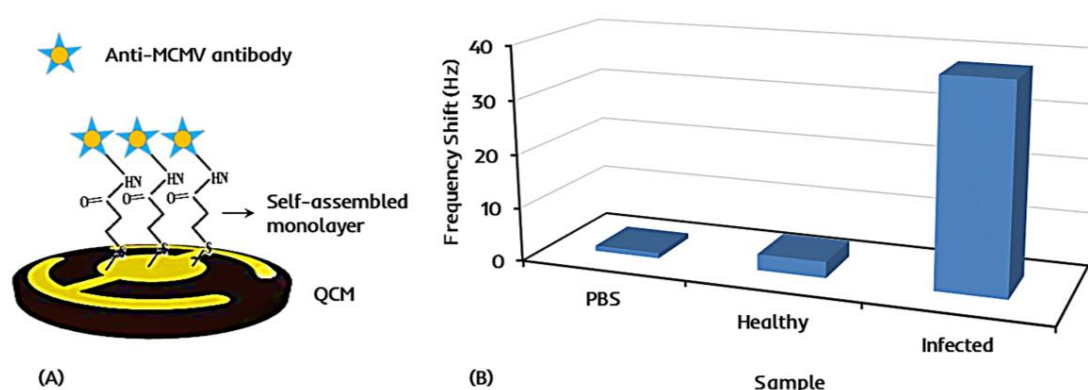
This setup was also used to quantify viruses from chicken tracheal swab samples. Authors did not observe any significant interference with AIV subtypes H3N2, H2N2, and H4N8 [93]. It is imperative to mention here that by increasing the number of active binding sites at sensor interfacial coatings, amplified mass loading can be achieved by QCM based immunosensors. In this perspective, the fabrication of nanomaterials with NAb could lead to enhanced mass deposition on electrode surface thus, amplifying sensor response. This has also been demonstrated in a report [98], where authors developed Au nanoparticles functionalized with antibodies to amplify the recognition process. It has been shown that Au modified receptors increase the detection limit to three orders of magnitude higher as compared to direct QCM sensing without amplification.

Huang et al. [94] reported a NAb-QCM biosensor for the selective recognition of maize chlorotic mottle virus (MCMV). They mixed 3-mercaptopropanoic acid and 11-mercaptoundecanoic acid (10:1 ratio) to prepare a self-assembled monolayer on QCM gold electrodes. Then, anti-MCMV antibody was used as the cross-linking agent for specific recognition of MCMV. The MCMV was cultivated in corn, and the infected tissues were collected after 14 days for tests. Figure 6A shows the surface modification of QCM electrodes to fabricate NAb-QCM biosensor for MCMV detection.

The anti-MCMV coated NAb-QCM biosensor successfully detected MCMV in the concentration range of 250 ng/mL to 10 µg/mL. The detection limit was reported to be 250 ng/mL, which was very close to that achieved by conventional ELISA method. The device was also found to be 45 fold more selective towards MCMV as compared to other viruses such as maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV), and wheat streak mosaic virus (WSMV) at the same concentration. Furthermore, the NAb-QCM biosensor was not only capable to recognize MCMV in a mixture of MCMV, MDMV, WSMV, and SCMV, but also distinguished between healthy and infected corn leaf samples with high accuracy [94], as shown in Figure 6B.

The NAb-virus interactions can be used to identify and differentiate both human and non-human primates. This approach was extended to highly virulent species like Ebola virus (EBOV). Yu et al. [99] raised both polyclonal and monoclonal antibodies in rabbits against soluble EBOV envelope glycoprotein (GP) to study EBOV envelope diversity and develop diagnostics. Three regions were used for the generation of anti-EBOV polyclonal antibodies, namely: Sudan-Gulu, Zaire, and Ivory Coast, EBOV GP peptides to immunize rabbits.

