Screening for Viral Infections

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Introduction

Viral diagnostics are increasingly significant for public health and clinical medicine. Recognition of an infectious disease as viral was once important only as an insight that allowed practitioners to exclude the need for antibiotics. Tools for virus diagnosis, surveillance and discovery have become more urgent with the expansion of the inventory of effective and specific antiviral drugs, and of the emergence of new zoonotic agents that require assays to facilitate containment and to direct investments in vaccines. An additional incentive is the evidence to support new models of diseases wherein viral infections result in autoimmunity, neuro-developmental damage, or neoplasia, that manifest months to years after the infection has resolved.

In this article we will review the status of histopathology, culture, and molecular and serological assays in clinical and environmental microbiology.

Pathology

Before the advent of high throughput genetic methods for detection and characterization of viral sequences the majority of viruses were detected using electron microscopy. Prominent examples of viral discovery enabled by electron microscopy include tobacco mosaic virus, ebolavirus, variola major virus, norovirus, polyomavirus, and SARS coronavirus (Kausche et al., 1939; Breman et al., 2016; Van Rooyen and Illingworth, 1944; Long et al., 1970; Kapikian et al., 1972; Haycox et al., 1999; Nicholls et al., 2003). Electron microscopy continues to be an important tool in virology where it is increasingly employed for confirmation of a molecular finding rather than as the first step in investigating outbreaks of infectious disease. Readers may wish to see Goldsmith and Miller for an excellent review of the history and use of electron microscopy in virology (Goldsmith and Miller, 2009). Histopathology and immunohistochemistry, too, have shifted from primary roles in viral identification to applications for viral biology and pathogenesis. In the earliest applications of polymerase chain reaction (PCR) in viral discovery, primer selection was guided by findings in analyses of clinical materials using panels of antisera to a wide range of viruses or hyperimmune sera from patients with specific diseases. In our own work with Sherif Zaki for example, immunological cross reactivity facilitated the discovery of West Nile virus NY99 (Briese et al., 1999). The finding of a viral nucleic acid sequence in an extract from an individual with disease is insufficient to demonstrate a causal relationship. An argument for a causal relationship is strengthened if footprints of the agent in an affected organ can be demonstrated through immunohistochemistry or in situ hybridization for viral proteins or nucleic acids, respectively (Lipkin, 2010).

Culture

The focus of clinical diagnostic virology has shifted from culture to molecular methods. This largely reflects the introduction of commercial assays that allow rapid, inexpensive detection of viral pathogens using multiplex PCR panels, and quantitative PCR assays that can be used to monitor the efficiency of interventions by tracking viral burden. Culture nonetheless remains critical to viral discovery, and basic and translational viral research. Virus amplification through culture enhances the efficiency of detection through molecular methods. SARS coronavirus, for example, was readily characterized using sequencing, microarrays decorated with oligo-nucleotide probes representing viral sequences, and consensus PCR. Proof-of-causation through fulfillment of Koch's Postulate requires propagation of an agent isolated from an individual with a disease, and recapitulation of the disease following introduction of the agent into a naïve host. Although alternative strategies for proving a causative relationship have been established, Koch's Postulate remains the most persuasive. Virus isolation is also essential for understanding how it enters a cell, replicates itself, evades innate immune responses, and causes disease in animal models. Finally, virus isolation is imperative for developing and testing the efficacy of antiviral drugs and vaccines.

Viruses can be isolated and propagated in cell culture systems or in live animals. The choice of an in vitro versus an in vivo strategy can have a substantive impact even for closely related viruses. Whereas inoculation of suckling mice is useful in detecting human enterovirus A, tissue culture is better for detection of human enterovirus B. Success depends on the presence of cell surface receptors that enable entry; intracellular factors required for trafficking of viral nucleic acids and proteins to appropriate sites for transcription, replication, translation, assembly and egress; and the ability of the virus to evade innate immune responses that inhibit viral replication. Accordingly, many clinical microbiology laboratories maintain multiple continuous cell lines for virus isolation. Some viruses cannot be propagated in continuous cell lines. Cytomegaloviruses, for example, require immature cells and are typically grown in primary fibroblast cultures. Other viruses require specific receptors that can be introduced through transfection, or more complex systems comprising multiple cell types that resemble organs (organotypic cultures). Still others are even more fastidious and require live animals such as suckling or transgenic mice. Culture in primary cells, transfected cells, organotypic cultures, and live animal systems is typically restricted to public health reference and research laboratories due to the need for substantial investment in resources and expertize.

