Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER

15

Molecular Testing in Emerging Infectious Diseases

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BACKGROUND AND CATALOGUE OF EMERGING INFECTIOUS AGENTS

By the late 1960s there was a widespread opinion that the era of infectious diseases was finished and that vaccines and antibiotics had controlled microbial pathogens. Indeed, it was commonly believed that we had discovered the important agents of infections and that there was little left to do in this scientific field. "…The war on infectious diseases is over and we have won…" was an often repeated conclusion. Yet in the quarter of a century between 1967 and 1992 more than 30 previously unrecognized pathogens were discovered as the etiologic agents of human infectious diseases (Table 15.1). Some of the diseases were well characterized, but the causes had been unknown. Other novel syndromes were recognized and the etiologic agents identified including acquired immunodeficiency syndrome (AIDS) and human immunodeficiency virus (HIV). Nevertheless, the general belief was that infectious diseases were less important than cardiovascular diseases and cancer, and they were not favored for research support and public health attention.

In 1992, the concept of emerging infectious diseases was defined and brought to the attention of physicians and scientists by a very widely distributed and read publication from the Institute of Medicine of the National Academies of Sciences, *Emerging Infections: Microbial Threats to Health in the United States*. The emergence of at least 16 novel infectious agents over the following 12 years (Table 15.2) emphasized that this phenomenon would be a continued series of events. The causes of awareness of the presence of an unknown pathogen are the abrupt onset of a cluster of severe illness (eg, *Legionella* pneumonia at a convention of the American Legion), recognition of distinct gross or microscopic pathologic lesions (eg, pseudomembranous colitis caused by *Clostridium difficile*), and clinical laboratory microscopy (eg, intramonocytic inclusions of *Ehrlichia chaffeensis* in patients with human monocytotropic ehrlichiosis). In numerous other instances application of an advanced technologic method identified the etiology of a well-defined syndrome (eg, noroviruses in Norwalk diarrheal illness; an outbreak had occurred and samples retained from years earlier).

DISCOVERY OF EMERGING INFECTIOUS AGENTS USING MOLECULAR METHODS

Many methods have been employed for the initial detection and identification of novel emerging pathogens including microscopy, bacterial culture, cell culture, animal inoculation, electron microscopy, archaic serologic tests, cross-reactive serologic tests, serendipitous serologic testing, and immunohistochemistry. However, currently molecular methods including probe hybridization, polymerase chain reaction (PCR) that amplifies the target or the signal, and nucleic acid sequencing are the most prominent methods for detection and characterization of newly emerging pathogens, both for discovering the agent and for determining that it is truly novel [1–11].

An example of the application of molecular methods to the identification of previously unidentified

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TABLE 15.1 Chronological List of Emerged Infectious Agents/Diseases 1967–1992

Year	Agent	Agent characteristics	Disease	CDC molecular test name (test code)[a]	FDA-approved/cleared molecular test (manufacturer)[b]	Reference
1967	Marburg virus	Enveloped, single-stranded, negative sense RNA filovirus	Hemorrhagic fever	Marburg Identification (CDC-10349)	NA	1–5
1969	Lassa virus	Enveloped, single-stranded, bisegmented, ambisense RNA arenavirus	Hemorrhagic fever	Lassa Fever Identification (CDC-10343)	NA	1,6–8
1972	Norovirus	Nonenveloped, single-stranded RNA, viruses in the *Caliciviridae* family	Gastroenteritis	Norovirus Molecular Detection (CDC-10357), Norovirus Genotyping (CDC-10356), Norovirus Molecular Detection and Genotyping (CDC-10358)	NA	9
1973	Rotavirus	Double-stranded RNA virus. Five groups (A, B, C, D, and E); group A is the main human pathogen	Gastroenteritis	Rotavirus Molecular Detection and Genotyping (CDC-10410), Rotavirus Genotyping (CDC-10409)	NA	10–12
1975	Parvovirus B19	Nonenveloped, single-stranded DNA virus	Fifth disease or erythema infectiosum	Parvovirus B19 Molecular Detection (CDC-10363)	NA	13–15
1976	*Vibrio vulnificus*	Gram-negative, motile, curved, rod-shaped bacterium of the genus *Vibrio*	Vomiting, diarrhea, abdominal pain, and a blistering, cellulitis or septicemia	*Vibrio*, *Aeromonas*, and Related Organisms Study (CDC-10121), *Vibrio*, *Aeromonas*, and Related Organisms Identification (CDC-10120), *Vibrio* Subtyping (CDC-10122)	NA	16,17
1976	*Cryptosporidium parvum*	A protozoan	Cryptosporidiosis with symptoms including acute, watery, and nonbloody diarrhea	*Cryptosporidium* Special Study (CDC-10491)	NA	18–20
1977	Ebola virus	Enveloped, linear, single-stranded, negative-sense RNA filovirus	Hemorrhagic fever	Ebola Identification (CDC-10309)	FilmArray Biothreat-E test. Emergency Use Authorization (EUA) (Idaho Technology, Inc.)	2,5
1977	*Clostridium difficile*	A gram-positive bacterium	Colitis, diarrhea	*Clostridium difficile* Identification (CDC-10228), *Clostridium difficile* Outbreak Strain Typing (CDC-10229)	ICEPlex *C. difficile* Kit (PrimeraDx), IMDx *C. difficile* for Abbott m2000 (Intelligent Medical Devices, Inc.), BD Diagnostics BD MAX Cdiff Assay, (GeneOhm Sciences Canada Inc.), Quidel Molecular Direct *C. difficile* Assay, (Quidel Corporation), Verigene *C. difficile* Nucleic acid Test (Nanosphere, Inc.), Portrait Toxigenic *C. difficile* Assay (Great Basin Scientific, Inc.), Simplexa *C. difficile* Universal Direct Assay (Focus Diagnostics, Inc.), Xpert *C. difficile*/Epi (Cepheid),	21–23

					Illumigene *C. difficile* DNA Amplification Assay (Meridian Bioscience, Inc.), llumigene C. difficile Assay (Meridian Bioscience, Inc.), Xpert *C. difficile* (Cepheid), ProGastro Cd Assay (Prodesse, Inc.), BD GeneOhm Cdiff Assay (BD Diagnostics/GeneOhm Sciences, Inc.)	
1977	*Legionella pneumophila*	A thin, aerobic, pleomorphic, flagellated, non-spore forming, gram-negative bacterium of the genus *Legionella*	Legionnaires' disease	*Legionella* species Identification and Typing (CDC-10159), *Legionella* species Molecular Detection (CDC-10160), *Legionella* species Study (CDC-10161)	NA	24,25
1977	Hantaan virus	Single-stranded, enveloped, negative sense RNA viruses in the Bunyaviridae family	Hantavirus hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS)	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	26,27
1977	Hepatitis delta virus	A small circular enveloped RNA virus	Superimposed on conditions of hepatitis with HBV	Hepatitis D Serology, NAT, and Genotyping (CDC-10328)	NA	28,29
1977	*Campylobacter* sp. (or *jejuni*)	Curved, helical-shaped, non-spore forming, gram-negative, and microaerophilic bacteria	Campylobacteriosis, Guillain-Barré syndrome (GBS)	*Campylobacter* and *Helicobacter* Study (CDC-10125), *Campylobacter*, *Helicobacter*, and Related Organisms Identification (CDC-10126), *Campylobacter*, *Helicobacter*, and Related Organisms Identification and Subtyping (CDC-10127)	NA	30,31
1979	*Cyclospora cayetanensis*	An apicomplexan, cyst-forming coccidian protozoan	Cyclosporiasis, gastroenteritis	Cyclospora Molecular Detection (CDC-10477)	NA	32,33
1980	HTLV-1	A retrovirus of the human T-lymphotropic virus (HTLV) family	Adult T-cell lymphoma (ATL), HTLV-I-associated myelopathy, uveitis, *Strongyloides stercoralis* hyper-infection	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	34–37
1981	*Staphylococcus aureus* toxin	Exotoxins secreted by *S. aureus* that are compact, ellipsoidal proteins sharing a characteristic folding pattern with superantigen	Toxic shock syndrome	Staphylococcal Toxic Shock Syndrome Toxin (TSST-1) (CDC-10426)	NA	38–40
1982	*Borrelia burgdorferi*	A bacterial species of the spirochete class of the genus *Borrelia*	Lyme disease	*Borrelia* Culture and Identification (CDC-10299), *Borrelia* Special Study (CDC-10300)	NA	41,42

(*Continued*)

TABLE 15.1 (Continued)

Year	Agent	Agent characteristics	Disease	CDC molecular test name (test code)[a]	FDA-approved/cleared molecular test (manufacturer)[b]	Reference
1982	*Escherichia coli* O157:H7	An enterohemorrhagic serotype of the bacterium *E. coli*	Hemolytic-uremic syndrome (HUS)	*Escherichia* and *Shigella* Identification, Serotyping, and Virulence Profiling (CDC-10114), Bacterial Select Agent Identification and AST (CDC-10224)	NA	43,44
1983	HIV-1	A lentivirus (a subgroup of retrovirus)	Acquired immune deficiency syndrome (AIDS)	HIV Molecular Surveillance Study (International Only) (CDC-10332), HIV-1 Drug Resistance Special Study (International Only) (CDC-10334), HIV-1 Genotype Drug Resistance (International Only) (CDC-10335), HIV-1 Nucleic Acid Amplification (Qualitative) (CDC-10275), HIV-1 Nucleic Acid Amplification (Viral Load) (CDC-10276), HIV-1 PCR (International Only) Qualitative (CDC-10336), HIV-1 PCR (International Only) Quantitative Viral Load (CDC-10337)	Abbott RealTi*m*e HIV-1 Assay (Abbott Molecular, Inc.), COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche Molecular Systems), APTIMA HIV-1 RNA Qualitative Assay (Gen-Probe, Inc.), ViroSeq HIV-1 Genotyping System (Abbott Molecular, Inc.), TRUGENEHIV-1 genotyping Kit and OpenGeneDNA Sequencing System (Siemens Healthcare Diagnostics)	45,46
1983	*Helicobacter pylori*	A gram-negative, microaerophilic bacterium	Peptic ulcer, MALT lymphoma, gastric cancer	*Helicobacter pylori* Special Study (CDC-10117)	NA	47,48
1984	*Haemophilus influenzae* biogroup *aegyptius*	Phylogenetically the same as *H. influenzae*, a gram-negative, coccobacillary, facultatively anaerobic bacterium belonging to the *Pasteurellaceae* family	Acute and often purulent conjunctivitis (pink eye)	*Haemophilus influenzae* Identification and Serotyping (CDC-10221), *Haemophilus influenzae* Study (CDC-10222), *Haemophilus* species (Not *H. influenzae*/*H. ducreyi*) ID (DC-10141)	NA	49,50
1985	*Enterocytozoon bieneusi*	A unicellular, obligate intracellular eukaryote, a species of the order microsporida	Diarrhea	Microsporidia Molecular Identification (CDC-10481), Enteric Isolation—Primary Specimen (CDC-10106)	NA	20,51
1986	*Chlamydophila pneumoniae*	An obligate intracellular bacterium in the species of *Chlamydophila*	Pneumonia	*Chlamydophila pneumoniae* Molecular Detection (CDC-10152)	FilmArray Respiratory Panel (RP) (Idaho Technology, Inc.)	52,53
1988	Human herpesvirus 6	Double-stranded DNA virus within the betaherpesvirinae subfamily and of the genus *Roseolovirus*	Neuroinflammatory diseases such as multiple sclerosis, exanthem subitum (also known as roseola infantum or sixth disease), and encephalitis, bone marrow suppression and pneumonitis in transplant recipients	Human Herpes Virus 6 (HHV6) Detection and Subtyping (CDC-10266)	NA	54,55

