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Viral surveillance and discovery

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The field of virus discovery has burgeoned with the advent of high throughput sequencing platforms and bioinformatics programs that enable rapid identification and molecular characterization of known and novel agents, investments in global microbial surveillance that include wildlife and domestic animals as well as humans, and recognition that viruses may be implicated in chronic as well as acute diseases. Here we review methods for viral surveillance and discovery, strategies and pitfalls in linking discoveries to disease, and identify opportunities for improvements in sequencing instrumentation and analysis, the use of social media and medical informatics that will further advance clinical medicine and public health.

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Introduction

The changing dynamics of emerging viruses has resulted in an increasingly important role for viral discovery and diagnostics in clinical medicine and public health. Globalization of travel and trade in domestic animals and animal products, bush meat trafficking, political instability and bioterrorism, as well as climate change and its effects on vector distribution have all facilitated the emergence and reemergence of zoonoses [1–3]. Viruses previously restricted to one host species or geographic region can now appear in unexpected locations, confounding clinicians who are unprepared to recognize new syndromes or to detect new pathogens with their existing diagnostic tests. Immunosuppression associated with organ transplantation, chemotherapy or HIV/AIDS has enabled viruses with limited virulence to become more pathogenic [4–7]. The increased appreciation for the potential role of viruses in chronic cardiovascular, endocrine, neurodevelopmental and neoplastic disorders coupled with a growing repertoire of diagnostic tools and techniques has resulted in a re-energized quest for associated pathogens (Figure 1) [8–10]. This burgeoning interest in discovery and diagnostics now also has practical applications given recent improvements in drugs and vaccines. The lack of effective therapies once made the diagnosis of viral infection primarily an academic exercise; however, the expanding armamentarium of countermeasures for specific viruses promises unprecedented opportunities to reduce morbidity, mortality and the economic costs of viral infections.

