REPORT

USE OF POLYMERASE CHAIN REACTION IN LABORATORY TESTING

HEALTH TECHNOLOGY ASSESSMENT UNIT
MEDICAL DEVELOPMENT DIVISION
MINISTRY OF HEALTH MALAYSIA

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Head, Health Technology Assessment Unit,
Medical Development Division,
Ministry of Health Malaysia
21st Floor, PERKIM Building.
Jalan Ipoh, 51200 Kuala Lumpur.
Malaysia.

Tel: 603-40457639
Fax: 603-40457740
e-mail: htamalaysia@hotmail.com

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MEMBERS OF EXPERT COMMITTEE

Dato' (Dr.) Mohd Ismail Merican - Chairman
Consultant Physician
Kuala Lumpur Hospital

Dr. Yasmin Ayob
Consultant Haematologist
Blood Bank Services Centre
Kuala Lumpur Hospital

Dr. Shalini Kumar
Consultant Pathologist
Seremban Hospital

Dr. Zubaidah Abdul Wahab
Clinical Microbiologist
Kuala Lumpur Hospital

Dr. Alan Khoo
Molecular Pathologist
Institute for Medical Research
Kuala Lumpur

Dr. Zubaidah Zakaria
Consultant Haematologist
Institute for Medical Research
Kuala Lumpur

Project Co-ordinators

Dr. S Sivalal
Medical Development Division
Ministry of Health Malaysia

Dr. Rusilawati Jaudin
Medical Development Division
Ministry of Health Malaysia

EXECUTIVE SUMMARY

Polymerase Chain Reaction (PCR) is in vitro amplification of specific DNA sequences by simultaneous primer extension of complementary strands of DNA where numerous copies of small fragments of genetic material can be produced to facilitate the analysis of genetic material.

This study was done to assess the efficacy, efficiency and cost implications of PCR technology in medical care.

The results shows that PCR can assist in early diagnosis of TB, identify mycobacterium in clinical specimens even with smaller number of bacteria, evaluate response to therapy, detect cases of relapse and differentiate mixed infections. PCR is recommended for specific indications like disseminated TB or TB meningitis. In HIV, PCR can be used to predict progression and survival, as well as the risk of vertical transmission. It can also assist in detecting the viral load including pregnancy and in staging of HIV. PCR thus, should be used to assess HIV risk and therapy. PCR is also able to detect low level of HBV viraemia in asymptomatic HbsAg positive subjects and in evaluating the degree of response to therapy. Similarly, it can be used in identifying HBs Ag-negative patients with liver disease, HBV infection in liver transplantation, and in identifying mutant strains of HBV. PCR is recommended for evaluation of anti-viral therapies and for detection of HBV in high-risk patients.

PCR can be used for qualitative and quantitative measurement of viraemeia in diagnosis of acute HCV infection and anti-HCV-negative chronic hepatitis C carriers. It can also be used in the evaluation of HCV viraemia in asymptomatic blood donors with normal liver enzymes, assessing virological response to treatment, predicting treatment response to alpha-interferon and assessing severity of disease. PCR is thus recommended in assessing treatment efficacy.

Typing by PCR is simpler, accurate and reliable and is recommended as the method of choice for a transplant programme, especially in unrelated bone marrow and cord blood transplants. PCR testing is also recommended in the identification of post-transfusion purpura, zygosity testing, antenatal diagnosis and thrombocytopenic patients, and to distinguish between homozygous and heterozygous individuals. Similarly, PCR is recommended for testing patients with haemgolinopathies especially paediatric patients as well as in prenatal diagnosis, for carrier detection and for detection of alpha and beta thalassaemias In congenital disorders PCR is recommended as an alternative method for confirmatory diagnosis of Duchenne Muscular Dystrophy and Fragile X syndrome

In addition, PCR is recommended for screening high-risk populations for genetic predisposition for cancer, monitoring of residual tumour cells in peripheral blood, paternity testing in situations where there are only minute quantities of tissue or where the tissue has been damaged, identification of unknown skeletal remains in mass disasters, sexual assaults against women, establishment of a forensic database, and to establish patient identity in cases of mix-up.

The cost per test ranges from RM 40.00 to RM150.00. Thus, PCR is a rapid, safe and cost-effective method for selective testing

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USE OF POLYMERASE CHAIN REACTION IN LABORATORY TESTING

1. INTRODUCTION

Polymerase Chain Reaction (PCR) is a gene amplification technique developed in 1985 by Mullis and colleagues at Cetus Corporation. It is currently the best known of the genetic diagnostic methods. PCR is essentially a technique for in vitro amplification of specific DNA sequences by simultaneous primer extension of complementary strands of DNA. Using PCR numerous copies of small fragments of genetic material can be produced to facilitate the analysis of genetic material.

Recently, PCR has been found to be beneficial in predicting severity and treatment outcomes for infections like HIV and hepatitis. Other uses include the tracking of genetic diseases, identification of bacteria or viruses not possible with the usual diagnostic techniques, and customizing antibodies for cancer treatment. PCR may also be used in forensic medicine.

1.1 Infectious Diseases

The rapid and accurate detection and identification of an infectious agent is of high priority in the treatment of infectious diseases. The gold standard for detecting the causative agent is *in vitro* culture techniques. Serology, on the other hand, provides an indirect measure of infection. However, there are several infectious agents for which there are no reliable *in vitro* culture systems or serologic techniques. Since PCR directly detects minimal quantities of pathogenic genetic material, they can provide high sensitivity diagnosis in the acute phase without the need to await antibody formation.

PCR has been used for the detection and identification of a number of specific bacterial pathogens causing infectious diseases. These include those that are difficult to grow e.g. *Mycobacterium leprae*, *Borrelia burgdorferi*, or slow-growing pathogens like *Mycobacterium tuberculosis*, where rapid identification is needed. The traditional *in vitro* culture techniques for these might take 4-8 weeks. Related to this are identifying pathogens such as *Shigella spp* from a dense background of normal flora. In addition, it can be used for antibiotic susceptibility testing, especially for those pathogens where the spectrum of resistance mechanisms is restricted and it is important to be able to predict drug resistance e.g. MRSA, DNA gyrase A mutation, that confers resistance to quinolones.

Tuberculosis (TB) is a persistent problem in developing countries and one of the main causes of mortality. There has been an increase in drug-resistant organisms in TB and the increasing number of high-risk immuno-suppressed patients further exacerbates it recently. Thus, a rapid and timely diagnosis of TB would aid in combating this disease. Currently, the laboratory diagnosis of mycobacterium infections relies on microscopic examination and culture of specimens. However, microscopic examination is only positive in 50-60% of patients with this disease, and hence, the identification of mycobacteria in culture is required for confirmation. The culturing of organisms has nearly 100% specificity and in addition it permits susceptibility testing of the isolates. When using conventional culture methods, however, results are only available after 3-6 weeks, since most pathogenic mycobacteria are slow growing organisms. Thus, this delays the diagnosis of this infection.

PCR also has been extensively used in viral diagnosis and monitoring due to the advantage of speed compared to culture, and also because of the greater sensitivity relative to viral antigen testing. For example, plasma HIV RNA determination or viral load is used as a prognostic marker of disease progression and provides a valuable tool to predict clinical response to therapy. PCR is also useful for early diagnosis of vertically transmitted HIV in infants less than 15 months of age and among seronegative patient of high risk for the acquired immunodeficiency syndrome.

Apart from this it has begun to be used in the identification and diagnosis of both fungal and parasitic infections such as *Cryptococcus neoformans, Pneumocystis carinii, Toxoplasma gondii* and Leishmaniasis. It allows rapid and sensitive diagnosis of fungal sepsis especially among immuno-compromised patients.

