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Clinical uses of Molecular Biology in the Treatment of Infectious Diseases

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Abstract

Molecular biological technologies for microbe identification and characterisation have revolutionized diagnostic microbiology and are now standard specimen processing. Polymerase chain reaction (PCR) procedures have ushered in this new age by rapidly detecting microbes that were previously impossible to identify. Molecular approaches can now discover infections of public health relevance faster than fastidious microbes. Molecular technologies now identify antimicrobial resistance genes and give public health information like strain genotyping. Viral resistance identification and viral load measurement for antiviral therapy monitoring have enhanced microorganism treatment. Multiplex PCR, real-time PCR, and automation are lowering molecular technique costs, increasing their use. This review will discuss the clinical efficacy of clinical microbiology laboratory molecular technologies and how they have transformed infectious illness diagnosis and therapy.

Keywords: Molecular biology; Virology; Polymerase chain reaction; Genotype

Introduction

Nucleic acid amplification and detection have changed the way labs work. Instead of looking at how antibodies or metabolic products show up in the body, labs now use molecular tools to quickly identify infectious agents. The clinical microbiology laboratory uses genetic methods to find and describe viral diseases and figure out what's wrong with bacteria that are hard to detect. People want a quick response time and high sensitivity and precision, but they also

need detailed proof and quality control. The PCR method, which was first used with single-round or stacked methods and gel electrophoresis, has become the most common way for clinical microbiology labs to do molecular detection. But technology for DNA or RNA extraction, amplification, product identification, and real-time PCR will make molecular labs more efficient and cost-effective. DNA chip microarray technology could be useful in clinical microbiology labs [1].

This study will talk about how genetic methods can be used to treat viral diseases. In virology, it is used to test for tolerance, determine a virus's type, measure the amount of virus in a sample, and find viruses in general. In bacteriology, genetic methods have been used to test for resistance, find infections that are hard to find, find dangerous infections faster, and find infections that happened after antibiotics. Improvements in parasitology and mycology include making it easier to find fungus infections in people with low neutrophil counts. Other uses include finding biosecurity agents, studying statistics, stopping infections, and figuring out what goes wrong with genetic methods.

Virology

For many years, it has been hard to find out if someone has a viral illness because cell culture systems are expensive, take a lot of time in the lab, and require trained staff. Many viruses also grow slowly in artificial medium, which makes them hard to find. Serology is useless in the early stages of an infection, specific antisera are hard to get, and clinical antibody detection is not accurate enough for many viruses. PCR technology has made it easier to find viruses. Due to the limited accuracy of cerebrospinal fluid (CSF) culture and serology, HSV encephalitis was usually identified by taking a sample of the brain.1 PCR can find HSV DNA in CSF with a 95% accuracy rate2, so a brain biopsy is not needed. Enteroviruses or HSV can cause viral meningitis, which can be found faster and more accurately by PCR than by culture3 (one day vs. five days). Multiplex PCR for HSV and other bacteria that cause meningitis [2].

Both PCR and molecular methods that don't use PCR make it easier to find blood-borne viruses. Since HCV antibodies can't tell the difference between a past infection and a current one, HCV RNA is used to find ongoing cases. Only people with HCV RNA can pass the virus to a child through a blood transfer, an organ gift, a needle-stick injury, or from a parent to a child. HIV pro-viral DNA may show early HIV infection before Western Blot serology

confirms it.6 HIV pro-viral DNA identifies vertical spread in babies. The Chiron Procleix HIV-1/HCV transcription driven amplification assay, used by the Australian Red Cross Blood Service, checks samples from all donors for HIV and HCV. This makes the possibly infectious window shorter, from 22 to 66 days to 9 and 7 days.

Monitored Treatment

Monitoring viral DNA or RNA is now needed for people with long-term viral illnesses. Branched chain DNA signal amplification, real-time PCR, and competitive PCR are all ways to measure the amount of virus. HIV treatment needs viral load tests. It is the main way to check how well antiretroviral drugs are working and see if the virus is becoming resistant, which is shown by a rise in viral load even though treatment is going on. HIV viral loads influence development and outcome.18 Tests that are very sensitive, like the Cobas Amplicor HIV1 Monitor Ultrasensitive Test, lower the amount of virus that can be found to 50 copies per millilitre [3].