Nucleic Acid Tests

Polymerase Chain Reaction Assays

The discovery of PCR by Mullis in 1983 transformed clinical microbiology and public health (Saiki et al., 1985) by enabling rapid, inexpensive, tools for microbial differential diagnosis, surveillance, and discovery, as well cDNA cloning. In viral diagnostics, PCR can be broadly divided into assays that target single agents versus those that target more than one agent (multiplex PCR assays), assays that are designed to detect only specific viral species versus those that also detect related viruses (consensus PCR assays), and assays that are quantitative (qPCR assays). The amplification products of single agent PCR can be visualized after size fractionation through gel electrophoresis using dyes like ethidium bromide or the newer and safer stains (e.g., SYBRsafe or GelGreen among others) that also intercalate with nucleic acids. Alternatively, products can be quantitated using complementary oligonucleotide probes that contain both a fluorescent reporter dye and quenching molecule. In Taqman assays, probe bound to the template is degraded by the 5′ to 3′ exonuclease activity of the polymerase, thereby freeing the reporter molecules for detection. In molecular beacon assays, the quencher and reporter molecule are in close proximity to one another in a hairpin probe structure and become separated when PCR results in a product that is complementary to the hairpin loop sequence between them. Through use of a standard curve and known concentrations of template one can measure the amount of target in an experimental sample. Taqman and molecular beacon assays are exquisitely sensitive and typically can detect as few as 10 target molecules per reaction.

Multiplex PCR

The majority of multiplex PCR assays are based on the Taqman system; however, gel based, mass spectrometric, and Luminex-bead based assays have also been established. At the time of writing, only five fluorescence reporter dyes can be unequivocally separated; thus, multiplex Taqman assays are limited to five targets (typically four viral and 1 host gene target control). The sensitivity is similar to single agent Taqman PCR. Two platforms combine PCR with mass spectroscopy to detect either the product itself or tags attached to the primers. The Ibis system, adopted by Abbott, uses matrix-assisted laser desorption–ionization (MALDI) MS to directly measure the molecular weights of PCR products in a sample and to compare them with a database of known or predicted product weights (Van Ert et al., 2004; Ecker et al., 2005; Sampath et al., 2007; Ecker et al., 2008). The Ibis system discriminates products of PCR amplification using universal bacterial 16S rRNA primers. There is no correlate universal viral primer set; thus, the platform is better suited to bacteriology than virology. MassTag PCR can accommodate up to 50 different primer sets. It uses atmospheric pressure chemical ionization (APCI) MS to detect small molecular weight reporter tags attached to PCR primers (Briese et al., 2005). Syndrome-specific MassTag PCR panels have been established for the detection of viruses, bacteria, fungi, and parasites associated with acute respiratory diseases, diarrheas, encephalitides/meningitides, and hemorrhagic fevers (Briese et al., 2005; Lamson et al., 2006; Palacios et al., 2006; Renwick et al., 2007; Dominguez et al., 2008; Tokarz et al., 2009; Palacios et al., 2009; Tokarz et al., 2010; Tokarz et al., 2011; Tokarz et al., 2012; Al-Samarrai et al., 2013; Hsu et al., 2013). Another highly multiplexed platform, established by Luminex Corporation uses flow cytometry to detect PCR amplification products bound to matching oligonucleotides on fluorescent beads (Brunstein and Thomas, 2006; Han et al., 2006; Li et al., 2007). Signal in multiplex PCR and Luminex assays requires the presence of three independent genetic targets (the forward primer, the reverse primer, and the probe). This provides high confidence in the fidelity of a finding. In contrast, mass spectrometric assays require the presence of only two genetic targets. Accordingly, we use them as screening tools and follow-up presumptive findings with single agent PCR or Sanger sequencing.

DNA Microarrays

Microarrays are typically $750 \times 250 \times 1$ mm glass slides that have been modified to bind oligonucleotides or peptides for detection of host or microbial nucleic acids or antibodies, respectively. This section of the article will focus on nucleic acid detection. Arrays for serology will be discussed below. Arrays comprise hundreds to millions of probes and can be designed to detect virtually any DNA or RNA target sequence. By varying the length of probes one can differentiate between truly complementary or less closely related sequences. Whereas probes less than 25 nt are specific, those more than 60 nt in length tend to be more promiscuous. Two longer probe array platforms were established for viral surveillance and discovery: the GreeneChip and the Virochip (Wang et al., 2002; Wang et al., 2003; Palacios et al., 2007; Quan et al., 2007). Both employ random amplification strategies prior to hybridization. Because host and viral sequences are amplified with similar efficiencies, the sensitivity for viral detection in tissues is lower than for targeted PCR assays. Host nucleic acid can be reduced by enzymatic digestion (DNA) or ribosomal RNA (rRNA) subtraction; nonetheless, these platforms are most successful with acellular template sources, such as virus cell culture supernatant, serum, plasma, cerebrospinal fluid, or urine. Viral nucleic acid that is hybridized to oligonucleotides on arrays can also be eluted from microarrays to enrich for relevant template prior to sequencing (SARS) (Wang et al., 2003).