1.2 Haematological diseases

1.2.1 Human Leucocytes Antigen (HLA) Typing

The HLA antigens, which constitute the major histocompatibilty complex (MHC) in man, are the most important alloantigens in determining the compatibility of tissue grafts and haemopoeitic cells. This MHC gene are located on the short arm of chromosome 6, and encodes two classes of proteins - MHC Class I and Class II (or HLA class I and class II) - that are involved in the immune response. In addition to serological typing, cellular assays have served as a method for definition of class II diversity. During the past 10 years, more than 760 new HLA alleles have been identified by DNA sequence analysis. Serological specificity therefore are frequently not homologous with allelic types and comprise multiple undetected molecular subtype e.g. 18 HLA-A types are recognised by routine serology, whereas currently there are 85 recognised alleles for this locus. Characterization of alloantisera depends on the availability of a comprehensive cell panel. Laboratories in Europe, North America and Japan have provided the majority of alloantisera and the individual cell types. Consequently, these reagents may not be appropriate for the identification of new HLA specificity that may exist in other ethnic populations. The introduction of DNA based techniques for the detection of many HLA alleles has provided an opportunity to investigate the relationship between HLA disparity and transplant complication.

HLA typing is used for the following:

- allogeneic bone marrow transplant
- other organ transplants e.g. renal, heart , liver etc
- blood transfusion induced HLA alloimmunisation
- in identifying patients developing NHFTR,TRALI
- immunological refractoriness to platelet transfusion
- diagnosis of Human Immune Deficiency syndrome

- major histocompatibility complex class II
- bone marrow registry

1.2.2 Human Platelet Antigen (HPA) Typing

Platelets carry a number of antigenic structures on their surface, some of which are shared with other blood cells (ABH, HLA Class I). In addition, more than 10 separate platelet-specific alloantigen systems are recognised, nine of which have been included in the HPA 1-9 system nomenclature. In each antigen system, the difference between the 2 alleles is a single base change in the relevant gene, giving rise to a single amino acid difference in the glycoprotein. This substitution causes change in the tertiary structure of membrane glycoproteins giving rise to differing epitopes, which can allow alloimmune recognition.

Clinical conditions in which alloantibodies to platelet-specific antigens are produced are the following:

- ***platelet transfusion***
- febrile reactions to platelets and refractoriness to random platelets usually due either to non-immune causes, HLA Class I antibodies or platelet specific alloantibodies
- ***fetal/neonatal alloimmune thrombocytopenia***
- ***post-transfusion purpura***

An essential component of the diagnosis and subsequent treatment of patients with these conditions is rapid and accurate platelet typing. Traditionally, the platelet phenotype has been investigated using human sera, but good quality typing sera are scarce due to the low immunogenicity of HPA with a skewed distribution of alleles in the population. Thrombocytopenia is a major limiting factor in any method used for platelet phenotyping. Serological typing is reserved for large-scale donor typing, with genotyping methods being routinely used for investigation of patients.

1.2.3 Thalassaemia(Haemoglobinopathies)

Haemoglobinopathies represent one of the major health problems in Malaysia. In areas with a large population of persons at risk for haemoglobin(Hb) disorders, screening is therefore of outmost importance. Beta thalassaemia trait (the heterozygote carrier state) is present in about 3 to 9% of South East Asians, and is the most common trait in Malaysia. Alpha thalassaemia consists of four different syndromes, depending on the number of alpha globin chain gene deletions. Hydrops fetalis is a result of four gene deletion. These infants suffer from severe anemia, and die before or immediately after birth.

Screening for thalassaemia aims to offer testing to the population prior to having children, so as to be able to identify thalassaemia carrier couples. They can be informed of their risk, and the options for avoiding birth of severe forms of thalassaemia. The prime target in Malaysia is screening for alpha α, βHbE and Hb Constant Spring.

Hb E trait is the third most common haemoglobinopathy in the world, and the most common in South East Asians where prevalence is estimated to be 30%. Hb H disease is present in about 1% of South East Asians. Haemoglobin S and Haemoglobin C are found in sickle cell disease. The case fatality rate of sickle cell disease during infancy can be as high as 30-35% with inadequate or delayed treatment of infections.

1.3 Genetic Disorders

1.3.1 Duchenne Muscular Dystrophy

Duchenne muscular dystrophy *(DMD)* is a progressive lethal degenerative skeletal muscle wasting process, due to mutations of the dystrophin gene, which also affects the brain, heart and smooth muscles. It is an X chromosome-linked condition with an incidence of 1:3 500 male births. In 65% of cases, gross rearrangements (deletions or duplications) have been detected within the gene. Previously, carrier detection and prenatal diagnosis were performed using polymorphic markers within Xp211. The PCR technique has revolutionized deletion detection and haplotype analysis.

The primary aim is for the detection of DMD carriers in female relatives of patients with DMD. Mutations in DMD patients will be identified and the presence of these mutations in his relatives will be tested. For the diagnosis of DMD in the proband, specific diagnostic criteria are used. The DMD gene of the proband is then analysed for the mutation for the molecular confirmation of the condition. The mother of the affected child is then subjected to the DMD gene analysis to assess the carrier status.

1.3.2 Fragile X Syndrome

Fragile X Syndrome is a sex linked disorder and is the commonest cause of inherited mental retardation, affecting approximately 1 in 1 200 people worldwide. It is second only to Down's Syndrome as a chromosomal cause of mental retardation. Both males and females may be affected and the symptoms are so varied as to provide a spectrum of mental retardation ranging from a low normal I.Q. to mild, moderate or severe mental retardation. Fragile X syndrome appears in children of all ethnic and socioeconomic backgrounds.

It is caused by a mutation on the "X" chromosome at the site Xq27.3 that is near the end of the "X" chromosome that in a normal person contains between 6 to 50 repetitions of the genetic code CGG. For reasons, which remain unclear, the regulation of the code breaks down in some people, causing the number of CGG repeats to increase. An expansion of the sequence from 50 to 200 repeats is called a pre-mutation and generally causes few or no symptoms of Fragile X Syndrome. However, an increase of 200 - 2 000 CGG repeats is called a full mutation and results in the Fragile X Syndrome.

The physical features of the disease may be quite subtle, and because of this, many children and adults with Fragile X Syndrome have been known to, and may, appear fairly normal. About 80% of boys with Fragile X will have some degree of mental impairment,

ranging from low-normal intelligence to severe retardation. The majority are mildly to moderately retarded. Approximately 30% of girls with Fragile X are mentally impaired. The others have normal intelligence but may show subtle learning disabilities, particularly in mathematics.

1.4 Malignant Tumours

Most current cancer detection tests are based on either antibody assays to a marker protein with altered expression in cancer patients, or, on imaging studies to identify characteristic lesions. Generally, these assays require a significant volume of cancer cells. The molecular techniques of PCR and reverse transcriptase PCR (RT-PCR) are highly sensitive methods for detecting a small number of cancer cells, when the tumour burden is smaller and potentially more curable. PCR can complement current methods for diagnosing cancer especially with regards to classification of cancer cells and determining disease spread. PCR is especially useful in detecting cases where very few cancer cells are available for tissue testing. It is more sensitive and less expensive than existing methods

1.4.1 Breast Cancer

Breast cancer is an extremely common malignancy. About 5 to 10% of breast cancer patients have a hereditary form of the disease. The breast cancer susceptibility genes, BRCA1 and BRCA2, are altered in 30 to 70 percent of all inherited cases of breast cancer. A woman's lifetime risk to breast and ovarian cancer is elevated by mutations in the BRCA1 or the BRCA2 gene. Women with a BRCA1 mutation have a 80-90% risk of developing breast cancer and a 40-50% risk of developing ovarian cancer. Hence, testing of the BRCA1 and BRCA2 gene may indicate whether women with a family history of breast or ovarian cancer have increased chances of developing breast cancer.

1.5 Paternity Testing & Forensic Research

DNA patterns are inherited and are unique to an individual, with the exception of genetically identical twins. PCR-based DNA-analysis can be used to resolve paternity disputes especially in cases where the samples are small or partially degraded. Similarly, in forensic identification, the use of PCR in DNA fingerprinting is a reliable and robust method even when the tissue samples are exposed to extreme physical, thermal, and chemical insult.

2. OBJECTIVE

To assess the technical features, efficacy, efficiency and cost implications of PCR technology in medical care.

