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Emerging technologies for the detection of viral infections

Peter D. Burbelo¹, Michael J. Iadarola², Adrija Chaturvedi¹

¹Dental Clinical Research Core, National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD, USA

²Department of Perioperative Medicine, Clinical Center, National Institutes of Health, Bethesda, MD, USA

Abstract

Viruses represent one of the major environmental agents that cause human illness and disease. However, the ability to diagnose viral infections is limited by detection capability and scope. Here we describe several emerging technologies that provide rapid and/or high-quality viral diagnostic information. Two technologies, novel CRISPR-based diagnostics and a portable DNA sequencing instrument, are uniquely suited to increase the number of viral agents analyzed, even in point of care settings. We also discuss a phage-based method for generating comprehensive viral profiles of previous exposure/infection and a fluid-phase immunoassay that yields highly quantitative viral antibody analyses. Future applications of these approaches will accelerate on-site clinical diagnosis of viral infections and provide insights into the role viruses play in complex diseases.

Keywords

viral diagnosis; CRISPR; high-throughput DNA sequencing; nanopore sequencing; Luciferase Immunoprecipitation Systems; VirScan

Introduction

Human viral infections cause many acute illnesses and chronic diseases with varying effects, ranging from no obvious symptoms to acute, debilitating infections, chronic conditions and even lethal infections [1]. The symptoms of viral infections are diverse, including fever, diarrhea, encephalitis, immunodeficiency, tissue damage, and organ failure. In many cases, the clinical features of viral infection overlap other human diseases, such as bacterial infections. Obtaining a rapid, accurate differential diagnosis of the cause of a human illness is a critical component of diagnostic medicine, particularly in the case of viral infections, to improve the delivery of human healthcare. The range of human illnesses and disease associated with viral infection is vast, in part due to the differential ability of viruses to infect selected tissues and organ systems. Notable examples of human viruses that cause significant health problems include human immunodeficiency virus (HIV) causing severe immunodeficiency, and hepatitis B virus (HBV) and hepatitis C virus (HCV), causing liver damage. Other viruses targeting the digestive tract (rotavirus, astroviruses), nervous system (Zika virus; ZKV and West Nile virus; WNV), and lung (Influenza, respiratory syncytial virus; RSV) are associated with gastroenteritis, neurological and pulmonary symptoms, respectively. In addition, viruses such as human T-cell lymphotropic virus (HTLV-I), human

papilloma virus (HPV), Merkel virus and other viruses are associated with different types of cancer [2]. Due to wide spread role of viral infections in human illness and disease, improved methodologies for rapid point of care detection and profiling of these infectious agents in clinical samples could accelerate and improve patient outcomes.

The full spectrum of known viruses that infect human host cells (i.e. the human virome) and their impact on health remains largely incomplete [3]. The total number of human viruses is unknown but likely comprises over several hundred different viruses. Cataloguing human viruses can be challenging, because while some viral agents only cause acute infection and then are completely cleared, other viruses produce chronic infections and, in some cases, remain dormant for long periods of time, showing reactivation on occasion. Despite the many clinically defined features associated with different viral infections, a major gap exists in determining whether viruses, which are not generally recognized to cause chronic illnesses, contribute to disease susceptibility or even show complex contributions to chronic diseases [4]. This is in part because all viral infections stimulate the immune system and thus may be drivers of systemic inflammation or influence immune homeostasis. Unanswered questions include whether the timing or specific sequence of viral infections has an impact on immune function and overall health, or whether the overall number of viral infections or selected agents correspond in some individuals with greater morbidity or susceptibility to certain chronic diseases. Addressing these complex questions has been difficult until recently, because there were only a limited number of diagnostic tools available to document the presence of a select viral agent.

In this review, we focus on several emerging technologies that represent new approaches for diagnosis of infectious viral agents (Table I). We discuss strategies based on detecting or sequencing viral nucleic acids with an important emphasis on technologies that can quickly provide diagnostic information of viral infections in point of care settings. In addition, we describe two technologies that measure host antibody responses, which are not only useful for diagnostic information, but provide additional insights into the role viruses play in infection-associated and other complex diseases.

