# Nucleic acid technology and infectious diseases

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**The past decade has witnessed an explosion in the knowledge of microbial genetics, pathogenesis, and antimicrobial resistance as a result of advances in molecular technology. This has brought important breakthroughs in the management of patients with infectious diseases, as organisms that had previously been difficult to demonstrate in vitro can now be detected by molecular techniques such as the polymerase chain reaction. Not only is rapid diagnosis now possible, but old diseases of uncertain aetiology have been found to have an infective origin, for instance, Whipple's disease. Molecular technology has also contributed greatly to epidemiological studies of outbreaks, understanding antimicrobial resistance, developing new antimicrobial agents, the in vitro synthesis of immunomodulators, production of vaccines, and gene therapy. The limitations of these latest technologies, however, need to be remembered so that they yield meaningful information for patient care.**

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## Introduction

Infections arise from the interaction between microorganisms and the human host who may be colonised, latently infected, or develop clinical features of inflammation, cell death, immunosuppression, or neoplasia. The understanding of infectious diseases is largely achieved by epidemiological studies or laboratory techniques.

Traditional laboratory methods used to study the implicated microbes and the host response include (1) visualisation of microbes by microscopy; (2) conventional culture in broth or agar media, cell lines, and laboratory animal hosts followed by identification of the isolates; (3) detection of the host immune response by measurement of specific antibodies against the implicated microbes; and (4) studying pathogenesis with various chromatographic, enzymatic, and immunological methods at the level of cell biology, histology, and animal or human experimentation. All these techniques vary in their scope of application, sensitivity, specificity, turnaround time, and labour intensiveness in the detection of various aspects of the infectious process. But these tools alone cannot provide information at the subcellular level, that is, they do not tell us how the various attributes or components of the microbe function at different stages of infection and their effect on the host.

## Nucleic acid technology

Since the introduction of nucleic acid technology into the arena of infectious disease, a huge amount of new information has been accumulated to facilitate the diagnosis, treatment, and prevention of infections as well as the understanding of their pathogenesis. These include: the extraction and purification of microbial DNA and RNA, restriction endonuclease digestion, amplification of a specific fragment of DNA or RNA with a defined sequence by techniques such as polymerase chain reaction (PCR), ligase chain reaction, RNA polymerase amplification, Qβ replicase amplification and strand displacement amplification, hybridization

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of a specific probe to a known DNA or RNA sequence, and cloning of genes by screening genomic or cDNA libraries for the production of specific antigens or DNA sequences used in serological assays or as probes.

These protein antigens and DNA sequences may also be candidate recombinant or naked DNA vaccines. Cloned DNA fragments can further be characterised by DNA sequencing to identify homologous sequences in the gene bank. The expression of these sequences at different stages of the cell cycle and their binding to transactivators/repressors can be studied by Northern blotting in pulse chase experiments and DNA footprinting. Furthermore, the importance or strategic function of these cloned sequences, in the pathogenesis of infection, can be determined by site-directed mutagenesis in which a transposon is inserted into the sequence, which renders the gene non-functional.

With this armamentarium of nucleic acid techniques, it is now possible to develop rapid, sensitive, and specific methods for the detection of microbes and their antimicrobial resistance profiles, reproducible typing techniques for outbreak investigation, new antimicrobials for treatment, safe and effective vaccines for the prevention of infections, and immunomodulators to enhance the host defence mechanism. It is also possible to insert a normal gene into host immune cells to reconstitute a lost immune function or to insert a lethal gene to kill a cancer cell infected by an oncogenic virus. There are numerous publications on the use of nucleic acid technology in the understanding and management of infectious diseases. Only those with a direct impact on patient care are used as examples here.

## The polymerase chain reaction

By far the most important nucleic acid technique with a direct impact on the diagnosis of infections is the PCR. The gold standard for the diagnosis of many infectious diseases has traditionally been the isolation of the implicated microbes by culture from representative clinical samples. The difficulty of culturing many microbes due to their fastidious growth requirements, slow rate of growth, or lack of culturing facilities led to the development of laboratory methods to detect specific antigens, metabolic products, DNA sequences, or antibody responses of the patient. Besides replacing the culture process and improving the turnaround time, these non-culture tests may circumvent the problems associated with the previous administration of antibiotics (which renders the culture negative due to residual antibiotics in the specimen), requirement for stringent conditions of specimen transport or storage, the need for an invasive tissue biopsy, and the lower sensitivity of culture in some cases.