Tools for the hunt

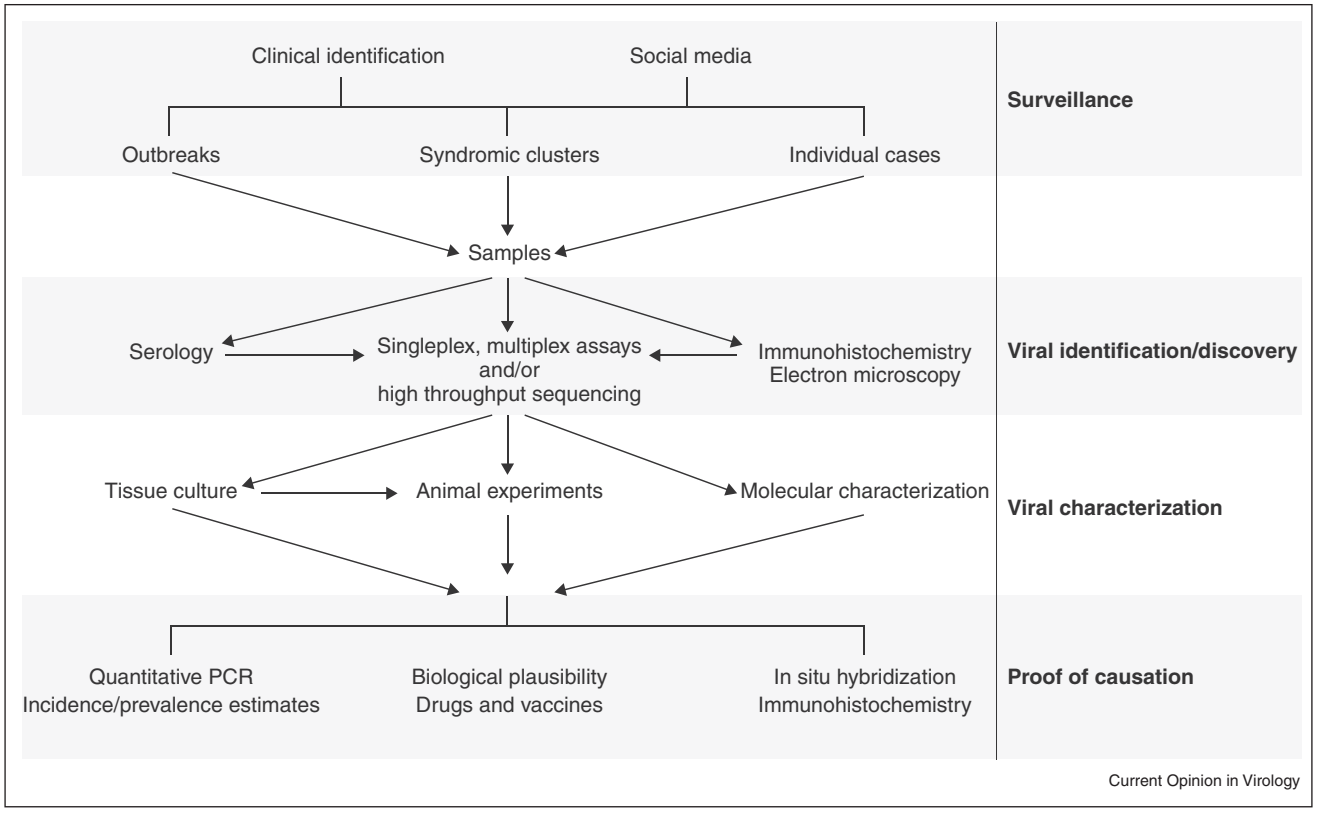
Tissue culture

Although this classical method for viral discovery is dismissed by some investigators as cumbersome, expensive and quaint, we continue to use it for several reasons. First, the growth of an agent in culture provides an excellent source of enriched template for molecular characterization. Additionally, having an inoculum for animal model studies, a cell-based assay for serology or a titered stock for neutralization tests greatly aids the pursuit of the proof of disease causation [11–14]. Such stocks are also needed for *in vitro* assessment of vaccines and to test drug candidates. Finally, visualization of an agent by electron microscopy is an intellectually satisfying coup de grace for even the most jaded microbe hunter [15,16]. The challenge is to find a cell line that allows both entry and replication. The former requires expression of the appropriate receptors (and in some instances, co-receptors), while the latter requires host factors that support virus transcription, replication, assembly and egress [17,18]. Another impediment can be the induction of innate immune responses that impede transcription, replication and/or protein synthesis. Of these challenges, innate immunity is the most straightforward to address, as responses can be depressed through the use of antibodies or other molecules that target alternate pathways. The only current solution for the receptor and factor challenge is to test multiple cell lines, both continuous and primary, from a wide range of host species. Unfortunately, in some instances, these efforts fail and the only system that may prove useful for virus amplification is animal inoculation [17].

Imaging

Until the advent of rapid, inexpensive unbiased high throughput sequencing, immunohistochemistry was frequently the first step towards identifying candidates for molecular assays. In 1999, for example, our decision to pursue flavivirus consensus PCR with brain extract of victims of the encephalitis outbreak in New York was guided by immunoassays performed by Sherif Zaki at the Centers for Disease Control in Atlanta [19–21]. More recently, imaging has been used primarily for examining the strength of the association between an infectious

Figure 1



Staged strategy for pathogen discovery and proof of causation.

agent and a disease by testing for its presence at the site of pathology [22–24]. Although rapid protocols for electron microscopy exist that allow the visualization of viruses in a few hours, they require substantial operator expertise and a high agent concentration. Immunohistochemical assays can be pursued using serum from either the affected individual or one with a similar disease, or with antibodies generated in animal models inoculated with the virus candidate or immunized with viral proteins [25]. Where serum is already available these approaches can be implemented rapidly; however, with truly novel agents the delay required to create immunological reagents is typically measured in weeks to months. An alternative is to pursue *in situ* hybridization. In as little as a few days, genetic sequence of the pathogen candidate can be used to design probes with which to examine the distribution of that candidate in tissues [26].

Multiplex assays

Clinical syndromes are only rarely pathognomonic of infections with specific agents. Thus, although singleplex assays are more common in clinical microbiology, multiplex assays can be more useful not only in differentiating amongst likely candidate pathogens, but also in excluding them, so that efforts can be focused on discovery [27–30]. Multiplex PCR assays in current application detect products using fluorescence, mass spectroscopy or capillary electrophoresis [31–35]. The most highly multiplexed of these can address as many as 50 candidate agents with the sensitivity required for analysis of clinical materials [36–38]. Even higher levels of multiplexing can be obtained with DNA microarrays that can comprise millions of individual probes [39–42]. However, sensitivity may be insufficient for clinical applications. This drawback may ultimately be overcome through the use of nanofluidics and electronic nanocircuitry [43–45•].

