# The molecular biology in infectious diseases biology essay

Science, Biology



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\n[/toc]\n \nWith the advent of polymerase chain reaction technique, microbiologists were quick to realise that this new diagnostic modality had potential to make microbiological diagnosis easier, cheaper and faster. Instead of conventional laboratory methods that rely on phenotypic expression of antigens or measurement of biochemical products, use of molecular methods helps in rapid identification of a number of infectious agents. Molecular methods have increasingly been incorporated into the clinical microbiology laboratory, particularly for the detection and characterisation of viral infections, and for the diagnosis of diseases that occur due to infection with fastidious bacteria. The advantages are obvious. There is a rapid turnaround time meaning that the time taken from the receipt of a sample to despatch of the results is minimal. These tests have a high sensitivity and specificity but it is difficult to ensure quality control and avoid contamination during the use of such methods. Usually molecular methods involve the use of PCR, which may be either straightforward PCR or the nested variety. In future, it is expected that the use of microarray would further increase the utility of the molecular methods.\n

# **MOLECULAR BIOLOGY IN VIROLOGY**

\nViruses perhaps pose real challenge for amicrobiologist, as they can neither be cultured nor can they be seen under an ordinary microscope. The responses that they generate in human body are erratic and may not correlate with the type or severity of the infection. All these impediments can create lot of problems in diagnosis. That is where the role of molecular medicine comes in.\n\nCNS infectionsThe advent of polymerase chain reaction technique has revolutionised the diagnosis of CNS infections. Previously, the diagnosis of such infections was a cumbersome and insensitive process. However, with the advent of the PCR technique, their diagnosis has become relatively simple. To understand this better, let us have a look at the application of PCR in the diagnosis of HSV, which is the most common cause of acute sporadic focal encephalitis. The sensitivity and specificity of CSF PCR for the diagnosis of HSV are 96% and 99% respectively. About the same percentages characterise the molecular methods that are used to detect enteroviruses and Epstein-Barr virus and human cytomegalovirus in the CSF. The sensitivity and specificity rates are lesser for the VZV, HHV6, HIV and rabies viruses. JCV is a slow-growing virus (takes up to five weeks to grow in culture), so a diagnostic test other than culture is needed. Previously, the JCV virus used to be spotted by means of a nested PCR which had 92% sensitivity and 100% specificity. However, in cases with concomitant HIV infection, after the introduction of HAART therapy, the PCR technique showed a strong decrease in the sensitivity, because of the lower amount of the virus in the CSF, due to the restoration of the immune system. However, with the help of the real time PCR, sensitivity

and specificity has been restored to the previous levels.\n\nHPV infectionEarly diagnosis of papillomavirus infection is a key issue for the prevention of HPV-related cancers. In some developing countries, these

prevention of HPV-related cancers. In some developing countries, these cancers represent the most prevalent neoplastic pathology. Screening programmes have been, and are, mainly based on the examination of cytologic smears from the cervical canal stained by Papanicolau staining technique (Paptest). Since antibody response is unreliably detected and does not necessarily correlate with current viral presence, the diagnostic virologic assays for HPV infection are based on HPV DNA detection and typing. Different PCR assays, mostly using general primers for the conserved L1 or E1 regions have been developed. In some cases, nested protocols using combinations of the mentioned set of primers can be used. Typing is achieved by sequencing, restriction endonuclease digestion or probe hybridization, all performed on the amplified product. In spite of these advances, none of these methods can be considered a golden standard. Microarray based assays are currently being developed to improve specificity and type range. Unlike cytologic screening, a vaginal swab is adequate for obtaining a reliable result, since infected cells and virions with their high load of viral DNA are spread throughout the genital tract.\n\nHepatitis virusesNucleic acid detection techniques are more sensitive than immunoassays which detect viral antigen in samples of different origins for diagnosis of HAV. HAV has been detected with techniques such as PCR -RFLP, SSCP, Southern blotting, sequencing, nucleic acid hybridization and reverse transcription-PCR (RT-PCR). Amplification of viral RNA by RT-PCR is currently the most sensitive and widely used method for detection of HAV

