Hormozgan University of Medical Sciences

Health Institute

Hormozgan University of Medical Sciences

REVIEW ARTICLE

Molecular diagnosis of Viral infections

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Accepted 28 July 2015; Autumn-Winter 2015

ABSTRACT

Context: Detection of causative pathogen is crucial for treatment strategies in most infections. Molecular diagnosis as a more sensitive and specific method, has become the best option to investigate certain pathogens, mainly viruses. Recent scientific and technological approaches resulted in changing the area of diagnostic microbiology.

Evidence Acquisition: A comprehensive online search of data bases was done and all appropriate articles and documents retrieved were reviewed thoroughly and systematically.

Results: Many new user-friendly commercial assays have been developed for this purpose. During recent decades, remarkable efforts have been made for genomes sequencing of micro organisms. Investigation of nucleic acids in detection of variety of organisms related to infectious diseases such as nosocomial infections, sexually transmitted diseases, and immunocompromised host infections.

Introduction: The most application of molecular techniques is in epidemiological and clinical investigations in viral infections such as HPV, genotyping for HCV and HIV resistance to viral polymerase inhibitors due to structural origin. Recent approaches such as microarrays and next-generation sequencing are expected to have a huge impact in the next revolution of pathogen identification. In this review, we focused on the most recently molecular approaches in diagnosis of viral infection and their characteristics.

Key words: Molecular Diagnosis, Viral Infections, Microarrays, Next-Generation Sequencing

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Context:

Detection of causative pathogen is crucial for treatment strategies in most infections. Molecular diagnosis as a more sensitive and specific method, has become the best option to investigate certain pathogens, mainly viruses. Recent scientific and technological approaches resulted in changing the area of diagnostic microbiology (1). Many new user-friendly commercial assays have been developed for this purpose. During recent decades, remarkable efforts have been made for genomes sequencing of micro organisms (2,3). Investigation of nucleic aids in the detection of variety of organisms related to infectious diseases such as nosocomial infections, sexually transmitted diseases, and immunocompromised host infections (4). The most application of molecular techniques is in epidemiological and clinical investigations in viral infections such as HPV in cervical (5,6) and genotyping for HCV (7). HIV resistance to viral polymerase inhibitors due to structural origin has been excluded in recent studies (8,9). Some point mutations in the HPV-encoded thymidine kinase gene has been shown as a cause for the occurrence of acyclovir resistance (10) which is very important in patients AIDS, immunosuppressed conditions or whom undergoing long-term therapy (11,12). Recent approaches such as microarrays and next-generation sequencing, are expected to have a huge impact in the next revolution of pathogen identification (13). There is no need For a molecular diagnostic method to support the replication of an organism. The most necessity to identify a specific molecule in a reaction is a proper molecular probe (14). Nucleic acid amplification technology has reduced the time of Viral detection and characterization (15). In this review, we focused on the most recently molecular approaches in diagnosis of viral infection and their characteristics.

Evidence Acquisition:

Using keywords such as molecular diagnosis, viral infections, microarrays, next-generation sequencing and methods, a comprehensive online search was done of data bases including google scholar, pubmed, science direct and all eligible articles and documents retrieved were reviewed thoroughly and systematically.

Results:

There have been considerable improvements in the methods used to detect viral nucleic acid in the diagnosis and supervision of patients with different kinds of viral infections. These methods are now included into a wide-ranging of diagnostic and management strategy and in a range of clinical settings.

1. Non Amplified Nucleic Acid Probes

Nucleic acid probes are small fragments of Deoxyribonucleic acid or ribonucleic acid that have being labeled with enzymes, chemiluminescent molecules or radioisotopes. Complementary sequences are targets for these in micro organisms. Nucleic acid probes can be used to recognize some viruses. liquid- phase, solid-phase and in situ hybridization are the most common used formats for probe hybridization. Due to poor analytical sensitivity, these techniques might be used only to detection a large scale microorganisms (16). Regardless of advances in this technology, there are number of challenges in application of viral testing related to precision, accuracy and standardization (17).