To record sensor responses, a freshly prepared sensor was exposed to each species of EBOV GP and the binding events were monitored in real-time for 12 min [99]. In this study, the QCM device could measure low concentration of EBOV GP with the lowest detection limits of 14 nM and 56 nM for the Zaire and Sudan-Gulu EBOV GPs, respectively. The minimal detectable mass was found to be 11 ng, which was comparable to the ELISA results. Besides, these low detection limits could be achieved quickly as compared to hours required for conventional detection strategies, e.g., ELISA and surface plasmon resonance.



**Figure 6.** (A) A schematic diagram showing modification of the gold electrode surface of a QCM for the detection of maize chlorotic mottle virus (MCMV); (B) The sensor response of anti-MCMV natural antibody (NAb)-coated QCM biosensors towards phosphate-buffered saline (PBS, pH 7.4, 0.01 M) and the crude extractions from healthy and infected maize plants. Figure 6B is reproduced with permission from Huang et al. [94]. Copyright by the Royal Society of Chemistry, 2014.

More recently, a few reports revealed the rapid diagnosis of H3N2 canine influenza virus (CIV) and canine parvovirus (CPV) with the help of NAb-QCM biosensors [95,100]. Kim et al. [95] immobilized antibodies on gold-coated QCM surface using a calixcrown derivative, ProLinker™ B. The resulting device was able to detect H3N2 CIV species at lower concentrations as compared to commercial chromatography Ag kit. In addition, H3N2 CIV-positive reference samples were subjected to an anti-CIV nucleoprotein (NP) monoclonal antibody deposited on QCM surface. This QCM assay exhibited 97.1% sensitivity and 94.7% specificity against 73 field saliva samples.

Zhang et al. [96] used a different approach for selective electrogravimetric immunoassay of hepatitis B virus (HepBV) surface antigen. They prepared a sandwich-type biosensor using hyper-branched polymer as a bridge to link multiple secondary antibodies to amplify QCM immunosensor signal. HepBV surface antigen-antibody interactions were used to recognize HepBV with a detection limit of 2 ng/mL. The sensor response on a QCM chip against HepBV surface antigen was observed to be 5 times higher when compared to other conventional methods.

### 3.3. DNA and Aptamers for QCM Virus Sensors

In the following sections of this article, DNA and aptamer based QCM biosensors for the selective recognition and quantification of different viruses are reviewed. The strategies for immobilization of DNA and aptamers, fabrication of QCM sensing devices, and the performance in terms of sensitivity, selectivity, and limit of detection are discussed.

One of the most important challenges in utilizing DNA and aptamers as the selective receptor layers in QCM-based virus sensors is their anchoring to the surface of QCM electrode. This requires the modification of Au-electrodes by functional thiols which can bind to the electrode surface forming a self-assembled monolayer at thiol-end [101,102], while the next-end functionality, e.g., a carboxylic acid, can bind to the receptor DNA and aptamers.

In another approach, DNA or aptamer is first attached to biotin and the biotinylated-DNA or biotinylated-aptamer is then immobilized on avidin or streptavidin layer anchored to the surface of QCM electrodes [103,104]. Although both of these approaches are well-established for years and efficiently work in biosensors, this additional step in fabrication process is somewhat tedious and requires extra chemicals. Nonetheless, the performance of these sensors shows that the results are rewarding with high selectivity and excellent sensitivity. Table 3 provides the selected examples of DNA and aptamer based QCM biosensors for different viruses.

**Table 3.** The selected examples of the DNA and the aptamers as receptors for QCM-based viral diagnostics.

Receptor	Target	Fabrication/Immobilization Method	Detection Limit	Reference
Immobilized DNA probe	HepBV	Thiolated-ssDNA probe	1 f.mol/cm <sup>2</sup>	[105]
		Thiolated-DNA probe	10 <sup>4</sup> copies/mL	[106]
		DNA immobilized via poly(ethyleneamine)-glutaraldehyde method	0.01 µg/mL	[107]
	Vaccinia virus	Biotinylated-DNA immobilized via NeutrAvidin protein	1 nM	[108]
	VHSV	Biotinylated-DNA immobilized via avidin	0.0016 µM	[109]
	HPV	Biotinylated-oligonucleotides immobilized via streptavidin	30 nM	[110]
	CymMV and ORSV	Mercaptohexyl modified ssDNA-CymMV coat proteins and ssDNA-ORSV coat proteins	0.05 ng/µL	[111]
Immobilized aptamer	AIV H5N1	Thiolated aptamer	2 HAU	[112]
		Streptavidin-biotin complexation plus particle label for amplification	1 HAU	[113]
		Aptamer embedded in hydrogel	0.4 HAU	[114]
		Aptamer embedded in hydrogel	0.0128 HAU	[115]
	HIV-1 Tat protein	Thiol monolayer and streptavidin-biotin complexation	0.25 ppm	[116]