High throughput sequencing

The advent of rapid, inexpensive platforms for DNA sequencing has revolutionized microbiology by providing culture-independent methods for pursuing virus discovery and characterization, surveillance and outbreak investigation. Over the past 10 years, the per base cost of sequencing has decreased 10 000-fold, from $5000 per megabase using capillary electrophoresis to $0.5 per megabase using the Illumina platform [46,47]. Accordingly, as the volume of sequence data has grown exponentially in individual experiments and international data repositories, the challenge has shifted from sequence acquisition to analysis. A key determinant in the complexity of bioinformatics

analysis is the read length, which ranges from 150 bp on the Illumina HiSeq or MiSeq instruments to 700 bp on the Roche GS FLX Titanium pyrosequencer [46,48]. The shorter the read length, the greater the number of calculations needed to assemble continuous strings of genomic sequence. An additional challenge on some instruments (e.g. GS FLX and Ion Torrent Personal Genome Machine [PGM]) is the uncertainty of base identification caused by nucleotide homopolymers [46,49–52]. Although single molecule sequencers are in development that promise to provide both fidelity and long strings of sequence data, the current strategy for many investigators is to use the GS FLX or PGM platforms to generate a scaffold for assembling and mapping sequence data from Illumina instruments [53,54••,55••]. Irrespective of platform, the process following sequence acquisition is similar for pathogen discovery: contiguous sequences are assembled, host sequence is subtracted, and the residual sequences are examined for similarity to known microbial sequences at the nucleotide or amino acid level [56,57]. Where no similarities are found, one can examine nucleotide composition and/or order for patterns consistent with viral genera and host species. This approach can also help in deciding which sequences merit further analysis, an important advantage particularly in examining fecal specimens where sequences may represent viruses infecting the host, or a plant or animal consumed by host [58–63]. Although investigations of individual cases or outbreaks of disease still typically focus first on testing for the presence of specific agents using singleplex or syndrome-specific multiplex assays, as the costs of sequencing continues to decrease, instruments become more portable and methods for sample preparation and sequence analysis become more user friendly, we anticipate that sequencing will move to the forefront of clinical diagnosis as well as discovery.

Serology

Although a wide range of genetic methods have been established for multiplex detection of viruses – oligonucleotide microarrays, multiplex PCR and sequencing – serology is largely confined to the singleplex methods that have been employed for decades, including immunofluorescence, enzyme linked immunosorbent (ELISA), Western blot, lateral flow and neutralization assays. Thus, with the notable exception of the Luminex system wherein individual bead populations are decorated with panels of different antigens that are simultaneously queried, investigators must commit to testing specific pathogen hypotheses using individual tests rather than pursuing syndromic surveillance for a wide range of agents [64]. An additional challenge is that the development of immunoassays is typically a time consuming and labor intensive process that requires preparation of antigen and production or characterization of antisera for validation. It is impossible to estimate rates of morbidity and mortality associated with infection unless one can determine not only the presence of an agent in individuals with disease but also the number of individuals who have been infected but have no or only mild disease. Despite these drawbacks, serology can be useful when molecular methods are unsuccessful. For example, direct means of genetic detection are not useful when viral nucleic acids are not present in an accessible sample (e.g. cerebrospinal fluid in most cases of encephalitis) or where disease may be triggered by an agent that is no longer present (e.g. chronic or degenerative diseases). The risk of exposure to a new infectious agent is modulated by an individual's previous exposures to similar agents or vaccines. Such exposures may confer complete or partial protection or result in increased risk for more severe disease owing to antibody-mediated enhancement as in dengue [65•]. Thus, knowledge of an individual's immunological history may influence decisions concerning his/her treatment, vaccination or deployment as a first responder or health care worker in areas with increased probability of encountering specific pathogens. There is urgent need, therefore, to develop serological platforms that can be rapidly deployed as new agents are discovered and to simultaneously detect evidence of exposure to more than one agent. One such platform is Luciferase immunoprecipitation systems (LIPS), a quantitative high-throughput liquid phase format assay wherein viral protein antigens are fused to an enzyme reporter, Renilla luciferase (ReL), expressed in mammalian cells, harvested and then incubated with serum and staphylococcal protein A/G magnetic beads [12,66••]. Following precipitation, luciferase activity is determined as an index to antibody concentration. Establishment of a LIPS assay for a new virus can be developed within several days of the acquisition of genomic sequence data. Another promising platform builds on the microarray technology currently used for detection of the nucleic acids of pathogens [67–69]. Early versions based on spotted peptides have been supplanted by 25 mm × 75 mm arrays onto which peptides are synthesized *in situ* at densities that exceed 1 million features. This density already allows designs that tile the proteomes of hundreds of viruses. As the printing density increases and efficiencies of scale drive down costs we envision that high throughput multiplex serology will become feasible in clinical as well as research laboratories.