1989	*Rickettsia japonica*	A genus of nonmotile, gram-negative, non-spore forming, highly pleomorphic bacteria	Japanese spotted fever	*Rickettsia* Molecular Detection (CDC-10402), *Rickettsia* Special Study (CDC-10405)	NA	56,57
1989	Hepatitis C virus	A small, enveloped, positive-sense single-stranded RNA virus of the family *Flaviviridae*	Hepatitis C	Hepatitis C Serology, NAT and Genotyping (CDC-10327)	Abbott RealTime HCV Genotype II (Abbott Molecular, Inc.), Abbott Realtime HCV Assay (Abbott Molecular, Inc.), COBAS AmpliPrep/ COBAS TaqMan HCV test (Roche Molecular Systems), Versant HCV 3.0 Assay (bDNA) (Siemens Healthcare Diagnostics), Versant HCV RNA Qualitative Assay (Gen-Probe, Inc.), COBAS AMPLICOR Hepatitis C Virus (HCV) Test (Roche Molecular Systems, Inc.), AMPLICOR HCV Test, v2.0 (Roche Molecular Systems, Inc.)	58,59
1990	Hepatitis E virus	A single-stranded positive-sense RNA, nonenveloped	Hepatitis	Hepatitis E Serology, NAT and Genotyping (CDC-10329)	NA	60,61
1990	*Balamuthia mandrillaris*	A free-living leptomyxid amoeba	Amoebiasis including granulomatous amoebic encephalitis (GAE)	*Balamuthia* Molecular Detection (CDC-10474), Ameba Identification (*Acanthamoeba*, *Balamuthia*, *Naegleria*) (CDC-10286)	NA	62,63
1990	Human herpesvirus 7	A member of Betaherpesviridae, a subfamily of the Herpesviridae	Exanthema subitum, acute febrile diseases	Human Herpes Virus 7 (HHV7) Detection (CDC-10267)	NA	64,65
1991	Guanarito virus	Enveloped, single-stranded, bisegmented RNA viruses with ambisense genomes	Venezuelan hemorrhagic fever	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	66,67
1991	*Encephalitozoon hellem*	A unicellular, intracellular microsporidian species	Keratoconjunctivitis, infection of respiratory and genitourinary tract, and disseminated infection	Microsporidia Molecular Identification (CDC-10481)	NA	68,69
1991	*Ehrlichia chaffeensis*	An obligately intracellular gram-negative rickettsial bacterium	Human monocytotropic ehrlichiosis	*Anaplasma* and *Ehrlichia* Molecular Detection (CDC-10290), *Anaplasma* and *Ehrlichia* Special Study (CDC-10291)	NA	70–72

[a]*CDC molecular test name (test code) are available from the Center for Disease Control and Prevention Test Directory, http://www.cdc.gov/laboratory/specimen-submission/list.html#M (last accessed 12/19/2014).*
[b]*FDA-approved/cleared molecular test (manufacturer) are available from the US Food and Drug Administration at http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm (last accessed 12/19/2014).*
NA, not available.
References:

1. Drosten C, Gottig S, Schilling S, et al. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. *J Clin Microbiol*. 2002;40(7):2323–2330.
2. Koehler JW, Hall AT, Rolfe PA, et al. Development and evaluation of a panel of filovirus sequence capture probes for pathogen detection by next-generation sequencing. *PLoS One*. 2014;9(9):e107007.
3. Muhlberger E, Trommer S, Funke C, Volchkov V, Klenk HD, Becker S. Termini of all mRNA species of Marburg virus: sequence and secondary structure. *Virology*. 1996;223(2):376–380.
4. Towner JS, Khristova ML, Sealy TK, et al. Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola. *J Virol*. 2006;80(13):6497–6516.

(*Continued*)

TABLE 15.1 (Continued)

5. Euler M, Wang Y, Heidenreich D, et al. Development of a panel of recombinase polymerase amplification assays for detection of biothreat agents. *J Clin Microbiol*. 2013;51(4):1110–1117.
6. Asogun DA, Adomeh DI, Ehimuan J, et al. Molecular diagnostics for Lassa fever at Irrua specialist teaching hospital, Nigeria: lessons learnt from two years of laboratory operation. *PLoS Negl Trop Dis*. 2012;6(9):e1839.
7. Djavani M, Lukashevich IS, Sanchez A, Nichol ST, Salvato MS. Completion of the Lassa fever virus sequence and identification of a RING finger open reading frame at the L RNA 5′ end. *Virology*. 1997;235 (2):414–418.
8. Ehichioya DU, Asogun DA, Ehimuan J, et al. Hospital-based surveillance for Lassa fever in Edo State, Nigeria, 2005–2008. *Trop Med Int Health*. 2012;17(8):1001–1004.
9. Cotten M, Petrova V, Phan MV, et al. Deep sequencing of norovirus genomes defines evolutionary patterns in an urban tropical setting. *J Virol*. 2014;88(19):11056–11069.
10. Martinez MA, Soto-Del Rio MD, Gutierrez RM, et al. DNA microarray for detection of gastrointestinal viruses. *J Clin Microbiol*. 2014.
11. Moore NE, Wang J, Hewitt J, et al. Metagenomic analysis of viruses in feces from unsolved outbreaks of gastroenteritis in humans. *J Clin Microbiol*. 2014.
12. Ye S, Lambert SB, Grimwood K, et al. Comparison of test specificity in commercial antigen and in-house PCR methods for rotavirus detection in stool specimens. *J Clin Microbiol*. 2014.
13. Bonvicini F, Manaresi E, Bua G, Venturoli S, Gallinella G. Keeping pace with parvovirus B19 genetic variability: a multiplex genotype-specific quantitative PCR assay. *J Clin Microbiol*. 2013;51 (11):3753–3759.
14. Maple PA, Hedman L, Dhanilall P, et al. Identification of past and recent parvovirus B19 infection in immunocompetent individuals by quantitative PCR and enzyme immunoassays: a dual-laboratory study. *J Clin Microbiol*. 2014;52(3):947–956.
15. Plentz A, Wurdinger M, Kudlich M, Modrow S. Low-level DNAemia of parvovirus B19 (genotypes 1-3) in adult transplant recipients is not associated with anaemia. *J Clin Virol: the official publication of the Pan American Society for Clinical Virology*. 2013;58(2):443–448.
16. Cruz CD, Win JK, Fletcher GC. An improved method for quantification of *Vibrio vulnificus* in oysters. *J Microbiol Methods*. 2013;95(3):397–399.
17. Wei S, Zhao H, Xian Y, Hussain MA, Wu X. Multiplex PCR assays for the detection of *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio cholerae* with an internal amplification control. *Diagn Microbiol Infect Dis*. 2014;79(2):115–118.
18. Mazurie AJ, Alves JM, Ozaki LS, Zhou S, Schwartz DC, Buck GA. Comparative genomics of cryptosporidium. *Int J Genomics*. 2013;2013:832756.
19. Silva SO, Richtzenhain LJ, Barros IN, et al. A new set of primers directed to 18S rRNA gene for molecular identification of *Cryptosporidium* spp. and their performance in the detection and differentiation of oocysts shed by synanthropic rodents. *Exp Parasitol*. 2013;135(3):551–557.
20. Rubio JM, Lanza M, Fuentes I, Soliman RH. A novel nested multiplex PCR for the simultaneous detection and differentiation of *Cryptosporidium* spp., *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. *Parasitol Int*. 2014;63(5):664–669.
21. Dingle KE, Elliott B, Robinson E, et al. Evolutionary history of the *Clostridium difficile* pathogenicity locus. *Genome Biol Evol*. 2014;6(1):36–52.
22. Eyre DW, Cule ML, Wilson DJ, et al. Diverse sources of *C. difficile* infection identified on whole-genome sequencing. *N Engl J Med*. 2013;369(13):1195–1205.
23. Leibowitz J, Soma VL, Rosen L, Ginocchio CC, Rubin LG. Similar proportions of stool specimens from hospitalized children with and without diarrhea test positive for *Clostridium difficile*. *Pediatr Infect Dis J*. 2014.
24. Gomez-Valero L, Rusniok C, Rolando M, et al. Comparative analyses of *Legionella* species identifies genetic features of strains causing Legionnaires inverted question mark disease. *Genome Biol*. 2014;15 (11):505.
25. Sanchez-Buso L, Comas I, Jorques G, Gonzalez-Candelas F. Recombination drives genome evolution in outbreak-related *Legionella pneumophila* isolates. *Nat Genet*. 2014;46(11):1205–1211.
26. Noh JY, Cheong HJ, Song JY, et al. Clinical and molecular epidemiological features of hemorrhagic fever with renal syndrome in Korea over a 10-year period. *J Clin Virol: the official publication of the Pan American Society for Clinical Virology*. 2013;58(1):11–17.
27. Wang ML, Lai JH, Zhu Y, et al. Genetic susceptibility to haemorrhagic fever with renal syndrome caused by Hantaan virus in Chinese Han population. *Int J Immunogenet*. 2009;36(4):227–229.
28. Karatayli E, Altunoglu YC, Karatayli SC, et al. A one step real time PCR method for the quantification of hepatitis delta virus RNA using an external armored RNA standard and intrinsic internal control. *J Clin Virol: the official publication of the Pan American Society for Clinical Virology*. 2014;60(1):11–15.
29. Le Gal F, Gault E, Ripault MP, et al. Eighth major clade for hepatitis delta virus. *Emerg Infect Dis*. 2006;12(9):1447–1450.
30. Taboada EN, Clark CG, Sproston EL, Carrillo CD. Current methods for molecular typing of *Campylobacter* species. *J Microbiol Methods*. 2013;95(1):24–31.
31. Vondrakova L, Pazlarova J, Demnerova K. Detection, identification and quantification of *Campylobacter jejuni*, *coli* and *lari* in food matrices all at once using multiplex qPCR. *Gut Pathog*. 2014;6:12.
32. Riner DK, Nichols T, Lucas SY, Mullin AS, Cross JH, Lindquist HD. Intragenomic sequence variation of the ITS-1 region within a single flow-cytometry-counted *Cyclospora cayetanensis* oocysts. *J Parasitol*. 2010;96(5):914–919.
33. Zhou Y, Lv B, Wang Q, et al. Prevalence and molecular characterization of *Cyclospora cayetanensis*, Henan, China. *Emerg Infect Dis*. 2011;17(10):1887–1890.
34. Brunetto GS, Massoud R, Leibovitch EC, et al. Digital droplet PCR (ddPCR) for the precise quantification of human T-lymphotropic virus 1 proviral loads in peripheral blood and cerebrospinal fluid of HAM/TSP patients and identification of viral mutations. *J Neurovirol*. 2014;20(4):341–351.
35. Firouzi S, Lopez Y, Suzuki Y, et al. Development and validation of a new high-throughput method to investigate the clonality of HTLV-1-infected cells based on provirus integration sites. *Genome Med*. 2014;6(6):46.
36. Pessoa R, Watanabe JT, Nukui Y, et al. Molecular characterization of human T-cell lymphotropic virus type 1 full and partial genomes by Illumina massively parallel sequencing technology. *PLoS One*. 2014;9(3):e93374.
37. Ratner L, Philpott T, Trowbridge DB. Nucleotide sequence analysis of isolates of human T-lymphotropic virus type 1 of diverse geographical origins. *AIDS Res Hum Retroviruses*. 1991;7(11):923–941.
38. Hait J, Tallent S, Melka D, Keys C, Bennett R. Prevalence of enterotoxins and toxin gene profiles of *Staphylococcus aureus* isolates recovered from a bakery involved in a second staphylococcal food poisoning occurrence. *J Appl Microbiol*. 2014;117(3):866–875.
39. Hait JM, Tallent SM, Bennett RW. Screening, detection, and serotyping methods for toxin genes and enterotoxins in *Staphylococcus* strains. *J AOAC Int*. 2014;97(4):1078–1083.