3. TECHNICAL FEATURES

A PCR test involves a series of 20 to 40 amplification cycles. Each cycle consists of three phases – denaturation of nucleic acid, annealing of primers and extension of primers. Primers are short oligonucleotides (20-30 bases long). The primers are annealed to the target DNA after they have been denatured. These primers are then extended using a DNA polymerase that synthesizes complementary DNA molecules. The use of *Taq* polymerase (a thermoresistant DNA polymerase) allows the procedure to be performed at a high temperature (72^{o} C) and thereby increase the specificity of the reaction.

After a single cycle, both the original target strands would have been copied. With PCR, there are repetitive cycles of denaturation, annealing and primer elongation. Each cycle doubles the amount of target DNA, so that with 30 to 35 consecutive cycles, million-fold amplification can be accomplished.

If the substrate is RNA, there is a supplementary step whereby a strand of cDNA complementary to the target RNA molecule is synthesized from one primer. This is followed by the normal PCR procedure after the addition of the second primer. Alternatively the cDNA can be synthesized using random priming, with subsequent addition of the two primers.

There are two types of PCR-based tests -qualitative and quantitative. The qualitative tests are used for screening purposes, for example, screening for infection e.g. Chlamydia infection, HIV, Parvo B19 etc. The quantitative tests, on the other hand, are used to measure the viral or bacterial load, monitor the effect of treatment (e.g. the elimination of TB bacteria), or to predict the prognostic value of a particular therapy.

3.1 Preparation of DNA and RNA samples.

DNA and RNA are usually prepared by standard techniques and only small quantities are required. Adequate amounts of nucleic acid are provided by needle biopsy, peripheral blood mononuclear cell (PBMC), and serum or plasma samples.

3.2 Primer design

Optimal designing of the primers is critical for the success of the PCR technique.

3.3 Analysis of amplification products

After completion of polymerase chain reaction, the sample can be analyzed in a variety of ways. The most common method is to electrophorese an aliquot on an agarose gel, stain with ethidium bromide or another DNA-binding fluorescent dye, and then visualize the

amplified material under UV light. The band should be as large as the distance between the two primers.

The identity of post-PCR amplification products must be established by traditional hybridization techniques such as the Southern blotting with radiolabelled oligonucleotides complementary to a region of the amplified DNA or, if available, a full-length cDNA probe, to check the specificity of the procedure.

3.4 Studies on genetic variability

Besides identifying DNA or RNA fragments, PCR allows nucleotide sequences to be rapidly defined.

3.5 Components of a PCR Laboratory

The PCR laboratory should ideally consist of three distinct work areas - specimen preparation area, reagent preparation and PCR set-up area, and amplification and detection area. In order to avoid problems of contamination, each area should be dedicated to a single procedure. These facilities are operated by personnel trained in molecular biotechnology, while in infectious diseases; interpretation is done by clinical microbiologists or a physician (possibly an infectious disease physician).

3.6 Indications

Many different types of tissues or fluids can be analyzed by PCR. Fresh, frozen or even formalin-fixed paraffin-embedded tissues have been successfully used.

Any target sequence can be analyzed by PCR for which the nucleotide sequence is known. The following are some of the pathogens that can be identified by PCR:

VIRUSES	BACTERIA	PARASITES	FUNGI
HIV 1 & 2	*Mycobacterium tuberculosis*	*Toxoplasma gondii*	*Cryptococcus neoformans*
HTLV 1 & 2	**Mycobacterium. Leprae**	Trypanosoma	Aspergillus
HPV	*Borrelia burgdorferi*	**Leishmania spp**	
CMV	**Treponema pallidum**	Plasmodium	
EBV	*Leptospira spp*	**Pneumocystis carinii**	
Rabies	*Escherichia coli*		
Hepatitis A,B,C	*Shigella spp*		
HSV 1 & 2	*Bordetella pertussis*		
Parvovirus B19	*Listeria pneumophilia*		

Rotavirus	*Clostridium difficile*
Rubella	*Mycobacterium pneumoniae*
Enterovirus	*Chlamydia spp*
Measle virus	
Japanese encephalitis	
Influenzae	
Mumps	

3.7 DNA analysis of DMD

For diagnosis of DMD, a double PCR multiplex test is performed on the patient's DNA. If a deletion is detected, the female relative (beginning with the mother of the patient) will be tested for carrier status by quantitative multiplex PCR and loss of heterozygosity of polymorphic loci. When no living patient is available for DNA analysis, or no deletion is found using the multiplex test, haplotype analysis is performed on the DNA of family members using highly polymorphic CA-repeat markers of restriction fragment length polymorphism.

At present, the dystrophin gene is known to harbour some 43 polymorphic sites (GBD-Online Genome Database), 10 of which are CA-repeats with high information content. Routinely used loci include 3′DYSI, STR50, 5′DYSIII, 5′DYSII and 5′DYSI. If necessary, this panel can be extended to other loci, either within or flanking the gene.

3.8 Screening for breast cancer

PCR testing is carried out using lymphocyte DNA. Screening for the presence of mutations across the whole gene with 23 exons for BRCA1 and 27 exons for BRCA2, is performed by analysis of the PCR products with denaturing gradient gel electrophoresis, or single strand conformation polymorphism, or protein truncation test and confirmed with direct sequencing.

3.9 Limitations of PCR

In order to understand the strengths and limitations of these techniques, the procedures for the identification of restriction fragment length polymorphism (RFLP) resulting from variation in the number of tandem repeats (VNTR) of a short DNA segment, or even simple tandem repeats (STRs) such as dinucleotide repeats require scrutiny. To minimize analytical pitfalls, particular attention should be given to the composition of DNA probes.

Many variables affect PCR. These include cycling temperatures chosen for the denaturation of synthesized DNA strands, annealing and primer extension steps, the

type of thermocyclers used to achieve temperature cycling, requirements for primers, to name just a few. Particular attention should be paid to trouble shooting PCR products.

A multiplicity of kits is currently available for the isolation of DNA and RNA. These kits while simplifying the isolation procedure could in some instances introduce a pre-analytical variable depending on the type of detergent used in cell lysis, which may impact on the amplification of DNA, by the PCR.

The anticoagulants used for blood collection could also affect digestion with restriction enzymes and amplification reactions, such as has been with heparin under certain conditions. Residual red blood cells can inhibit taq polymerase enzyme used in PCR amplification. The type of tissue fixative and the duration of fixation can affect the efficiency of amplification reactions. Finally, contamination from the working environment should be controlled to minimize both pre-analytical and post-analytical error.

3.9.1 Technical limitations

a) *False positives*

Due to its extreme sensitivity, PCR will detect minute contamination and give rise to exaggerated false positive reactions.

Some of the precautions to prevent PCR contamination are as follows:

- using separate rooms for DNA-RNA extraction, PCR Reaction and Electrophoresis: Hybridization
- duplicating samples
- dispensing reagents into single-use aliquots
- using pipettes with disposable plungers
- using controls (performing PCR without the addition of DNA, referencing negative samples extracted in parallel with analyzed samples, referencing positive and negative DNA and RNA)

b) *False negatives*

False negative results may arise from poor quality of nucleic acid preparations with absence of standardized measurements for RNA extraction. It could also be caused by differing methods being used for RNA extraction. PCR requires the presence of the organism, whereas serology can determine whether a patient has been exposed to a disease even in the absence of organisms.

c) *Problems of primer design*

It is important to choose the right region of the virus or agent under study

d) *Problems of methodology*
There may be problems encountered in amplification using the single step assay, a single tube assay or a "nested" PCR

e) *Problems of controls*

f) *Problems with quantification*: issues of specificity and sensitivity

3.9.2 Limitations of the results of interpretations

a) PCR does not provide direct information on the state of the viral DNA under study (free or integrated). For example, in the study of peripheral blood mononuclear cells, it may be difficult to distinguish between serum viral particles adhering on to the cell membranes and viral DNA contained within the cells themselves, unless the serum PCR is negative.

b) PCR is unlikely to discriminate between colonization and infection.

c) There may be "errors" of Taq polymerase, and these must be considered when interpreting nucleotide sequence data obtained after PCR

d) PCR assays can detect residual DNA molecules whose clinical relevance may be unclear

e) Complex ethical problems need consideration, especially in cases of antenatal screening e.g. amniocentesis, confidentiality and protecting personal integrity.

f) Problems in breast cancer detection:
- The large size disease genes and the scattered distribution of mutations throughout the gene complicate the task of mutation detection and make rapid screening for mutations a major technical challenge. The test cannot predict the severity of the manifestations.
- A woman planning to have genetic testing should be fully aware of the genetic, clinical and psychological implications of the test as well as the limitations of the existing test.