CRISPR-based diagnostics for point of care viral nucleic acid detection

Numerous methods for detecting viral infections have been developed for clinical diagnostic purposes, but most of these tests require lengthy molecular amplification strategies and significant instrumentation for detection and readout [5]. At present there is a lack of robust tests for rapid diagnosis of most viral infections. However, multiple new technologies are being explored in point of care settings, including for acute infections (Epstein Barr virus, EBV), global outbreaks (Ebola virus; EBOV, Dengue virus; DNV and ZKV), and emerging pandemic viruses (Middle East respiratory syndrome virus; MERS and H5N1 influenza). One emerging technology that has the potential to significantly simplify nucleic-acid based viral detection involves diagnostic tests built on the gene editing CRISPR technology [6]. Extraordinarily high analytical sensitivity is achieved by employing CRISPR effector enzymes, such as Cas9, Cas13a or Cas13B, along with single guide RNA (called sgRNA) which specifically recognizes the target viral nucleic acid of interest. In a 2016 study, the specific endonuclease activity of Cas9 was used to detect pathogenic viruses such as ZKV

and DNV with femtomolar detection sensitivity, including distinguishing between the American and African version of ZKV [7]. This approach relied on gene circuits embedded in paper. Cas9-mediated enzymatic activation by specific detection of ZKV and DNV nucleic acid sequences turned on a gene-protein expression cascade producing a colorimetric readout, but still requiring an electronic device for measurement.

CRISPR-based diagnostics underwent rapid improvement from the discovery that certain CRISPR enzymes such as Cas13a show high levels of promiscuous RNAase activity upon recognition of their molecular target [8]. In a new technology called SHERLOCK, this CRISPR RNAase activity is exploited for signal amplification and nucleic acid detection [9]. Isothermal amplification of DNA and RNA present in the samples is first accomplished using recombinase polymerase amplification (RPA) and reverse transcriptase (RT) to increase the amount of potential viral RNA target. The Cas13a enzyme with specific sgRNA for the virus of interest is then added. If the viral RNA of interest is present, the Cas13a binds to the viral RNA and is further activated to collaterally cleave a fluorescently labeled reporter, producing a light signal which can be measured by a fluorescent plate reader. These steps provide a definitive detection of the presence of the RNA in question. In only a few hours, SHERLOCK detected ZKV RNA in clinical urine and serum samples with 20 femtomolar sensitivity.

Beyond these findings, a newer version called SHERLOCKv2 incorporates further improvements, including increasing sensitivity to 2 attomolar, and reducing the speed of detection to 2 hours [10]. Importantly, SHERLOCKv2 was configured into a paper-based lateral flow assay, allowing detection of viral infection through simple visual inspection. One additional advance for making the technology field deployable has been the development of HUDSON, an easy and accelerated method to extract nucleic acid involving heat and chemical reduction for SHERLOCKv2 analysis [11]. This combination of HUDSON and SHERLOCKv2 techniques allowed DNV and ZKV to be detected directly in unprocessed bodily fluids such as whole blood, serum and saliva and urine in less than 2 hours in a field-deployable format.

Several advantages need to be highlighted about the CRISPR-based diagnostics. First, the ability of all the enzymes to work at low temperatures obviates the need for special equipment for nucleic acid amplification. Second, with the paper-based system, no special equipment is needed for detection, and the cost of the reagents per test is quite low. The ability to freeze-dry reagents on paper will allow the diagnostic tests to be deployed in remote locations. Lastly, the unique specificity of the CRISPR system allows for sensitive diagnostic tests to be developed in a modular and rapid fashion for any virus, allowing a large panel of infections to potentially be assessed simultaneously.