40. Leopold SR, Goering RV, Witten A, Harmsen D, Mellmann A. Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. *J Clin Microbiol*. 2014;52(7):2365–2370.
41. Clark KL, Leydet BF, Threlkeld C. Geographical and genospecies distribution of *Borrelia burgdorferi sensu lato* DNA detected in humans in the USA. *J Med Microbiol*. 2014;63(Pt 5):674–684.
42. Jacquot M, Gonnet M, Ferquel E, et al. Comparative population genomics of the *Borrelia burgdorferi* species complex reveals high degree of genetic isolation among species and underscores benefits and constraints to studying intra-specific epidemiological processes. *PLoS One*. 2014;9(4):e94384.
43. Brewster JD, Paoli GC. DNA extraction protocol for rapid PCR detection of pathogenic bacteria. *Anal Biochem*. 2013;442(1):107–109.
44. Rump LV, Gonzalez-Escalona N, Ju W, et al. Genomic diversity and virulence characterization of historical *Escherichia coli* O157 strains isolated from clinical and environmental sources. *Appl Environ Microbiol*. 2014.
45. Casabianca A, Orlandi C, Canovari B, et al. A real time PCR platform for the simultaneous quantification of total and extrachromosomal HIV DNA forms in blood of HIV-1 infected patients. *PLoS One*. 2014;9(11):e111919.
46. Di Giallonardo F, Zagordi O, Duport Y, et al. Next-generation sequencing of HIV-1 RNA genomes: determination of error rates and minimizing artificial recombination. *PLoS One*. 2013;8(9):e74249.
47. Kao CY, Lee AY, Huang AH, et al. Heteroresistance of *Helicobacter pylori* from the same patient prior to antibiotic treatment. *Infect Genet Evol*. 2014;23:196–202.
48. Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G. Diagnosis of *Helicobacter pylori*: What should be the gold standard? *World J Gastroenterol*. 2014;20(36):12847–12859.
49. Quentin R, Ruimy R, Rosenau A, Musser JM, Christen R. Genetic identification of cryptic genospecies of *Haemophilus* causing urogenital and neonatal infections by PCR using specific primers targeting genes coding for 16S rRNA. *J Clin Microbiol*. 1996;34(6):1380–1385.
50. Strouts FR, Power P, Croucher NJ, et al. Lineage-specific virulence determinants of *Haemophilus influenzae* biogroup aegyptius. *Emerg Infect Dis*. 2012;18(3):449–457.
51. Subrungruang I, Mungthin M, Chavalitshewinkoon-Petmitr P, Rangsin R, Naaglor T, Leelayoova S. Evaluation of DNA extraction and PCR methods for detection of *Enterocytozoon bienuesi* in stool specimens. *J Clin Microbiol*. 2004;42(8):3490–3494.
52. Benitez AJ, Thurman KA, Diaz MH, Conklin L, Kendig NE, Winchell JM. Comparison of real-time PCR and a microimmunofluorescence serological assay for detection of *Chlamydophila pneumoniae* infection in an outbreak investigation. *J Clin Microbiol*. 2012;50(1):151–153.
53. Ravindranath BS, Krishnamurthy V, Krishna V, C SK. In silico synteny based comparative genomics approach for identification and characterization of novel therapeutic targets in *Chlamydophila pneumoniae*. *Bioinformation*. 2013;9(10):506–510.
54. Debaugnies F, Busson L, Ferster A, et al. Detection of Herpesviridae in whole blood by multiplex PCR DNA-based microarray analysis after hematopoietic stem cell transplantation. *J Clin Microbiol*. 2014;52(7):2552–2556.
55. Sedlak RH, Cook L, Huang ML, et al. Identification of chromosomally integrated human herpesvirus 6 by droplet digital PCR. *Clin Chem*. 2014;60(5):765–772.
56. Hanaoka N, Matsutani M, Kawabata H, et al. Diagnostic assay for *Rickettsia japonica*. *Emerg Infect Dis*. 2009;15(12):1994–1997.
57. Matsutani M, Ogawa M, Takaoka N, et al. Complete genomic DNA sequence of the East Asian spotted fever disease agent *Rickettsia japonica*. *PLoS One*. 2013;8(9):e71861.
58. Fevery B, Susser S, Lenz O, et al. HCV RNA quantification with different assays: implications for protease-inhibitor-based response-guided therapy. *Antivir Ther*. 2014.
59. Quer J, Gregori J, Rodriguez-Frias F, et al. High-resolution hepatitis C virus (HCV) subtyping, using NS5B deep sequencing and phylogeny, an alternative to current methods. *J Clin Microbiol*. 2014.
60. Vollmer T, Knabbe C, Dreier J. Comparison of real-time PCR and antigen assays for detection of hepatitis E virus in blood donors. *J Clin Microbiol*. 2014;52(6):2150–2156.
61. Zhou X, Wang Y, Metselaar HJ, Janssen HL, Peppelenbosch MP, Pan Q. Rapamycin and everolimus facilitate hepatitis E virus replication: revealing a basal defense mechanism of PI3K-PKB-mTOR pathway. *J Hepatol*. 2014;61(4):746–754.
62. da Rocha-Azevedo B, Tanowitz HB, Marciano-Cabral F. Diagnosis of infections caused by pathogenic free-living amoebae. *Interdiscip Perspect Infect Dis*. 2009;2009:251406.
63. Lares-Jimenez LF, Booton GC, Lares-Villa F, Velazquez-Contreras CA, Fuerst PA. Genetic analysis among environmental strains of *Balamuthia mandrillaris* recovered from an artificial lagoon and from soil in Sonora, Mexico. *Exp Parasitol*. 2014.
64. Donaldson CD, Clark DA, Kidd IM, Breuer J, Depledge DD. Genome sequence of human herpesvirus 7 strain UCL-1. *Genome Announc*. 2013;1(5).
65. Oakes B, Hoagland-Henefield M, Komaroff AL, Erickson JL, Huber BT. Human endogenous retrovirus-K18 superantigen expression and human herpesvirus-6 and human herpesvirus-7 viral loads in chronic fatigue patients. *Clin Infect Dis: an official publication of the Infectious Diseases Society of America*. 2013;56(10):1394–1400.
66. Fulhorst CF, Cajimat MN, Milazzo ML, et al. Genetic diversity between and within the arenavirus species indigenous to western Venezuela. *Virology*. 2008;378(2):205–213.
67. Vieth S, Drosten C, Charrel R, Feldmann H, Gunther S. Establishment of conventional and fluorescence resonance energy transfer-based real-time PCR assays for detection of pathogenic New World arenaviruses. *J Clin Virol: the official publication of the Pan American Society for Clinical Virology*. 2005;32(3):229–235.
68. Hester JD, Varma M, Bobst AM, Ware MW, Lindquist HD, Schaefer FW, 3rd. Species-specific detection of three human-pathogenic microsporidial species from the genus *Encephalitozoon* via fluorogenic 5′ nuclease PCR assays. *Mol Cell Probes*. 2002;16(6):435–444.
69. Pombert JF, Selman M, Burki F, et al. Gain and loss of multiple functionally related, horizontally transferred genes in the reduced genomes of two microsporidian parasites. *Proc Natl Acad Sci USA*. 2012;109(31):12638–12643.
70. Breitschwerdt EB, Hegarty BC, Qurollo BA, et al. Intravascular persistence of *Anaplasma platys*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii* DNA in the blood of a dog and two family members. *Parasit Vectors*. 2014;7:298.
71. Doyle CK, Labruna MB, Breitschwerdt EB, et al. Detection of medically important *Ehrlichia* by quantitative multicolor TaqMan real-time polymerase chain reaction of the *dsb* gene. *J Mol Diagn*. 2005;7(4):504–510.
72. Walker DH, Paddock CD, Dumler JS. Emerging and re-emerging tick-transmitted rickettsial and ehrlichial infections. *Med Clin North Am*. 2008;92(6):1345–1361, x.

TABLE 15.2 Chronological List of Emerged Infectious Agents/Diseases Since 1992

Year	Agent	Agent characteristics	Disease	CDC molecular test name (test code)[a]	FDA-approved/cleared molecular test (manufacturer)[b]	Reference
1992	Barmah Forest virus	An *Alphavirus* (small, spherical, enveloped viruses with a genome of a single-strand positive-sense RNA)	Epidemic polyarthritis (fever, malaise, rash, joint pain, and muscle tenderness)	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	1,2
1992	*Vibrio cholerae* O139	A gram-negative, comma-shaped bacterium	Watery diarrhea and vomiting	*Vibrio cholerae* Identification (CDC-10119), *Vibrio* Subtyping (CDC-10122), *Vibrio*, *Aeromonas*, and Related Organisms Identification (CDC-10120), *Vibrio*, *Aeromonas*, and Related Organisms Study (CDC-10121)	NA	3,4
1992	*Bartonella henselae*	A proteobacterium	Cat-scratch disease, subacute regional lymphadenitis	*Bartonella* Molecular Identification (CDC-10295), *Bartonella* Special Study (CDC-10297)	NA	5,6
1992	*Rickettsia honei*	Nonmotile, obligately intracellular, gram-negative, non-spore forming bacteria	Flinders Island spotted fever	*Rickettsia* Molecular Detection (CDC-10402), *Rickettsia* Special Study (CDC-10405)	NA	7–9
1992	Sabia virus	An arenavirus (round, pleomorphic, and enveloped virus containing a beaded nucleocapsid with two single-stranded RNA segments)	Hemorrhagic fever	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	10,11
1993	*Encephalitozoon intestinalis*	A parasite	Diarrhea	Microsporidia Molecular Identification (CDC-10481), Enteric Isolation—Primary Specimen (CDC-10106)	NA	12,13
1993	Sin Nombre virus	A single-stranded RNA negative-strand virus	Hantavirus cardiopulmonary syndrome (HCPS)	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	14,15
1994	Human herpesvirus 8	A double-stranded DNA virus	Kaposi sarcoma	Human Herpes Virus 8 (HHV8) Detection (CDC-10268)	NA	16,17
1994	*Anaplasma phagocytophilum*	An obligately intracellular gram-negative bacterium	Human granulocytic anaplasmosis	*Anaplasma* and *Ehrlichia* Molecular Detection (CDC-10290), *Anaplasma* and *Ehrlichia* Special Study (CDC-10291)	NA	18,19
1994	*Rickettsia felis*	Nonmotile, obligately intracellular, gram-negative, non-spore forming bacteria	Flea-borne spotted fever	*Rickettsia* Molecular Detection (CDC-10402), *Rickettsia* Special Study (CDC-10405)	NA	7–9

1994	*Rickettsia africae*	Nonmotile, obligately intracellular, gram-negative, non-spore forming bacteria	African tick bite fever	*Rickettsia* Molecular Detection (CDC-10402), *Rickettsia* Special Study (CDC-10405)	NA	7–9
1995	Hendra virus	Nonsegmented, single-stranded negative-sense RNA	Edema and hemorrhage of the lungs, encephalitis	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	20,21
1995	Alkhumra virus	Enveloped virus with monopartite, linear, single-stranded RNA genomes	Tick-borne hemorrhagic fever	Alkhumra Identification (CDC-10274)	NA	22,23
1997	*Rickettsia slovaca*	Nonmotile, obligately intracellular, gram-negative, non-spore forming bacteria	Tick-borne lymphadenopathy	*Rickettsia* Molecular Detection (CDC-10402), *Rickettsia* Special Study (CDC-10405)	NA	7–9
1999	Nipah virus	Nonsegmented, single-stranded negative-sense RNA	Respiratory, gastrointestinal and neurologic symptoms, encephalitis	Nipah Virus Identification (CDC-10354)	NA	20,24
1999	West Nile virus	A positive-sense, single-stranded RNA virus	West Nile fever, encephalitis	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	25,26
1999	*Ehrlichia ewingii*	An obligately intracellular gram-negative rickettsial bacteriaum	Ehrlichiosis ewingii infection	*Anaplasma* and *Ehrlichia* Molecular Detection (CDC-10290), *Anaplasma* and *Ehrlichia* Special Study (CDC-10291)	NA	9,27,28
2001	Human metapneumovirus	A negative-sense, single-stranded RNA virus	Pneumonia	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	Quidel Molecular RSV + hMPV Assay (Quidel Corporation), Quidel Molecular hMPV Assay (Quidel Corporation), Pro hMPV + Assay (Prodesse, Inc.), FilmArray Respiratory Panel (RP) (Idaho Technology, Inc.), xTAG Respiratory Viral Panel (RVP) (Luminex Molecular Diagnostics, Inc.), xTAG Respiratory Viral Panel Fast (RVP FAST) (Luminex Molecular Diagnostics, Inc.) eSensor Respiratory Viral Panel (RVP) (GenMark Diagnostic), ProFlu + Assay (Gen-Probe Prodesse, Inc.)	29,30

(*Continued*)

TABLE 15.2 (Continued)

Year	Agent	Agent characteristics	Disease	CDC molecular test name (test code)[a]	FDA-approved/cleared molecular test (manufacturer)[b]	Reference
2003	Monkeypox virus	A double-stranded DNA virus	Febrile enanthem	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	31,32
2003	SARS coronavirus	A positive-sense and single-stranded RNA virus	Severe acute respiratory syndrome (SARS)	SARS Molecular Detection (CDC-10412)	NA	33–38
2004	*Rickettsia parkeri*	Nonmotile, obligately intracellular, gram-negative, non-spore forming bacteria	American tick bite fever	*Rickettsia* Molecular Detection (CDC-10402), *Rickettsia* Special Study (CDC-10405)	NA	7–9
2005	Human retroviruses (HTLV-3/4)	Human retroviruses	Unclear association with disease	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	39,40
2005	Human bocavirus	A linear, nonsegmented single-stranded DNA viruses	Unclear association with disease	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	41,42
2008	*Plasmodium knowlesi*	A primate malaria parasite	Malaria	Malaria Surveillance (CDC-10235)	NA	43,44
2008	Lujo virus	A bisegmented RNA arenavirus	Viral hemorrhagic fever (VHF)	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	45,46
2008	Chapare virus	Enveloped, single-stranded, bisegmented, ambisense RNA arenavirus	Hemorrhagic fever	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	47,48
2009	*Ehrlichia muris*–like	An obligate intracellular gram-negative rickettsial bacteriaum	Ehrlichiosis	*Anaplasma* and *Ehrlichia* Molecular Detection (CDC-10290), *Anaplasma* and *Ehrlichia* Special Study (CDC-10291), Bacterial ID of Unknown Isolate (Not Strict Anaerobe) (CDC-10145), Bacterial ID from Clinical Specimen (16S rRNA PCR) (CDC-10146)	NA	49,50
2009	Pandemic H1N1 influenza virus	A new influenza A subtype H1N1 RNA virus, having hemagglutinin (HA) of the H1 subtype and neuraminidase (NA) of the N1 subtype	Flu, pneumonia, acute respiratory distress syndrome (ARDS)	Pathologic Evaluation of Influenza and Other Viral Infections (CDC-10366)	Prodesse ProFAST Assat (Gen-Probe Prodesse, Inc.), Quidel Molecular Influenza A + B Assay (Quidel Corporation), IMDx Flu A/B and RSV for Abbott m2000 (Intelligent Medical Devices, Inc.), CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (CDC), Xpert Flu Assay (Cepheid),	51,52