RNA. Diagnosis of hepatitis C is based on serological and molecular assays which detect HCV-specific antibodies (anti-HCV) and HCV RNA respectively. The molecular assays currently available are reverse transcriptase RT PCR. Third-generation anti-HCV enzyme-linked immunosorbent assays (ELISAs) are highly sensitive as well as specific and represent the primary diagnostic assay. The recombinant immunoblot assay (RIBA) is a supplemental assay that can be used to confirm a positive ELISA, particularly in low-risk populations. HCV infection can be diagnosed by RT-PCR as early as 1-2 weeks after infection or 4-6 weeks before anti-HCV seroconversion. The determination of HCV RNA is, in principle, important for the selection of patients for antiviral therapy and for the assessment of its efficacy. In cases with positive ELISA, RT PCR allows to discriminate between patients with chronic hepatitis C and those with resolved HCV infection that can remain anti-HCV positive for years or decades. Discrimination of genotype 1 from genotypes 2 and 3 as well as guantitative determination of viremia levels has become important for the selection of an optimal treatment regimen. In general, however, genotyping and quantitative RT PCR tests should be used only in the context of a defined therapy protocol and not for the initial diagnosis of HCV infection.\n\nRespiratory infectionsOf the common viruses causing respiratory infections, the use of molecular methods in the diagnosis of adenovirus, influenza virus, parainfluenza virus, and respiratory syncytial virus (RSV) infections has not been clearly established. Although PCR techniques are available for the diagnosis of these viruses, other rapid conventional techniques are also available: influenza virus and RSV can be detected in the clinical specimens by immunofluorescence; parainfluenza

virus and adenovirus can be detected by immunofluorscence after incubation for 48 h in shell vial cultures. In these cases, nucleic acid amplification techniques have no added value in terms of sensitivity or rapidity. Rhinoviruses and coronaviruses grow poorly in cell culture and rapid detection techniques like immunofluorscence and culture techniques are not available for these viruses. In such cases, use of molecular methods presents a distinct advantage. The PCR technique is rapid and fairly sensitive. Hantavirus pulmonary syndrome is characterized by fever, myalgias, headache, and cough, followed rapidly by respiratory failure. Antibodies against heterologous hantavirus antigens were initially used to identify the causative agent. After the advent of PCR technique, PCR was used to detect the hantavirus genome in autopsy specimens. The virus cannot be cultured, hence, PCR remains the best diagnostic possibility for this syndrome. Besides these viruses, PCR based assays have also been developed for the SARS and H5N1 strains of influenza.\n\nGastrointestinal diseaseViruses are responsible for more cases of infectious diarrhoea as compared to bacteria and other pathogens. The method of choice for microbiological diagnosis of rotavirus from stool samples is PCR. Norovirus, a calicivirus formerly known as Norwalk virus, can be diagnosed by electron microscopy, enzyme immunoassay and PCR; but PCR is the most sensitive and rapid method. PCR is also the most sensitive method for the diagnosis of astroviruses and enteric adenoviruses (serotypes 40 and 41).\n\nHIVAlthough human immunodeficiency virus (HIV) infection is routinely diagnosed by serology, early HIV infection can be detected by HIV pro-viral DNA detection much before HIV antibodies can be confirmed by Western Blot serology. Vertical transmission of HIV infection

can also be detected in the neonate using the HIV pro-viral DNA detection technique. These methods are capable of reducing the potentially infectious window period. The real time PCR can also be used to quantitate the viral load; molecular techniques can thus be used to prognosticate in cases with HIV infection. Finally, HIV genotyping for the detection of drug resistance can guide antiretroviral therapy and complements viral load assessment.\n