Social media

Recent advances in the portability and accessibility of the Internet affords new opportunities for viral surveillance and discovery. Individuals can rapidly and inexpensively share reports of outbreaks of disease as well as primer sequences, protocols and insights, or request assistance. The first program to build such a presence was ProMED-mail (Programme for Monitoring Emerging Infectious Diseases), created in 1994 to provide a free, interactive international forum for reporting and discussing outbreaks of disease [70]. It remains active to the present

day and serves more than 60 000 subscribers in 185 different countries. Two other popular programs are GPHIN (Global Public Health Intelligence Network) [71] and HealthMap [72]. GPHIN is a subscription news service that reviews media reports and web entries for information concerning outbreaks [71]. HealthMap is a hybrid of the passive and active surveillance strategies used by ProMED-mail and GPHIN, respectively, which provides user-friendly maps of disease emergence with informative hyperlinks [72]. Although none of these programs yet integrates the full range of data sources that have the potential to contribute to global awareness (e.g. medical or veterinary service use, purchases of prescription drugs and over the-counters remedies), they have dramatically enhanced communication amongst scientists, clinicians, public health practitioners, and laid the foundation for a global warning system that enables focused investments in surveillance and discovery.

Mechanisms of pathogenesis and proof of causation

In the most straightforward examples of pathogen discovery the investigator encounters an outbreak of what appears to be a transmissible disease. Many individuals present with similar signs and symptoms and there is: (1) a pattern consistent with either direct human-to-human transmission or exposure to a common source whether aerosol, enteric or arboviral; (2) a high concentration of the agent at the site of pathology; (3) an acute presentation of disease. However, limiting microbe hunting to such ideal scenarios would eliminate opportunities for actionable insights into chronic or sporadic disorders or early zoonotic transmission events. Host factors including age, nutrition, genetic context, co-infection, and previous exposures to related agents can have a profound impact on susceptibility to infection and the expression of disease. Viruses can cause damage at the site of infection as a direct result of replication, or as an indirect effect of innate or adaptive immune responses to microbial gene products. They may also induce neoplasia through cell cycle interference. The gold standard for proof of causation are the criteria established by Loeffler and Koch known as Koch's postulates [73]. They require that an agent be present in every case of the disease, specific for the disease and sufficient to reproduce the disease after culture and inoculation into a naïve host. Others including Rivers, Fredericks and Relman, and the current authors have proposed alternative criteria to allow for instances where an agent cannot be propagated in culture or there is no suitable animal model with which to test for transmissibility of disease as well as infection [74–76]. Detailed strategies for pursuing causation are described in recent reviews [56,77]. They rely upon demonstrating strength of association through assessment of adaptive immunity to the candidate agent, quantitation of viral burden, demonstration of footprints of the agent at the site of pathology, biological plausibility by analogy to disease associated with similar agents in the same or other hosts, and prevention or mitigation of disease through use of candidate-specific drugs or vaccines (Figure 1).

Conclusions

The introduction of new tools for surveillance, diagnosis and discovery is timely given the increasing globalization of travel and trade and other factors that enhance the emergence and re-emergence of pathogenic viruses. These tools will have application beyond control of acute disease. We anticipate that they will lead to insights into the pathogenesis of a wide range of idiopathic conditions not typically ascribed to infection such as neurodevelopmental, autoimmune and neoplastic disorders. Concomitant advances in methods for vaccine and drug development suggest the potential for dramatic improvements in health and welfare enabled by enhanced viral intelligence.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cleaveland S, Laurenson MK, Taylor LH: **Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence**. *Philos Trans R Soc Lond B Biol Sci* 2001, **356**:991-999.

2. Parrish CR, Holmes EC, Morens DM, Park EC, Burke DS, Calisher CH, Laughlin CA, Saif LJ, Daszak P: **Cross-species virus transmission and the emergence of new epidemic diseases**. *Microbiol Mol Biol Rev* 2008, **72**:457-470.

3. Woolhouse ME, Haydon DT, Antia R: **Emerging pathogens: the epidemiology and evolution of species jumps**. *Trends Ecol Evol* 2005, **20**:238-244.

4. Kumar D: **Emerging viruses in transplantation**. *Curr Opin Infect Dis* 2010, **23**:374-378.

5. Schulz TF: **Cancer and viral infections in immunocompromised individuals**. *Int J Cancer* 2009, **125**:1755-1763.

6. Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, Comer JA, Guarner J, Paddock CD, DeMeo DL *et al.*: **Transmission of lymphocytic choriomeningitis virus by organ transplantation**. *N Engl J Med* 2006, **354**:2235-2249.