A portable, rapid DNA sequencing device for viral diagnostics

DNA sequencing has long been the centerpiece technology for virus characterization, as it provides foundational information for molecular diagnosis. Although advances in DNA sequencing technologies have allowed whole genomes to be sequenced within a few days, DNA sequencing has only recently been utilized for viral diagnosis in clinical settings [12].

In 2008, high-throughput sequencing was applied to diagnose an unknown viral agent in clinical samples for the first time [13]. In this seminal study, three patients who had received transplant tissue derived from a single donor died of febrile illness 4 to 6 weeks later. Multiple diagnostic methods failed to identify the causative agent of their fatal illness, but unbiased high-throughput sequencing of RNA isolated from liver and kidney tissue identified a novel arenavirus. One year later, unbiased sequencing was also used to discover a fatal human disease outbreak caused by Lujo virus, another novel arenavirus [14]. Despite these promising findings, the sequencing data acquired in these studies required substantial investment in time and dedicated sequencing instruments in a centralized laboratory. The newest breakthrough technology, Nanopore sequencing, is a third generation sequencing technology that is bringing DNA sequencing into the forefront for rapid viral diagnostics in point of care settings [15]. Nanopore sequencing technology is based on the translocation of DNA through a protein pore, generating electrical conductivity changes for individual base calls. The prototypical device, the MinION, is a remarkably small, portable DNA sequencing instrument that is essentially the size of a USB memory stick. The MinION provides sequence information in real time, with typical sequence runs accomplished in about an hour. As with other sequencing technologies, additional time is required for extracting the nucleic acid from the clinical sample and for performing the amplification reactions necessary to construct the amplified cDNA library.

In one study, the MinION was used for unbiased detection of different viral infections in human blood samples in a well-equipped laboratory facility [16]. The RNA extracted from the clinical blood samples was used to generate metagenomic cDNA libraries, allowing for amplification of multiple candidate viruses, and further processed for nanopore sequencing. Following MinION sequencing, a laptop with internet access was used for bioinformatics analysis via restricted nucleotide search algorithms and this analysis was performed in real time by continually scanning the assembled DNA sequence to identify different viruses. With < 6 hours overall turnaround time, several different viruses were detected in clinical blood samples, including EBOV in two patients with acute hemorrhagic fever, chikungunya virus in an asymptomatic blood donor and a patient with HCV infection [16]. The relatively short time required to detect the viruses highlights the potential use of the MinION for rapid clinical diagnostics. However, it is important to note that the blood samples used had moderate to high viral titers (10^5 to 10^8 copies per ml), and this unbiased approach will require further improvements to address the sensitivity needed for detection of samples harboring fewer viral particles.

The MinION was also utilized for diagnostic analysis of EBOV on site in patients from Guinea, West Africa [17]. In this study, instead of construction of an unbiased library, targeted EBOV-specific amplification was employed. Approximately one hundred and forty EBOV-infected subjects were analyzed, often in less than 24 hours. Although as little as 15–60 minutes was needed for the sequencing process itself, additional time was required for RNA extraction from the blood samples, the RT-PCR reactions, and bioinformatic analysis of the sequence data. In this outbreak, two genetically different EBOV genomes were identified, in which one lineage reflected the initial outbreak in Guinea, while the other EBOV lineage crossed the border from a different outbreak in Sierra Leone. In a further separate study, the MinION was also successfully deployed as an outbreak tool in a similar

way for studying EBOV in Liberia, Africa [18]. These findings highlight how rapid DNA sequencing, even in resource-limited settings, can be used to monitor the spread of viral infections across countries. To fully employ diagnostic DNA sequencing in resource poor settings, additional development of battery powered thermocyclers or isothermal approaches for nucleic acid amplification are needed to generate the prerequisite libraries. Based on the rapid progress in sequencing technology, it is likely that further improvements will make DNA sequencing an important component of future unbiased viral diagnostics.