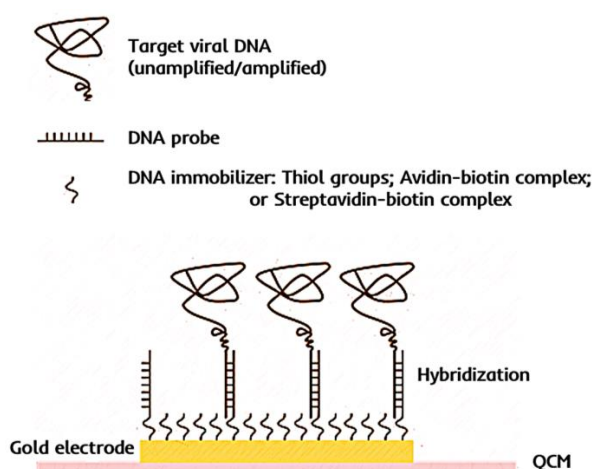
### 3.3.1. DNA

As stated above, the fabrication of DNA-QCM biosensors requires the immobilization of specific DNA probe on gold electrode surface of QCM. Once the device is ready, different viruses can be selectively recognized and quantified via hybridization of their genomic sequence with DNA probe. Hereby, some of the most promising results of DNA-QCM biosensors for different viruses are discussed. For instance, DNA-QCM biosensors have been successfully tested for the detection of hepatitis B virus (HepBV) [105–107], hepatitis C virus (HepCV) [117], vaccinia virus [108], viral haemorrhagic septicaemia virus (VHSV) [109], human papilloma virus (HPV) [110], dengue virus [118], and orchid viruses such as cymbidium mosaic virus (CymMV) and odontoglossum ringspot virus (ORSV) [111].

The most recent example in this case is the development of a single-step, label-free QCM-based HepBV biosensor by Giamblanco et al. [105]. The HepBV genome consists of a double stranded DNA. Giamblanco et al. [105] fabricated a device for detecting HepBV DNA by immobilizing thiolated single stranded DNA (ssDNA) oligonucleotide probes on the surface of QCM gold electrode. The detection of HepBV genome was achieved via hybridization between ssDNA probe and viral DNA. This DNA-QCM biosensor was capable of detecting fmol/cm<sup>2</sup> of HepBV virus with ssDNA probe

density of  $\sim 4 \times 10^{12}$  molecules per  $\text{cm}^2$  without using any amplification or labeling technique. The ssDNA probe density determines the sensitivity and selectivity of the fabricated QCM-DNA biosensor, because the diffusion of target HepBV genome to the hybridization site, its conformation, and spacing between the ssDNA probes are dependent on probe density [119,120]. Thus, the receptor surfaces with lower DNA probe density may produce a strongly selective and irreversible adsorption and vice versa.

Earlier, Skladal et al. [117] fabricated a QCM-DNA biosensor by immobilizing biotinylated-DNA (via interaction with avidin or streptavidin) on QCM surface for detecting HepCV in serum. The immobilization of DNA probe was achieved by modifying gold electrode with cysteamine and subsequent activation with glutaraldehyde followed by anchoring streptavidin or avidin. They found out that immobilization efficiency of avidin was significantly higher as compared to streptavidin, and the biotinylated-DNA-avidin based QCM was regenerated 30 times without any loss of binding capacity. Figure 7 summarizes the mechanism for the detection of viruses using DNA-QCM biosensors.