					Simplexa Flu A/B & RSV Direct (Focus Diagnostics, Inc.), FilmArray Respiratory Panel (RP) (Idaho Technology, Inc.), artus Infl A/B RG RT-PCR Kit (Qiagen GmbH), JBAIDS Influenza A Subtyping Kit (US Army Medical Materiel Development Activity), JBAIDS Influenza A&B Detection Kit (US Army Medical Materiel Development Activity), eSensor Respiratory Viral Panel (RVP) (GenMark Diagnostic), ProFlu + Assay (Gen-Probe Prodesse, Inc.), Verigene Respiratory Virus Plus Nucleic Acid Test (RV +) (Nanosphere, Inc.), Simplexa Flu A/B & RSV (Focus Diagnostics, Inc.), CDC Influenza 2009 A (H1N1) pdm Real-Time RT-PCR Panel (CDC), Simplexa Influenza A H1N1 (2009) (Focus Diagnostics, Inc.)	
2010	*Candidatus* Neoehrlichia mikurensis	An obligately intracellular gram-negative rickettsial bacteriaum	Ehrlichiosis-like syndrome	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	53,54
2011	Severe fever with thrombocytopenia virus	Negative-stranded, enveloped RNA virus	Severe fever with thrombocytopenia syndrome (SFTS)	Pathologic Evaluation of Unexplained Illness Due to Possible Infectious Etiology (CDC-10372)	NA	55,56
2012	Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV)	Positive-sense, single-stranded RNA coronavirus	Middle East respiratory syndrome	MERS-CoV PCR 9 (CDC-10488)	NA	57,58
2013	Novel H7N9 influenza virus (China)	A new influenza A subtype H7N9 RNA virus, having HA of the H7 subtype and NA of the N9 subtype	Flu, pneumonia, acute respiratory distress syndrome (ARDS)	Pathologic Evaluation of Influenza and Other Viral Infections (CDC-10366)	NA	59

[a]*CDC molecular test name (test code) are available from the Center for Disease Control and Prevention Test Directory, http://www.cdc.gov/laboratory/specimen-submission/list.html#M (last accessed 12/19/2014).*
[b]*FDA-approved/cleared molecular test (manufacturer) are available from the US Food and Drug Administration at http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm (last accessed 12/19/2014).*
NA, not available.

(*Continued*)

TABLE 15.2 (Continued)

References:

1. Lee E, Stocks C, Lobigs P, et al. Nucleotide sequence of the Barmah Forest virus genome. *Virology*. 1997;227(2):509–514.
2. Poidinger M, Roy S, Hall RA, et al. Genetic stability among temporally and geographically diverse isolates of Barmah Forest virus. *Am J Trop Med Hyg*. 1997;57(2):230–234.
3. Pang B, Zheng X, Diao B, et al. Whole genome PCR scanning reveals the syntenic genome structure of toxigenic *Vibrio cholerae* strains in the O1/O139 population. *PLoS One*. 2011;6(8):e24267.
4. Zhao J, Kang L, Hu R, et al. Rapid oligonucleotide suspension array-based multiplex detection of bacterial pathogens. *Foodborne Pathog Dis*. 2013;10(10):896–903.
5. Lantos PM, Maggi RG, Ferguson B, et al. Detection of *Bartonella* species in the blood of veterinarians and veterinary technicians: a newly recognized occupational hazard? *Vector Borne Zoonotic Dis*. 2014;14 (8):563–570.
6. Psarros G, Riddell Jt, Gandhi T, Kauffman CA, Cinti SK. *Bartonella henselae* infections in solid organ transplant recipients: report of 5 cases and review of the literature. *Medicine (Baltimore)*. 2012;91 (2):111–121.
7. Renvoise A, Rolain JM, Socolovschi C, Raoult D. Widespread use of real-time PCR for rickettsial diagnosis. *FEMS Immunol Med Microbiol*. 2012;64(1):126–129.
8. Sekeyova Z, Roux V, Raoult D. Phylogeny of *Rickettsia* spp. inferred by comparing sequences of 'gene D', which encodes an intracytoplasmic protein. *Int J Syst Evol Microbiol*. 2001;51(Pt 4):1353–1360.
9. Walker DH, Paddock CD, Dumler JS. Emerging and re-emerging tick-transmitted rickettsial and ehrlichial infections. *Med Clin North Am*. 2008;92(6):1345–1361, x.
10. Gonzalez JP, Bowen MD, Nichol ST, Rico-Hesse R. Genetic characterization and phylogeny of Sabia virus, an emergent pathogen in Brazil. *Virology*. 1996;221(2):318–324.
11. Vieth S, Drosten C, Charrel R, Feldmann H, Gunther S. Establishment of conventional and fluorescence resonance energy transfer-based real-time PCR assays for detection of pathogenic New World arenaviruses. *J Clin Virol: the official publication of the Pan American Society for Clinical Virology*. 2005;32(3):229–235.
12. Galvan A, Magnet A, Izquierdo F, Fenoy S, Henriques-Gil N, del Aguila C. Variability in minimal genomes: analysis of tandem repeats in the microsporidia *Encephalitozoon intestinalis*. *Infect Genet Evol*. 2013;20:26–33.
13. Rubio JM, Lanza M, Fuentes I, Soliman RH. A novel nested multiplex PCR for the simultaneous detection and differentiation of *Cryptosporidium* spp., *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. *Parasitol Int*. 2014;63(5):664–669.
14. Black WCt, Doty JB, Hughes MT, Beaty BJ, Calisher CH. Temporal and geographic evidence for evolution of Sin Nombre virus using molecular analyses of viral RNA from Colorado, New Mexico and Montana. *Virol J*. 2009;6:102.
15. Henderson WW, Monroe MC, St Jeor SC, et al. Naturally occurring Sin Nombre virus genetic reassortants. *Virology*. 1995;214(2):602–610.
16. Dollard SC, Roback JD, Gunthel C, et al. Measurements of human herpesvirus 8 viral load in blood before and after leukoreduction filtration. *Transfusion*. 2013;53(10):2164–2167.
17. Speicher DJ, Johnson NW. Detection of human herpesvirus 8 by quantitative polymerase chain reaction: development and standardisation of methods. *BMC Infect Dis*. 2012;12:210.
18. Chan K, Marras SA, Parveen N. Sensitive multiplex PCR assay to differentiate Lyme spirochetes and emerging pathogens *Anaplasma phagocytophilum* and *Babesia microti*. *BMC Microbiol*. 2013;13:295.
19. Stuen S, Granquist EG, Silaghi C. *Anaplasma phagocytophilum*—a widespread multi-host pathogen with highly adaptive strategies. *Front Cell Infect Microbiol*. 2013;3:31.
20. Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. *Curr Top Microbiol Immunol*. 2012;359:179–196.
21. Yu M, Hansson E, Shiell B, Michalski W, Eaton BT, Wang LF. Sequence analysis of the Hendra virus nucleoprotein gene: comparison with other members of the subfamily Paramyxovirinae. *J Gen Virol*. 1998;79 (Pt 7):1775–1780.
22. Madani TA, Abuelzein el TM, Azhar EI, Al-Bar HM, Abu-Araki H, Ksiazek TG. Comparison of RT-PCR assay and virus isolation in cell culture for the detection of Alkhumra hemorrhagic fever virus. *J Med Virol*. 2014;86(7):1176–1180.
23. Madani TA, Azhar EI, Abuelzein el TM, et al. Complete genome sequencing and genetic characterization of Alkhumra hemorrhagic fever virus isolated from Najran, Saudi Arabia. *Intervirology*. 2014;57 (5):300–310.
24. Harcourt BH, Tamin A, Ksiazek TG, et al. Molecular characterization of Nipah virus, a newly emergent paramyxovirus. *Virology*. 2000;271(2):334–349.
25. Lim SM, Koraka P, Osterhaus AD, Martina BE. Development of a strand-specific real-time qRT-PCR for the accurate detection and quantitation of West Nile virus RNA. *J Virol Methods*. 2013;194 (1–2):146–153.
26. Pisani G, Pupella S, Cristiano K, et al. Detection of West Nile virus RNA (lineages 1 and 2) in an external quality assessment programme for laboratories screening blood and blood components for West Nile virus by nucleic acid amplification testing. *Blood Transfus*. 2012;10(4):515–520.
27. Breitschwerdt EB, Hegarty BC, Qurollo BA, et al. Intravascular persistence of *Anaplasma platys*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii* DNA in the blood of a dog and two family members. *Parasit Vectors*. 2014;7:298.
28. Doyle CK, Labruna MB, Breitschwerdt EB, et al. Detection of medically important *Ehrlichia* by quantitative multicolor TaqMan real-time polymerase chain reaction of the *dsb* gene. *J Mol Diagn*. 2005;7 (4):504–510.
29. Klemenc J, Asad Ali S, Johnson M, et al. Real-time reverse transcriptase PCR assay for improved detection of human metapneumovirus. *J Clin Virol: the official publication of the Pan American Society for Clinical Virology*. 2012;54(4):371–375.
30. Roussy JF, Carbonneau J, Ouakki M, et al. Human metapneumovirus viral load is an important risk factor for disease severity in young children. *J Clin Virol: the official publication of the Pan American Society for Clinical Virology*. 2014;60(2):133–140.
31. Grant RJ, Baldwin CD, Nalca A, et al. Application of the Ibis-T5000 pan-Orthopoxvirus assay to quantitatively detect monkeypox viral loads in clinical specimens from macaques experimentally infected with aerosolized monkeypox virus. *Am J Trop Med Hyg*. 2010;82(2):318–323.
32. Li Y, Olson VA, Laue T, Laker MT, Damon IK. Detection of monkeypox virus with real-time PCR assays. *J Clin Virol: the official publication of the Pan American Society for Clinical Virology*. 2006;36(3):194–203.