2. Amplified Nucleic Acid Techniques

Since the 1980s, the growing accessibility of nucleic acid amplification methods has improved understanding about epidemiology, pathogenesis, laboratory and clinical aspects of known and novel viral pathogens. Polymerase chain reaction is the most commonly applied qualitative diagnostic method (18). Nucleic acid testing for infectious diseases is almost completely performed in centralized laboratories using high-end instrumentation and expert personnel (19). Development of the PCR has initiated the beginning of molecular diagnostic techniques (20). Though this method is the most generally used nucleic acid amplification technique, other methodologies have been developed but they are not PCR based techniques. The biochemical mechanisms of these methods are based on amplification of signal, target or probes. One of these methods is NASBA (nucleic acid sequence-based amplification). This is a strong amplification method which has been used to identify a number of pathogens, including RNA viruses such as HIV and HCV (21,22).

3. Microarrays

A DNA array is a set of spots attached to a slide where each spot contains one or more single-stranded DNA oligonucleotide fragment (23). In high density arrays technology, hundred or thousands of oligonucleotides are attached which are referred to as microarrays. Signals resulted from hybridization of a labeled amplification product are mapped to a number of positions inside the array. The sequence of product can be identified using the pattern of hybridization, if there are sufficient large amount of probes. The result of hybridization between the bound probe and labeled sequences in the sample is revealed by imaging or scanning the array surface. The chip can be scanned using the confocal microscopy and fluorescent signals that reveal hybridization at specific locations on the chip are detected. Since many DNA sequences can be present on a slide, concurrent testing for several viruses is possible for microarray analysis for instance, to study polymorphisms of the HIV protease gene for detection of drug resistance (13,24,25).

4. Multiplex methods: nucleic acid-based

Multiplexing technologies enable the simultaneous identification of multiple viruses in a single assay which can significantly decrease the time, cost and labor-input related to conventional single reaction detection methods (26).

4.1. Multiplexed Microsphere-Based Array

Microsphere-based suspension array technologies present a stage for nucleic acid identification which have some advantages as well as rapid data achievement, high specificity and sensitivity and multiplexed analysis potential. Compared to planar microarrays, advantages of these techniques are including being user friendly, higher statistical power, low cost, faster hybridization kinetics and more flexibility in preparation (27). A multiplexed assay for diagnosis of viral infection using this system has been developed for HCV, HSV, HIV and HPV typing with high specific outcome (24,28).

5. WHOLE-GENOME SEQUENCING

Subsequent to electron microscopy, cell culture and Polymerase chain reaction, whole genome sequencing (WGS) is one of these techniques which is now changing the way of understanding viruses, mainly in the fields of genome sequencing, development, ecology, detection and transcriptomics (29). Whole genome sequencing is the basis for the inclusive understanding of an organism's function. (30). Recent studies showed that characterization of complex mixture of microflora in environmental context can be done using high-throughput sequencing (31). Next-generation sequencing (NGS) techniques present an unique "step-change" raise in the quantity of sequence data which can be generated from a sample. Although this method used for de novo sequencing of large genomes, to achieve ultra deep coverage, NGS can be applied for resequencing of small viral genomes. Thus, NGS potential gives information further than the consensus for a viral sample through revealing present nucleotide substitutions in just a small portion of the population (32) such as sequencing of new influenza viruses, detection of viral genome inconsistency and evolution within the host, such as assessment of human immunodeficiency virus and human hepatitis C virus species, and monitoring of low-abundance antiviral drug-resistance mutations. For stratagies based on metagenomics in detection of unforeseen disease-associated viruses and for the identification of novel human viruses, for instance cancer-related viruses, and description of the human virome in healthy and disease conditions, NGS techniques have been applied. (33). Detection of Fatal arenavirus infection associated with organ transplantation (LCMV) and Merkel cell polyomavirus (MCV) are two examples of NGS application in viral infection (13). Summary of comparision these techniques are given in Table 1.

Table 1. Some main characteristics of recent molecular techniques and examples of their application

Technique	Advantages/ Disadvantages	Example of viruses	Refrences
Non Amplified Nucleic Acid Probes	poor analytical sensitivity Necessity for large number of microorganisms	HPV, CMV	(16)
Amplified Nucleic Acid Techniques	highly purified nucleic acids easy to use in clinical settings real-time detection during amplification	Most of viruses: HCV, HBV, HPV, CMV, VZV, BK virus, HIV-1, Influenza A and B, , HSV 1/2	(23, 34)
Microarrays **Multiplexed Microsphere-Based Array**	simultaneous testing for multiple viruses high sensitivity and specificity multiplexed analysis potential simplicity of use low cost higher statistical power faster hybridization kinetics more flexibility in array preparation	Respiratory viruses, HCV, HPV, HIV, CNS, HSV, SARS	(23, 27, 35)
Whole-Genome Sequencing (based on third generation sequencing technique)	No amplification of template DNA required, realtime monitoring of nucleotide incorporation Nonbiased DNA sequence, High error rates and low reads High NTP incorporation error rates Generates long-read lengths 800-1000 bp, Single molecule sequencing	LCMV, MCV	(13, 36, 37)