High Throughput Sequencing

The introduction in 2005 of automated pyrosequencing by 454 Corporation provided the first insights in the potential power of what has become known as 'Next Generation Sequencing' (NGS). Before the platform was replaced in 2013 by the more efficient Illumina system, pyrosequencing resulted in the discovery and implication of novel viruses in outbreaks of disease in humans,

domestic animals and wildlife including an astrovirus associated with fatal encephalitis in X-linked agammaglobulinemia, disseminated arenavirus infection after organ transplantation, a polyomavirus causing vasculitis with myositis and retinal blindness, LuJo and Bundigbuyo virus associated hemorrhagic fever, and a reovirus that decimated wild and domestic salmon worldwide (Palacios et al., 2010). Whereas 454 pyrosequencing required three days to yield 50,000 sequence reads, hundreds of millions of reads can now be obtained in less than 24 h. The pace of viral discovery has accelerated accordingly, and sequencers are moving into clinical microbiology and public health laboratories worldwide.

In the past several years, improvements in single molecule sequencing platforms have begun to address the potential drawback of the Illumina sequencing system concerning its limited read length (~350–700 bp). Recent sequencing technologies aim at fewer but longer reads, such as the single molecule real-time (SMRT) sequencing from PacBio (Eid et al., 2009), or the MinION from Oxford Nanopore (Lu et al., 2016). The latest Sequel sequencer by PacBio generates ~2 million reads in a mean length range of 15–50 kb (up to ~200 kb). However, it is quite a sizable instrument and takes ~30 h to run. In contrast is the MinION by Oxford Nanopore, a tiny device of ~100 g that is connected to a common USB port of a laptop computer. Data can be assessed in real time after a few minutes of run-time and downloaded once sufficient data for the application purpose are collected, commonly after 12–20 h. The device generates ~10 million reads ranging in length from approx. 10 kb to several hundred kb. The long-read platforms are currently used mostly in combination with short read data to generate the most accurate assemblies with regard to sequence duplications or repetitive elements (Wick et al., 2017; Giddins et al., 2018).

VirCapSeq-VERT

Host and viral nucleic acids compete as template in unbiased high throughput sequencing. In complex samples, such as tissue, blood, nasopharyngeal secretions, and feces, host sequences outnumber viral sequences by orders of magnitude. The relative sparsity of viral sequences in complex samples can lead to detection failure where the number of sequence reads is insufficient to include rarer templates. Thus, to enhance the probability for recovery of viral sequences, investigators typically obtain a minimum of 200 million reads per sample. This approach is resource intensive. It requires not only a substantial investment in sequencing but also in bioinformatic analysis. One strategy to focus and increase sensitivity for viral diagnosis and discovery has focused on enrichment of viral template through subtraction of host nucleic acid via nuclease digestion and depletion of rRNA. Another strategy is positive selection. VirCapSeq-VERT is a positive selection system designed to increase the sensitivity of NGS for detection of vertebrate viral sequences based on viral sequence capture (Briese et al., 2015). The platform comprises a library of biotinylated oligonucleotide probes that tile the coding sequences of all known vertebrate viruses with an inter-oligonucleotide distance of 100–150 nucleotides. cDNA libraries prepared from samples containing viral genetic material are hybridized with the oligonucleotides. The resulting viral nucleic acid/viral probe hybrids are collected using avidin coated magnetic beads and taken forward for sequencing. When compared with unbiased NGS, VirCapSeq-VERT enables a 100 to 1000-fold increase in sensitivity and improved depth of coverage. We find that 2.5 million reads with VirCapSeq-VERT provides similar sensitivity and genome coverage to that obtained with 200 million reads after subtraction (Williams et al., 2018). Bioinformatic complexity and computational resources are reduced accordingly.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPER) Platforms

Bacteria and archaebacteria have evolved a response to invading bacteriophages that entails specific degradation of viral nucleic acid. Infection results in the integration of DNA fragments of bacteriophages into the bacterial genome as clustered repeats known as CRISPR arrays. Genes encoding CRISPER-associated (Cas) proteins with endonuclease (and in some instances integrase) activity are located in close proximity to CRISPR arrays. Subsequent infection with a similar bacteriophage results in expression from CRISPER arrays and Cas genes of RNA transcripts that target and degrade viral nucleic acid. CRISPER arrays can be genetically engineered to represent virtually any sequence. Thus, when joined with the appropriate Cas enzyme the system can be used to degrade vertebrate viral sequences in addition to bacteriophage sequences. CRISPER Cas systems can also facilitate gene editing by including Cas proteins with both endonuclease and integrase activities. For diagnostic purposes CRISPER Cas systems have been employed in conjunction with isothermal amplification in lateral flow assays and in CMOS arrays for multiplex detection of viruses in environmental and clinical samples (Gootenberg et al., 2018; Freije et al., 2019).