4 SCOPE OF ASSESSMENT

The assessment includes the following clinical conditions:
- Infectious diseases – Tuberculosis and HIV infection, Hepatitis B and C
- Investigation of Haematology disorders – Human Leucocyte Antigen (HLA) Typing, Human Platelet Antigen (HPA) Typing, Thalassemia
- Genetic Disorders – Duchenne Muscular Dystrophy, Fragile X Syndrome
- Malignant tumours
- Paternity testing

- Forensic medicine

5 METHODOLOGY

5.1 Tuberculosis

Journals from 1990 to 1998 were hand-searched, especially the Journal of Infectious Diseases. A total of 5 200 articles were found, of which 8 articles were used as reference and summarised in the evidence table.

5.2 HIV

Journals from 1990 to 1998 were hand-searched. A total of 1 400 articles were found, of which 9 articles were used as reference and are in the evidence table.

5.3 Hepatitis B

A search using MEDLINE was carried out and 3 366 articles were found under *Hepatitis B /diagnosis* as MESHword. A total of 75 710 articles had *polymerase chain reaction* as the MESHword. Of these, 140 articles had both *Hepatitis B/diagnosis* and *polymerase chain reaction* as MESHwords. A total of 23 957 articles had the term *serological (*all fields*)*. 20 articles had *hepatitis B/diagnosis (*MESH), *polymerase chain reaction (*MESH) and *serological (*all fields). All 20 articles had English abstracts. 10 of these were excluded for the following reasons: review articles (1); articles not on hepatitis B as the main study (3); sample size of less than 30 (2); study not on PCR (2); not using PCR on all samples (2). The remaining 10 articles were selected and tabulated in the evidence table.

5.4 Hepatitis C

Literature search on Hepatitis C was carried out based on "The National Institutes Of Health Consensus Development Conference: Management Of Hepatitis C" taken from Hepatology, Volume 26 number 3 supplement 1 September 1997 (Official Journal of the American Association for the Study of Liver Disease). These articles were fully reviewed. The titles of all articles quoted were checked and those related to PCR and Hepatitis C was identified. The original articles were searched manually and a total of 6 articles were retrieved. The information was tabulated in the evidence table.

5.5 HLA Typing

MEDLINE search was carried out. 1 344 articles on HLA were found. Of these, 161 were related to PCR. From these, 40 articles were selected as these involved the use of HLA typing in bone marrow transplants, cord blood transplants, organ transplants and diagnosis of graft versus host disease. Once selected, each abstract of the articles were studied. If found to be relevant, the full articles were obtained (where available) and these were analysed. The results are tabled in the evidence table below.

5.6 HPA Typing

A search of MEDLINE was carried out. 32 citations were found. 14 articles were selected, as these articles addressed HPA typing using PCR in clinical practice, and are in the evidence table. The other articles focussed mainly on research and or the techniques were old.

5.7 Thalassemia

A MEDLINE search was carried out. 26 articles were selected as being relevant for this issue which is as tabulated in the evidence table.

5.8 Duchenne Muscular Dystrophy

A MEDLINE search was carried out. Under *polymerase chain reaction* (all fields), 82 402 articles and *Duchenne Muscular Dystrophy* (all fields), 1 629 articles record was found. There were 2 320 articles listed under *multiple* (all fields). Out of these, 64 articles had *polymerase chain reaction* (all fields), *Duchenne Muscular Dystrophy* (all fields) and *multiplex* (all fields). The abstracts of all these articles were downloaded and reviewed. Only 14 abstracts on the use of multiplex PCR in Duchenne Muscular Dystrophy were selected for the evidence table. The rest were excluded for the following reasons: sample size less than 30 or uncertain number of cases (18), review articles (5), abstracts from non-English articles (9), inadequate information (4), letter (1) and use of other methods (13).

5.9 Fragile X Syndrome

A MEDLINE search was carried out using the keyword *polymerase chain reaction* and *fragile X*. All the titles and abstracts were reviewed. 9 original articles related to PCR and Fragile X were selected and obtained as can be seen in the evidence table.

5.10 Solid Tumours, Paternity Testing, Forensic Pathology

The MEDLINE, Royal College of Pathology, The Centre for Reviews and Dissemination databases were searched from 1993 to 1998. The key words used were *paternity testing* (98 citations, 16 were appraised), *forensic pathology*, *polymerase chain reaction application* (2 187 citations, 218 appraised), *breast cancer*, *colon cancer and solid tumours* (534 citations, 32 appraised) including MESH titles. HSTAT, Centre for Evidence Based Medicine, HSRProj, and HealthStar databases were also used to verify the completeness of the search. The selected evidence is indicated in the evidence table.

5.11 Ranking of evidence

The committee ranked the evidence using the ranking system of the Catalan Agency for Health Technology Assessment (Appendix). Where there was difficulty rating the evidence on a scale of 1-9, the evidence was ranked as good, fair or poor. This system of grading is not totally applicable for all types of evidence, being more applicable for therapeutic use. However, the intention was to indicate the strength of the evidence obtained using a common grading scale.

6 RESULTS & DISCUSSION

6.1 Infectious Diseases

6.1.1 Tuberculosis

a) Early diagnosis of pulmonary tuberculosis

There is evidence that PCR is able to provide early diagnosis of pulmonary tuberculosis *(TB)* - up to within 6 hours of specimen decontamination - leading to earlier institution of anti-TB treatment, earlier application of isolation procedures, and hence reduction in transmission of the disease (Rajalahti, 1990; Lang, 1998; Eing, 1998; Reischl, 1998). PCR is also able to identify mycobacterium in those patients of *extra-pulmonary TB*, (Bonington, 1998; Sjobring, 1990) especially those with tuberculous meningitis (the most serious form of extra-pulmonary TB).

b) Increased sensitivity of detecting mycobacterium

PCR increases the sensitivity of detecting mycobacterium - the sensitivity ranges from 83-96% while the specificity is said to be 100% (Bonington, 1998; Sjobring, 1990; Lang 1998). In one study, 4 out of 19 confirmed TB cases were PCR positive although both cultures and smears were negative. In addition, PCR was able to detect a single mycobacterium whereas a minimum of 5 mycobacteria is needed for a culture to be positive. In the case of tuberculous meningitis less than 10% of direct smears are positive due to the low level of mycobacteria in the cerebro-spinal fluid (CSF) of these patients.

c) Evaluation of response to anti-TB therapy
PCR can be used for evaluation of response to anti-TB therapy - patients' samples are usually negative for mycobacterium at the end of successful therapy by PCR, and these results are available much earlier than culture results, obviating any gap in treatment (Musser, 1995; Reischl, 1998; Kennedy, 1998). PCR has been used in the rapid identification of resistant organisms especially in relapsed cases or in cases of failure of treatment (Musser, 1995; Reischl, 1998). PCR is able to differentiate mixed TB infections among HIV patients, *Mycobacterium-avium* complex (MAC) being among the common mycobacteria isolated (Rajalahti, 1998; Eing, 1998; Reischl, 1998). Treatment regimens are different in these cases and most of the MAC TB cases are drug-resistant.