Discussion:

Advances in the area of bioinformatics, nanotechnology and genomics have been changed the views of the interaction between viral and host transcriptomes (33). Possibilities for these techniques are only limited by current scientific thoughts and, somewhat, by their cost, which leads to restricted applications to relatively small amount of samples. Remaining challenges are including the storage and analysis of the large number of data obtained. An exciting era of viral investigation has begun which causes new challenges to realize the role of recently revealed viral diversity in both disease and health. A variety of molecular methods based on nucleic acid including amplified and non-amplified probes, microarrays, and target amplification are at the present applied for detection of organisms, their virulence factors and drug resistance determinants (29). The prospect of the molecular diagnostics of viral infections will indisputably be focused on a significant enhance in the number of information detected with significant basic, rapid platforms that will need complex software analysis to resolve the data for apply in clinical strategies. These highly developed techniques will probably focus on differences of microarrays and whole genome sequencing to improve clinical management. The success and introduction of these new methods through virology laboratories in order to establish diagnostic tests for the viruses implicated in human infections, will be mainly depend on numerous factors (26). The needs for special circumstances are such as modern equipments and trained personnel to enhance the efficacy of these new technologies in diagnostic area.

References:

1. Robertson BH, Nicholson JK. NEW MICROBIOLOGY TOOLS FOR PUBLIC HEALTH AND THEIR IMPLICATIONS 1. Annu Rev Public Health. 2005;26:281-302.
2. Robertson BH, Nicholson JK. NEW MICROBIOLOGY TOOLS FOR PUBLIC HEALTH AND THEIR IMPLICATIONS 1. Annu Rev Public Health. 2005;26:281-302.
3. Markowitz VM. Microbial genome data resources. Current opinion in biotechnology. 2007;18(3):267-72.
4. Pallen MJ, Wren BW. Bacterial pathogenomics. Nature. 2007;449(7164):835-42.
5. Sra KK, Torres G, Rady P, Hughes TK, Payne DA, Tyring SK. Molecular diagnosis of infectious diseases in dermatology. Journal of the American Academy of Dermatology. 2005;53(5):749-65.
6. Reid R, Greenberg M, Jenson AB, Husain M, Willett J, Daoud Y, et al. Sexually transmitted papillomaviral infections: I. The anatomic distribution and pathologic grade of neoplastic lesions associated with different viral types. American journal of obstetrics and gynecology. 1987;156(1):212-22.
7. Fuchs P, Girardi F, Pfister H. Human papillomavirus DNA in normal, metaplastic, preneoplastic and neoplastic epithelia of the cervix uteri. International journal of cancer. 1988;41(1):41-5.
8. Choo Q, Richman K, Han J, Berger K, Lee C, Dong C, et al. Genetic organization and diversity of the hepatitis C virus. Proceedings of the National Academy of Sciences. 1991;88(6):2451-5.
9. D'Aquila RT. HIV-1 drug resistance. Molecular pathogenesis and laboratory monitoring. Clinics in laboratory medicine. 1994;14(2):393-422.
10. Frost SD, McLean AR. Quasispecies dynamics and the emergence of drug resistance during zidovudine therapy of HIV infection. Aids. 1994;8(3):323-32.
11. Palù G, Gerna G, Bevilacqua F, Marcello A. A point mutation in the thymidine kinase gene is responsible for acyclovir-resistance in herpes simplex virus type 2 sequential isolates. Virus research. 1992;25(1):133-44.
12. Pease AC, Solas D, Sullivan EJ, Cronin MT, Holmes CP, Fodor S. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. Proceedings of the National Academy of Sciences. 1994;91(11):5022-6.
13. Field AK, Biron KK. " The end of innocence" revisited: resistance of herpesviruses to antiviral drugs. Clinical microbiology reviews. 1994;7(1):1-13.
14. Liu Y-T. A technological update of molecular diagnostics for infectious diseases. Infectious disorders drug targets. 2008;8(3):183.
15. Viscidi R, Yolken R. Molecular diagnosis of infectious diseases by nucleic acid hybridization. Molecular and cellular probes. 1987;1(1):3-14.
16. Tang Y-W, Procop GW, Persing DH. Molecular diagnostics of infectious diseases. Clinical Chemistry. 1997;43(11):2021-38.
17. Arnold L, Hammond PW, Wiese W, Nelson N. Assay formats involving acridinium-ester-labeled DNA probes. Clinical Chemistry. 1989;35(8):1588-94.
18. Gullett JC, Nolte FS. Quantitative Nucleic Acid Amplification Methods for Viral Infections. Clinical Chemistry. 2015;61(1):72-8.
19. Somerville LK, Ratnamohan VM, Dwyer DE, Kok J. Molecular diagnosis of respiratory viruses. Pathology-Journal of the RCPA. 2015;47(3):243-9.
20. Niemz A, Ferguson TM, Boyle DS. Point-of-care nucleic acid testing for infectious diseases. Trends in biotechnology. 2011;29(5):240-50.
21. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. 1988;239(4839):487-91.
22. Gracias KS, McKillip JL. NUCLEIC ACID SEQUENCE-BASED AMPLIFICATION (NASBA) IN MOLECULAR BACTERIOLOGY: A PROCEDURAL GUIDE. Journal of Rapid Methods & Automation in Microbiology. 2007;15(3):295-309.
23. Chan AB, Fox JD. NASBA and other transcription-based amplification methods for research and diagnostic microbiology. Rev Med Microbiol. 1999;10:185-96.
24. Cobo F. Suppl 1: Application of Molecular Diagnostic Techniques for Viral Testing. The open virology journal. 2012;6:104.
25. Wang D, Urisman A, Liu Y-T, Springer M, Ksiazek TG, Erdman DD, et al. Viral discovery and sequence recovery using DNA microarrays. PLoS biology. 2003;1(2):e2.
26. Palacios G, Quan P-L, Jabado OJ, Conlan S, Hirschberg DL, Liu Y, et al. Panmicrobial oligonucleotide array for diagnosis of infectious diseases. Emerging infectious diseases. 2007;13(1):73.