33. Adachi D, Johnson G, Draker R, et al. Comprehensive detection and identification of human coronaviruses, including the SARS-associated coronavirus, with a single RT-PCR assay. *J Virol Methods*. 2004;122(1):29−36.
34. Huang JL, Lin HT, Wang YM, et al. Rapid and sensitive detection of multiple genes from the SARS-coronavirus using quantitative RT-PCR with dual systems. *J Med Virol*. 2005;77(2):151−158.
35. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med*. 2003;348(20):1967−1976.
36. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med*. 2003;348(20):1953−1966.
37. Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet*. 2003;361(9366):1319−1325.
38. Rota PA, Oberste MS, Monroe SS, et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science*. 2003;300(5624):1394−1399.
39. Mahieux R, Gessain A. HTLV-3/STLV-3 and HTLV-4 viruses: discovery, epidemiology, serology and molecular aspects. *Viruses*. 2011;3(7):1074−1090.
40. Moens B, Lopez G, Adaui V, et al. Development and validation of a multiplex real-time PCR assay for simultaneous genotyping and human T-lymphotropic virus type 1, 2, and 3 proviral load determination. *J Clin Microbiol*. 2009;47(11):3682−3691.
41. Christensen A, Dollner H, Skanke LH, Krokstad S, Moe N, Nordbo SA. Detection of spliced mRNA from human bocavirus 1 in clinical samples from children with respiratory tract infections. *Emerg Infect Dis*. 2013;19(4):574−580.
42. Proenca-Modena JL, Gagliardi TB, Paula FE, et al. Detection of human bocavirus mRNA in respiratory secretions correlates with high viral load and concurrent diarrhea. *PLoS One*. 2011;6(6):e21083.
43. Foster D, Cox-Singh J, Mohamad DS, Krishna S, Chin PP, Singh B. Evaluation of three rapid diagnostic tests for the detection of human infections with *Plasmodium knowlesi*. *Malar J*. 2014;13:60.
44. Lucchi NW, Poorak M, Oberstaller J, et al. A new single-step PCR assay for the detection of the zoonotic malaria parasite *Plasmodium knowlesi*. *PLoS One*. 2012;7(2):e31848.
45. Atkinson B, Chamberlain J, Dowall SD, Cook N, Bruce C, Hewson R. Rapid molecular detection of Lujo virus RNA. *J Virol Methods*. 2014;195:170−173.
46. Ishii A, Thomas Y, Moonga L, et al. Molecular surveillance and phylogenetic analysis of Old World arenaviruses in Zambia. *J Gen Virol*. 2012;93(Pt 10):2247−2251.
47. Cajimat MN, Milazzo ML, Rollin PE, et al. Genetic diversity among Bolivian arenaviruses. *Virus Res*. 2009;140(1−2):24−31.
48. Delgado S, Erickson BR, Agudo R, et al. Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. *PLoS Pathog*. 2008;4(4):e1000047.
49. Pritt BS, Sloan LM, Johnson DK, et al. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. *N Engl J Med*. 2011;365(5):422−429.
50. Thirumalapura NR, Qin X, Kuriakose JA, Walker DH. Complete genome sequence of *Ehrlichia muris* strain AS145T, a model monocytotropic *Ehrlichia* strain. *Genome Announc*. 2014;2(1).
51. Bermudez de Leon M, Penuelas-Urquides K, Aguado-Barrera ME, et al. In vitro transcribed RNA molecules for the diagnosis of pandemic 2009 influenza A(H1N1) virus by real-time RT-PCR. *J Virol Methods*. 2013;193(2):487−491.
52. Tellez-Sosa J, Rodriguez MH, Gomez-Barreto RE, et al. Using high-throughput sequencing to leverage surveillance of genetic diversity and oseltamivir resistance: a pilot study during the 2009 influenza A (H1N1) pandemic. *PLoS One*. 2013;8(7):e67010.
53. Pekova S, Vydra J, Kabickova H, et al. *Candidatus* Neoehrlichia mikurensis infection identified in 2 hematooncologic patients: benefit of molecular techniques for rare pathogen detection. *Diagn Microbiol Infect Dis*. 2011;69(3):266−270.
54. Welinder-Olsson C, Kjellin E, Vaht K, Jacobsson S, Wenneras C. First case of human "*Candidatus* Neoehrlichia mikurensis" infection in a febrile patient with chronic lymphocytic leukemia. *J Clin Microbiol*. 2010;48(5):1956−1959.
55. Wen HL, Zhao L, Zhai S, et al. Severe fever with thrombocytopenia syndrome, Shandong Province, China, 2011. *Emerg Infect Dis*. 2014;20(1):1−5.
56. Yu XJ, Liang MF, Zhang SY, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. *N Engl J Med*. 2011;364(16):1523−1532.
57. Lu X, Whitaker B, Sakthivel SK, et al. Real-time reverse transcription-PCR assay panel for Middle East respiratory syndrome coronavirus. *J Clin Microbiol*. 2014;52(1):67−75.
58. Memish ZA, Al-Tawfiq JA, Makhdoom HQ, et al. Respiratory tract samples, viral load, and genome fraction yield in patients with middle East respiratory syndrome. *J Infect Dis*. 2014;210(10):1590−1594.
59. Lam TT, Wang J, Shen Y, et al. The genesis and source of the H7N9 influenza viruses causing human infections in China. *Nature*. 2013;502(7470):241−244.

agents of human infection is that of hepatitis C virus (HCV). After the discoveries of hepatitis A and B viruses, it was clear that the majority of cases of post-transfusion hepatitis were due to a condition designated non-A non-B hepatitis. The disease was transmissible to chimpanzees. In 1989, plasma from an infected chimpanzee was pelleted by ultracentrifugation and nucleic acids extracted from the pellet. cDNA was synthesized from both RNA and DNA with random primers and reverse transcriptase. Screening identified an RNA-encoded clone that expressed an antigen that reacted with antibodies of infected subjects. Eventually the complete genomes of all of the genotypes of HCV were determined, and a novel species most closely related to flaviviruses was established [12,13].

Another dramatic emergence of a viral disease occurred in 1993 in the Four Corners region of the southwestern United States. A mysterious highly lethal respiratory illness was investigated by a team from the Centers for Disease Control and Prevention (CDC). Extensive serologic screening of numerous antigens revealed unexpected reactivity with antigen of hantaviruses from other parts of the world that caused renal disease and hemorrhagic fever, and immunohistochemistry detected hantaviral antigen in pulmonary endothelium. Regions within the M segment of the RNA hantaviral genomes encoding G2 protein that are highly conserved were targeted by primers for nested PCR after reverse transcriptase generation of cDNA. Tissues from infected patients were analyzed, and the PCR products sequenced revealing a novel hantavirus subsequently named Sin Nombre virus. Viral sequences were identified in other patients and in *Peromyscus maniculatus* rodents, the reservoir. The story of hantaviral pulmonary syndrome unfolded to reveal related agents in many locations in North, Central, and South America [14,15].

A novel coronavirus in association with cases of severe acute respiratory syndrome (SARS-CoV) emerged in southern China in late 2002 and spread to 37 countries in five continents with 8273 confirmed cases and 775 deaths. No further cases have been reported since July 2003 [16]. RT-PCR, cloning, and sequencing contributed to identification of the SARS-CoV within weeks of the first cases reported in 2003 [17–20] and enabled rapid development of effective molecular diagnostic assays for routine clinical use [21,22]. SARS-CoV is associated with high mortality. Thus, timely and accurate diagnosis is needed to prevent the spread of this contagious disease. SARS-CoV spreads by respiratory secretions and airborne transmission. Early in the illness, SARS cannot be distinguished from common respiratory infections based on clinical symptoms [16]. During the SARS epidemic, PCR-based molecular testing was helpful because of its ability to rapidly screen for many viruses. After the identification of SARS-CoV, specific RT-PCR and serological assays were developed, and RT-PCR detected infection before the appearance of antibodies when the risk of transmission is greatest [16–22].

The bacterial *rrs* gene encoding 16S rRNA was recognized as a valuable phylogenetic tool for discrimination and identification of bacterial species. David Relman crafted this tool into an approach to identify an unknown etiologic agent by PCR of the *rrs* gene with primers that corresponded to genomic regions that were conserved among eubacteria. Using this approach, he amplified and determined bacterial DNA sequences from bacillary angiomatosis lesions of patients with AIDS. Comparison with a bacterial gene database revealed that the DNA sequences matched bacteria that are currently named *Bartonella henselae* and *B. quintana*. Serendipitous testing of a patient who also had been diagnosed with cat scratch disease led to the recognition that *B. henselae* was also the long sought-after etiology of this well-characterized disease [23,24].

The same approach to discovery using *rrs* gene amplification and DNA sequencing led to the identification of what is currently classified as *Anaplasma phagocytophilum* as the etiologic agent of tick-transmitted human granulocytotropic anaplasmosis [25,26]. Subsequently *Ehrlichia ewingii* was recognized as another human tick-borne pathogen among patients evaluated in a molecular diagnostics laboratory who tested negative for *E. chaffeensis* infection [27–29]. More recently, Bobbi Pritt at Mayo Clinic noted that the melting curve of the DNA amplicons in a real-time PCR assay for Anaplasmataceae differed from the expected curves of known pathogens for a group of patients in Wisconsin and Minnesota. Sequence analysis identified another novel tick-borne pathogen tentatively designated *Ehrlichia muris*-like agent [8,9].

The discovery of a novel bunyavirus that has caused thousands of human infections with a case fatality rate of 12% in 15 provinces in China relied upon a molecular approach to identify the viral agent. Xue-Jie Yu investigated an outbreak in China that was thought to be due to severe infection with *A. phagocytophilum*. He noted that some of the clinical manifestations differed from those of anaplasmosis. He observed cytopathic effect in DH-82 cells inoculated with clinical samples rather than the typical morulae formed by *Anaplasma* species in infected cells. Ultrastructural analysis suggested that the pathogen causing the outbreak was a virus that belongs to the family of bunyaviruses. Based on the known sequence of bunyaviruses, PCR primers were designed, which yielded no amplicons. Subsequently, he began sequencing the RNA of

heavily infected cells and discarded the sequences of the culture host species, *Canis familiaris*. This approach enabled him to determine that he had recovered a novel *Phlebovirus* of the family Bunyaviridae. He accomplished this feat without the use of next-generation sequencing (NGS) [10]. The application of NGS now allows us to obtain an abundance of viral gene sequences from infected host cells and the discovery of further novel viral and bacterial agents.

MOLECULAR EPIDEMIOLOGICAL STUDIES OF EMERGING INFECTIOUS PATHOGENS

Molecular technologies have been critical in the initial discovery of agents of emerging infectious diseases. These methods have also been routinely used for further characterization of pathogen strains and sequence variations. Molecular data are now widely used in molecular epidemiological studies and phylogenetic analyses, and sequence comparisons have been performed to facilitate the specific detection of genetically diverse strains/sequences and investigate the origin, transmission, distribution, biology, and diversity of these pathogens [12,13,21,30–33], which are fundamentally important in the prevention and tracking of disease outbreaks. Knowledge of sequence variations is used in the development of accurate diagnostic assays and for the design of effective treatment strategies of diseases caused by these agents. Molecular epidemiological studies are critical for public health surveillance [14,15,34–65]. We provide here examples of how molecular tests contributed to public health surveillance and patient care.

Influenza A

Seasonal and pandemic influenza A represents one of the greatest threats to global health [66–68]. Continuing challenges in influenza include the sporadic human cases of highly pathogenic avian H5N1 influenza, emergence of pandemic H1N1 influenza in 2009 [62,69], and human infections with avian H7N9 influenza in 2013 [11]. Influenza A virus undergoes continuous antigenic drift and sporadic antigenic shifts in the viral surface glycoproteins, hemagglutinin (H) and neuraminidase (N). Influenza A has 15 H and 9 N subtypes. Antigenic H and N subtypes to which humans lack immunity are introduced by reassortment of virus genes and cause pandemics, whereas H and N antigenic variants determined by point mutations cause seasonal influenza epidemics [66,67].

Molecular assays are the preferred method for identification and surveillance of new strains of influenza A infections [11,62,67]. Influenza A has no pathognomonic symptoms, and diagnosis based on clinical signs is correct in only two-thirds of patients [68,70]. Therefore, sensitive and rapid laboratory tests are required to diagnose and guide antiviral treatment. Recently, multiplex molecular assays for respiratory viruses including influenza viruses have been developed, and several have received approval/clearance by the US Food and Drug Administration (FDA) for routine clinical use (Table 15.2, listed under pandemic H1N1 influenza virus). These assays provide rapid and sensitive tests for respiratory viral infections.

Human Immunodeficiency Virus 1

Human immunodeficiency virus 1 (HIV-1) was discovered in 1983 (Table 15.1). It is a single-stranded, positive-sense, enveloped RNA retrovirus (http://www.hiv.lanl.gov/). HIV-1 can cause AIDS, a chronic disease leading to immunodeficiency and susceptibility to opportunistic infections (http://www.who.int/hiv/en/). Three groups of HIV-1 have been identified based on sequence similarity, including M (main), O (outlier), and N (non-M/non-O) (http://www.hiv.lanl.gov/). Of the three groups of HIV-1, group M dominates the global epidemic and is further classified into subtypes A, B, C, D, F, G, H, J, and K. In addition, circulating recombinant forms (CRFs), mosaic viruses formed between subtypes during co- or superinfection, have also been recognized (http://www.hiv.lanl.gov/). Although subtype B is predominant in North America and Europe, non-B variants represent more than 90% of HIV-1 circulating globally [71]. In recent years, the prevalence of non-B subtypes and CRFs in the United States is steadily increasing due to increased international travel and immigration [72–74]. Sequencing data of HIV-1 genomes have been used for tracking HIV epidemics and for the design of accurate viral detection, viral load, and HIV-1 drug-resistance genotype assays to guide clinical use of antiretroviral treatment [38,75]. The recent availability of the NGS approach has greatly facilitated generation of HIV-1 sequences and detection of quasispecies, which can improve understanding of HV-1 infection, pathogenesis, and epidemics [38,76,77].