Serology

Serology complements direct detection methods by providing a history of exposure to a viral agent. To date most serology assays have been performed using single agent enzyme-linked immunosorbent assays (ELISA), neutralization assays (NT), western blots (WB), immunofluoroscence assays (IFA), haemagglutination assay (HA), or hemagglutination inhibition assay (HAI).

Hemagglutination Inhibition Assays

Serum agglutination, perhaps the first routine diagnostic test, was introduced in the 1800s for diagnosis of thypoid fever (Widal, 1896). Later, agglutination of blood cells was introduced by Landsteiner to determine ABO blood groups (Landsteiner, 1901).

In virology, the hemagglutination (HA) assay is based on the capacity of some viruses responsible for prominent human and animal diseases to bind and cross-link red blood cell surface receptors. The resulting lattice formation deposits as a diffuse reddish layer on the surface of the reaction container. The presence of virus-specific antibodies (Abs) bind the virus and thus inhibit agglutination of red blood cells. Antibodies are quantitated by incubating fixed numbers of red blood cells and virus stock with dilutions of a test serum. Hemagglutination inhibition (HAI) assay are commonly used in typing and subtyping of influenza viruses (Hirst, 1942). Other agglutination assays include latex agglutination (LA) systems where microbeads are coated with Abs or viral antigens.

Plaque Reduction Neutralization (PRNT) Assays

Antibodies can be detected and quantitated based on their capacity to prevent viruses from binding to and infecting their target cells (Pedersen and Spackman, 2014). The readout is a reduction in the number of lytic plaques in a culture of susceptible cells inoculated with the virus of interest. In PRNT as in hemagglutination assays, the requirement for infectious virus poses challenges for many clinical microbiology laboratories, particularly with assays for highly pathogenic viruses. Accordingly, in some instances investigators used less pathogenic viruses such as vesicular stomatitis virus (VSV) where the VSV glycoprotein gene has been exchanged for the glycoprotein of a more pathogenic virus such as ebola (Lee et al., 2017). Because of its native Ag presentation and biological signal readout PRNT is the still the method of choice for measuring functional (protective) immunity. Since even minor variation in protein structure result in changed neutralization behavior, it is also superior to the other methods in distinguishing closely related viruses via cross-neutralization studies. The specificity of PRNT assays is frequently exploited in differentiating immunological reactivity to related viruses. For example, PRNT is considered the gold standard in the differential serodiagnosis of infection with flaviviruses including Zika and dengue viruses (Kuno, 2003; Musso and Gubler, 2016). Only recently, Serochip analyses enabled the characterization of specific peptides that include distinguishing epitopes that now can be transferred other platforms such a peptide ELISA or lateral flow format with the promise of specific identification (Mishra et al., 2018). PRNT assays can also be employed to identify virus isolates through use of panels of hyperimmune sera generated in experimentally infected animals, and to group viruses taxonomically (Casals, 1957; The Enteroviruses, 1957; Schmidt et al., 1961; Muir et al., 1998).

Immunofluorescent Assays (IFA), Enzyme-Linked Immunosorbent Assays (ELISA) and Immunoblot Assays

Each of these methods build on the specific binding between Ab and its matching Ag. In IFA, cultured cells are infected with virus, fixed on glass slides or in multi-well plates with acetone or formaldehyde, and incubated with the test serum, plasma or CSF. A reporter dye linked to a secondary Ab directed against the primary (test) Ab is then introduced prior to fluorescence microscopy. Secondary antibodies can also be conjugated to enzymes that yield products that can be visualized by light microscopy.

Enzyme-based dye formation is also the basis for the ELISA (Engvall and Perlmann, 1971; Van Weemen and Schuurs, 1971), the most common assays used for antibody detection. Amongst the various platforms, the ELISA is the most versatile and popular. This is in part because of the multitude of ways to present Ags or Abs and the comparatively high throughput in 96-well format. Another advantage is the ability to present targets in native form supporting the detection of a wide variety of epitopes. The ELISA is typically performed in 96-well plates wherein wells coated with a viral antigen are incubated with serially diluted patient body fluid, a reporter enzyme (usually peroxidase or alkaline phosphatase) directly conjugated to the serum immunoglobulins (direct ELISA) or a secondary antibody (indirect ELISA), and finally a dye system that generates upon enzymatic catalysis a soluble colored product that can be read spectrophotometrically. A common variation is the sandwich ELISA wherein the well-surface is first coated with Abs specific for the Ag (capture Ab) before the antigen is applied. Next, the detection Ab is applied, either in direct or in indirect format. The advantage of the sandwich ELISA is that it increases the sensitivity and specificity of the assay.