### **MOLECULAR BIOLOGY IN BACTERIOLOGY**

\nApplication of molecular biology for diagnosis of bacterial infections should be considered in three scenarios, namely (a) for the identification of an organism already isolated in pure culture, (b) for the rapid identification of an organism in diagnostic setting from clinical specimens and (c) for the identification of an organism from non-culturable specimens, e. g. culture negative endocarditis. Most modern clinical microbiology diagnostic laboratories rely on a combination of colonial morphology, physiology and identification of biochemical/serological markers for identification and successful classification of the pathogen at the genus and species levels. This is important because it is necessary to correctly type bacteria for epidemiological purposes and for infection control. The rapid identification of an organism already isolated in pure culture needs no elaboration. Molecular methods can rapidly isolate the nucleic acid from the cultured organism and type it accurately and rapidly. For rapid identification of organisms in a diagnostic setting from clinical specimens, molecular biological techniques play an important role. The culture report may take a long time to come and so, in many cases, patients maybe managed empirically. In today's world,

the importance of rapid diagnosis is also evident in the setting of a bioterrorist attack. In cases where the organism cannot be cultured, molecular biology has an important role to play. Take a situation where antibiotic therapy has already been instituted and the culture is negative. Even in such cases, the organism's DNA remains intact and this DNA can be exploited in molecular diagnostics. By and large, molecular techniques in bacteriology use DNA as the starting material. DNA has the advantage of being stable and bacteria contain DNA as their genetic material. Usually, molecular biology is required to answer one question; whether or not the target DNA is present. Quantitation has little meaning unless some specific problem like food contamination is being studied. Under these circumstances, DNA is adequate as the starting material. At times, when there is no indication regarding the identity of a bacterial organism, amplification of DNA encoding ribosomal RNA genes in conjunction with DNA sequencing of the amplified product can be done. In bacteria, there are three genes which decide the rRNA functionality. These are 5S, 16S and 23S rRNA gene. The 16S rRNA gene has been most commonly employed for identification purposes due to it being highly conserved and having a moderate copy number depending on the genus. 16S rRNA genes are found in all bacteria and they accumulate mutations at slow and constant rate over time, hence, they may be used as " molecular clocks". Highly variable portions of the 16S rRNA sequence provide unique signature to any bacterium and useful information about relationships between different bacteria. More recently, employment of the 16S-23S rRNA intergenic spacer region has become popular due to its large copy number, and more

importantly, because of its high sequence variability. The 23S rRNA subunit has also been used to identify bacterial species. In future, it is likely that the bacteriological diagnosis will look beyond just the 16S rRNA sequence. Additionally, there are situations where the 16S rRNA sequence may not be able to differentiate between species. Under these circumstances, sequences of essential genes such as that of heat shock proteins maybe employed. This is particularly useful in differentiating between species such as Burkholderia cenocepacia and B. multivorans.\n\nFastidious BacteriaThe diagnosis of infections due to fastidious bacteria has benefited greatly from molecular detection methods. Many fastidious bacteria, such as Mycobacterium tuberculosis, Chlamydia trachomatis, Neisseria gonorrheae and Bordetella pertussis have important public health implications. Non-culture-based molecular testing has the advantage of avoiding delays of days to weeks over conventional culture techniques. This is important for early recognition and treatment. Fastidious bacteria usually transmit tuberculosis and sexually transmitted diseases. Collection of samples in cases of sexually transmitted diseases can be a source of intense embarrassment. Again, the chances of contamination are quite high and most of the organisms are highly fastidious and require special conditions for growth. Under these circumstances, molecular detection is useful, since non invasive specimens that are unsuitable for traditional culture, such as initial stream of urine and selfcollected vaginal swabs can be used. These are more convenient and acceptable and increase the patient's compliance with testing. Molecular testing methods provide sensitivity and specificity which is equivalent to what is seen with standard culture methods. In remote areas, molecular