27. Boonham N, Kreuze J, Winter S, van der Vlugt R, Bergervoet J, Tomlinson J, et al. Methods in virus diagnostics: from ELISA to next generation sequencing. Virus research. 2014;186:20-31.
28. Dunbar SA. Applications of Luminex® xMAP™ technology for rapid, high-throughput multiplexed nucleic acid detection. Clinica Chimica Acta. 2006;363(1):71-82.
29. Smith PL, WalkerPeach CR, Fulton RJ, DuBois DB. A rapid, sensitive, multiplexed assay for detection of viral nucleic acids using the FlowMetrix system. Clinical Chemistry. 1998;44(9):2054-6.
30. Radford AD, Chapman D, Dixon L, Chantrey J, Darby AC, Hall N. Application of next-generation sequencing technologies in virology. Journal of General Virology. 2012;93(Pt 9):1853-68.
31. Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, et al. Whole-genome random sequencing and assembly of Haemophilus influenzae Rd. Science. 1995;269(5223):496-512.
32. Palacios G, Druce J, Du L, Tran T, Birch C, Briese T, et al. A new arenavirus in a cluster of fatal transplant-associated diseases. New England journal of medicine. 2008;358(10):991-8.
33. Wright CF, Morelli MJ, Thébaud G, Knowles NJ, Herzyk P, Paton DJ, et al. Beyond the consensus: dissecting within-host viral population diversity of foot-and-mouth disease virus by using next-generation genome sequencing. Journal of virology. 2011;85(5):2266-75.
34. Barzon L, Lavezzo E, Militello V, Toppo S, Palù G. Applications of next-generation sequencing technologies to diagnostic virology. International journal of molecular sciences. 2011;12(11):7861-84.
35. Deiman B, van Aarle P, Sillekens P. Characteristics and applications of nucleic acid sequence-based amplification (NASBA). Molecular biotechnology. 2002;20(2):163-79.
36. 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(5624):1394-9.
37. Roberts RJ, Carneiro MO, Schatz MC. The advantages of SMRT sequencing. Genome Biol. 2013;14(6):405.
38. Harris TD, Buzby PR, Babcock H, Beer E, Bowers J, Braslavsky I, et al. Single-molecule DNA sequencing of a viral genome. Science. 2008;320(5872):106-9