Viral load testing checks how well treatments for chronic HCV and HBV infections are working. During treatment with interferon-alpha and ribavirin, HCV patients with genotype 1 have their HCV RNA virus loads checked. Patients with HCV infection who have been treated with a mixture of drugs for 6 months and still don't have HCV RNA have usually gotten rid of the virus for good. This is called a lasting virological response. If there is no HCV genotype 1 RNA after 12 weeks, there is a 75% chance that the virus will not go away. Even if HCV RNA can still be found after 12 weeks of treatment, there is a 33% chance of a sustained virological reaction if the viral load has gone down 100-fold. HBV carriers with active liver disease have their HBV DNA loads checked to see if they need antiviral drugs like interferon-alpha or lamivudine and to see how well they work. HBV viral load rises show lamivudine-resistant viral types. Traditional culture methods are not accurate enough to find cytomegalovirus infections in people who have HIV and have received a bone marrow or solid organ donation. Quantitative PCR is the standard way to check for CMV infection in people whose immune systems aren't working well, and it gives people medicine to take before they get sick.

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Viral Genotyping and Resistance

HIV testing finds out if a person is resistant to a drug and helps direct antiretroviral treatment. In a library like the Stanford reverse transcriptase and protease database (http://hivdb.stanford.edu), sequences can be checked for resistance changes. For treatment of chronic viral hepatitis, testing is needed. Six types of HCV are found all over the world. The most important factor in how well a combination treatment works is the HCV genotype. Chronic HCV cases with genotypes 2 or 3 were more likely to get better after 6 months of treatment than those with genotype 1 were after 12 months. Variants of the virus that are resistant to lamivudine must be watched for in HBV cases that don't go away. After one year of lamivudine treatment, 14–32% of phase III patients have YMDD variations in the active site of the polymerase gene. After two and three years, 42% and 52% of Asian patients have YMDD variations. The amount of HBV virus and the sequence of the DNA polymerase gene active site show that lamivudine resistance is present [4].

Bacteriology

Fastidious Bacteria

The hard-to-find bacteria illnesses can be diagnosed with the help of molecular identification and virology. Some of the difficult bacteria that hurt public health are *Mycobacterium TB, Chlamydia trachomatis, Neisseria gonorrheae, and Bordetella pertussis*. Traditional culture-based molecular testing can take days or weeks, but non-culture-based molecular testing can find and fix health problems quickly. Assays for *M. tuberculosis, C. trachomatosis, and N. gonorrhoeae* are available for sale. The Q replicase system, strand displacement amplification, ligase chain reaction, and transcription-based amplification are all ways to find nucleic acids. Due to its higher sensitivity and better ability to track down contacts, molecular identification has greatly increased the number of sexually transmitted bacteria that have been proven in the lab. For a sexual health check, women need speculum checks and men need urinary swabs. These hurt and embarrass, which makes people less likely to obey. For genetic testing, you can use urine from the first stream and vaginal swabs that you take yourself. These are easier to use and help more people pass tests. Even though C. trachomatis. Urine testing for *C. gonorrhoeae* is as sensitive and specific as invasive specimens for *C. trachomatis* and *N. gonorrhoeae* in men and urethral swabs. The PCR tests for N. But vaginal tests that you take yourself may help. The specificity of vaginal and cervical swabs and a U.S.-approved transcription-mediated amplification test for C. PCR. trachomatis is the same.

Vaginal gonorrhea is being tested by the FDA for C.N. trachomatis. Molecular processes that use dry swabs are easier to transport to faraway places than samples in specialized transport medium. This is because samples in specialized transport medium are harder to keep alive during transport. Molecular methods can use the same test to find different vaginal diseases, like C. trachomatis N [5].

Mycobacteriology

Mycobacteriology is helped by molecular methods. But M molecule identification is very important. The normal society is good at picking up TB. The separation method might not be able to get DNA out of bacterial cells easily. Even so, M. TB confirms acid-fast bacilli seen under a microscope with up to 98% accuracy in lung tuberculosis in a day, whereas culture takes two weeks or more. Smear-negative specimens have a 40% sensitivity for genetic proof. Molecular methods, on the other hand, can prove a good culture in just one day. Even if the test is clear but the culture is positive, TB may be proven faster [6].