6.1.2. HIV

a) Management of HIV
PCR can stage HIV, predict HIV progression & survival (Saag, 1996; Mellors, 1995; Galetto-Lacour, 1996). It helps to provide a decision on when to start therapy (Saag, 1996; Lin, 1994), measures response to anti-HIV drugs (Saag, 1996; Galetto-Lacour, 1996; O' Brien, 1996; Coombs, 1997).

b) Detection of viral load
PCR can also be used to detect the HIV viral load, and also determine the likelihood of vertical transmission of HIV through measurement of HIV RNA levels (Saag, 1996, Dickover, 1996; Alimenti, 1991; Weiser, 1994).

6.1.3 Hepatitis B Infection

Several studies have shown its usefulness in demonstrating viraemias, the identification of seronegative viral carriers and studies on mother-child transmission. PCR has also been found useful in the selection of patients for treatment with alpha-INF, including monitoring of therapy to determine response or non-response.

a) Diagnosis of Hepatitis B status

PCR provides a sensitive test to detect hepatitis B virus (HBV) infection as it can detect 10 to 10^2 particles/ml compared to the 10^5 to 10^6 particles/ml range possible

with a regular spot assay, although there is a variation of sensitivity with different brands (Schottstedt, 1998; Aspinall, 1995). It is also able to identify those who are capable of transmitting infection by detecting HBV DNA in HB Ag-positive subjects, those who are HBV DNA-negative by standard hybridization technique or who have no other evidence for other causes of liver diseases. HBV DNA provides a more accurate indication of the status of hepatitis B virus replication in patients who have seroconverted from HB_eAg to anti-Hbe, and from HB_sAg to anti-HBs. PCR techniques also enable detection of carriers, mutant strains, detection in delta hepatitis patients (Hofer, 1998; Jlg, 1995; Gerken, 1991; Villa, 1995; Lozano, 1994, Shih, 1990; Feray, 1990; Carmen, 1989).

b) Role of HBV in aetiology of hepatocellular carcinoma (HCC)

Detection of HBV DNA and RNA molecules in tumour cells of seronegative patients with HCC would suggest HBV as the cause of HCC (Villa, 1995; Paterlini, 1990).

c) Assessing effect of treatment

HBV DNA level can be used to predict treatment response to interferon as well as relapses (Perrilo, 1990; Fattovich, 1992; Brunetto, 1989). HBV DNA levels can help in assessing the severity of the disease thus enabling better selection of patients for treatment - patients with low HBV DNA levels are likely to respond to alpha-interferon compared with those who have a higher viral load (Jlg, 1995).

6.1.4 Hepatitis C Infection

a) Diagnosis of acute HCV infection
PCR can detect HCV RNA as early as 1-2 weeks from clinical onset and is therefore useful in anti-HCV negative patients strongly suspected to have acute hepatitis C based on epidemiological considerations, in defining infectivity, in asymptomatic blood donors, in immunocompromised patients (Farci, 1991; Gregory, 1997; Chan, 1993; Alter 1997).

a) Evaluation of anti-viral therapy
PCR can be used to assess the efficacy of treatment and virological response to alpha-interferon, and assessing association with genotype(Lindsay, 1997; Davis, 1997).. A meta-analysis reported a greater end-of treatment response (ETR) in those with HCV RNA levels $< 1 \times 10^6$ genomes/ml achieved compared to those with higher levels (Lindsay, 1997).

6.2 Investigation Of Haematological Diseases.

6.2.1 HLA Typing

HLA typing can be carried out by serological and molecular methods. However, the serological method has many drawbacks like unavailability of anti sera, requirement for large quantities of sample, and non-specific antisera (polyvalent). Hence, with this, in HLA B typing there is an error rate of 25%, which may result in poor engraftment in transplants in general.

PCR testing in HLA typing provides more accuracy (William, 1997; Cavanagh, 1997; Santasath, 1997; Bettinotti, 1997; Hemmatpour, 1996; Bozon, 1996; Woszezek, 1997; Olerup, 1992; Bunce, 1993; Hvid, 1992) is more reliable (Otten, 1995; Darke, 1998; Westman, 1993) provides results with no false positives and no false negatives (Bunce, 1993; Olerup, 1992), carry out DNA typing, and detect CD 34+ cells that cannot be diagnosed by flow cytometry (Thoma, 1994; Lucotte, 1997).

The method is simple (Kyung, 1995; Pollack, 1994; Erlich, 1993), rapid (Tan, 1996; Bunce, 1995; Fuji, 1993, Kyung, 1995; Hayakawa, 1993), practical (Kyung, l995) and precise (Lynas, 1994; Madsen, 1992; Bozon, 1996; Woszezek, 1997; Erlich, 1993).

It can be used in typing of cadaver donors (Bunce, 1995; Olerup, 1992), for umbilical cord blood donor evaluation (Pollack, 1994), kidney and bone marrow transplant (Mytilineos, 1997; Darke, 1998; Mercier, 1994), detection of DNA polymorphism (Aldener-Cannava, 1994; Jungerman, 1997), various alleles.(Petzel-Erler, 1994, Eberle, 1997; Zetterquist, 1997; Bunce,1997), sequencing of genes (Santamaria, 1992). These are elaborated in the various studies in the evidence table below.

6.2.2 HPA

Donors and recipients should be typed in HPA-1 system at least for post-transfusion purpura and platelet refractoriness (Merieux, 1997; Mazzucato, 1996; Simsek, 1994). PCR is effective in zygosity testing, antenatal diagnosis and thrombocytopenic patients (Tazzari, 1998; Porreti, 1997).

It is rapid and thus has application in blood banking for genotyping in large samples (Muller, 1997; Legler, 1996), which have proven to be in total concordance with results from conventional testing (Zotz, 1997,). There is a high degree of correlation between newer methods and conventional testing methods (Zotz, 1997; Legler, 1996; Kluter, 1996; Metcalfe, 1995; Bray, 1994), and no discrepancies between serological testing and testing with PCR (Kekomaki, 1995; Holensteiner, 1995; Fujiwara, 1995).

The evidence is presented in the evidence table.

6.2.3 Thalassaemias (Haemoglobinopathies)

The PCR technique is simple, rapid, flexible, cost effective and accurate and can be used routinely in prenatal diagnosis (Ahmed, 1996; Chang, 1995; Old, 1990; Aliza, 1998; Savage, 1990; George, 1996) However, misdiagnosis of homozygous α thalassaemia can occur if PCR is used alone in prenatal diagnosis (Ko, 1997). There are a number of PCR - based procedures like reverse dot analysis, primer specific amplification followed by denaturing gradient gel electrophoresis (DGGE), and direct sequencing (Antonio, 1997; Shi-ping, 1994). PCR is useful in the development of a screening programme to identify carriers of α thalassaemia (Bowden, 1992; Chan, 1996; Savage, 1990; Winichagoon, 1995, Saxena, 1997) and α2 globin DNA detection (Kropp, 1989; Thonglairoam, 1991).

Reverse dot blotting is a rapid, accurate method for detecting β thalassaemia mutations and antenatal diagnosis (Sutcharitchan, 1995; Muralitharan, 1996). The amplification refractory mutation detection system (ARMS) should have wide application to the carrier detection and prenatal diagnosis (Old, 1990; Saxena, 1997; Kanavakis, 1997). Denaturing gradient gel electrophoresis (DGGE) and ARMS are useful in countries where there are very heterogenous β thalassaemia mutations (Losekoot, 1990; Tan, 1994). PCR can also be used in combination with other methods like silver stained single strand conformational polymorphism (SSCP) for β mutation in pre-implantation (El Hashimite, 1997; Trent, 1998).

Chorionic villi sampling (CVS) procedure was found to be uneventful and would play a key role in the prevention of the disease (Ng, 1996). PCR provides a comprehensive basis for carrying out prenatal diagnosis of thalassaemia in an area where it is frequently found (Ahmed, 1996). The diagnosis of thalassaemia and sickle cell anaemia is possible with early fetal sampling and PCR based techniques (Tuzmen, 1996). Regional collaborative studies are to be encouraged as an indispensable tool in providing better health care (Thong, 1996).