Hepatitis C Virus

It is believed that 150 million people worldwide are infected with HCV (http://www.who.int/mediacentre/factsheets/fs164/en/). Between 70% and 80% of people infected with HCV will develop chronic

infection. Chronic hepatitis C is closely associated with the development of cirrhosis and hepatocellular carcinoma and is the most common cause of adult liver transplantation in the United States and the world (http://www.cdc.gov/hepatitis/hcv/). A comparison of HCV genomic sequences from around the world revealed substantial heterogeneity of nucleotide sequences. Phylogenetic analyses have shown that HCV strains can be classified into six genotypes (numbered 1–6) and a large number of subtypes within each genotype [78]. HCV genotypes 1, 2, and 3 appear to have a worldwide distribution, but their relative prevalence varies from one geographic area to another. HCV genotype 1 is reported to be the most common in the United States [79–81]. HCV virus genome sequencing has been used to study HCV genotypes, subtypes, quasispecies, and mutations. The information is important for epidemiological studies, to trace the source of infection, for development of direct acting antiviral (DAA) therapy, and for understanding of susceptibility and resistance to antiviral treatment [82–85].

MOLECULAR DIAGNOSTICS OF EMERGING INFECTIOUS PATHOGENS

Many methods have been employed for the clinical diagnostics of emerging pathogens including microscopy, bacterial culture, cell culture, and serologic tests. However, each of these methods has its own limitations that must be considered by the clinical laboratory. For example, even though cell culture could be considered as the gold standard in diagnosis of infection with emerging obligate intracellular bacteria such as *Rickettsia* or *Ehrlichia*, the requirement for biological safety laboratory level 3 (BSL-3) (for *Rickettsia)* or BSL-2 (for *Ehrlichia*) makes this test difficult to implement in many conventional clinical microbiology laboratories. Further, the prolonged turnaround time (TAT) (eg, detection by culture at 7–10 days after sample processing) makes this approach impractical. Results from such a test are not clinically useful due to failure to guide therapy during the early stages of infection when appropriate antibiotic treatment is highly effective. Similar to culture, serologic tests such as indirect immunofluorescence assays, which rely on detection of antigen-specific antibodies, have several limitations such as low sensitivity during the early stages of infection when there is a low level of specific antibodies and false-positive results due to cross-reaction of antibodies to antigens from closely-related bacterial species. In addition, diagnosis of acute infection by IgG serology using single or paired (acute and convalescent) serum samples has the limitation of lack of a standardized cutoff titer among laboratories if a single sample is obtained, or the frequent inability to obtain convalescent serum when paired samples are required. In the latter case, while IgG serology could be useful for epidemiologic surveillance, paired sera are not optimal for timely diagnosis and treatment of acute infection. Thus, the emergence of molecular methods including probe hybridization, target or signal amplification, and sequencing provides better diagnostic advantages compared to microscopy, culture, and serology such as rapid TAT, higher sensitivity and higher specificity in different patient populations, and using different specimen types (eg, blood, plasma, cerebrospinal fluid, tissues, fluids). These molecular tests have become the gold standards due to their high negative and positive predictive values and their ability to detect and characterize newly emerging pathogens for clinical purposes [1–11].

Molecular assays are routinely used in clinics for the diagnosis, prognosis, and treatment decisions of various emerging infectious diseases [12,13,38,75,86–91] (Tables 15.1 and 15.2). As listed in Tables 15.1 and 15.2, there are US FDA-approved/cleared tests for some of these pathogens, and CDC has tests for all these agents. There are also laboratory-developed tests brought to clinical use after significant research and development and validation studies by individual laboratories [12,13,38,75,86–91]. As in other infectious diseases, clinical molecular tests for emerging infectious diseases include (1) nucleic acid detection assays with defined limit of detection cutoffs, (2) quantitative methods with broad dynamic ranges, lower and higher limit of quantification values, (3) genotyping and subtyping assays, and drug resistance mutation assays at even single base-pair resolution are used for disease prognosis and guiding treatment strategies [71,81,92]. General quality management protocols that cover preanalytic, analytic, and postanalytic phases also apply to molecular tests of emerging pathogens.

Following the discovery of HIV-1 in 1983 and HCV in 1989, molecular tests were developed and implemented for routine clinical use to detect viral infection, monitor viral load, and examine specific HIV-1 drug-resistant mutations and HCV genotypes to guide patient management. Several practice guidelines have incorporated HIV-1 and HCV molecular tests (eg, http://www.who.int/hiv/pub/guidelines/en/; http://www.hcvguidelines.org/full-report-view). For example, because detection of HCV RNA, not IgG antibody, is diagnostic of current HCV infection, and HCV genotype 1 is more difficult to treat than genotype 2 or 3, testing for HCV genotype is recommended to guide selection of the most appropriate treatment regimen. HCV RNA detection and genotyping assays are routinely performed in clinical diagnostics laboratories (http://www.hcvguidelines.org/full-report-view).

Over the years, with advances in molecular technology, HIV-1 and HCV clinical molecular tests have improved significantly with respect to performance characteristics including sensitivity, specificity, and dynamic range. Currently, there are several FDA-approved/cleared molecular tests for HIV-1 and HCV (Table 15.1), and new methods are continuously developed and evaluated for better care of patients with HIV-1 and HCV infection [38,75,89,93].

LIMITATIONS OF CURRENT TESTING AND FUTURE PROSPECTS

A high portion of emerging infectious diseases are vector-borne zoonoses that have emerged from natural cycles. The underlying causes of their emergence are a combination of environmental changes, such as increased populations and geographic distribution of their reservoir hosts and vectors, and development of new scientific tools that contribute to their detection and identification. For example, PCR-based molecular methods have enabled the discovery of a large number of bacterial and viral organisms in ticks, which preceded the identification of these organisms as etiologic agents of emerging infectious diseases.

Among these emerging infectious diseases are two contrasting tick-borne infections, Lyme borreliosis [37,47], and human monocytotropic ehrlichiosis (HME) [27–29]. Lyme disease is well known, feared, at times inappropriately diagnosed, and very rarely fatal. HME is largely unknown, frequently misdiagnosed as another tick-borne disease such as Rocky Mountain spotted fever or a viral infection, and is often life-threatening. Lyme borreliosis occurs particularly in suburban populations in the northeastern United States and has been investigated extensively in prominent academic medical institutions in this region. HME occurs particularly in the rural southeastern United States and has not been the focus of in-depth clinical studies in academic medical centers in this region. Both Lyme borreliosis and HME have high incidence although that of HME is not well recognized.

The effects of these conditions on the development and application of diagnostic tests including molecular diagnostics are far from satisfying. Diagnosis of Lyme borreliosis depends heavily on serological assays. Patients with Lyme disease frequently have developed antibodies to *Borrelia burgdorferi* by the time in their course of illness when they present for medical attention. These patients and those with a classic bulls-eye appearing rash are diagnosed, treated effectively with appropriate antibiotics and recover. As with other infections antibodies take time to be stimulated and produced. Thus, some patients' diagnoses may be delayed. Molecular methods seldom provide a diagnosis owing to the paucity of organisms in the blood and other readily obtained clinical samples [94].

A tremendous problem is the large number of persons with atypical symptoms of a wide range that includes those similar to chronic fatigue syndrome or fibromyalgia who are convinced that they are suffering from chronic Lyme disease but whose results of validated tests do not support the diagnosis. Many of them are convinced that the tests are inadequate and that better tests are needed [94]. In contrast, patients with HME often have not developed antibodies to the etiologic agent, *E. chaffeensis*, at the time when they present for medical attention. The bacteria can infect mononuclear phagocytes and are present in circulating monocytes providing an often effective target for molecular diagnostics at a time when appropriate antibiotic treatment results in rapid recovery from an otherwise life-threatening infection [29]. Yet HME, which likely has an incidence similar to Lyme disease, lacks a readily available point-of-care diagnostic test. Effective molecular target genes have been identified, and in-house assays provide proof-of-concept that molecular diagnostics offer an effective approach [28,95]. Moreover, low-cost instrument-free devices for nucleic acid amplification and specific identification have been developed that would be appropriate for point-of-care diagnosis.

Why have no more effective efforts been made to devise, develop, and commercialize molecular approaches to these two important emerging infections? For Lyme borreliosis, molecular diagnostics may not possess the solution when too few or no *Borrelia* are present. For HME, the issues lie in the realms of clinical practice, public health, and business. Physicians who are unaware of HME and note that febrile illnesses during the tick season often respond to doxycycline therapy are not inclined to order send out tests that would cost the patient. Serology that is based on comparing IgG antibody levels in paired sera often fails to provide a diagnosis of acute infection as it relies on the seldom-obtained convalescent serum. Public health agencies are powerless to address effectively a disease that is not diagnosed, and if diagnosed, is not reported. The epidemiologic reports depend on the data obtained by passive surveillance. In fact, active, prospective, population-based surveillance in endemic regions such as Missouri suggested that HME is a highly prevalent disease [96]. The combination of nonspecific clinical manifestations of HME, test underutilization, lack of a gold standard test that is effective when therapeutic decisions are made, and problems in interpretation of diagnostic tests such as serology, and misleading epidemiologic data have accounted for reported low incidence of HME. This situation has

failed to stimulate interest in commercial development and marketing of a useful point-of-care assay, although there could be an adequate pull from the potential users of the test.

The advances of sequencing technology, nanotechnology, and bioinformatics have driven molecular tests including assays for emerging pathogens to be more comprehensive and precise. For example, the availability of various sequence databases permits quick identification of sequence identity and variations. For example, the HIV database http://www.hiv.lanl.gov/ contains data on HIV genetic sequences and drug resistance associated mutations. It is valuable for HIV epidemiological studies, research, development, and clinical validation studies of HIV clinical assays [38,71]. It is well known that there are significant variations of clinical phenotypes in the presence of emerging infections ranging from asymptomatic carrier to lethal infection. Recently, assays to examine multiple pathogen panels have been developed [97–102], which should increase the diagnostic yield for many pathogens. A critical need for emerging pathogen analysis is quicker, easier, cost-effect assays that can be used in a point-of-care setting. New assays that are performed on platforms with a small footprint and detect pathogens quickly (in minutes instead of hours or days) have entered clinical use. For example, the FilmArray (BioFire Diagnostics, Inc.) and Simplexa (Focus Diagnostics, Inc.) molecular assays can generate results in approximately 60 min. The user-friendly Alere i (Alere Inc.) and Cobas Liat (Roche Molecular Systems) platforms are compact and portable, generate rapid molecular results in 15–20 min, can use electricity or rechargeable battery, and therefore are completely mobile and suited for point-of-care testing. It is obvious that the current rapid development of new technologies will further enhance the utility of molecular diagnostics in various emerging infectious diseases.

The advancement of molecular methods for emerging infections comes hand-in-hand with other areas including general infectious diseases, genetics and genomics, and oncology. There are needs to develop unified sequence databases for the input and search of emerging pathogens and other sequences, to understand pathogen/genotype/sequence correlation with phenotypes (eg, lethality or carrier with an emerging infection), to develop panels to more effectively diagnose patients based on shared clinical signs and symptoms, and to develop point-of-care molecular platforms and assays for emerging infectious diseases.

Over the last two decades, sequencing technology has evolved from labor-intensive and time-consuming methodologies to automatic and real-time sequence detection. Recent development and use of NGS has revolutionized the landscape of microbiology and infectious disease. The availability of sequencing data has speeded up pathogen discovery, and also helped improve diagnosis, typing of pathogens, detection of virulence and drug resistance, and development of new vaccines and targeted treatment [103–106].

With the ever-extending use of NGS on a variety of clinical samples, rapid progress on determining the composition of the human microbiome and its impact upon human health are to be expected in the coming years. This deluge of sequencing data requires a consolidated and curated database to input and search sequences, sequence variations, associated symptoms and diseases, available tests, and treatment options. A unified reporting guideline for molecular epidemiology has been proposed recently [107]. Adoption of this guideline by the research and clinical communities should help to integrate the effort for the comprehension of genomics and metagenomics relevant to the field of medical microbiology, and to improve management of infectious diseases.

Traditional pathogen detection methods in infectious diseases rely upon the identification of agents associated with a particular clinical syndrome. The availability of a significant amount of sequence information and the emerging field of metagenomics using NGS have the potential to revolutionize pathogen detection by allowing the simultaneous detection of all microorganisms in a clinical sample, without *a priori* knowledge of their identities. This can identify new sequences and organisms that may be initially considered nonpathogenic and may cause infections in different human populations and health conditions. They may cause diseases not previously thought to have a microbial component, and the methods may determine previously unknown etiology of infections. For example, infection with certain emerging pathogens may only cause disease symptoms in patients with AIDS or immune suppression after organ transplantation, or in travelers not previously exposed to the agents. Further biological and clinical studies are necessary to categorize sequence information and interpret clinical relevance when a pathogen sequence is detected, which is critical for diagnosis, treatment, and public health surveillance of emerging infectious diseases.