Mycobacteriology has made success through genetic classification of mycobacterial species that don't cause tuberculosis. Phenotypic methods take a long time and don't have the tests needed to tell the different species apart. The 16S rRNA gene sequencing method is used by many labs to make this process easier. Some species, like the fast-growing group, can't be told apart by reading the 16S rRNA gene alone. Instead, they need a multi-gene method that uses the hsp65, rpoB, and sod genes.34 Because PCR is more sensitive, it has replaced direct fluorescent antibody and culture as the "gold standard" for detecting B. early whooping cough. In a school outbreak of pertussis [7], nasopharyngeal aspirates found 48% of clinical cases, while culture only found 5%. To find the most cases of this public health agent, PCR is used to find cases early in the disease and serology is used to find potential cases late in the disease. Molecular methods can also be used to quickly find out if someone has *Legionella spp., Mycoplasma pneumoniae, or Chlamydia pneumoniae.*

Fast Diagnosis of Bacteria

To treat meningococcal disease, it is important to get a quick evaluation and give close friends chemoprophylaxis. When combined with culture and other lab processes, PCR may now be able to offer same-day identification from clean site material. Genotyping B/C types of *N. meningitidis* and finding *N. meningitis*. Meningitidis and genosero grouping of clinical

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cases of *N. meningitidis*. Other germs that can cause meningitis have also been quickly found. 90% of bacterial meningitis is caused by *N. meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae* type B. Multiplex PCR can find them [8].

Drug Resistance

Genotypic antibiotic resistance identification is appealing because it avoids problems like resistance that changes in how it looks. It might be helpful to be able to quickly and consistently identify the genotype of bacteria that cause infections, like MRSA and VRE. The mecA gene of MRSA and other *S. aureus* strains proves this resistance. S can be found quickly by looking at both the mecA and nuc genes at the same time. *aureus* and MRSA from blood cultures that come back positive. Since MRSA infections kill more people than those caused by methicillin-sensitive S, it is important to know how to choose a drug early on. *aureus.* DNA-based means of amplification can also find VRE faster and more accurately. There are 44 Real-time PCR kits on the market right now that can find MRSA and VRE. Plasmids and transposons carry Escherichia coli and *Klebsiella pneumoniae's* extended spectrum β-lactamases (ESBL). In hospitals, bacteria with ESBL can spread quickly and cause wounds, urinary tract infections, and septicemia. Since drug sensitivity tests might not find organisms with the gene for resistance, lab tests are needed to find them. Most clinical microbiology labs use quantitative methods to find ESBL, but molecular identification of these point changes at the active site of the -lactamase gene can prove ESBL and define its type for global tracking [9].

Parasitology and Mycology

Molecular tests can sometimes help with illnesses caused by fungi. Pneumocystis jiroveci, which used to be called Pneumocystis carinii, can cause dangerous pneumonia in HIV-positive and immune-compromised people, but it can only be found by looking at samples from the respiratory tract under a microscope. When forced phlegm or bronchoalveolar lavage samples are marked with calcofluor white or methenamine silver, *P. jiroveci* is often found. Immunofluorescence is better than these stains because it is more sensitive, but it is more expensive and requires special tools. PCR is reliable, especially for people without HIV, but it is not very specific because this bacteria is a common partner and can be found without illness. With 18S rRNA gene PCR, diseases caused by *Aspergillus spp*. that affect the blood can be found. This disease is hard to find because culture isn't good at picking up on

early signs of illness, and it's hard to get tissue samples from people with low platelet counts. The earlier you treat it, the better the effects, but antifungal drugs are generally expensive and dangerous. *Aspergillus* PCR might make it easier to figure out what's wrong, but it's not clear how it will improve treatment and prognosis. Most parasites can't be grown in a lab, so genetic methods are used to find them. To prove a foetal infection49, PCR can find Toxoplasma gondii in amniocentesis fluid, and it can find it in CSF to identify encephalitis. *Plasmodium spp*. On the other hand, PCR is more accurate than microscopy and can find malaria in people whose thick and thin blood films are clear because of chemoprophylaxis or partial protection. The Plasmodium species PCR test can also show mixed results [10].