**Figure 7.** A schematic representation of the DNA-QCM virus sensor. DNA probe is attached to the QCM gold electrodes through an immobilizer that often uses thiol linkages, avidin-biotin, or streptavidin-biotin complexation. DNA probe then selectively binds to the target.

Kleo et al. [108] fabricated a DNA-QCM biosensor for indirect determination of vaccinia virus DNA using polymerase chain reaction (PCR) amplification. The biotinylated-capture-DNA was immobilized via affinity binding to NeutrAvidin, while NeutrAvidin was anchored to the QCM electrode by thiol moieties. The synthetic vaccinia virus complementary ssDNA generated by PCR technique was hybridized with recognition sites of capture-DNA probe. The change in frequency of QCM triggered by hybridization of ssDNA complementary strand onto the ssDNA capture strand was monitored. The DNA-QCM biosensor signal was significantly enhanced by denaturation of PCR amplified target DNA due to improved hybridization efficiency. The sensor response and specificity of sensor were further increased by using a gold nanoparticles tagged enhancer sequence. In this method, the analysis time was reduced to 15 min in comparison to classical techniques.

Dell'Atti et al. [110] also coupled the DNA-QCM multi-sensor (based on three biosensors) with PCR amplification for the simultaneous detection HPV genotypes in human samples. They detected sixteen strains of the high-risk HPV by immobilizing a degenerate DNA probe on QCM electrodes. The degenerate DNA probe was selected in a conserved region of the viral genome for detecting different viral strains. The DNA-QCM multi-sensor by immobilizing degenerate HPV, HPV 16 and HPV 18 biotinylated-DNA probes for simultaneously detecting and genotyping various HPV strains. The sensor exhibited excellent sensitivity and specificity with a detection limit of 30 nM.



### 3.3.2. Aptamers

Aptamers are pieces of single stranded nucleic acids (DNA or RNA) typically 20–90 nucleotides in length [121]. They are particularly attractive for sensor applications since they are relatively easily to produce with selectivity for a wide variety of analytes. These range from ions [122,123] or small molecules [124] to a wide range of proteins [125] or even entire cells [126,127]. As a result, aptamers can be used for detecting viruses directly as well as molecules produced by the affected host in response to the viral infection (as for instance antibodies or certain molecules produced on the host cell surface [128]). Thus, they are potentially useful sensing molecules for both late and early stages of a viral disease. Aptamers have already been produced for a wide variety of viruses. A few prominent examples are HIV [129,130], HepBV [131], EBOV [132], severe acute respiratory syndrome (SARS) [133,134], norovirus [135], rabies virus [136], vaccinia virus [137,138], dengue virus [128], and influenza viruses [139,140].

Aptamers are produced in a process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) in vitro [141,142]. A schematic representation of the process is shown in Figure 8. The SELEX process for production of aptamers offers the advantage over natural antibodies that the time required for aptamers' selection is only a few weeks rather than a few months needed to produce monoclonal antibodies [121]. Aptamers are also much more stable than antibodies making them suitable in applications requiring harsh conditions, (e.g., high temperature or extreme pH) [143]. General review articles about aptamer-based sensors [144] and aptamer-based sensors for viruses are available in literature [145–147].

Combining aptamers with QCMs as transducers is useful: for instance, an advantage that is specific for QCM or mass-sensitive detection systems, but is also positive for other transduction applications, is that aptamers are relatively small. Thus, there are no problems with swelling of the selective layer after the aptamers are attached to the surface of a QCM device. Despite these advantages, the QCM aptasensors are not widely studied and reported yet for viruses. However, there are some promising results, mainly for sensing AIV H5N1 and HIV-1, which indicate the potential of aptamer-QCM viral diagnostic systems. A few such examples are discussed below.