Western blot assays (also known and immunoblots) are typically performed including complex Ag preparations such as crude infected cell extracts that are size fractionated by electrophoresis in sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gels, transferred onto nitrocellulose or polyvinylidene difluoride membranes, and then incubated with sera. Immunoreactivity of antibodies in sera are detected using a species-specific secondary antibody that is linked to an enzyme that results in either a dye or chemiluminescence signal. Proteins subjected to SDS-PAGE are denatured. Thus, they are not designed to detect conformational epitopes that may be critical in viral neutralization.

Lateral Flow Assays (LFA)

These assays are designed for point of care serodiagnosis and field applications. In LFAs. the liquid sample (plasma, serum, urine or CSF) flows by capillary action through a membrane where the antigen or antibody in the sample binds to a dye-labeled cognate marker (antigen or antibody). The antigen-antibody-dye marker complex continues to migrate through the membrane until further migration is blocked by binding of the complex to an antibody to a component of the complex that is fixed in position, the appearance of a line of dye indicates that the antigen or antibody was present in the sample.

Multiplex Platforms for High Throughput Serology

In contrast to molecular diagnostics where advances in technology such as PCR and HTS have dramatically improved sensitivity, specificity, and capacity for multiplex analyses, serologic methods remained largely unchanged. This lag is important given the role of serology in establishing the distribution and frequency of infection, testing the significance of association between the finding of an agent and disease, focusing efforts in pathogen discovery and surveillance, and for monitoring humoral responses to vaccines and immunomodulatory drugs. Most serological tests target only one agent; however, Luminex-based systems have been employed that can address up to 100 targets simultaneously.

Phage display systems and arrays that comprise spotted recombinant proteins expressed in vitro in *E. coli*, *S. cerevesiae*, baculo-viruses, and cell-free, coupled transcription-translation, have also been established. The VirScan platform comprehensively tests for the presence of antiviral antibodies through immunoprecipitation of viral peptides in bacteriophage followed by DNA sequencing. The current version screens 93,904 200-mer oligonucleotides, encoding 56-residue peptide that tile the proteome of 206 viral species with 28 residue overlap (Xu *et al.*, 2015). The phage library is incubated with serum, plasma, or CSF. Bound antibodies are recovered by using a mixture of protein A and G coated magnetic beads. The bound phage DNA is sequenced and quantitated to identify immunoreactive epitopes. These assays are powerful but require expertize and resources for cDNA cloning, expression, sequencing, and bioinformatic analysis. An additional challenge is that VirScan may not have the resolution required to differentiate immune responses to closely related viruses. Peptide arrays (Serochips) are an alternative high throughput platform for unbiased serology. Serochips comprise up to 6 million distinct linear peptide sequences attached to a solid support. Like the VirScan platform, Serochips display only linear peptides; thus, neither platform will detect conformational or glycosylated epitopes. To ensure our ability to detect immune responses to discriminant as well as common viral epitopes we tile the entire proteome of the relevant viruses using 12 aa peptides with 11 aa overlap. After incubation with serum, plasma or CSF, arrays are incubated with fluorescence-labeled secondary antibodies (IgG or IgM). Finally, high-resolution image scans are obtained and relative fluorescence signal intensity is used to map immunoreactivity to specific viruses and identify peptides that can be transferred to less expensive and simpler methods for serodiagnosis such as ELISA, western blot, Luminex assays, or smaller peptide arrays.

The detection of an IgG versus an IgM response is important to certain aspects of functional serology. During the challenge of the immune system with a new Ag, stimulated B-cells produce their membrane-presented Ab first as pentameric immunoglobulin class M (IgM) isotype antibodies, that usually exhibit limited specificity. During the next 2–4 weeks plasma B-cells transition after isotype switching and hypermutation to the production of highly specific monomeric immunoglobulin G (IgG) isotype antibodies (Nossal, 2007; Tonegawa, 1983). The ratio of IgM to IgG response may therefore be helpful in determining the timing of the infection. IgM vs IgG determination is easily achieved in all indirect assay systems through the choice of the secondary Ab, applying either an anti-human IgM or an anti-human IgG-specific Ab. There are many other considerations directing the choice of a particular serologic platform. This often includes the classical paradigm of confirming a positive result in one assay in a second technically independent assay, as it is still common practice in Lyme diagnosis; after initial screening by ELISA positive results are confirmed by immunoblot reaction patterns (Centers for Disease Control and Prevention, 1995; Tokarz *et al.*, 2018).