LIPS profiling of viral antibodies

In addition to nucleic acid detection, antibody testing for viral infection remains a major diagnostic tool. ELISA and Western blotting have classically been used to measure antibody response, but several newer technologies have substantial advantages. One technology, Luciferase Immunoprecipitation Systems (LIPS), utilizes luciferase-tagged antigens in a highly quantitative immunoprecipitation assay to provide a robust tool for viral diagnosis, virus discovery, monitoring of anti-viral treatments and disease stratification of virus-associated infections [19]. In LIPS, mammalian expression vectors are used to express chimeric luciferase-viral fusion proteins. Different luciferases, including *Gaussia* luciferase, Nanoluciferase and *Renilla* luciferase, have all been used as reporters that provide high analytical sensitivity and low assay background values. Extracts containing the recombinant luciferase-tagged viral antigen are used without purification and are incubated with sera/plasma or other bodily fluids containing antibodies. The immune complexes containing anti-viral antibodies bound to the luciferase-tagged viral antigen are then immunoprecipitated by protein A/G beads and washed. The resulting luciferase activity of the sample is measured using a luminometer [19]. The amount of luciferase activity is proportional to the amount of antibody directed against the virus. By employing appropriate buffer blanks and other controls, the light output from luciferase activity is highly interpretable for diagnostic purposes, even when gold-standard verified samples for specific viral infections are not available [20].

Using LIPS, immunoreactivity has been assessed against whole or partial proteomes for viruses including HIV [21], HCV [22], and HTLV-I [23]. In many circumstances, only one specific viral protein is required to diagnose a viral infection, but in other situations, a few specific viral proteins are needed. For example, gG-1 [24], gG-2 [24] and the gE [25] viral proteins alone are sufficient in LIPS to diagnose with high sensitivity and specificity for herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2) and varicella zoster virus (VZV) infection, respectively. On the other hand, Kaposi's sarcoma herpesvirus (KSHV; also known as HHV-8) requires multiple viral proteins for high diagnostic sensitivity [26]. Because LIPS can detect conformational antibodies, it can measure immunoreactivity against viral proteins that are poorly or not at all useful in other assays, including solid phase ELISA, Western Blot and protein arrays.

Due to its dynamic range of antibody detection and ability to assess antibodies for multiple antigens against a single virus, LIPS has been useful to stratify subsets of subjects with different viral infections, including for EBV [27], KSHV [28], HIV [29], and HTLV-I [23]. Tracking antibody levels through LIPS in longitudinal samples can be used to monitor viral

treatment. In one HIV study, LIPS was able to detect the impact of anti-retroviral treatment (ART) based on the time treatment was initiated after infection [21]. Compared to long-term ART of chronic HIV infection, drug initiation in early HIV infection significantly reduced the production of antibodies by 43-fold in cerebral spinal fluid and by 7-fold in systemic blood. Through LIPS, it was found that early initiation of ART after infection had a markedly greater impact on selectivity, sparing the central nervous system from acquiring HIV antibodies; information that could not be found by molecular analysis of HIV. This finding highlights the important clinical findings that can be obtained by LIPS.

In addition to studying humoral responses against single viruses at one time, LIPS has provided new insight into whether viruses that are not generally recognized to cause long-term disease may influence complex interactions in chronic illness [30]. LIPS antibody profiles against eleven different viruses in healthy subjects and patients with three chronic diseases revealed not only differences in the prevalence of viral infections, but also in the altered levels of antibodies against certain viruses among the different diseases. Findings from HIV subjects, one of the groups studied, demonstrated a higher prevalence of viral infections for cytomegalavirus (CMV) and HSV-2 compared to uninfected control subjects, but also revealed altered antibody levels against several other viral agents including EBV and several enteroviruses. Determining whether these viruses or other viruses contribute to specific features of patient subsets or represent biomarkers of disease requires further study.