Assays to examine multiple pathogen panels have been developed [97–102]. These assays are designed either to detect many infections that can cause similar symptoms (eg, FDA-approved/cleared respiratory viral panels as listed in Table 15.2, multiple viruses that can trigger gastrointestinal symptoms) [100,101], pathogens that share homologous sequences, for example, 16S rRNA sequencing [98,99,102,108] or are expected to occur under the circumstances of biothreat [97]. The availability of more pathogen sequences and further understanding of their correlation with clinical

symptoms are necessary for the rational design of panels that can fit various needs.

New technological developments including microfluidics, nanotechnology, and lab-on-a-chip technologies have enabled development of user-friendly, easy, and quick point-of-care molecular tests including Alere i (Alere Inc.) and Cobas Liat (Roche Molecular Systems). In the setting of emerging infectious diseases, rapid and accurate identification of the causative agent is critical to facilitate effective patient management and enable prompt initiation of infection controls. Point-of-care assays are especially needed in resource-limited settings and in situations with lack of access to centralized medical facilities. Further development of point-of-care molecular tests for emerging pathogens is critical to timely diagnosis, treatment, and subsequent control of emerging infectious disease.

References

[1] Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albarino CG, et al. Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. PLoS Pathog 2008;4:e1000047.

[2] Djavani M, Lukashevich IS, Sanchez A, Nichol ST, Salvato MS. Completion of the Lassa fever virus sequence and identification of a RING finger open reading frame at the L RNA 5′ End. Virology 1997;235:414–18.

[3] Harcourt BH, Tamin A, Ksiazek TG, Rollin PE, Anderson LJ, Bellini WJ, et al. Molecular characterization of Nipah virus, a newly emergent paramyxovirus. Virology 2000;271:334–49.

[4] Lee E, Stocks C, Lobigs P, Hislop A, Straub J, Marshall I, et al. Nucleotide sequence of the Barmah Forest virus genome. Virology 1997;227:509–14.

[5] Madani TA, Azhar EI, Abuelzein el TM, Kao M, Al-Bar HM, Farraj SA, et al. Complete genome sequencing and genetic characterization of Alkhumra hemorrhagic fever virus isolated from Najran, Saudi Arabia. Intervirology 2014;57(5):300–10.

[6] Matsutani M, Ogawa M, Takaoka N, Hanaoka N, Toh H, Yamashita A, et al. Complete genomic DNA sequence of the East Asian spotted fever disease agent *Rickettsia japonica*. PLoS One 2013;8:e71861.

[7] Pekova S, Vydra J, Kabickova H, Frankova S, Haugvicova R, Mazal O, et al. Candidatus Neoehrlichia mikurensis infection identified in 2 hematooncologic patients: benefit of molecular techniques for rare pathogen detection. Diagn Microbiol Infect Dis 2011;69:266–70.

[8] Pritt BS, Sloan LM, Johnson DK, Munderloh UG, Paskewitz SM, McElroy KM, et al. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. N Engl J Med 2011;365:422–9.

[9] Thirumalapura NR, Qin X, Kuriakose JA, Walker DH. Complete genome sequence of *Ehrlichia muris* strain AS145T, a model monocytotropic *Ehrlichia* strain. Genome Announc 2014;2:e01234–13.

[10] Yu XJ, Liang MF, Zhang SY, Liu Y, Li JD, Sun YL, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. N Engl J Med 2011;364:1523–32.

[11] Lam TT, Wang J, Shen Y, Zhou B, Duan L, Cheung CL, et al. The genesis and source of the H7N9 influenza viruses causing human infections in China. Nature 2013;502:241–4.

[12] Houghton M. The long and winding road leading to the identification of the hepatitis C virus. J Hepatol 2009;51:939–48.

[13] Houghton M. Discovery of the hepatitis C virus. Liver Int 2009;29:82–8.

[14] Black WC, Doty JB, Hughes MT, Beaty BJ, Calisher CH. Temporal and geographic evidence for evolution of Sin Nombre virus using molecular analyses of viral RNA from Colorado, New Mexico and Montana. Virol J 2009;6:102.

[15] Henderson WW, Monroe MC, St Jeor SC, Thayer WP, Rowe JE, Peters CJ, et al. Naturally occurring Sin Nombre virus genetic reassortants. Virology 1995;214:602–10.

[16] Payne B, Bellamy R. Novel respiratory viruses: what should the clinician be alert for? Clin Med 2014;14:s12–16.

[17] Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 2003;348:1967–76.

[18] Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003;348:1953–66.

[19] Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003;361:1319–25.

[20] Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 2003;300:1394–9.

[21] Adachi D, Johnson G, Draker R, Ayers M, Mazzulli T, Talbot PJ, et al. Comprehensive detection and identification of human coronaviruses, including the SARS-associated coronavirus, with a single RT-PCR assay. J Virol Methods 2004;122:29–36.

[22] Huang JL, Lin HT, Wang YM, Yeh YC, Peck K, Lin BL, et al. Rapid and sensitive detection of multiple genes from the SARS-coronavirus using quantitative RT-PCR with dual systems. J Med Virol 2005;77:151–8.

[23] Lantos PM, Maggi RG, Ferguson B, Varkey J, Park LP, Breitschwerdt EB, et al. Detection of *Bartonella* species in the blood of veterinarians and veterinary technicians: a newly recognized occupational hazard? Vector Borne Zoonotic Dis 2014;14:563–70.

[24] Psarros G, Riddell IV J, Gandhi T, Kauffman CA, Cinti SK. *Bartonella henselae* infections in solid organ transplant recipients: report of 5 cases and review of the literature. Medicine 2012;91:111–21.

[25] Chan K, Marras SA, Parveen N. Sensitive multiplex PCR assay to differentiate Lyme spirochetes and emerging pathogens *Anaplasma phagocytophilum* and *Babesia microti*. BMC Microbiol 2013;13:295.

[26] Stuen S, Granquist EG, Silaghi C. *Anaplasma phagocytophilum*—a widespread multi-host pathogen with highly adaptive strategies. Front Cell Infect Microbiol 2013;3:31.

[27] Breitschwerdt EB, Hegarty BC, Qurollo BA, Saito TB, Maggi RG, Blanton LS, et al. Intravascular persistence of *Anaplasma platys*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii* DNA in the blood of a dog and two family members. Parasit Vectors 2014;7:298.

[28] Doyle CK, Labruna MB, Breitschwerdt EB, Tang YW, Corstvet RE, Hegarty BC, et al. Detection of medically important *Ehrlichia* by quantitative multicolor TaqMan real-time polymerase chain reaction of the *dsb* gene. J Mol Diagn 2005;7:504–10.

[29] Walker DH, Paddock CD, Dumler JS. Emerging and re-emerging tick-transmitted rickettsial and ehrlichial infections. Med Clin North Am 2008;92:1345–61.

[30] Hadjinicolaou AV, Farcas GA, Demetriou VL, Mazzulli T, Poutanen SM, Willey BM, et al. Development of a molecular-

beacon-based multi-allelic real-time RT-PCR assay for the detection of human coronavirus causing severe acute respiratory syndrome (SARS-CoV): a general methodology for detecting rapidly mutating viruses. Arch Virol 2011;156:671–80.
[31] Lan YC, Liu TT, Yang JY, Lee CM, Chen YJ, Chan YJ, et al. Molecular epidemiology of severe acute respiratory syndrome-associated coronavirus infections in Taiwan. J Infect Dis 2005;191:1478–89.
[32] Tang JW, Cheung JL, Chu IM, Ip M, Hui M, Peiris M, et al. Characterizing 56 complete SARS-CoV S-gene sequences from Hong Kong. J Clin Virol 2007;38:19–26.
[33] Tang JW, Cheung JL, Chu IM, Sung JJ, Peiris M, Chan PK. The large 386-nt deletion in SARS-associated coronavirus: evidence for quasispecies? J Infect Dis 2006;194:808–13.
[34] Bonvicini F, Manaresi E, Bua G, Venturoli S, Gallinella G. Keeping pace with parvovirus B19 genetic variability: a multiplex genotype-specific quantitative PCR assay. J Clin Microbiol 2013;51:3753–9.
[35] Cajimat MN, Milazzo ML, Rollin PE, Nichol ST, Bowen MD, Ksiazek TG, et al. Genetic diversity among Bolivian arenaviruses. Virus Res 2009;140:24–31.
[36] Christensen A, Dollner H, Skanke LH, Krokstad S, Moe N, Nordbo SA. Detection of spliced mRNA from human bocavirus 1 in clinical samples from children with respiratory tract infections. Emerg Infect Dis 2013;19:574–80.
[37] Clark KL, Leydet BF, Threlkeld C. Geographical and genospecies distribution of *Borrelia burgdorferi* sensu lato DNA detected in humans in the USA. J Med Microbiol 2014;63:674–84.
[38] Di Giallonardo F, Zagordi O, Duport Y, Leemann C, Joos B, Kunzli-Gontarczyk M, et al. Next-generation sequencing of HIV-1 RNA genomes: determination of error rates and minimizing artificial recombination. PLoS One 2013;8:e74249.
[39] Dingle KE, Elliott B, Robinson E, Griffiths D, Eyre DW, Stoesser N, et al. Evolutionary history of the *Clostridium difficile* pathogenicity locus. Genome Biol Evol 2014;6:36–52.
[40] Donaldson CD, Clark DA, Kidd IM, Breuer J, Depledge DD. Genome sequence of human herpesvirus 7 strain UCL-1. Genome Announc 2013;1 e00830–13.
[41] Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, et al. Diverse sources of *C. difficile* infection identified on whole-genome sequencing. N Engl J Med 2013;369:1195–205.
[42] Fulhorst CF, Cajimat MN, Milazzo ML, Paredes H, de Manzione NM, Salas RA, et al. Genetic diversity between and within the arenavirus species indigenous to western Venezuela. Virology 2008;378:205–13.
[43] Galvan A, Magnet A, Izquierdo F, Fenoy S, Henriques-Gil N, del Aguila C. Variability in minimal genomes: analysis of tandem repeats in the microsporidia *Encephalitozoon intestinalis*. Infect Genet Evol 2013;20:26–33.
[44] Gomez-Valero L, Rusniok C, Rolando M, Neou M, Dervins-Ravault D, Demirtas J, et al. Comparative analyses of *Legionella* species identifies genetic features of strains causing Legionnaires inverted question mark disease. Genome Biol 2014;15:505.
[45] Gonzalez JP, Bowen MD, Nichol ST, Rico-Hesse R. Genetic characterization and phylogeny of Sabia virus, an emergent pathogen in Brazil. Virology 1996;221:318–24.
[46] Ishii A, Thomas Y, Moonga L, Nakamura I, Ohnuma A, Hang'ombe BM, et al. Molecular surveillance and phylogenetic analysis of Old World arenaviruses in Zambia. J Gen Virol 2012;93:2247–51.
[47] Jacquot M, Gonnet M, Ferquel E, Abrial D, Claude A, Gasqui P, et al. Comparative population genomics of the *Borrelia burgdorferi* species complex reveals high degree of genetic isolation among species and underscores benefits and constraints to studying intra-specific epidemiological processes. PLoS One 2014;9:e94384.
[48] Lares-Jimenez LF, Booton GC, Lares-Villa F, Velazquez-Contreras CA, Fuerst PA. Genetic analysis among environmental strains of *Balamuthia mandrillaris* recovered from an artificial lagoon and from soil in Sonora, Mexico. Exp Parasitol 2014;145:S57–61.
[49] Le Gal F, Gault E, Ripault MP, Serpaggi J, Trinchet JC, Gordien E, et al. Eighth major clade for hepatitis delta virus. Emerg Infect Dis 2006;12:1447–50.
[50] Mazurie AJ, Alves JM, Ozaki LS, Zhou S, Schwartz DC, Buck GA. Comparative genomics of cryptosporidium. Int J Genomics 2013;2013:832756.
[51] Muhlberger E, Trommer S, Funke C, Volchkov V, Klenk HD, Becker S. Termini of all mRNA species of Marburg virus: sequence and secondary structure. Virology 1996;223:376–80.
[52] Pang B, Zheng X, Diao B, Cui Z, Zhou H, Gao S, et al. Whole genome PCR scanning reveals the syntenic genome structure of toxigenic *Vibrio cholerae* strains in the O1/O139 population. PLoS One 2011;6:e24267.
[53] Pessoa R, Watanabe JT, Nukui Y, Pereira J, Kasseb J, de Oliveira AC, et al. Molecular characterization of human T-cell lymphotropic virus type 1 full and partial genomes by Illumina massively parallel sequencing technology. PLoS One 2014;9:e93374.
[54] Poidinger M, Roy S, Hall RA, Turley PJ, Scherret JH, Lindsay MD, et al. Genetic stability among temporally and geographically diverse isolates of Barmah Forest virus. Am J Trop Med Hyg 1997;57:230–4.
[55] Pombert JF, Selman M, Burki F, Bardell FT, Farinelli L, Solter LF, et al. Gain and loss of multiple functionally related, horizontally transferred genes in the reduced genomes of two microsporidian parasites. Proc Natl Acad Sci USA 2012;109:12638–43.
[56] Ratner L, Philpott T, Trowbridge DB. Nucleotide sequence analysis of isolates of human T-lymphotropic virus type 1 of diverse geographical origins. AIDS Res Hum Retroviruses 1991;7:923–41.
[57] Riner DK, Nichols T, Lucas SY, Mullin AS, Cross JH, Lindquist HD. Intragenomic sequence variation of the ITS-1 region within a single flow-cytometry-counted *Cyclospora cayetanensis* oocysts. J Parasitol 2010;96:914–19.
[58] Rump LV, Gonzalez-Escalona N, Ju W, Wang F, Cao G, Meng S, et al. Genomic diversity and virulence characterization of historical *Escherichia coli* O157 strains isolated from clinical and environmental sources. Appl Environ Microbiol 2014;81:569–77.
[59] Sanchez-Buso L, Comas I, Jorques G, Gonzalez-Candelas F. Recombination drives genome evolution in outbreak-related *Legionella pneumophila* isolates. Nature Genet 2014;46:1205–11.
[60] Sekeyova Z, Roux V, Raoult D. Phylogeny of *Rickettsia* spp. inferred by comparing sequences of 'gene D', which encodes an intracytoplasmic protein. Int J Syst Evol Microbiol 2001;51:1353–60.
[61] Strouts FR, Power P, Croucher NJ, Corton N, van Tonder A, Quail MA, et al. Lineage-specific virulence determinants of *Haemophilus influenzae* biogroup *aegyptius*. Emerg Infect Dis 2012;18:449–57.
[62] Tellez-Sosa J, Rodriguez MH, Gomez-Barreto RE, Valdovinos-Torres H, Hidalgo AC, Cruz-Hervert P, et al. Using high-throughput sequencing to leverage surveillance of genetic diversity and oseltamivir resistance: a pilot study during the 2009 influenza A(H1N1) pandemic. PLoS One 2013;8:e67010.
[63] Towner JS, Khristova ML, Sealy TK, Vincent MJ, Erickson BR, Bawiec DA, et al. Marburgvirus genomics and association with