samples in specialised transport medium to preserve viability. In addition, molecular methods can test for multiple genital pathogens such as C. trachomatis, N. gonorrhoeae, the Donovanosis agent and the genital mycoplasmata from the same swab. Mycobacteriology has been aided by the introduction of molecular methods. However, it is important to note that molecular detection of M. tuberculosis is one of the few examples where conventional culture remains more sensitive. This is possibly due to the difficulty in releasing the DNA from the bacterial cells during the extraction process. Yet, molecular methods are important in detection of M tuberculosis since it allows confirmation of acid-fast bacilli seen on microscopy with up to 98% sensitivity in pulmonary tuberculosis. Moreover, the results are obtained within a day compared to two weeks or more for culture. Specimens that are smear-negative have a much lower chance of molecular confirmation. Molecular biology can also speciate the organisms grown on a culture plate in a day as compared to the four weeks it would normally take by standard methods.\n\nAntibiotic resistance markersDetection of antibiotic resistance is of utmost importance in today's scenario where bacteria are developing resistance faster than the antibiotics can be discovered. Applying rapid and reliable genotypic detection to bacteria with infection control implications such as methicillin resistant Staphylococcus aureus (MRSA) and vancomycin resistant enterococci (VRE) is of great potential benefit. The differentiation of MRSA from other S. aureus is done by detection of the mecA gene responsible for methicillin resistance. It is important to detect

MRSA early, not only for epidemiological purposes but also for early initiation of the patient's treatment, which is more aggressive and specific in such cases. Similarly, detection of VRE is rapid using DNA-based amplification techniques, which are highly sensitive as compared to traditional methods. Extended spectrum  $\beta$ -lactamases (ESBL) are found in Escherichia coli and Klebsiella pneumoniae and are readily transmitted to plasmids and transposons. ESBL containing bacteria can spread rapidly in health care facilities to cause wound infections, urinary tract infections and septicaemia. Molecular detection of these point mutations at the active site of the  $\beta$ lactamase gene can confirm the ESBL and allows for epidemiological typing. Multi-drug resistant tuberculosis (defined as the presence of both rifampicin and isoniazid resistance) is a serious problemin many parts of the world. Rather than employing traditional culture methods which deliver results after several days, detection of the rpoB and hsp65 gene targets can detect the resistance genes in a single day.\n

# MOLECULAR BIOLOGY IN MYCOLOGY AND PARASITOLOGY

\nAlthough not frequently applied for detection of eukaryotic infections, molecular testing can be helpful in a number of clinical circumstances. PCR can be used to detect Pneumocystis jiroveci infection in HIV patients. The specificity of PCR is however limited in such cases, as this organism is a ubiquitous commensal and can be detected even in the absence of pneumonia. Aspergillus spp. can be detected by PCR especially in neutropenic patients. It is difficult to culture Aspergillus early in the disease and so molecular testing can be of great benefit. Parasitological diagnosis is aided by molecular methods, since most parasites are not cultured in routine laboratory settings, and therefore, their diagnosis relies mostly on the relatively less sensitive methods, such as microscopy and serology. Toxoplasma gondii can be detected by PCR in amniocentesis fluid (to confirm foetal infection) and in CSF (to diagnose toxoplasma encephalitis). In cases of malaria, PCR can diagnose malaria even after chemoprophylaxis and/ or treatment has been given. It can also diagnose mixed infections which are difficult to diagnose on microscopy.\n

#### LIMITATIONS OF MOLECULAR METHODS

\nDespite so many advantages of molecular diagnostics, conventional diagnostic methods cannot be replaced, since many common tests performed in the clinical microbiology laboratory are rapid and inexpensive. Culture methods have advanced considerably and the modern automated culture systems allow rapid identification of bacteria and susceptibility testing. Bacterial culture methods can detect a large number of bacteria and speciation can be done rapidly. In contrast, PCR technique can detect only those organisms whose DNA is complementary to the primers used. Therefore, covering a similar breadth of possible organismsas culture would require introduction of inexpensive and simple microarray technologies, which are not yet available.\n\nFalse Positive and False Negative ResultsContamination remains the bugbear of molecular testing methods. The problems of contamination and laboratory management have been dealt with elsewhere in this book (Chapter 13 – Establishing a Molecular Biology Laboratory), suffice to say that scrupulous attention to avoid contamination