LIPS has also been employed to characterize and discover new viruses, because the detection of host antibodies provides evidence that a virus is a true infectious agent. In one study, LIPS was used to formally define the astrovirus, HMOAstV-C, as a prevalent new infectious agent in children and adults [20]. Remarkably, subsequent independent molecular DNA sequencing studies of viruses found in cerebral spinal fluid from patients with unexplained brain inflammation have shown that HMOAstV-C is the causative agent of some cases of viral encephalitis [31–33]. These findings shed light on the utility of having new tools to define the full spectrum of viral infections, and how this information can potentially impact the elucidation of previously unknown causes of human disease. In addition to human viruses, LIPS has been used to discover and characterize new animal viruses. In one study, LIPS identified a reservoir of HCV-like viruses (NPHV) in horses, which represented the first discovery and validation of HCV-like viruses outside of primates [34]. Other insightful studies have come from analysis of antibody responses against a novel parvovirus causing hepatitis in horses [35], and animal model of HCV-like virus in rats following experimental infection [36]. LIPS tests also have important applications for characterizing and diagnosing emerging viral agents including MERS, a potential lethal viral infection in humans, [37] and a new coronavirus of bat origin, HKU2, which causes a lethal infection in pigs [38]. As noted by the authors of this HKU2 study, once the new pandemic viral sequence was known, LIPS technology was rapidly able to provide a diagnostic test for studying this emerging viral pathogen [38].

These diverse findings highlight the value of LIPS for generating antibody profiles for personalized diagnosis and potentially monitoring human health. A streamlined version of LIPS, called LIPSTICKS, has recently been developed to detect HIV infection in humans and NPHV infections in horses in less than one minute per sample [39]. Future advances

with LIPSTICKS and the development of LIPS arrays against panels of viral proteins [40] for antibody profiling are likely to further accelerate insight into the role viruses play in human and animal diseases.

VirScan serological profiling of the human virome

The human virome, or the full spectrum of viruses that infect humans, is still not completely known. However, many different human viruses, along with their corresponding DNA and protein sequences, have already been characterized. Despite this available information, current antibody-based methods of detecting viral infections typically involve testing a few viral proteins from a limited number of viruses at a time. One new technology for simultaneously profiling hundreds of human viruses involves a phage immunoprecipitation sequencing approach called VirScan [41]. This method is based on a T7 bacteriophage system, in which relatively short polypeptide sequences are displayed on bacteriophage surfaces and captured by immobilized antibodies (Fig. 1). For VirScan, 206 different viruses known to infect human cells were first bioinformatically analyzed and used to generate a reference viral protein sequence data set. Using a programmable, DNA microsynthetic platform, 93,904 200-mer oligonucleotides were synthesized, encoding 56 amino acid peptides that encompassed every protein sequence of all 206 reference viruses. This large pool of oligonucleotides was then subcloned into a phage T7 vector, allowing this massive library of viral peptides to be expressed individually on the surface of phage particles. To analyze serologic responses, the viral phage library is used in an immunoprecipitation format with human serum samples. Anti-viral antibodies present in serum samples are incubated with phage expressing the viral peptides, allowing the antibody-antigen immune complexes to be captured by immobilized protein A/G magnetic beads. Following stringent washing, the corresponding bacteriophages are eluted and analyzed directly by high-throughput DNA sequencing. Subsequent bioinformatics analysis is then used to match and assemble the sequences for each virus and ultimately elucidate the viruses present in a given individual. This technique provides the advantage of possibly identifying humoral responses to hundreds of different viruses at one time to determine the full range of viruses that have infected a given individual.

In one application, VirScan showed high diagnostic sensitivity and specificity for identifying HCV and HIV infected individuals [41]. This is not unexpected, as both viruses are known to induce robust humoral responses. However, VirScan also revealed that individuals with untreated HIV infection showed a higher number of enriched HIV peptides than subjects with treated HIV infection, demonstrating additional information regarding the immunoreactive peptides. Other insights from VirScan came from cataloguing and comparing the seroprevalence of multiple viruses present in different human populations. Comparison of uninfected individuals to HIV-infected subjects revealed a much higher seroprevalence of antibodies in HIV patients against KSHV, HSV-2, CMV and other viruses, consistent with previous studies [42, 43].