Discussion/Summary

Viral diagnostics is no longer a discipline that requires resources and expertize available only in reference laboratories. Although culture and electron microscopy remain important tools for basic and translational research, molecular methods have moved to the forefront of diagnostics in clinical microbiology and public health. This reflects not only lower costs and ease of use but also the capacity of molecular methods to yield actionable information in hours rather than days. Serology too is becoming more robust and convenient with the advent of multiplexed assays that provide specific information without virus cultivation and neutralization assays. Advances in molecular and serological methods allow clinicians and public health practitioners to simultaneously consider a wide range of probable candidate viral pathogens, and to detect the presence of pathogens that are not typically anticipated in a differential diagnosis. These include viruses known to cause disease in other geographic regions or in other species, or viruses not previously known to medicine or science. We have not addressed in this article the potential for host genetic, proteomic, metabolomic or microbiome analyses to provide diagnostic and prognostic information independent of virus identification that will impact interventions. This is an area of active research that we anticipate will be featured in future editions of this encyclopedia. We also anticipate that insights from wider application of molecular and serological tools will encourage the development of drugs and vaccines that reduce the burden of viral infections in both acute and chronic disease.

References

Al-Samarrai, T., Wu, W., Begier, E., *et al.*, 2013. Evaluation of a pilot respiratory virus surveillance system linking electronic health record and diagnostic data. Journal of Public Health Management & Practice 19, 322–329.

Breman, J.G., Heymann, D.L., Lloyd, G., *et al.*, 2016. Discovery and description of Ebola Zaire virus in 1976 and relevance to the West African epidemic during 2013–2016. The Journal of Infectious Diseases 214 (suppl 3), S93–S101. doi:10.1093/infdis/jiw207.

Briese, T., Jia, X.Y., Huang, C., Grady, L.J., Lipkin, W.I., 1999. Identification of a Kunjin/West Nile-like flavivirus in brains of patients with New York encephalitis. Lancet 354 (9186), 1261–1262.

Briese, T., Kapoor, A., Mishra, N., et al., 2015. Virome capture sequencing enables sensitive viral diagnosis and comprehensive virome analysis. mBio 6 (5), e01491-15.

Briese, T., Palacios, G., Kokoris, M., et al., 2005. Diagnostic system for rapid and sensitive differential detection of pathogens. Emerging Infectious Diseases 11 (2), 310–313.

Brunstein, J., Thomas, E., 2006. Direct screening of clinical specimens for multiple respiratory pathogens using the Genaco respiratory panels 1 and 2. Diagnostic Molecular Pathology 15, 169–173.

Casals, J., 1957. The arthropod-borne group of animal viruses. Transactions of the New York Academy of Sciences 19 (3), 219–235.

Centers for Disease Control and Prevention, 1995. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. Morbidity and Mortality Weekly Report 44, 590–591.

Dominguez, S.R., Briese, T., Palacios, G., et al., 2008. Multiplex MassTag-PCR for respiratory pathogens in pediatric nasopharyngeal washes negative by conventional diagnostic testing shows a high prevalence of viruses belonging to a newly recognized rhinovirus clade. Journal of Clinical Virology 43, 219–222.

Ecker, D.J., Sampath, R., Blyn, L.B., et al., 2005. Rapid identification and strain-typing of respiratory pathogens for epidemic surveillance. Proceedings of the National Academy of Sciences of the United States of America 102 (22), 8012–8017.

Ecker, D.J., Sampath, R., Massire, C., et al., 2008. Ibis T5000: A universal biosensor approach for microbiology. Nature Reviews Microbiology 6 (7), 553–558.

Eid, J., Fehr, A., Gray, J., et al., 2009. Real-time DNA sequencing from single polymerase molecules. Science 323 (5910), 133–138.

Engvall, E., Perlmann, P., 1971. Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. Immunochemistry 8 (9), 871–874.

Freije, C.A., Myhrvold, C., Boehm, C.K., et al., 2019. Programmable inhibition and detection of RNA viruses using Cas13. Molecular Cell 76 (5), 826–837.

Giddins, M.J., Macesic, N., Annavajhala, M.K., et al., 2018. Successive emergence of ceftazidime-avibactam resistance through distinct genomic adaptations in blaKPC-2-Harboring Klebsiella pneumoniae sequence type 307 isolates. Antimicrobial Agents and Chemotherapy 62, 3.