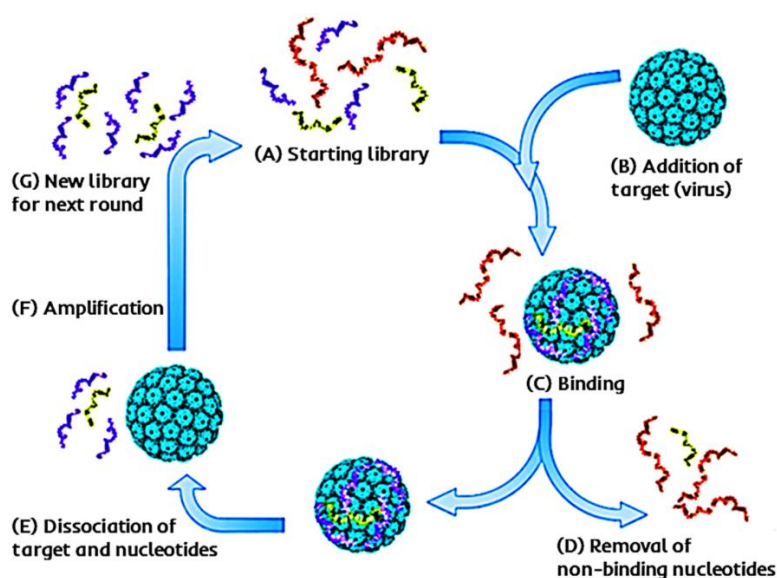
To utilize QCM as a transducer, the aptamer has to be attached to the electrode (usually gold) surface of the QCM. Before coating, it is common to clean the QCM surface with a (1:1:5) solution of H<sub>2</sub>O<sub>2</sub>, NH<sub>3</sub> and distilled water. Due to the high affinity between gold and sulfur, attaching a thiol is generally a method of choice for immobilization of aptamers. Then, streptavidin-biotin binding is exploited to attach and immobilize the aptamer (ala DNA, as discussed earlier). Such an approach was established by Tombelli and coworkers [116,148,149] for the detection of HIV-1 Tat (trans-activator of transcription) protein.

They first attached 11-mercaptopundecanol and carboxylated dextran to the gold surface. Then the surface of the crystal was activated with *N*-hydroxysuccinimide (50 mM) and 1-ethyl-3-(dimethylaminopropyl)carbodiimide (200 mM) in water. After 5 min, the activating solution was replaced by a streptavidin solution in acetate buffer. After blocking the remaining active sites with ethanolamine hydrochloride, the biotinylated aptamer solution was added to immobilize the aptamers. The QCM aptasensor exhibited excellent specificity with a detection limit of 0.25 ppm for HIV-1 Tat protein [116]. Furthermore, the authors directly compared QCM aptasensor and monoclonal anti-Tat antibody based immunosensor for HIV-1 Tat protein and received almost identical results (same sensitivity, slightly better dynamic range for antibodies, same regeneration procedure) [116].

A slightly different approach was used by Wang et al. [112] for label-free detection of AIV H5N1 in which they increased the active surface area by nanostructuring. By doing so they successfully achieved improved aptamer attachment and thus, sensitivity. Instead of using streptavidin-biotin complexation, they bound the aptamer directly to a thiol monolayer via an NHS linker. The resulting QCM-aptasensor demonstrated  $2^{-4}$  HAU [97] per 50  $\mu$ L detection limit for AIV H5N1, and did not exhibit noticeable interference with non-target AIV sub-types H1N1, H2N2, H7N2 and H5N3.

Brockman et al. [113] established a method to amplify the signal generated by a QCM-aptasensor for AIV H5N1. They first immobilized streptavidin directly to the QCM surface and then bound biotinylated aptamers to it to detect the viruses. Finally, the QCM-aptasensor signal was amplified by adding aptamer coated magnetic nanobeads. The nanobead amplification of the sensor signal was effective at low AIV H5N1 concentrations.

Recently, a slightly more complex approach was developed and utilized by some scientists [114,115], who implemented the aptamers for AIV detection into a hydrogel. The hydrogel approach has the inherent advantages: for example, the hydrogel can enhance the measuring effect; the aptamer is more protected against degradation inside a hydrogel; and some other functionalities can also be implemented in the hydrogel. Wang et al. [115] demonstrated that the developed hydrogel-based QCM aptasensor was capable of detecting AIV H5N1, and the device achieved high sensitivity with the detection limit of 0.0128 HAU [97]. Thus, it is confirmed that highly specific and label-free QCM aptasensors have great potential for fast and selective recognition of different viruses, e.g., AIV H5N1 and HIV.