VirScan serologic analysis of children compared to adults demonstrated that adults had higher seroprevalence of viral antibodies for EBV, Influenza A and Influenza B, HSV-1 and HSV-2, while children had higher seroprevalence for Rhinovirus-A [38]. The lower

seroprevalence of Rhinovirus-A in adults likely reflects the loss of B-cell responses over time in the absence of stimulation by these viral antigens. VirScan also provided evidence that the prevalence of different viruses was geographically different between Peru, South Africa, Thailand and the US. For example, almost 100% of individuals in Peru had HSV-1 infection, versus 85% in South Africa and only 50% in Thailand and the US. Similarly, antibodies against the related herpes virus, CMV were present in almost 100% of individuals in Peru and South Africa, but only 50% of individuals in the US.

Comparison of VirScan with existing technologies showed that it had high, but not perfect diagnostic sensitivity for detecting HIV1, HSV1 and HSV2-infected individuals [41]. VirScan showed limited sensitivity for detecting other viral infections. For example, it underestimated the prevalence of VZV infection (only 25% prevalence vs. the near 100% actual prevalence), which was likely due to the inability of the technique to detect antibodies directed against conformational epitopes of viral proteins. It is important to point out that there has only been one published report with VirScan, so additional studies are needed to independently validate the results and technology. Despite these issues, VirScan clearly has the potential to be a potent tool for untangling the roles of viral infection in overall health and complex diseases. One of the most interesting aspects of VirScan technology is its ability to determine the number and types of viral infections found in a given person. This line of inquiry studying 569 human donors revealed quite heterogeneous rates of virus exposure and infection. On average, VirScan detected serum antibodies against ten different viruses per person [38]. Antibody responses were detected against 62 of the 206 species of virus in the library in at least five individuals, highlighting the complexity of viral infection observed in some individuals. Remarkably, two individuals showed antibodies against 84 species of virus in the library. It would be highly informative in future studies to use VirScan to study individuals with complex diseases to determine whether specific viral infections or the number of infectious agents may be associated with or are drivers of disease. This technology could even elucidate changes in humoral responses and viral infectivity over time by analyzing longitudinal biobanked serum samples, which can often span over twenty years. By cataloguing virus exposure over time in disease subsets, VirScan may yield novel insights into viral infection that may correlate with many chronic illnesses including cancer, neurodegenerative and autoimmune diseases, where the risk factors and pathoetiology are currently not known.

Conclusions

In this review, we describe applications of innovative viral diagnostic methods. One technological focus involves development of point of care viral diagnostics, including CRISPR-based diagnostics and a portable, deployable DNA sequencer. Although these technologies are still in early stages and require further validation and technical refinement, they appear particularly promising. CRISPR-based paper-strip tests with simple visual readout have the potential to provide low cost diagnostics and portable tools to simultaneously detect many different viral agents in clinical samples. These rapid viral diagnostic tests will likely have a major impact on public health and influence how we track and treat different viral outbreaks. One immediate benefit will be the ability to rapidly test for unknown causes of pulmonary, gastrointestinal and neurological symptoms, aiding in the

differentiation between viral and bacterial infections in small hospitals and clinics, thereby helping deliver the appropriate treatment. In other situations, the ability to use unbiased sequencing approaches, rather than relying on predesigned detection methods, will further increase the utility of personalized analysis of current viral infections and discovery aspects when the viral agent is unknown.