7. Palacios G, Druce J, Du L, Tran T, Birch C, Briese T, Conlan S, Quan PL, Hui J, Marshall J *et al.*: **A new arenavirus in a cluster of fatal transplant-associated diseases**. *N Engl J Med* 2008, **358**:991-998.

8. Molyneux EM, Rochford R, Griffin B, Newton R, Jackson G, Menon G, Harrison CJ, Israels T, Bailey S: **Burkitt's lymphoma**. *Lancet* 2012, **379**:1234-1244.

9. Schrama D, Ugurel S, Becker JC: **Merkel cell carcinoma: recent insights and new treatment options**. *Curr Opin Oncol* 2012, **24**:141-149.

10. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S: **Human papillomavirus and cervical cancer**. *Lancet* 2007, **370**:890-907.

11. Bowen ET, Lloyd G, Harris WJ, Platt GS, Baskerville A, Vella EE: **Viral haemorrhagic fever in southern Sudan and northern Zaire. Preliminary studies on the aetiological agent**. *Lancet* 1977, **1**:571-573.

12. Burbelo PD, Dubovi EJ, Simmonds P, Medina JL, Henriquez JA, Mishra N, Wagner J, Tokarz R, Cullen JM, Iadarola MJ *et al.*: **Serology-enabled discovery of genetically diverse hepaciviruses in a new host**. *J Virol* 2012, **86**:6171-6178.

13. Glaser L, Conenello G, Paulson J, Palese P: **Effective replication of human influenza viruses in mice lacking a major alpha2,6 sialyltransferase**. *Virus Res* 2007, **126**:9-18.

14. Qiao M, Ashok M, Bernard KA, Palacios G, Zhou ZH, Lipkin WI, Liang TJ: **Induction of sterilizing immunity against West Nile Virus (WNV), by immunization with WNV-like particles produced in insect cells**. *J Infect Dis* 2004, **190**:2104-2108.

15. Nasar F, Palacios G, Gorchakov RV, Guzman H, Da Rosa AP, Savji N, Popov VL, Sherman MB, Lipkin WI, Tesh RB *et al.*: **Eilat virus, a unique alphavirus with host range restricted to insects by RNA replication**. *Proc Natl Acad Sci U S A* 2012, **109**:14622-14627.

16. Arslan D, Legendre M, Seltzer V, Abergel C, Claverie JM: **Distant mimivirus relative with a larger genome highlights the fundamental features of Megaviridae**. *Proc Natl Acad Sci U S A* 2011, **108**:17486-17491.

17. Ren RB, Costantini F, Gorgacz EJ, Lee JJ, Racaniello VR: **Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis**. *Cell* 1990, **63**:353-362.

18. Martina Y, Marcucci KT, Cherqui S, Szabo A, Drysdale T, Srinivisan U, Wilson CA, Patience C, Salomon DR: **Mice transgenic for a human porcine endogenous retrovirus receptor are susceptible to productive viral infection**. *J Virol* 2006, **80**:3135-3146.

19. Centers for Disease Control and Prevention (CDC), Outbreak of West Nile-like viral encephalitis–New York, 1999. MMWR Morb Mortal Wkly Rep 1999, 48:845–849.

20. Shieh WJ, Guarner J, Layton M, Fine A, Miller J, Nash D, Campbell GL, Roehrig JT, Gubler DJ, Zaki SR: **The role of pathology in an investigation of an outbreak of West Nile encephalitis in New York, 1999**. *Emerg Infect Dis* 2000, **6**:370-372.

21. Briese T, Jia XY, Huang C, Grady LJ, Lipkin WI: **Identification of a Kunjin/West Nile-like flavivirus in brains of patients with New York encephalitis**. *Lancet* 1999, **354**:1261-1262.

22. Hammerstad SS, Tauriainen S, Hyoty H, Paulsen T, Norheim I, Dahl-Jorgensen K: **Detection of enterovirus in the thyroid tissue of patients with graves' disease**. *J Med Virol* 2013, **85**:512-518.

23. Bhatnagar J, Jones T, Blau DM, Shieh WJ, Paddock CD, Drew C, Denison AM, Rollin DC, Patel M, Zaki SR: **Localization of pandemic 2009 H1N1 influenza A virus RNA in lung and lymph nodes of fatal influenza cases by in situ hybridization: new insights on virus replication and pathogenesis**. *J Clin Virol* 2013, **56**:316-321.

24. Li L, Zhao Q, Ge X, Teng K, Kuang Y, Chen Y, Guo X, Yang H: **Chinese highly pathogenic porcine reproductive and respiratory syndrome virus exhibits more extensive tissue tropism for pigs**. *Virol J* 2012, **9**:203.