Since the introduction of PCR into the arena of diagnostic microbiology, the test has been extensively used in the diagnosis of many viral, bacterial, fungal, and parasitic infections. Examples include tests for cytomegalovirus (CMV),[1] *Mycobacterium tuberculosis*,[2] the Whipple's disease agent,[3] *Pneumocystis carinii*,[4] and *Toxoplasma gondii*,[5] which are either slow-growing, difficult to culture, or non-cultivable. Its application in the direct detection of infectious agents from clinical specimens is based on three key principles: (1) all microbes contain a genome composed of DNA or RNA; (2) unique DNA or RNA sequences can be identified in each genome that are

**Table 1. Effect of the decontamination procedure for pulmonary specimens on the sensitivity of the polymerase chain reaction assays and standard culture[6]**

| | Limiting dilution of broth culture | | | | |
|---|---|---|---|---|---|
| **Decontamination procedure** | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| No decontamination | | | | | |
| mean CFU/10 μL* | Confluent | >200 | 30 | 3 | 0 |
| PCR† assays | + | + | + | ± | - |
| After decontamination | | | | | |
| mean CFU/10 μL | >200 | 36 | 4 | 0 | 0 |
| PCR assays | + | + | + | ± | - |

*CFU colony forming units on agar medium by Miles and Misra count
†PCR polymerase chain reaction

**Table 2. Performance of polymerase chain reaction assay in pulmonary specimens taken from different patient populations[6]**

| Sample type | Percentage of positive cultures | | Percentage of negative cultures | |
|---|---|---|---|---|
| | PCR + | PCR- | PCR + | PCR - |
| Serial follow up specimens from patients treated for tuberculosis (n=1169) | 90.8 | 9.2 | 15.8* | 84.2 |
| Specimens from patients not previously known to have tuberculosis (n=329) | 71.4 | 28.6 | 0 | 100 |

* All are from patients with culture-documented pulmonary tuberculosis while receiving antituberculous treatment

specific for that particular microbe; and (3) complementary primers to these specific sequences can be synthesised and used to bind to the target sequences present in the clinical specimen. In the presence of suitable reaction conditions, trillions of copies of the specific segment of gene sequences flanked by the two primer sequences can be generated within a few hours in a temp-cycler.

## Using the polymerase chain reaction to diagnose tuberculosis

One of the areas in which PCR has proved to be invaluable is the diagnosis of tuberculosis. Using a single-tube nested PCR assay, Chan et al showed that the test is highly sensitive when evaluated in an in vitro system, even after the NaOH decontamination process (Table 1).[6] Compared with traditional culture, which remains the gold standard for diagnosing tuberculosis, the overall sensitivity of PCR in respiratory specimens was 89%. The PCR positivity rate, however, was influenced by the smear positivity rate of the specimens (Table 2). Although the value of PCR has generally been recognised in the diagnosis of pulmonary tuberculosis, its value in extrapulmonary disease has not been universally accepted (Table 3). Some investigators have shown that the sensitivity of PCR in extrapulmonary specimens (cerebrospinal fluid, pleural fluid, etc.) is sometimes inferior to standard culture because of the small volume of specimens received, low number of bacteria present, or the presence of inhibitors.[6]

## Detecting cytomegalovirus infection in immunocompromised patients

Due to its exquisite sensitivity, PCR has been used as a marker for pre-emptive therapy against CMV disease in bone marrow transplant recipients.[7] Traditional culture for CMV from saliva and urine in immunocompromised patients is of limited value as it has a poor positive predictive value for disease. The culture of CMV from peripheral blood leukocytes is an important indicator for CMV disease but the turnaround time ranges from two days using shell VIAL culture to more than two weeks using conventional culture. In a longitudinal study of 101 bone marrow transplant recipients monitored weekly for CMV viraemia by culture and by PCR, the latter turned out to be a rapid, sensitive, and specific method for guiding the initiation of pre-emptive therapy (Table 4).[1] There was excellent correlation between at least two consecutive positive leukocyte CMV PCRs and subsequent development of graft-versus-host disease and CMV disease (Table 5).

## Other uses

Besides the detection of infectious agents, PCR has also been shown to provide rapid information on drug resistance[8] and clonality in epidemiological investigations of outbreaks.[9] By using evolutionarily conserved areas of 16S ribosomal DNA, PCR-directed DNA sequencing has enabled scientists to discover the existence of previously unknown or non-cultivable

**Table 3. Correlation among results of polymerase chain reaction assay, standard culture, and clinical treatment in extrapulmonary specimens[6]**

| No. and type of specimens | Culture positive for *M. tuberculosis* (%) | PCR positive (%) | No. of patients subsequently treated for tuberculosis (%) |
|---|---|---|---|
| CSF (n=373) | 4.6 | 2.4* | 12 |
| Pleural fluid (n=101) | 25 | 11* | 37 |
| Tissues (n=33) | 36 | 15 | 54.5 |
| Peritoneal and synovial fluids (n=14) | 14.3 | 0 | 35.7 |
| Urine (n=15) | 26.7 | 20 | 33.3 |
| Total (n=536) (%) | 12.3† | 6* | 20.3 |

* Five false positive results (four CSF, one pleural fluid). All are related to the use of re-used specimen bottles and the use of non-guarded pipette tips for aliquoting specimens.