Wide-range PCR

When it comes to diagnosing infectious diseases, broad-range PCR is more like fishing than focused PCR. Both the 16S rRNA gene in bacteria and the 18S rRNA gene in fungi use primers that match an area that is always the same. Any result from amplification is put into a sequence and compared to more than 9,000 species in Internet sources. GenBank (www.ncbi.nlm.nih.gov/Genbank), EMBL Data Library (www.ebi.ac.uk/embl), and the DNA Data Bank of Japan (www.ddbj.nig.ac.jp) share data every day, and RIDOM (www.ridom-rdna.de) is a high-quality library for mycobacterial speciation using bacterial rDNA sequences. Using 16S rRNA sequences and broad-range PCR, bacteria can be found in clean samples like blood or cerebrospinal fluid, a "molecular petri dish." It was used to find out that *B. henselae* was the bacteria that caused Whipple's disease in *T. whipplei*. It can be used to find out what caused infectious endocarditis. For this dangerous disease to be treated with antibiotics, the bacterial aetiology must be known. This can be hard to do when standard blood cultures come back negative because of antibiotic use. Broad-range PCR on heart valves and plants that have been removed or on blood from the periphery could show a missed diagnosis. Broad-range PCR is used to diagnose bacterial meningitis. Broad-range PCR was used to find the new SARS virus. Unknown viruses were found in SARS clinical samples by using broad-based primers to find them. The sequences showed that they were similar to the coronavirus genus, which backs up earlier lab results that led to a specific SARS CoV PCR within weeks of the first report of illness. Broad-range PCR runs the risk of amplifying DNA from the sample or the PCR tools, especially the Taq DNA polymerases, which can lead to false positives. When comparing an unknown sequence, the quality of the

sequences posted to a public library affects how accurate the data is. It's possible that the matching code is wrong or that it is shared with another species that has no data [11].

Public Health

Molecular tests help control infectious diseases by quickly and accurately figuring out what caused them. To quickly find and isolate people who might have SARS, you should rule out other respiratory illnesses like influenza. Even though there wasn't a way to tell if someone had the disease for a long time, this stopped it. If the SARS CoV comes back, we will have a lot of PCR-based testing kits to help us find the virus quickly. Diarrhea viruses like noroviruses spread quickly in hospitals and residential care homes, but they can now be found quickly so that cases can be isolated. Molecular testing can tell if a virus that spreads through the blood is dangerous. PCR testing lets health care workers with hepatitis B or C know if they can do things like surgery that put them at risk of being exposed. Molecular methods make it easier to deal with bacterial infections in the public health system. Early sign of *B. Pertussis, M. TB, and N. meningitidis* need both traditional and genetic tests to stop early spread.

Biosecurity

Bacillus anthracis, variola major virus (smallpox), *Clostridium botulinum*, and *Yersinia pestis* (plague) are all dangerous biological weapons because they can be invisible, don't show signs for days, and can spread to many people with very small amounts. A ton of sarin could kill as many people as 10g of anthrax germs. Since this kind of event could get worse quickly, we need monitoring tools that are fast, accurate, and sensitive. Real-time PCR works well for these things because standard methods are slow, picky, or need people with a lot of skill. It can be hard for PCR devices to get DNA out of cells without stopping the PCR process. New hardware lets spores be broken up and PCR done in 15 minutes, and battery-powered, compact TaqMan real-time PCR equipment with processing speeds of 30 minutes is being made. In this area, microarray technologies have a lot of potential, but they are restricted by the need to prepare samples for microfluidic devices. These problems might be solved by new technology.

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Problems with Molecular Methods

Even though it has benefits, molecular diagnostics can't yet replace traditional ways of treating many infectious diseases because many clinical microbiology laboratory tests are quick and inexpensive. Modern automatic culture methods and fast antigen assays make it possible to identify and test for sensitivity in minutes. All PCR tests except for broad-range PCR can only identify the organism whose DNA matches the primers. This is different from bacterial culture, which can find many bacteria that can be grown without knowing which one. So, to cover a similar range of species, we would need microarray methods that are easy to use and don't cost much.

False positive or negative results

Molecular testing is limited by both false positive and false negative results. To stop lab contamination from leading to fake positive results, you need big labs with physically separate areas for reagent preparation, material preparation, and product detection, as well as highly trained employees. Reagents are less likely to be contaminated in a lab when they are exposed to UV light and the surface is treated with sodium hypochlorite. amplicons are destroyed by using dUTP instead of dTTP for amplification and UNG treatment of starting reactions that have already been put together. Inter sample contamination can be stopped with disposable hats, gowns, gloves, and filter tips made of cotton. Even if you follow the steps carefully, broad-range PCR might not work because the chemicals contain foreign DNA. To detect pollution, PCR runs must contain suitable negative standards. Real-time PCR reaction and detecting methods that only use one tube have cut down on this risk.

Molecular identification is hard because different labs use different kinds of in-house PCR tests. HIV, hepatitis C, and hepatitis B testing is done for money. trachomatis, N. because they are so rare. Comparisons of sensitivity and specificity are hard to make because of differences in primer selection (different genes or sequences within genes), amplification format (single round, nested, real-time, or other nucleic amplification methods), and product detection (ethidium bromide gel electrophoresis, DNA probes, or sequencing).