Goldsmith, C.S., Miller, S.E., 2009. Modern uses of electron microscopy for detection of viruses. Clinical Microbiology Reviews 22 (4), 552–563.

Gootenberg, J.S., Abudayyeh, O.O., Kellner, M.J., et al., 2018. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science 360 (6387), 439–444.

Han, J., Swan, D.C., Smith, S.J., et al., 2006. Simultaneous amplification and identification of 25 human papillomavirus types with Templex technology. Journal of Clinical Microbiology 44, 4157–4162.

Haycox, C.L., Kim, S., Fleckman, P., et al., 1999. Trichodysplasia spinulosa – A newly described folliculocentric viral infection in an immunocompromised host. Journal of Investigative Dermatology Symposium Proceedings 4, 268–271.

Hirst, G.K., 1942. The quantitative determination of Influenza virus and antibodies by means of red cell agglutination. Journal of Experimental Medicine 75 (1), 49–64.

Hsu, C.C., Tokarz, R., Briese, T., et al., 2013. Use of staged molecular analysis to determine causes of unexplained central nervous system infections. Emerging infectious Diseases 19, 1470–1477.

Kapikian, A.Z., Wyatt, R.G., Dolin, R., et al., 1972. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. Journal of Virology 10 (5), 1075–1081.

Kausche, G.A., Pfankuch, E., Ruska, H., 1939. Die Sichtbarmachung von pflanzlichem Virus im Übermikroskop. Naturwissenschaften. 27 (18), 292–299. doi:10.1007/BF01493353.

Kuno, G., 2003. Serodiagnosis of flaviviral infections and vaccinations in humans. Advances in Virus Research 61, 3–65.

Lamson, D., Renwick, N., Kapoor, V., et al., 2006. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004–2005. Journal of Infectious Diseases 194, 1398–1402.

Landsteiner, K., 1901. Ueber Agglutinationserscheinungen normalen menschlichen Blutes. Wiener klinische Wochenschrift 14, 1132–1134.

Lee, S.S., Phy, K., Peden, K., Sheng-Fowler, L., 2017. Development of a micro-neutralization assay for ebolaviruses using a replication-competent vesicular stomatitis hybrid virus and a quantitative PCR readout. Vaccine 35 (41), 5481–5486.

Li, H., McCormac, M.A., Estes, R.W., et al., 2007. Simultaneous detection and high-throughput identification of a panel of RNA viruses causing respiratory tract infections. Journal of Clinical Microbiology 45, 2105–2109.

Lipkin, W.I., 2010. Microbe hunting. Microbiology and Molecular Biology Reviews 74 (3), 363–377.

Long, G.W., Nobel Jr, J., Murphy, F.A., Herrmann, K.L., Lourie, B., 1970. Experience with electron microscopy in the differential diagnosis of smallpox. Journal of Applied Microbiology 20 (3), 497–504.

Lu, H., Giordano, F., Ning, Z., 2016. Oxford nanopore MinION sequencing and genome assembly. Genomics Proteomics. Bioinformatics. 14 (5), 265–279.

Mishra, N., Caciula, A., Price, A., et al., 2018. Diagnosis of Zika virus infection by peptide array and enzyme-linked immunosorbent assay. mBio 9, e00095. [18].

Muir, P., Kämmerer, U., Korn, K., et al., 1998. Molecular typing of enteroviruses: current status and future requirements. The European Union concerted action on virus meningitis and encephalitis. Clinical Microbiology Reviews 11 (1), 202–227.

Musso, D., Gubler, D.J., 2016. Zika virus. Clinical Microbiology Reviews 29 (3), 487–524.

Nicholls, J.M., Poon, L.L., Lee, K.C., et al., 2003. Lung pathology of fatal severe acute respiratory syndrome. Lancet 361 (9371), 1773–1778.

Nossal, G., 2007. One cell-one antibody: Prelude and aftermath. Nature Immunology 8 (10), 1015–1017.

Palacios, G., Briese, T., Kapoor, V., et al., 2006. MassTag polymerase chain reaction for differential diagnosis of viral hemorrhagic fever. Emerging Infectious Diseases 12, 692–695.

Palacios, G., Hornig, M., Cisterna, D., et al., 2009. Streptococcus pneumoniae coinfection is correlated with the severity of H1N1 pandemic influenza. PLOS One 4, e8540.

Palacios, G., Lovoll, M., Tengs, T., et al., 2010. Heart and skeletal muscle inflammation of farmed salmon is associated with infection with a novel reovirus. PLOS One 5 (7), e11487.

Palacios, G., Quan, P.L., Jabado, O.J., et al., 2007. Panmicrobial oligonucleotide array for diagnosis of infectious diseases. Emerging Infectious Diseases 13 (1), 73–81.