In addition to viral nucleic acid analysis, serological profiling also has important diagnostic applications. The quantitative analysis of viral antibodies by LIPS and comprehensive detection of viruses by VirScan are likely to yield many new insights into the role human viruses play in disease. The development of additional serological rapid formats is likely to complement the diagnosis of viral agents by molecular methods. The finding that certain anti-viral antibodies are associated with future onset of a human disease is particularly intriguing. For example, human papilloma virus antibodies against the E6 and E7 oncoproteins are only found in patients harboring HPV-associated cancers and are not found in subjects with HPV-infection alone without malignancy or healthy controls [44]. Interestingly, HPV E6 antibodies have been detected ten years before future onset of head and neck cancer [45], highlighting their potential important role as a predictive biomarker for early cancer screening. By extension, additional viral antibody responses will likely be found for other chronic diseases.

Besides the key applications for viral diagnosis, another area these technologies will be applied to involves combining them with other existing genomic technologies, including whole genome sequencing. For example, both the large-scale analysis of viruses found in a given individual by VirScan or unbiased sequencing, along with the corresponding catalogue of gene variants by whole exon sequencing in a given individual, may yield important information about gene-virus infection susceptibility patterns. It is already known that certain viral infections, including severe influenza and HSV-1-associated encephalitis infection, are linked to unique gene variants in susceptible individuals [46]. Any unusual infection patterns discovered might be further explored in detail with the LIPS technology, in which dynamic antibody levels might provide additional insight into disease evolution and patient stratification. Overall, the ability of these and other emerging technologies to potentially catalogue viral infections in individuals over time are likely to reveal disease drivers and new prognostic biomarkers for many diseases.

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Executive Summary

- Until recently, only a limited number of viral diagnostic tools were generally available, leading to major gaps in clinical tests and knowledge regarding viral contributions to acute and complex disease.
- One emerging diagnostic tool, CRISPR effector enzymes in combination with virus-specific guide RNAs can be used to detect viral nucleic acids with high sensitivity. One promising format, SHERLOCKv2 configures these elements onto a paper-based lateral flow assay, allowing the possibility for visual diagnosis of a wide range of pathogenic viral agents in clinical samples in point of care settings.
- A new portable DNA sequencer is providing rapid information for diagnosis and monitoring viral infections. Future advances in sequencing technology may ultimately allow for the unbiased detection and diagnosis of viral agents, including in field deployable situations.
- Technological advances in antibody-based profiling of viral agents including by VirScan and LIPS are likely to continue to provide tools for viral diagnosis, monitoring and deciphering their role in human disease.
- Future applications of some of these technologies with existing personalized genomic technologies may shed important information about gene-virus susceptibility patterns.

Fig. 1. VirScan Overview.

a.) A comprehensive viral library of T7 phages that individually encode and express viral peptides on their surface are incubated with serum samples containing anti-viral antibodies. The immune complexes containing phage viral peptides on T7 phage are bound by serum antibodies and captured on immobilized protein AG beads, followed by unbound phage being removed through stringent washing **c.)** Amplification and high-throughput sequencing of insert DNA of the antibody-bound T7 phage reveals the DNA sequences of the bound viral peptides. Bioinformatic analysis is then used to assemble the identity of the viruses corresponding to the captured phage, thereby providing a comprehensive viral profile for any given individual.

Table I.

Emerging Viral Diagnostic Technologies

Technology	Findings
CRISPER-Based Viral Diagnostics	SHERLOCKv2 can detect viruses such as DNY and ZKV with 2 attomolar sensitivity in bodily fluids using a paper-based, field deployable format [10]. Highly adaptable for other agents by employing virus-specific guide KNAs.
MinION, a Portal DNA sequencer	The MinION unbiasedly detected viruses including EBOV, chikungunya virus, and HCV in less than 6 hours in clinical blood samples [16], The MinION along with EBOV-specific amplification was used in Africa to diagnose and track outbreaks [17,18].
LIPS	LIPS, using luciferase-tagged viral antigens in an immunoprecipitation format is a powerful method for antibody-based viral diagnosis, treatment monitoring and stratification of patients with viral-associated diseases [19].
VirScan	VirScan, a comprehensive phage based serological test can catalogue the presence of hundreds of viruses in different individuals [41].