Virus vs. bacteria

In contrast to culture, PCR results can sometimes be misread because nucleic acid does not always mean that a live organism is present. Meningococcal DNA found in a place that is

normally sterile is a good indicator of invasive meningitis. Since *P. jiroveci* can spread and cause illness, immunocompromised people who think they have PCP may only have a 50% chance of being right. After the first infection, EBV, CMV, and HSV are sometimes shed without causing symptoms. Since more disease-specific viruses cause higher viral counts, quantitative PCR may be more accurate than culture. For HIV, CMV, EBV, HBV, and HCV, it is clear how to measure the seriousness of the disease or how well a treatment is working. The positive prediction value of clinical CMV sickness can be improved by using viral load tests to track viral load rises to cut-off levels or rates of viral load growth. If you find RNA species that break down within minutes after a cell dies, this could mean that the virus is still alive and can reproduce [12].

The Future of Biotechnology

PCR and sequencing could find bugs that haven't been found yet and help epidemiologists study new contagious diseases. Molecular methods have shown that over 30 different types of bacteria can change into versions that can't be grown in bad conditions. Koch's ideas can't be used to figure out if germs like T make people sick or not. Whipple's disease is caused by whipplei. The study of how pathogens change over time, how infectious cycles stay in place in nature, the causes and processes of new pathogens, the susceptibility of different target groups, and the creation of DNA and RNA banks of genes that code for harmful factors all depend on molecular technology. This can be done with the help of microarrays, microchips, in situ PCR, and molecular robotics. Microarray and gene chip tests were first talked about in 1991. They combine downsizing, the use of industrial robots to make the chips, and the ability to read large amounts of exact genetic information quickly and accurately.

Up to 106 probes per cm2 can fit on the microarray platform. Pathogens that cause diseases like infectious diarrhea, asthma, and meningitis may be found with microarrays, as well as genetic signs of toxicity and drug susceptibility. Originally, an array was used to find out which HIV genes were resistant to protease. After the outbreak of 2003, microarrays were used to sequence the genome of the SARS virus. The US sequenced hundreds of types of variola major to find and describe biosecurity agents. They are the CDC. However, it is mostly used for study and is pretty expensive.

Repeatability problems need to be dealt with because the technology is sensitive and strict working conditions must be followed. Since each array makes several data points, analyzing

data needs computer programs. In infectious disease labs, biotechnology companies are already looking into the market for genetic tests that use microarrays. In the beginning, people focused on how drugs were not working and how to find mycobacteria. Affymetrix GeneChip microarrays are used to find pathogens, virulence factors, how drugs work, and how to make vaccines. But Prodesse's multiplex real-time PCR kits for identifying lung pathogens will fight with these companies. Due to the economy, testing methods that are like clinical chemistry lab tests will be computerized and cheaper. Nucleic acid extraction and purification, as well as the physical loading of extracted nucleic acids and master mixes into PCR reaction tubes, are the most time-consuming parts of molecular technology.

These jobs are now done by automated extraction and cleaning systems and pipetting robots. The COBAS AmpliPrep from Roche was one of the first automatic extraction machines. Biotinylated oligonucleotide probes catch the DNA that is released and attach it to magnetic beads that are coated with streptavidin. Roche made the MagNA Pure LC System for labs that do medical testing. In this 60-minute system, cross-contamination is stopped by positive pressure pipetting, UV cleaning, and HEPAfiltration. But extraction methods that aren't as good as hand ones may make PCR less sensitive. The prices, sample sizes, and processing times of the QIAGEN BioRobot EZ1 and M48/9604 systems, the Abbott m1000 system, the ABI PRISMTM 6100 Nucleic Acid PrepStation and 6700 Automated Nucleic Acid Workstation, and the Corbett Robotics X-tractor Gene.TM vary [13].

The Corbett CAS-1200TM Automated DNA Sample Setup makes it easy to set up PCR, prepare reagents, make a series of dilutions, and pipette samples. If these methods work well, the molecular diagnostics lab will be able to test more data with less highly trained staff and better output. The clinical microbiology laboratory may be able to use molecular methods to solve more problems than just identifying and measuring microbes. With every new piece of technology, there are new questions, which lowers the practical value of the test. How long should DNA live after healing or medicine, and in what fluids or organs of the body? How do we tell the difference between being colonized and having a current infection? Is it normal to find DNA in bacteria from clean places? Some traditional methods have been replaced in the virology lab by molecular methods. However, until they can quickly and cheaply study many genetic markers to figure out the cause and susceptibility, they will still need to use traditional culture and susceptibility tests.

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