**Figure 8.** A schematic representation of the SELEX process for producing aptamers: (A) The process starts with a library containing nucleotides with varying length and sequences; (B) The target molecule (in this example a virus particle) is added and favorable conditions are provided for binding. (C) Once, the binding between the virus particles and the nucleotides is achieved; (D) the non-binding nucleotides are removed; (E) The bound nucleotides are subsequently separated from the virus particles, and (F) amplified; (G) Finally, the product is used as a new library for another cycle. The cycle is repeated (typically 5–20 times) until only strongly binding nucleotides are present.

#### 4. Summary and Outlook

This article presents a review of the gravimetric viral diagnostic systems consisting of a selective layer or receptor that captures viruses and a QCM transducer that translates viral binding events into legible sensor signals. This work provides a comparative study of the assembly and performance of different types of receptors such as synthetic antibodies, natural antibodies, DNA probes, and aptamers. In the past 15–20 years, a number of reports have been published on combining these receptors with QCM to develop rapid, low-cost, reliable, sensitive, and specific biosensors for label-free recognition of viruses, or viral DNA and surface proteins. In summary, we report the competing advantages and drawbacks of various receptors based on their nature, immobilization and fabrication strategies, cost-effectiveness, stability, and sensing performance.

Firstly, gravimetric or mass-sensitive biosensors based on QCM are one of the very few devices that can virtually detect and report anything that has mass ranging from small molecules to microorganisms. A QCM is particularly useful for sensing viruses, which are neither fluorescent nor electrically conducting and thus are difficult to detect by optical or electrochemical methods. QCM is also highly sensitive to very small changes in surface mass, i.e., in the range of  $\text{ng}/\text{cm}^2$  [37]. Moreover, QCM can be combined with synthetic or naturally occurring receptors easily for selective and specific recognition of any target, e.g., viruses in this case. Table 4 provides a qualitative comparison of the most commonly used virus recognition technologies with QCM based gravimetric viral diagnostics.

Apart from many advantages of QCM devices as a general gravimetric transducer, it suffers from certain inherent problems. For example, in order to enhance sensitivity, fundamental frequency of QCM has to be increased and correspondingly the thickness of quartz sheet has to be reduced. Decreasing thickness of quartz sheet would make QCM device more fragile and mechanically unstable thus, limiting its practical use when extremely high sensitivity is desired in chemical sensing. The viscosity of the in-contact medium also has strong influence on QCM damping as highly viscous analyte solution would lead to increase frequency fluctuations that make measurements difficult at trace levels. Furthermore, the integration of QCM with micro/nano electromechanical systems is still in its infancy due to material constraints and other issues. In future, some of these problems need to be addressed to achieve the true potential of QCM-based gravimetric virus sensors.

**Table 4.** A comparison of different virus recognition techniques with QCM based gravimetric sensors.

Technique	Detection Principle	Time	Cost	Remarks
Viral plaque assay	Measuring virus infective particles	Lengthy	Inexpensive	Conventional, Simple, Poor reproducibility
Hemagglutination assay	Virus protein assay	Lengthy	Inexpensive	Conventional, Simple, Poor reproducibility
Immunofluorescence assay	Virus protein assay	Moderately fast	Expensive	Modern, Sensitive, Poor reproducibility
ELISA	Virus Protein Binding with Enzymes	Rapid	Inexpensive	Modern, Highly Sensitive, Good reproducibility
PCR	Nucleic Acid Amplification	Rapid	Inexpensive	Modern, Highly Sensitive, Excellent reproducibility
Virus Flow Cytometry	Biophysical method of counting virus particles	Rapid	Expensive	Modern, Highly Sensitive, Excellent reproducibility
QCM gravimetric Sensor	Selective binding of virus particles or proteins	Fast	Inexpensive	Simple and reversible, Good reproducibility, Highly sensitive and selective