a large hemorrhagic fever outbreak in Angola. J Virol 2006;80:6497–516.
[64] Yu M, Hansson E, Shiell B, Michalski W, Eaton BT, Wang LF. Sequence analysis of the Hendra virus nucleoprotein gene: comparison with other members of the subfamily Paramyxovirinae. J Gen Virol 1998;79:1775–80.
[65] Zhou Y, Lv B, Wang Q, Wang R, Jian F, Zhang L, et al. Prevalence and molecular characterization of *Cyclospora cayetanensis*, Henan, China. Emerg Infect Dis 2011;17:1887–90.
[66] Nicholson KG, Wood JM, Zambon M. Influenza. Lancet 2003;362:1733–45.
[67] Webster RG, Govorkova EA. Continuing challenges in influenza. Ann NY Acad Sci 2014;1323:115–39.
[68] Ortiz JR, Neuzil KM, Shay DK, Rue TC, Neradilek MB, Zhou H, et al. The burden of influenza-associated critical illness hospitalizations. Crit Care Med 2014;42(11):2325–32.
[69] Bermudez de Leon M, Penuelas-Urquides K, Aguado-Barrera ME, Curras-Tuala MJ, Escobedo-Guajardo BL, Gonzalez-Rios RN, et al. In vitro transcribed RNA molecules for the diagnosis of pandemic 2009 influenza A(H1N1) virus by real-time RT-PCR. J Virol Methods 2013;193:487–91.
[70] Ebell MH, Afonso AM, Gonzales R, Stein J, Genton B, Senn N. Development and validation of a clinical decision rule for the diagnosis of influenza. J Am Board Fam Med 2012;25:55–62.
[71] Xu F, Schwab C, Liang X, Weaver S, Li A, Sanborn MR, et al. Low Prevalence of non-subtype B HIV-1 strains in the Texas prisoner population. J Mol Genet 2010;2:41–4.
[72] Brennan CA, Stramer SL, Holzmayer V, Yamaguchi J, Foster GA, Notari EP, et al. Identification of human immunodeficiency virus type 1 non-B subtypes and antiretroviral drug-resistant strains in United States blood donors. Transfusion 2009;49:125–33.
[73] Lin HH, Gaschen BK, Collie M, El-Fishaway M, Chen Z, Korber BT, et al. Genetic characterization of diverse HIV-1 strains in an immigrant population living in New York City. J Acquir Immune Defic Syndr 2006;41:399–404.
[74] Peeters M, Aghokeng AF, Delaporte E. Genetic diversity among human immunodeficiency virus-1 non-B subtypes in viral load and drug resistance assays. Clin Microbiol Infect 2010;16:1525–31.
[75] Casabianca A, Orlandi C, Canovari B, Scotti M, Acetoso M, Valentini M, et al. A real time PCR platform for the simultaneous quantification of total and extrachromosomal HIV DNA forms in blood of HIV-1 infected patients. PLoS One 2014;9:e111919.
[76] de Goede AL, Vulto AG, Osterhaus AD, Gruters RA. Understanding HIV infection for the design of a therapeutic vaccine. Part I: Epidemiology and pathogenesis of HIV infection. Ann Pharm Fr 2014;73:87–99.
[77] Young SD. A "big data" approach to HIV epidemiology and prevention. Prev Med 2014;70C:17–18.
[78] Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005;42:962–73.
[79] Nainan OV, Alter MJ, Kruszon-Moran D, Gao FX, Xia G, McQuillan G, et al. Hepatitis C virus genotypes and viral concentrations in participants of a general population survey in the United States. Gastroenterology 2006;131:478–84.
[80] Rustgi VK. The epidemiology of hepatitis C infection in the United States. J Gastroenterol 2007;42:513–21.
[81] Clement CG, Yang Z, Mayne JC, Dong J. HCV genotype and subtype distribution of patient samples tested at University of Texas Medical Branch in Galveston, Texas. J Mol Genet 2010;2:36–40.
[82] Aherfi S, Solas C, Motte A, Moreau J, Borentain P, Mokhtari S, et al. Hepatitis C virus NS3 protease genotyping and drug concentration determination during triple therapy with telaprevir or boceprevir for chronic infection with genotype 1 viruses, southeastern France. J Med Virol 2014;86:1868–76.
[83] Campo DS, Skums P, Dimitrova Z, Vaughan G, Forbi JC, Teo CG, et al. Drug resistance of a viral population and its individual intrahost variants during the first 48 hours of therapy. Clin Pharmacol Ther 2014;95:627–35.
[84] Irving WL, Rupp D, McClure CP, Than LM, Titman A, Ball JK, et al. Development of a high-throughput pyrosequencing assay for monitoring temporal evolution and resistance associated variant emergence in the hepatitis C virus protease coding-region. Antivir Res 2014;110:52–9.
[85] Svarovskaia ES, Dvory-Sobol H, Parkin N, Hebner C, Gontcharova V, Martin R, et al. Infrequent development of resistance in genotype 1-6 hepatitis C virus-infected subjects treated with sofosbuvir in phase 2 and 3 clinical trials. Clin Infect Dis 2014;59:1666–74.
[86] Benitez AJ, Thurman KA, Diaz MH, Conklin L, Kendig NE, Winchell JM. Comparison of real-time PCR and a microimmunofluorescence serological assay for detection of *Chlamydophila pneumoniae* infection in an outbreak investigation. J Clin Microbiol 2012;50:151–3.
[87] Ravindranath BS, Krishnamurthy V, Krishna V, Sunil Kumar C. In silico synteny based comparative genomics approach for identification and characterization of novel therapeutic targets in *Chlamydophila pneumoniae*. Bioinformation 2013;9:506–10.
[88] Fevery B, Susser S, Lenz O, Cloherty G, Perner D, Picchio G, et al. HCV RNA quantification with different assays: implications for protease-inhibitor-based response-guided therapy. Antivir Ther 2014;19:559–67.
[89] Quer J, Gregori J, Rodriguez-Frias F, Buti M, Madejon A, Perez-Del-Pulgar S, et al. High-resolution hepatitis C virus (HCV) subtyping, using NS5B deep sequencing and phylogeny, an alternative to current methods. J Clin Microbiol 2014;53:219–26.
[90] Klemenc J, Asad Ali S, Johnson M, Tollefson SJ, Talbot HK, Hartert TV, et al. Real-time reverse transcriptase PCR assay for improved detection of human metapneumovirus. J Clin Virol 2012;54:371–5.
[91] Roussy JF, Carbonneau J, Ouakki M, Papenburg J, Hamelin ME, De Serres G, et al. Human metapneumovirus viral load is an important risk factor for disease severity in young children. J Clin Virol 2014;60:133–40.
[92] Yang ZM, Morrison R, Oates C, Sarria J, Patel J, Habibi A, et al. HIV-1 genotypic resistance testing on low viral load specimens using the Abbott ViroSeq HIV-1 Genotyping System. LabMedicine 2008;39:671–3.
[93] Vollmer T, Knabbe C, Dreier J. Comparison of real-time PCR and antigen assays for detection of hepatitis E virus in blood donors. J Clin Microbiol 2014;52:2150–6.
[94] Aguero-Rosenfeld ME, Wormser GP. Lyme disease: diagnostic issues and controversies. Exp Rev Mol Diagn 2015;15:1–4.
[95] Killmaster LF, Loftis AD, Zemtsova GE, Levin ML. Detection of bacterial agents in *Amblyomma americanum* (Acari: Ixodidae) from Georgia, USA, and the use of a multiplex assay to differentiate *Ehrlichia chaffeensis* and *Ehrlichia ewingii*. J Med Entomol 2014;51:868–72.
[96] Olano J, Masters E, Hogrefe W, Walker DH. Human monocytotropic ehrlichiosis, Missouri. Emerg Infect Dis 2003;9:1579–86.
[97] Euler M, Wang Y, Heidenreich D, Patel P, Strohmeier O, Hakenberg S, et al. Development of a panel of recombinase polymerase amplification assays for detection of biothreat agents. J Clin Microbiol 2013;51:1110–17.

[98] Koehler JW, Hall AT, Rolfe PA, Honko AN, Palacios GF, Fair JN, et al. Development and evaluation of a panel of filovirus sequence capture probes for pathogen detection by next-generation sequencing. PLoS One 2014;9:e107007.

[99] Lindsay B, Pop M, Antonio M, Walker AW, Mai V, Ahmed D, et al. Survey of culture, goldengate assay, universal biosensor assay, and 16S rRNA gene sequencing as alternative methods of bacterial pathogen detection. J Clin Microbiol 2013;51:3263–9.

[100] Martinez MA, Soto-Del Rio MD, Gutierrez RM, Chiu CY, Greninger AL, Contreras JF, et al. DNA microarray for detection of gastrointestinal viruses. J Clin Microbiol 2014;53:136–45.

[101] Moore NE, Wang J, Hewitt J, Croucher D, Williamson DA, Paine S, et al. Metagenomic analysis of viruses in feces from unsolved outbreaks of gastroenteritis in humans. J Clin Microbiol 2014;53:15–21.

[102] Wei S, Zhao H, Xian Y, Hussain MA, Wu X. Multiplex PCR assays for the detection of *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio cholerae* with an internal amplification control. Diagn Microbiol Infect Dis 2014;79:115–18.

[103] Cox MJ, Cookson WO, Moffatt MF. Sequencing the human microbiome in health and disease. Hum Mol Genet 2013;22:R88–94.

[104] Lecuit M, Eloit M. The human virome: new tools and concepts. Trends Microbiol 2013;21:510–15.

[105] Miller RR, Montoya V, Gardy JL, Patrick DM, Tang P. Metagenomics for pathogen detection in public health. Genome Med 2013;5:81.

[106] Padmanabhan R, Mishra AK, Raoult D, Fournier PE. Genomics and metagenomics in medical microbiology. J Microbiol Methods 2013;95:415–24.

[107] Field N, Cohen T, Struelens MJ, Palm D, Cookson B, Glynn JR, et al. Strengthening the Reporting of Molecular Epidemiology for Infectious Diseases (STROME-ID): an extension of the STROBE statement. Lancet Infect Dis 2014;14:341–52.

[108] Zhao J, Kang L, Hu R, Gao S, Xin W, Chen W, et al. Rapid oligonucleotide suspension array-based multiplex detection of bacterial pathogens. Foodborne Pathog Dis 2013;10:896–903.