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needs to be given. To avoid false positive results due to laboratory contamination, relatively large and separate laboratory areas are required for reagent preparation, specimen preparation and product detection together with high level of staff training and skill. Amplicon laboratory contamination can be reduced by ultraviolet light irradiation of reagents and chemical inactivation of surface contamination with sodium hypochlorite. Intersample contamination can be reduced by the use of disposable equipments, cotton filter tips, and disposable personal protective equipments such as caps, gowns and gloves. Appropriate negative controls should be included in every PCR run to detect any kind of contamination. Poor primer design can also lead to erroneously positive results. Primers may be poorly designed such that incidental amplification of microorganisms other than those sought occurs. Also, primers are designed based on the known sequences available through international databases, but designing a primer wrongly may result in non-specific amplification. For further clarification on this subject, readers are requested to refer to primer design in the basics of PCR (Chapter 7 – The Polymerase Chain Reaction). False negative results may also be a problem. It maybe difficult to extract DNA from organisms like Mycobacterial spp. Substances in some clinical specimens such as sputum and faeces can degrade DNA and RNA, while other specimens may contain substances such as polysaccharides, haem and therapeutic drugs that inhibit the PCR enzymes. It is, therefore, important to include inhibitor checks for each specimen to ensure that the negative PCR reaction is not actually an inhibited reaction. Applying internal control can check for the presence of inhibitors as well as ensure successful DNA

extraction.\n\nLack of Uniformity in Molecular TestingMolecular diagnosis is also complicated by the vast array of in-house PCR tests used in different laboratories. Many tests are not available just because it is not commercially viable to manufacture their kits. Investigators then develop their own inhouse tests which use different primers amplifying different genes and/ or different sequences within genes. The PCR format maybe different (standard PCR, multiplex or nested PCR). These variables lead to a considerable lack of uniformity in testing.\n\nDifferentiation between Infection and DiseaseSince the presence of nucleic acid sequence does not necessarily mean the presence of viable organisms, a problem with interpretationof PCR results can emerge. For some infections, such as invasive meningococcal disease, the presence of meningococcal DNA at a sterile site has a very high positive predictive value. However, the detection of P. jiroveci in immunosuppressed patients may have only a 50% positive predictive value for PCP, since P. jiroveci may colonise as well as cause disease. In some cases, quantitative

PCR maybe helpful because higher organism loads are more specific for infection. Also, RNA can be used as a template. Since RNA degrades easily, the presence of RNA would indicate pathogen viability and replication.\n

## **MOLECULAR BIOLOGY AND BIOTERRORISM**

\nThe earlier belief that bioterrorism is not a serious threat has been proved wrong. It is evident from so many recent attacks since mid 1980's, that bioterrorism is not a myth but a real problem. Biotechnology can be used quite easily by committed terrorist groups to produce microorganisms that are capable of large scale morbidity and mortality.\n\nOrganism