† Nine specimens were smear positive.

bacteria.[10] Existing diseases of unknown aetiology and new syndromes may now be found to have a microbial cause with the approach to diagnosis redefined.

## Limitations

Despite the enormous number of publications on PCR in diagnostic microbiology in the past five years, the use of the PCR assay is still restricted to research centres that have the specialised reagents, equipment, and proper safeguards against cross-contamination with amplified DNA. Stringent precautions to prevent PCR product carryover and the inclusion of necessary positive and negative controls in each run are required.[11] However, commercial kits with simple operating procedures and good designs against cross-contamination have recently become available. Examples include the Amplicor (Roche Diagnostic Systems, Basel, Switzerland), LCx (Abbott Diagnostics, Abbott Park, Il, US) and the GenProbe (GenProbe Inc., San Diego, Ca, US) systems, which detect *M. tuberculosis* and respiratory pathogens.[12] These kits will allow for more extensive use of the PCR assay in clinical management. Sufficient in-use information will hopefully be generated in the near future to determine the exact role of PCR in the management of infectious diseases.

## The application of gene cloning

The technology of gene cloning has allowed for the development of specific gene probes and recombinant antigens. At present, commercially manufactured probes have been used routinely for the identification of *M. tuberculosis* and other non-tuberculous mycobacteria from positive cultures. Because of the low sensitivity, these probes cannot be used for direct detection in clinical specimens. Using a chemiluminescent signal system coupled to the DNA probe, various genotypes of human papillomavirus can be detected directly from cells harvested from endocervical swabs. This could identify women with a higher risk of progression from cervical intraepithelial neoplasia to invasive cancer.

Another important use of the DNA probe hybridization technique is in the field of DNA fingerprinting. Traditional typing techniques in bacteriology are phenotypic methods that include serotyping, immunobloting, phage typing, bacteriocin production, and antibiograms. Many of these are either poorly discriminatory or not highly reproducible. A reliable fingerprint can be generated by digestion of the extracted microbial DNA by restriction endonuclease, gel electrophoresis, and then hybridization with a DNA probe. This has been used in Hong Kong for the study of out-

breaks of *Vibrio cholerae* O1 and the source of an imported case of *V. cholerae* O139.[13]

Recombinant antigens are used extensively to detect a specific antibody response directed against the putative microbe. Many enzyme immunoassays designed for the detection of HIV, HBV, and HCV use a single or a pool of recombinant protein antigens. Fur-

**Table 4. Clinical characteristics of bone marrow transplant recipients and their disease correlations with leukocyte polymerase chain reaction or cytomegalovirus viraemia[1]**

| Characteristics | Value for group | | P value |
|---|---|---|---|
| | Patients with no positive or isolated single PCR* | Patients with consecutively positive PCR | |
| Total no. of patients | 43 | 58 | |
| Sex (F/M) | 18/25 | 28/30 | 0.52 |
| Median age (range, y) | 29 (14-48) | 32 (17-50) | |
| Diagnosis†: | | | |
| AML | 14 | 21 | 0.70 |
| CML | 12 | 18 | 0.73 |
| Others | 17 | 19 | 0.48 |
| CMV‡ status of donor/recipient: | | | |
| +/+ | 25 | 52 | 0.0002 |
| -/+ (including autologous BMT§) | 13 | 5 | 0.005 |
| +/- | 2 | 1 | 0.39 |
| -/- (including autologous BMT) | 3 | 0 | 0.04 |
| BMT type: | | | |
| Allogeneic | 29 | 51 | 0.012 |
| Autologous | 11 | 1 | 0.0002 |
| Matched unrelated donor | 2 | 6 | 0.29 |
| Syngeneic | 1 | 0 | 0.24 |
| No. of patients receiving total body irradiation | 23 | 40 | 0.11 |
| Acute GVHD‖ (grade II-IV) | 10 | 36 | 0.0001 |
| Blood samples monitored | 662 | 779 | |
| Positive PCR assays (%) | 22 (3.3) | 275 (35.3) | |
| Viraemic patients (CMV isolates) | 0 | 25 (49) | <0.00001 |
| No. of patients with CMV disease (%) | 3 (7.0) | 35 (60.3) | <0.00001 |

* PCR polymerase chain reaction
† AML acute myeloid leukaemia; CML chronic myeloid leukaemia
‡ CMV cytomegalovirus
§ BMT bone marrow transplant
‖ GVHD graft-versus-host disease

thermore, monoclonal antibodies can be raised against these recombinant antigens to produce immunoassays that can detect specific circulating antigens. Examples of these immunoassays include the p24 antigen of HIV, the surface antigen of HBV, and the pp65 antigen of CMV, which are present in the serum or buffy coat of infected patients. Using these specific recombinant antigens or the monoclonal antibody directed against such antigens, both the sensitivity and specificity of these assays are greatly enhanced because other cross-reactive components are excluded from the assay.