6.3 Genetic Disorders

6.3.1 Duchenne Muscular Dystrophy(DMD)

PCR is widely used to detect the deleted exons of the 2000kb dystrophin gene as seen in the evidence table on this aspect (Baranzini, 1998; Sertic, 1997; Banerjee, 1997; Dincer, 1996; Kruyer, 1994; Katayama, 1993; Gokgoz, 1993; Baranov, 1993; Coral-Vanquez, 1993). PCR was found to be very sensitive and specific (Low, 1996; Yau, 1996; Jeffrey, 1992).

Amplification using multiplex PCR is a useful alternative compared to the conventional Southern blot analysis for detecting deletions (Nieman-Seyde, 1992; Claustres, 1991; Sakubara, 1991).

6.3.2 *Fragile X Syndrome*

PCR is a simple and rapid diagnostic screening test for Fragile X syndrome for determining prevalence of Fragile X syndrome in various populations (Elbaz, 1998; Arvio, 1997). It has been found that a testing protocol combining routine karyotyping with PCR is the most cost-effective diagnostic strategy (Marini, 1997; Bussani, 1996). It is suggested that systematic genetic counseling and carrier screening (Ryynanen, 1994) or testing of children with learning difficulties in schools can assist in detection and prevention of the disease (Slaney, 1995. DNA testing of mentally impaired people with no family history of Fragile X can still identify a small percentage with mutation as well as other in whom the risk of the disease is unclear (Ramos, 1993). Public health programmes have been started in some countries including educational programmes and genetic counseling (Keenan, 1992; Trusler, 1985).

6.4 Malignant Tumours

6.4.1 Improvement of histological diagnosis through the detection of clonality and molecular markers.

In the differential diagnosis of early gastric mucosa-associated lymphoma, with *Helicobacter pylori* gastritis, PCR detected monoclonal B-cells before histology showed lymphoma (Rudolph, 1997). Small round cell tumours (SRCT) are a heterogenous group of highly malignant neoplasms, predominantly of infancy and early childhood, where precise and accurate histological diagnosis can be elusive in a proportion of SRCTs. Using PCR and FISH techniques, the characteristic molecular genetic abnormalities of the different SRCTs can be rapidly identified, thus establishing diagnosis, and having an impact on treatment and survival (Mcmanus, 1996). The distinction between reactive and cutaneous T-cell infiltrates is difficult histologically because the changes manifested are at the borderline between the two lesions. Using PCR, T-cell monoclonality was demonstrated in 50% of borderline biopsy samples in patients who later developed lymphoma (Ashton-Key1997).

6.4.2 Prognostic assessment at diagnosis with impact on therapeutic decisions.

Human papilloma virus (HPV) testing can be used to discriminate patients who will develop recurrent CIN 3 from those who will not. PCR analysis may be useful to monitor the therapeutic results (Chua and Hrerpe, 1997). Inverted papillomas of the paranasal sinuses are benign tumours; nevertheless, malignant transformation occurs in 13 % of cases. HPVs are accepted as the tumour inducing and promoting agents in their aetiology and types 16 and 18 are associated with increased risk of developing cancer. Amplification of HPV DNA fragments which were used as predictors for their biological behaviour were detected in 7 out of 21 cases (Bernauer, 1997).

6.4.3 Screening high-risk populations for genetic predisposition for cancer.

Hereditary Non-Polyposis Colorectal Cancer Syndrome (HNPCC) is the most common form of familial colorectal cancer and is estimated to account for up to 5 to 10 % of all colorectal ancers. 4 HNPCC genes have been identified in the pathogenesis. In Familial

adenomatous polyposis (FAP), which underlies about 1 % of colorectal cancer, mutation of the APC gene can be detected. Predictive testing for genetic susceptibility to cancer can be offered to patients' relatives, and allows early diagnosis and treatment, thus improving clinical management (Howell, 1994; Maher, 1994; Bennet, 1998; Whitelaw, 1996; Walpole, 1995).

Women carrying a BRCA1 (breast cancer 1) gene have a greater than 80 % lifetime risk of developing breast or ovarian cancer. There is a 2- to 4-fold increase in the risk of close relatives developing cancer at the same site. However, there is evidence to show that among women with a history of breast cancer and a family history of the disease, the percentage with BRCA1 coding-region mutations is less than 45%. These results suggest that even in a referral clinic specializing in screening women in high-risk families, the majority of tests for BRCA1 mutations will be negative and therefore uninformative (Couch, 1997). Early-onset breast cancer and strong family history may be useful guidelines for checking the BRCA1 status but findings drawn from the general population suggest it may be difficult to develop screening criteria for women with modest family history profiles (Malone, 1998). One benefit of the testing is the ability to make medical and lifestyle decisions while reducing the anxiety of not knowing their genetic background. Prophylactic mastectomy might increase the life expectancy of individual's aged 30 with BRCA1 or BRCA2 mutations from 2 to 8 years.

Similarly, in patients with ovarian cancer, screening for BRCA1, BRCA2, and HNPCC gene mutations yielded negative results in 90%, and were unhelpful (Rubin, 1998). A collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene showed that more data was needed to address specifically the specificity and sensitivity, before adopting this as a diagnostic test (Shattuck-Eidens, 1995).

6.4.4 Monitoring of residual tumour cells in peripheral blood

In patients with advanced hepato-cellular carcinoma (HCC), recurrence in the liver, after primary resection or even liver transplantation, occurs in a large percentage of cases. By using reverse-transcriptase PCR(RT-PCR), messenger RNA (mRNA) for human albumin gene, a liver cell marker, was detected in 16 out of 17 cases of advanced stage HCC in the peripheral blood (Kar, 1995). In 26 out of 31 patients with colorectal liver metastasis, RT-PCR recognition of the tumour marker carcinoembryonic antigen (CEA) was detected (Jonas, 1996). Similarly, an RT-PCR assay was successful in detecting tumour cells in peripheral blood and regional lymph nodes in patients with colorectal cancer, aiding in early diagnosis, assessing prognosis, and detecting residual disease after treatment (Wong, 1997). The use of immunobead-PCR to detect circulating tumour cells in colorectal cancer was shown to be a sensitive prognostic marker for relapse of the disease (Hardingham, 1995). The K-ras oncogene mutation is observed in about 50% of colorectal tumours and can be detected in stool samples; a method for robust PCR amplification and a reliable assay for K-ras gene mutation detection in stool samples has the potential to be specific, sensitive and cost-effective (Jen,1998).

Telomerase activity can be a sensitive and definitive analysis for cancer; using the RT-PCR method, small numbers of metastatic cancer cells in axillary lymph nodes can be detected (Iwase, 1998). The use of RT-PCR to increase detection of tumour cells in sentinel lymph node biopsy showed exceptional results for 2 markers, mammaglobin and CEA, in breast carcinoma (Min, 1998).

6.4.5 Gene therapy for cancer

Gene therapy for cancer is likely to fall into one of the following broad categories:

- those which evoke or amplify an immune response
- those which code for pro-drug activating enzymes, and,
- those which correct genetic changes responsible for the malignancy.

Using the armamentarium of molecular biologic tools, the third option is theoretically possible, but suffers practical difficulties including the need to target all stem cells within the tumour mass (Plautz et al., 1993). A novel method for the direct quantification of gene transfer into cells using PCR in-situ has the potential for use in a variety of gene therapy applications (Catzavelos, 1998).

6.5 Paternity Testing & Forensic Testing

6.5.1 Paternity testing

In paternity diagnosis length polymorphisms of variable number of tandem repeats loci are used together with DNA single locus systems. A child inherits half the DNA patterns from its true biological father. If an alleged father does not posses the specific DNA pattern in his profile, he is excluded from the paternity. In the case of non-exclusion, the probability exceeds 99.8% (the required value for positive proof of paternity), using PCR-based DNA systems (Kratzer, 1997). The successful use of nested PCR allowed a minute quantity of blood and charred human remains in a paternity testing yielded a 98% probability that the victim was a female. (Strom, 1998). Using DNA profiles from 8-10-week chorionic villus samples and blood samples from their parents, cases of disputed paternity could be resolved in very early pregnancy (Mingjun, 1993). In a paternity case where the putative father had received multiple blood transfusions, it was possible to establish probability of paternity of 72.33%. Using the DNA technique, it was demonstrated that the DNA patterns were not influenced by the transfusions and the possibility of paternity reached 99.9% (Huckenbeck, 1994). Similarly, paternity could be proved using DNA methodology in a man who had been treated for CML with bone-marrow transplantation and total body irradiation (Pakkala, 1994).