attributesThe five basic attributes that characterize a perfect military biological warfare (BW) agent are as follows: High virulence coupled with high host specificity; High degree of controllability; the organism should attack only specific groups or populations of people and should not attack the people initiating the bioterrorist attack. High degree of resistance to adverse environmental forces; Lack of timely countermeasures to the attacked population; Ability to camouflage the BW agent with relative ease. Some of these attributes might not be so important for BW agents that are applied for bioterrorism. For example, a terrorist group might not be concerned, whether the agents it uses can be controlled after release. Nevertheless, these criteria serve as useful considerations regarding the type of microorganisms which can possibly be used by the bioterrorists. In addition, to develop perfect bioterrorist agents, modern biotechnology techniques may be applied to enhance any or all of eight characteristics or traits of microorganisms i. e.- hardiness, resistance, infectiousness, pathogenicity, specificity, detection avoidance, senescence, and the viable but non-culturable state.\n\nUse of molecular biology in enhancing bioterrorist weaponsIn 2001, Australian scientists manipulated the mousepox virus to suppress the wild mouse population. The outcome was a modified virus that was far deadlier than the original one. This modified strain was also capable of killing mice naturally immune to mousepox or those immunized against the mousepox virus. Since the smallpox and the mousepox viruses are analogous to each other, it is entirely possible that the same experiment can be carried out in the smallpox virus. The smallpox virus is not readily available to terrorist organizations, however it is possible

for them to modify other viruses to subvert the human immune system. Again, it is not impossible to synthesize a new organism. In 2002, scientists in USA were successful in synthesizing polio virus from scratch using chemicals available in the open market. It is not only viruses that are prone to genetic manipulation. Bacteria and mycobacteria are also prone to genetic modification. Mycobacteria have been manipulated and a hypervirulent mutant strain of tuberculosis has been produced. Similar experiments have been carried out with protozoa such as Leishmania major. It is, therefore, possible to create lethal microorganisms by easily available methods. It would be wrong to assume that the access to these methods would be limited to research laboratories. Most of the techniques used are easily available and can be reproduced in an average laboratory.\n\nThe molecular basis of detectionIt is easy for the bioterrorist to manipulate the microscopic world for his benefits. However, it is equally easy for the biotechnologist to detect the organism and institute appropriate actions. Ideally, detection platforms should be capable of rapidly detecting and confirming biothreat agents, including modified or previously uncharacterized agents, directly from complex matrix samples, with no false results. Furthermore, the instrument should be portable, user-friendly, and capable of testing for multiple agents simultaneously. Such an instrument is as yet unavailable. The PCR can be used for the detection of bioterrorist weapons. However, it has inherent problems which have been alluded to earlier. It also requires a clean sample and is unable to detect protein toxins. It can also give a false positive result because of its inherent sensitivity. Q-PCR can be utilized to detect several targets simultaneously using different reporter dyes for

different targets. However, accurate characterization or identification of bacteria by Q-PCR is limited by the same bias and variations that are inherent in many nucleic acid techniques. Immunoassays have increasingly been used and developed for the detection of infectious diseases. Immunological detection has been successfully employed for detection of biothreat agents such as bacterial cells, spores, viruses, and toxins based on the concept that any compound capable of triggering an immune response can be targeted as an antigen. Immunoassays generally test for only one analyte per assay. The specificity of immunoassays is limited by the antibody guality, and sensitivity. The sensitivity is typically lower than with PCR and other DNA-based assays. As improvements are made in antibody quality (e. g., production of antibodies from recombinant libraries) and in assay parameters, it may be possible to increase immunoassay sensitivity and specificity. It is also proposed to use antibody fragments for detection of antigen fragments. Aptamers are small DNA or RNA ligands that recognize a target by shape and not by sequence. RNA aptamers include the ribozymes that can be engineered to generate a signal after target capture. DNA aptamers bind to a target after exposure to UV light. Aptamers can be used to detect the presence of entire organisms such as Bacillus anthracisspores or toxins like ricin. Finally, the microarray can be used to detecting a bioterrorist attack. Microarrays can detect several organisms at one go, therefore it potentially remains a tool for future in detecting biological warfare agents. To conclude, it is possible for committed terrorists to manipulate microorganisms using available molecular techniques to make them more virulent without much difficulty. However, alertness and access

to modern diagnostic methods can easily halt a bioterrorist attack. In conclusion, it can be stated that the key components to the fight against a bioterrorist attack are preparedness and awareness.