## Molecular technology and vaccines

The prevention of infectious diseases has also benefited from molecular technology. Recombinant vaccines such as the hepatitis B vaccine are extensively used and are effective in preventing hepatitis, and probably the sequelae of cirrhosis and hepatocellular carcinoma. The use of a mono-component recombinant antigen eliminates the theoretical risk of infection transmitted by the old hepatitis B vaccine. The risk of any autoimmune phenomena induced by other immunogenic components of the microbes is minimised since only a single or a few antigenic epitopes are used. The use of a recombinant protein vaccine completely prevents the possibility of reversion of an avirulent live vaccine to a wild strain capable of causing an iatrogenic infection.

An exciting development is the use of naked DNA vaccines, also known as somatic transgene vaccination. A cloned fragment of the gene of the target antigen is introduced into host cells, often the muscle cells. Naked DNA vaccines offer the advantages of high product purity, ease of production, stability of the episomal DNA, long-lasting humoral and cellular immunity, as well as efficient generation of CD8+ cytotoxic T cells and CD4+ Th1 cells.[14] This technique may prove to be useful in the future development of vaccines as well as for therapeutic purposes. Early studies on its use in experimental tuberculosis have been favourable.

## Making recombinant antimicrobials and cytokines

The rapidly increasing antimicrobial resistance in the hospital has called for the development of more effective antimicrobials. The cloning of antibiotic biosynthesis genes is actively being pursued by many investigators. New antibiotics can be produced as structural variants of existing ones by manipulation of the genes encoding the relevant enzymes involved in the biosynthetic pathway. These structural variants often have different spectra and potency of activity against various bacteria.

Besides antimicrobial therapy, important advances in the treatment of immunosuppressed patients have been achieved using recombinant cytokines. Patients with chemotherapy-induced neutropenia have benefited from GM-CSF or G-CSF, which shorten the duration of neutropaenia and reduce the risk of associated infectious complications. The incidence of infections in children with chronic granulomatous disease is decreased by the use of γ-interferon. In addition to using recombinant immunomodulators that

**Table 5. Correlation of risk of cytomegalovirus disease with viraemic load as expressed by the number of positive leukocyte polymerase chain reaction assays and cytomegalovirus isolation[1]**

| No. of patients | | | | |
|---|---|---|---|---|
| **Total** | **With CMV* disease** | **Number of positive PCR† assays** | **CMV isolation** | **Relative risk odds ratio (95% CI‡)** |
| 25 | 0 | 0 | - | 0 |
| 18 | 3 | 1 | - | 0.27 (0.06-1.12) |
| 33 | 17 | ≥2 | - | 2.4 (0.9-6.1) |
| 25 | 18 | ≥2 | + | 7.2 (2.4-22.6) |

* CMV cytomegalovirus
† PCR polymerase chain reaction
‡ CI confidence interval

augment the defence mechanism of the hosts, attempts to replace defective gene(s) causing primary immunodeficiencies have been made. Patients with defective T cell immunity due to adenosine deaminase (ADA) deficiency have been successfully treated by gene therapy. Using a retroviral vector, a cloned ADA gene can be transfected into the lymphocytes of the patient; the enzymatic and immune functions are thus restored.

Although the impact of gene therapy in infectious diseases is still not apparent,[15] much ongoing research is focused on its use in HIV infection and oncogenic virus-associated cancer. In HBV-associated hepatocellular carcinoma and HHV-8-associated Kaposi's sarcoma, attempts have been made to insert the thymidine kinase gene carried on a retroviral or adenoviral vector into the cancer cell through HBV- or HHV-8–encoded surface receptors. Once the thymidine kinase gene is integrated into the cancer cell, ganciclovir is given to the patient, with the drug then accumulating to a toxic level in those cancer cells.

It is important to remember that the battle against infectious diseases is far from being won. Many emerging and re-emerging microbial pathogens have been discovered in the past few years. Nevertheless, with the advances in nucleic acid technology, more rapid and effective control and understanding of these problems may hopefully be achieved.

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