6.5.2 Forensic Identification

In a mass disaster, the identification of bodies exposed to extreme thermal, physical and chemical degradation can be accomplished by short tandem repeat (STR) typing. In 66%

of the samples tested, all 4 STR loci were successfully identified using a PCR-based DNA-typing method. A PCR-based sex identification test also proved to be effective when applied to the degraded samples (Whitaker). In identification of unknown skeletal remains, genotyping may not necessarily provide proof of identification. Successful identification in 3 cases was possible, by comparing several STR loci in the remains with samples of personal articles of the respective individuals (Sasaki, 1997).

In sexual assaults against women, ABO blood-grouping genotyping of sperm DNA isolated from contaminated vaginal fluid in 4 cases was successful, using PCR-restriction fragment length polymorphism (Shiono, 1996). In sexual assault cases and missing person cases involving women, DNA samples extracted from stained pap smears, semen smears, and post-coital smears, were typed using PCR, and provided a valuable source of material for determining their genetic profiles (Roy, 1995). PCR analysis addresses the feasibility of detecting the DNA profiles of azoospermic semen, including those mixed with post-coital vaginal samples in suspected rape cases (Skinker, 1997).

In DNA typing for identification, there are 2 steps in the process. The first involves determining the profiles of the samples collected at the scene of crime and comparing them with that of the suspects and victims. In the case of a match that includes the suspect, the issue to be addressed is the likelihood of someone else's profile matching that of the sample studied. This likelihood is calculated by determining the frequency of the suspect's profile in the relevant population databases. The combined use of the various PCR-based polymorphic systems can provide a high power of discrimination and exclusion for establishment of population databases (Crespillo, 1997; Koh, 1994). Validation studies for genetic typing of a single locus (D1S80) have demonstrated that this system can be implemented into forensic casework, particularly analysis of mixtures (Gross, 1997). However, a meta-analysis pointer to genetic disequilibrium in south Poland showed that interpretation of the casework on the basis of D1S80 locus typing may be biased by inter-population differences (Turowska, 1995).

In a clinical setting, DNA typing of surgical specimens can be used to establish patient identity in cases of mix-up; furthermore the technique can be performed on paraffin-embedded tissues (Tsongalis, 1997; Romero, 1997).

Validation of mRNA sequencing for forensic casework, (Wilson, 1995) and multiplex polymorphic STR amplification sets developed for paternity identification (Alford, 1994) forensic cases (Mickaetal, 1996; Fildes, 1996; Wallin, 1998) prove that PCR-based systems are a reliable way for identification.

6.6 Cost-Effectiveness

The diagnosis of solid malignant tumours is based on morphology, complemented by immunohistochemical markers where necessary. The evidence reviewed indicates a very small percentage of solid tumours need molecular analysis for histological typing and that outcomes were influenced by utilising the test. However, in defined high-risk populations, PCR may have greater predictive power in preventing adverse outcomes e.g. in HPV screening for cervical cancer (van Ballagooijen, 1997), screening for micro metastatic disease in axillary lymph nodes of breast cancer patients (Lockett et al, 1998) and for HNPCC, it has the potential to reduce the cost of family management in a resource-poor country (Goldberg, 1998). For paternity testing and forensic identification, PCR has replaced the previous methods, namely ABO blood grouping, and morphological and odontological analysis respectively. Using this technique, laborious identification procedures are now near obsolete.

PCR is a rapid, safe and cost-effective method.

6.7 Ethical Considerations

Predictive testing for familial cancers can pose ethical dilemmas. However, if used with proper counselling and psychological support, screening could be targeted to relatives of patients at high risk. People exposed to potential bladder carcinogens (smokers) show a significant risk of slow NAT2 type polymorphism, which is associated with an increased risk of bladder cancer. The possibility of this information being used to discriminate against employees who smoke, and applicants for insurance, raises legal, ethical and social issues. In France, the introduction of DNA testing aroused concerns as to the unregulated use by police agencies and the widespread use in paternity or insurance use. Although legislation has been passed, enforcement is difficult, because especially for paternity testing, any neighbouring country can satisfy the need (Mangin, 1996).

6.8 Cost Implications

Items	Cost (RM)
Building costs	
Dedicated laboratory facilities (an existing facility could be renovated)	50,000.
Equipment	
Gene scanner*	500,000
- Thermal cycler] - Laminar hood] - EIA reader] - EIA wash system,etc]	100,000

***** at least 3 gene scanners currently available in the Ministries of Health and Education.

Staff requirements

To handle a work-load of 30 cases per week, the staff required is as follows:

1 Senior Scientific Officer
3 Scientific Officers (full-time)
6 Medical laboratory technologists

Cost estimations based on reagents/consumables

Screening
The reagent costs for 100 specimens are as follows:

Primers, probes, control DNA:	RM 2 500.00
Other reagents for PCR:	RM 1 000.00
Reagents for electrophoresis:	RM 500.00
Total estimated cost:	RM 4 000.00

Cost per test = **RM 40.00**

Screening using fluorescent labeled system on automated fragment analyzer
The reagent costs for 100 specimens are as follows:

Estimated cost of screening kit: RM 10 000.00

Capillaries, polymers,	RM 5 000.00
Buffers for electrophoresis:	
Total estimated cost:	RM 15 000.00

Cost per test = **RM 150.00**

Confirmatory testing
The costs of consumables for 100 reactions are as follows:

Primers + Control DNA:	RM 5 000.00
Other reagents for PCR:	RM 1 000.00
Reagents for electrophoresis	RM 500.00
Total cost	RM 6 500.00

Cost per reaction = **RM 65.00**

Note: Primer kits could be produced locally, so that it may be cheaper

Cost of purchasing services.

i. The charges imposed by the Singapore Institute for Science and Forensic Medicine are S$ 250/- per probe, assuming 4 probes are used for 1 sample, the cost per sample will be S$1 000.00.

ii. At University Malaya, the Institute of Biological Sciences charges RM1 000.00 per case, irrespective of the number of tests.

iii. The Chemistry department provides free services for medico-legal cases.

7. CONCLUSIONS

7.1 Infectious Diseases

7.1.1 Tuberculosis

PCR can assist in early diagnosis of TB, identify mycobacterium in clinical specimens even with smaller number of bacteria, evaluate response to therapy, detect cases of relapse and differentiate mixed infections.

7.1.2 HIV

PCR can be used to predict HIV progression and survival, as well as the risk of vertical transmission. It can also assist in detecting the viral load including pregnancy and in staging of HIV.

7.1.3 Hepatitis B

PCR is able to detect low level of HBV viraemia and can thus detect symptomatic Hb_sAg positive subjects, evaluate antiviral therapy by identifying those patients suitable for alpha-interferon treatment and evaluating degree of response to therapy, identify HB_sAg-negative patients with liver disease, identification of HBV infection in liver transplantation, and also to identify mutant strains of HBV.

7.1.4 Hepatitis C

In this infection, PCR can be used for qualitative and quantitative measurement of viraemia in diagnosis of acute HCV infection, diagnosis of anti-HCV-negative chronic hepatitis C carriers, evaluation of HCV viraemia in asymptomatic blood donors with normal liver enzymes, evaluation of anti-viral therapy by assessing the efficacy of treatment to alpha-interferon and assessing virological response to treatment, predicting treatment response to alpha-interferon and assessing severity of disease. PCR can also determine the genetic nature of HCV as predictor of treatment response.