25. Honkavuori KS, Shivaprasad HL, Briese T, Street C, Hirschberg DL, Hutchison SK, Lipkin WI: **Novel picornavirus in Turkey poults with hepatitis, California, USA**. *Emerg Infect Dis* 2011, **17**:480-487.

26. Anthony SJ, St Leger JA, Pugliares K, Ip HS, Chan JM, Carpenter ZW, Navarrete-Macias I, Sanchez-Leon M, Saliki JT, Pedersen J *et al.*: **Emergence of fatal avian influenza in New England harbor seals**. *MBio* 2012, **3** e00166-12.

27. Casas I, Tenorio A, Echevarria JM, Klapper PE, Cleator GM: **Detection of enteroviral RNA and specific DNA of herpesviruses by multiplex genome amplification**. *J Virol Methods* 1997, **66**:39-50.

28. Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, Sanchez A, Childs J, Zaki S, Peters CJ: **Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness**. *Science* 1993, **262**:914-917.

29. Shirato K, Nishimura H, Saijo M, Okamoto M, Noda M, Tashiro M, Taguchi F: **Diagnosis of human respiratory syncytial virus infection using reverse transcription loop-mediated isothermal amplification**. *J Virol Methods* 2007, **139**:78-84.

30. Tumpey TM, Basler CF, Aguilar PV, Zeng H, Solorzano A, Swayne DE, Cox NJ, Katz JM, Taubenberger JK, Palese P *et al.*: **Characterization of the reconstructed 1918 Spanish influenza pandemic virus**. *Science* 2005, **310**:77-80.

31. Brunstein JD, Cline CL, McKinney S, Thomas E: **Evidence from multiplex molecular assays for complex multipathogen interactions in acute respiratory infections**. *J Clin Microbiol* 2008, **46**:97-102.

32. Dominguez SR, Briese T, Palacios G, Hui J, Villari J, Kapoor V, Tokarz R, Glode MP, Anderson MS, Robinson CC *et al.*: **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**. *J Clin Virol* 2008, **43**:219-222.

33. Mahony J, Chong S, Merante F, Yaghoubian S, Sinha T, Lisle C, Janeczko R: **Development of a respiratory virus panel test for detection of twenty human respiratory viruses by use of multiplex PCR and a fluid microbead-based assay**. *J Clin Microbiol* 2007, **45**:2965-2970.

34. Nolte FS, Marshall DJ, Rasberry C, Schievelbein S, Banks GG, Storch GA, Arens MQ, Buller RS, Prudent JR: **MultiCode-PLx system for multiplexed detection of seventeen respiratory viruses**. *J Clin Microbiol* 2007, **45**:2779-2786.

35. Thaitrong N, Liu P, Briese T, Lipkin WI, Chiesl TN, Higa Y, Mathies RA: **Integrated capillary electrophoresis microsystem for multiplex analysis of human respiratory viruses**. *Anal Chem* 2010, **82**:10102-10109.

36. Han J, Swan DC, Smith SJ, Lum SH, Sefers SE, Unger ER, Tang YW: **Simultaneous amplification and identification of 25 human papillomavirus types with Templex technology**. *J Clin Microbiol* 2006, **44**:4157-4162.

37. Brunstein J, Thomas E: **Direct screening of clinical specimens for multiple respiratory pathogens using the Genaco Respiratory Panels 1 and 2**. *Diagn Mol Pathol* 2006, **15**:169-173.

38. Li H, McCormac MA, Estes RW, Sefers SE, Dare RK, Chappell JD, Erdman DD, Wright PF, Tang YW: **Simultaneous detection and high-throughput identification of a panel of RNA viruses causing respiratory tract infections**. *J Clin Microbiol* 2007, **45**:2105-2109.

39. Chiu CY, Alizadeh AA, Rouskin S, Merker JD, Yeh E, Yagi S, Schnurr D, Patterson BK, Ganem D, DeRisi JL: **Diagnosis of a critical respiratory illness caused by human metapneumovirus by use of a pan-virus microarray**. *J Clin Microbiol* 2007, **45**:2340-2343.

40. Chiu CY, Rouskin S, Koshy A, Urisman A, Fischer K, Yagi S, Schnurr D, Eckburg PB, Tompkins LS, Blackburn BG *et al.*: **Microarray detection of human parainfluenzavirus 4 infection associated with respiratory failure in an immunocompetent adult**. *Clin Infect Dis* 2006, **43**:e71-e76.

41. Lin B, Blaney KM, Malanoski AP, Ligler AG, Schnur JM, Metzgar D, Russell KL, Stenger DA: **Using a resequencing microarray as a multiple respiratory pathogen detection assay**. *J Clin Microbiol* 2007, **45**:443-452.