Pedersen, J.C., 2014. Hemagglutination-inhibition assay for influenza virus subtype identification and the detection and quantitation of serum antibodies to influenza virus. In: Spackman, E. (Ed.), Animal Influenza Virus. Methods in Molecular Biology (Methods and Protocols) 1161. New York, NY: Humana Press.

Quan, P.L., Palacios, G., Jabado, O.J., et al., 2007. Detection of respiratory viruses and subtype identification of influenza A viruses by GreeneChipResp oligonucleotide microarray. Journal of Clinical Microbiology 45 (8), 2359–2364.

Renwick, N., Schweiger, B., Kapoor, V., et al., 2007. A recently identified rhinovirus genotype is associated with severe respiratory-tract infection in children in Germany. Journal of Infectious Diseases 196, 1754–1760.

Saiki, R.K., Scharf, S., Faloona, F., et al., 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science. 230 (4732), 1350–1354.

Sampath, R., Hall, T.A., Massire, C., et al., 2007. Rapid identification of emerging infectious agents using PCR and electrospray ionization mass spectrometry. Annals of the New York Academy of Sciences 1102, 109–120.

Schmidt, N.F., Guenther, R.W., Lennette, E.H., 1961. Typing of ECHO virus isolates by immune serum pools. The "intersecting serum scheme. Journal of Immunology 87, 623–626.

The Enteroviruses, 1957. Committee on the Enteroviruses, National Foundation for infantile paralysis. American Journal of Public Health and the Nation's Health 47 (12), 1556–1566.

Tokarz, R., Firth, C., Madhi, S.A., et al., 2012. Worldwide emergence of multiple clades of enterovirus 68. Journal of General Virology 93, 1952–1958.

Tokarz, R., Jain, K., Bennett, A., Briese, T., Lipkin, W.I., 2010. Assessment of polymicrobial infections in ticks in New York state. Vector-Borne and Zoonotic Diseases 10, 217–221.

Tokarz, R., Kapoor, V., Samuel, J.E., *et al.*, 2009. Detection of tick-borne pathogens by MassTag polymerase chain reaction. Vector-Borne and Zoonotic Diseases 9, 147–152.

Tokarz, R., Kapoor, V., Wu, W., *et al.*, 2011. Longitudinal molecular microbial analysis of influenza-like illness in New York City, May 2009 through May 2010. Virology Journal 8, 288.

Tokarz, R., Mishra, N., Tagliafierro, T., *et al.*, 2018. A multiplex serologic platform for diagnosis of tick-borne diseases. Scientific Reports 8 (1), 3158.

Tonegawa, S., 1983. Somatic generation of antibody diversity. Nature. 302 (5909), 575–581.

Wang, D., Coscoy, L., Zylberberg, M., *et al.*, 2002. Microarray-based detection and genotyping of viral pathogens. Proceedings of the National Academy of Sciences of the United States of America 99 (24), 15687–15692.

Wang, D., Urisman, A., Liu, Y.-T., *et al.*, 2003. Viral discovery and sequence recovery using DNA microarrays. PLOS Biology 1 (2), e2.

Van Ert, M.S., Hofstadler, Y., Jiang, J., *et al.*, 2004. Mass spectrometry provides accurate characterization of two genetic marker types in Bacillus anthracis. BioTechniques 37 (4), 642.

Van Rooyen, C.E., Illingworth, R.S., 1944. A laboratory test for diagnosis of smallpox. British Medical Journal 2 (4372), 526–529. doi:10.1136/bmj.2.4372.526.

Van Weemen, B.K., Schuurs, A.H.W.M., 1971. Immunoassay using antigen-enzyme conjugates. FEBS Letters 15 (3), 232–236.

Wick, R.R., Judd, L.M., Gorrie, C.L., Holt, K.E., 2017. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLOS Computational Biology 13 (6), e1005595.

Widal, F.M., 1896. Serodiagnostic de la fiévre typhoide a-propos d'une modification par MMC Nicolle et al. Halipie. Bulletins et mémoires de la Société Médicale des Hôpitaux de Paris 13, 561–566.

Williams, S.H., Cordey, S., Bhuva, N., *et al.*, 2018. Investigation of the plasma virome from cases of unexplained febrile illness in Tanzania from 2013–2014: A comparative analysis between unbiased and VirCapSeq-VERT high-throughput sequencing approaches. mSphere 3 (4), e00311–e00318.

Xu, G.J., Kula, T., Xu, Q., *et al.*, 2015. Viral immunology. Comprehensive serological profiling of human populations using a synthetic human virome. Science. 348 (6239), aaa0698.