Secondly, positive and negative aspects of different receptors are briefly discussed. Synthetic antibodies based on MIPs can be fabricated easily using different approaches such as soft-lithography and epitope imprinting. The primary advantage of soft-lithographic surface imprinting procedure is to transmit complete geometrical and surface chemical characteristics into MIPs that leads to high specificity. On the other hand, typical challenge in this technique is to produce a good number of recognition centers for viruses that can lead to readable sensor signal. Another limitation of soft-lithography is the difficulty to imprint smaller biomacromolecules such as viral DNA or surface proteins, which can undergo conformational changes.

Conversely, the epitope approach can be used to imprint epitopes of viral surface proteins to detect different viruses. The difficulty in epitope imprinting however originates from the choice of specific fragments that can be imprinted and are capable of selectively capturing the target [150]. In addition, certain reports suggest that whole-protein imprinting is more efficient as compared to epitope imprinting [151]. Synthetic antibody replica designed via dual imprinting process present an exceptional case of selectivity and sensitivity for viruses and small molecules, but they have not

gained popular backing for their application to other viruses or bioanalytes. Thus, it is difficult to conclude about its future based on few examples.

Natural antibodies (NAb) are proteins with inherent sensitivity and specific binding capacity towards antigens, i.e., viruses. They are extremely selective and possess high binding affinity for target viruses. However, they have limited life span and cannot be used in harsh environments fearing their decomposition. For instance, the fragile structure of NAb limits their applications in real-life samples such as serum due to the presence of enzymes that may damage their structure [152]. Furthermore, it is believed that their protein character may lead to their deactivation during repeated binding-and-regeneration cycles [153,154].

When compared to fully synthetic antibodies (MIPs), DNA and aptamers are also less stable. DNA and aptamers are biomolecules, which consist of nucleic acids. They can be degraded by microorganisms. Also, the degradation by RNases and DNases can occur. RNases and DNases are problematic since they are an important part of the immune systems' defense against viruses in numerous organisms and thus are almost omnipresent. As a result, special care has to be taken to make them RNase/DNase free. Currently there are two strategies which have been developed to circumvent this problem. The first approach is to synthesize a "mirror" analog of the aptamer that retains the original properties, but is not cleaved by nucleases [155]. The second approach is to include local modifications of the ribose 2' sites in the aptamer chain which prevents cleavage by the enzymes [156,157].

3D structure of aptamers for instance in comparison with other NAb depends more on the surrounding medium. As a consequence, the sensing parameters and the quality of the sensors varies more when different solutions are used for sensing. Additionally, the high affinity of the NAb, DNA, and aptamers with their target can be challenging. Although high binding affinities are usually desired, it may lead to irreversible sensor signal preventing the receptor layer to be regenerated. Generally, longer regeneration times or harsher conditions are needed in that case than e.g., for MIP, which usually have a lower affinity for their target. Specifically, the aptamers are composed only of 4 different nucleic acids, while proteins have 20 amino acid building blocks and thousands of monomers are available commercially [158]. As a result there is less chemical flexibility, when using aptamers [159].

Finally, we believe that MIPs, NAb, DNA as well as aptamers are of great value for viral recognition systems due to their excellent sensitivity and selectivity towards a variety of disease-causing viruses. However, the NAb, DNA, and aptamers are worthy of further investigations to enhance their long-term stability, to develop new immobilization and fabrication technologies with the objective of lowering cost and effort, to optimize their concentration, orientation, binding efficiency for reversible binding, and to advance their easy regeneration preventing any functionality damages and-or degradation.

Synthetic antibodies based on MIPs, on the other hand, fairly deserve a more concentrated research effort to make them a viable alternative of natural receptors, and more so to commercialize MIP-QCM based gravimetric viral diagnostic systems because the MIPs are relatively more stable, selective and show comparable sensitivity, and are easy to fabricate and regenerate thus lowering their overall cost and analysis time. In future, these approaches may lead to commercial availability of cheaper and more reliable viral diagnostics.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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