42. Wong CW, Albert TJ, Vega VB, Norton JE, Cutler DJ, Richmond TA, Stanton LW, Liu ET, Miller LD: **Tracking the evolution of the SARS coronavirus using high-throughput, high-density resequencing arrays**. *Genome Res* 2004, **14**:398-405.

43. Rosenstein JK, Wanunu M, Merchant CA, Drndic M, Shepard KL: **Integrated nanopore sensing platform with sub-microsecond temporal resolution**. *Nat Methods* 2012, **9**:487-492.

44. Kaittanis C, Santra S, Perez JM: **Emerging nanotechnology-based strategies for the identification of microbial pathogenesis**. *Adv Drug Deliv Rev* 2010, **62**:408-423.

45. • Cherf GM, Lieberman KR, Rashid H, Lam CE, Karplus K, Akeson M: **Automated forward and reverse ratcheting of DNA in a nanopore at 5-A precision**. *Nat Biotechnol* 2012, **30**:344-348
This paper is one of the first to demonstrate that sequence information can be obtained by passing a DNA molecule through a protein nanopore, using Phi29 DNA polymerase to slow the speed of translocation. This work greatly advanced the use of nanopore technology for DNA sequencing..

46. Loman NJ, Misra RV, Dallman TJ, Constantinidou C, Gharbia SE, Wain J, Pallen MJ: **Performance comparison of benchtop high-throughput sequencing platforms**. *Nat Biotechnol* 2012, **30**:434-439.

47. Kircher M, Kelso J: **High-throughput DNA sequencing–concepts and limitations**. *Bioessays* 2010, **32**:524-536.

48. Metzker ML: **Sequencing technologies – the next generation**. *Nat Rev Genet* 2010, **11**:31-46.

49. Aird D, Ross MG, Chen WS, Danielsson M, Fennell T, Russ C, Jaffe DB, Nusbaum C, Gnirke A: **Analyzing and minimizing PCR amplification bias in Illumina sequencing libraries**. *Genome Biol* 2011, **12**:R18.

50. Kieleczawa J, Mazaika E: **Optimization of protocol for sequencing of difficult templates**. *J Biomol Tech* 2010, **21**:97-102.

51. Kozarewa I, Ning Z, Quail MA, Sanders MJ, Berriman M, Turner DJ: **Amplification-free Illumina sequencing-library preparation facilitates improved mapping and assembly of (G+C)-biased genomes**. *Nat Methods* 2009, **6**:291-295.

52. Dohm JC, Lottaz C, Borodina T, Himmelbauer H: **Substantial biases in ultra-short read data sets from high-throughput DNA sequencing**. *Nucleic Acids Res* 2008, **36**:e105.

53. Chan JZ, Pallen MJ, Oppenheim B, Constantinidou C: **Genome sequencing in clinical microbiology**. *Nat Biotechnol* 2012, **30**:1068-1071.

54. •• Croucher NJ, Harris SR, Fraser C, Quail MA, Burton J, van der Linden M, McGee L, von Gottberg A, Song JH, Ko KS *et al.*: **Rapid pneumococcal evolution in response to clinical interventions**. *Science* 2011, **331**:430-434.
This paper demonstrates the power of full genome sequencing to illuminate the response of pathogens to selection from host immune pressures or clinical interventions. By sequencing more than 200 complete genomes of a single strain of multi-drug resistant *Streptococcus pneumoniae*, the authors demonstrate the importance of widespread recombination for rapid adaptation.

55. •• Rohde H, Qin J, Cui Y, Li D, Loman NJ, Hentschke M, Chen W, Pu F, Peng Y, Li J *et al.*: **Open-source genomic analysis of Shiga-toxin-producing *E. coli* O104:H4**. *N Engl J Med* 2011, **365**:718-724.
This paper describes how unbiased high throughput sequencing and crowd-sourced analysis were used to determine the sequence information of the *E. coli* strain responsible for the 2011 German outbreak. This work illustrates how the increasing speed and decreasing cost of UHTS has transformed outbreak response efforts.

56. Mokili JL, Rohwer F, Dutilh BE: **Metagenomics and future perspectives in virus discovery**. *Curr Opin Virol* 2012, **2**:63-77.

57. Tang P, Chiu C: **Metagenomics for the discovery of novel human viruses**. *Future Microbiol* 2010, **5**:177-189.

58. Brzuszkiewicz E, Thurmer A, Schuldes J, Leimbach A, Liesegang H, Meyer FD, Boelter J, Petersen H, Gottschalk G, Daniel R: **Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: Entero-Aggregative-Haemorrhagic *Escherichia coli* (EAHEC)**. *Arch Microbiol* 2011, **193**:883-891.

59. Carroll IM, Ringel-Kulka T, Siddle JP, Klaenhammer TR, Ringel Y: **Characterization of the fecal microbiota using high-throughput sequencing reveals a stable microbial community during storage**. *PLoS One* 2012, **7**:e46953.

60. Hagen C, Frizzi A, Gabriels S, Huang M, Salati R, Gabor B, Huang S: **Accurate and sensitive diagnosis of geminiviruses through enrichment, high-throughput sequencing and automated sequence identification**. *Arch Virol* 2012, **157**:907-915.

61. Lamendella R, Domingo JW, Ghosh S, Martinson J, Oerther DB: **Comparative fecal metagenomics unveils unique functional capacity of the swine gut**. *BMC Microbiol* 2011, **11**:103.

62. Li RW, Wu S, Li W, Huang Y, Gasbarre LC: **Metagenome plasticity of the bovine abomasal microbiota in immune animals in response to Ostertagia ostertagi infection**. *PLoS One* 2011, **6**:e24417.

63. Shan T, Li L, Simmonds P, Wang C, Moeser A, Delwart E: **The fecal virome of pigs on a high-density farm**. *J Virol* 2011, **85**:11697-11708.

64. Krunic N, Merante F, Yaghoubian S, Himsworth D, Janeczko R: **Advances in the diagnosis of respiratory tract infections: role of the Luminex xTAG respiratory viral panel**. *Ann N Y Acad Sci* 2011, **1222**:6-13.

65. • Rothman AL: **Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms**. *Nat Rev Immunol* 2011, **11**:532-543.
This paper provides an excellent review of the complex nature of the immune response to dengue infection. It includes an in-depth discussion of the effects of cytokine storm, T cell response and antibody-dependent enhancement on the severity of infection and vaccination strategies.

66. •• Burbelo PD, Bren KE, Ching KH, Gogineni ES, Kottilil S, Cohen JI, Kovacs JA, Iadarola MJ: **LIPS arrays for simultaneous detection of antibodies against partial and whole proteomes of HCV, HIV and EBV**. *MolBiosyst* 2011, **7**:1453-1462.
With the creation of Luciferase immunoprecipitation systems (LIPS), serological identification of infectious agents became a rapid (~10 days from the generation of a small fragment of genomic sequence to a working assay) and highly sensitive technique that could be applied to any host organism. This paper demonstrates that LIPS assays can simultaneously profile antibody response to a broad panel of antigens.

67. McLoughlin KS: **Microarrays for pathogen detection and analysis**. *Brief Funct Genomics* 2011, **10**:342-353.

68. Panicker RC, Huang X, Yao SQ: **Recent advances in peptide-based microarray technologies**. *Comb Chem High Throughput Screen* 2004, **7**:547-556.

69. Robinson WH, DiGennaro C, Hueber W, Haab BB, Kamachi M, Dean EJ, Fournel S, Fong D, Genovese MC, de Vegvar HE *et al.*: **Autoantigen microarrays for multiplex characterization of autoantibody responses**. *Nat Med* 2002, **8**:295-301.

70. Madoff LC: **ProMED-mail: an early warning system for emerging diseases**. *Clin Infect Dis* 2004, **39**:227-232.

71. Mykhalovskiy E, Weir L: **The Global Public Health Intelligence Network and early warning outbreak detection: a Canadian contribution to global public health**. *Can J Public Health* 2006, **97**:42-44.

72. Freifeld CC, Mandl KD, Reis BY, Brownstein JS: **HealthMap: global infectious disease monitoring through automated classification and visualization of Internet media reports**. *J Am Med Inform Assoc* 2008, **15**:150-157.

73. Koch R: Ueber bakteriologische Forschung, Verhandl. des X. Interna. Med. Edited by Berlin, Germany: Congr, Berlin 1890; 1891 [August Hirschwald (Series Editor)]

74. Fredericks DN, Relman DA: **Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates**. *Clin Microbiol Rev* 1996, **9**:18-33.

75. Lipkin WI: **Microbe hunting**. *Microbiol Mol Biol Rev* 2010, **74**:363-377.

76. Rivers TM: **Viruses and Koch's Postulates**. *J Bacteriol* 1937, **33**:1-12.

77. Lipkin WI: **The changing face of pathogen discovery and surveillance**. *Nat Rev Microbiol* 2013, **11**:133-141.