7.2 Haematological Diseases

7.2.1 HLA Typing

It can be concluded that PCR is superior to the serological method, and its success rate can be maintained even under sub-optimal conditions. PCR is the method of choice for a transplant programme, especially in unrelated bone marrow and cord blood transplants.

7.2.2 HPA

PCR testing is used in the identification of post-transfusion purpura, zygosity testing, antenatal diagnosis and thrombocytopenic patients, and to distinguish between homozygous and heterozygous individuals.

7.2.3 Thalassemias

PCR testing can be used in patients with haemoglobinopathies, especially paediatric patients, as well as in prenatal diagnosis, for carrier detection and for detection of alpha and beta thalassaemias.

7.3 Genetic Disorders

7.3.1 Duchenne Muscular Dystrophy

PCR is able to provide a method for confirmatory diagnosis of DMD and it is also useful in the identification of the carrier status, compared to histopathological diagnosis of DMD, which requires a muscle biopsy

7.3.2 Fragile X Syndrome

PCR is more specific and sensitive compared to the conventional method, and has been used for prevalence testing as well as in patients with risk of the disease, and also makes the confirmatory diagnosis of Fragile X more effective.

7.4 Malignant Tumours

PCR has application in the following areas:

- improvement of histological diagnosis through the detection of clonality and molecular markers
- prognostic assessment at diagnosis with impact on therapeutic decisions
- screening high-risk populations for genetic predisposition for cancer e.g. hereditary non-polyposis colorectal cancer, breast cancer
- monitoring of residual tumour cells in peripheral blood
- gene therapy for cancer

7.5 Paternity Testing & Forensic Identification

PCR is a useful tool in paternity testing even with minute quantities of tissue and even where the tissue was damaged.

PCR is also a valuable tool in forensic testing including mass disasters.

8. RECOMMENDATIONS

8.1 Infectious diseases

8.1.1 Tuberculosis

PCR is recommended for specific indications like disseminated TB or TB meningitis.

8.1.2 HIV

PCR should be used to assess HIV risk & therapy.

8.1.3 Hepatitis B

It is recommended that PCR be used in evaluation of antiviral therapies and detection of HBV in high-risk patients.

8.1.4 Hepatitis C

It is recommended that PCR be used in assessing treatment efficacy.

8.2 Haematological disorders

8.2.1 HLA Typing

PCR is recommended as the method of choice for a transplant programme, especially in unrelated bone marrow and cord blood transplants.

8.2.2 HPA Typing

PCR is recommended for HPA typing.

8.2.3 Thalassaemia

PCR is recommended for testing patients with haemglobinopathies especially paediatric patients.

8.3 Genetic disorders

8.3.1 Duchenne Muscular Dystrophy

PCR is recommended as an alternative method for confirmatory diagnosis of DMD.

8.3.2 Fragile X Syndrome

PCR is recommended for the confirmatory diagnosis of Fragile X.

8.4 Malignant Tumours

PCR is recommended for screening high-risk populations for genetic predisposition for cancer and monitoring of residual tumour cells in peripheral blood.

8.5 Paternity Testing & Forensic Identification

PCR is recommended for a paternity testing in situations where there are only minute quantities of tissue or where the tissue was damaged.

PCR is recommended for forensic testing in specific instances e.g. mass disasters.

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191. Winichagoon P et al *Detection of α thalassaemia –1 (Southeast Asian type) and its application for prenatal diagnosis.* Clin Gent 1995; 47: 318-320.

192. Wong LS et al *Detection of circulating tumour cells and nodal metastases by RT-PCR technique.* Br J Surg 1997 Jun 84(6):834-9.

193. Woszezek G, Borowice M,Mis M, Gorska M, Kowalski ML. *Comparison of serological and molecular (PCR-SSP) techniques of HLA-DR typing in clinical laboratory routine.* Ann Transplant 1997; 2(1): 39 -42.

194. Yang KG et al *Molecular characterization of β globin gene mutations in Malay patients with HbE β thalassaemia and thalassaemia major.* Br J Haematology 1989 May; 72(1):73-80.

195. Yau et al. *Accurate diagnosis of carriers of deletions and duplications in Duchenne/Becker muscular dystrophy by fluorescent dosage analysis.* J Med Genet 1996. Jul: 33(7): 550-8.

196. Zabaleta ME et al. *Assessment of former and newly developed HBV assays in a Third World setting.* J Med Virol. 1992 Dec; 38(4): 240-5.

197. Zetterqiust -H; Bengston-M; Backstrom-g; Eagle-Jansson I et. *Report from the HLA class II typing by PCR Multicentre Study.* Eur- J-Immunogenet 1997Jun; 24(3): 191-9.

198. Zotz RB et al. *Genetic typing of human platelet antigen 1 by oligonucleotide ligation assay in a specific and reliable semi-automated system.* Br. Journal Haematology 1997 Jan; 96(1): 198.

10. EVIDENCE TABLE

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
TUBERCULOSIS				
1	*Use of Roche AMPLICOR Mycobacterium tuberculosis PCR in Early Diagnosis of Tuberculous meningitis.* Alec Bonington, J. I. George Strang, Paule E. Klapper, Steven V. Hood, William Rubomdora, Miranda Penny, Rose Willers, Edmund G.L. Wilkins; Journal of Clinical Microbiology, May 1998.	69 cases;	PCR for diagnosis of tuberculosis meningitis compared to Z-N staining of smears and radiometric culture.	Fair
2	*Polymerase Chain reaction for Detection of Mycobacterium tuberculosis.* Ulf Sjobring, Michael Mecklenburg, Ase Bengard Andersen, Hakan Miorner; Journal of Clinical Microbiology, Oct. 1990.	Not available	A nucleotide sequence of MTB (Pab gere) was used in the PCR technique for detection of mycobacterium tuberculosis.	Poor
3	*Detection of Mycobacterium tuberculosis Complex in Sputum Specimens by the Automated Roche Cobas Amplicor Mycobacterium Tuberculosis Test.* Iiris Rajalahti, Pauli Vuorinen, Markku M. Nieminen, Ari Miettinen; Journal of Clinical Microbiology, April 1998.	324 cases	Detection of MTB was compared between PCR, conventional method and Bactec radiometh. Sensitivity of PCR higher whereas sensitivity of acid-test smear remained unchanged with 3 consecutive sputum specimens.	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
4	*Clinical Evaluation of the Gen-Probe Amplified Direct Test for Detection of Mycobacterium tuberculosis Complex Organisms in Cerebrospinal Fluid.* Anne M. Lang, Jesus Feris-Iglesias, Chabela Pena, Jacqueline F. Sanchez, Leslie Stockman, Paul Rys, Glenn D. Roberts, Nancy K. Henry, David H. Persing, Franklin R. Cockerill III; Journal of Clinical Microbiology, August 1998.	84 samples	Gen-probe was able to detect 83% of TB meningitis compared to conventional laboratory techniques.	Fair
5	*Comparison of Roche Cobas Amplicor Mycobacterium tuberculosis Assay with In-House PCR and culture for Detection of M. tuberculosis*. Bodo R. Eing, Andrea Becker, Arthur Sohns, Ronald Ringelmann; Journal of Clinical Microbiology, July 1998.	1,681 samples;	PCR was used to detect MTB and showed 90% sensitivities.	Fair
6	*Antimicrobial Agent Resistance in Mycobacteria: Molecular Genetic Insights.* James M. Musser; Clinical Microbiology Reviews, October 1995.	Not applicable	DNA sequencing of PCR-amplified target was used to detect mutation in resistant strain and direct identification of resistant Organisms.	Poor
7	*Clinical Evaluation of the Automated COBAS AMPLICOR MTB Assay for Testing Respiratory and Non respiratory Specimens.* Udo Reischl, Norbert Lehn, Hans Wolf,	1054 samples.	Detection of MTB Complex using Bactec, PCR and conventional method were compare. In smear positive specimen, the sensitivities of the PCR	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	Ludmila Naumann; Journal of Clinical Microbiology, October 1998.		were 96%.	
8	*Polymerase Chain Reaction for Assessing Treatment Response in Patients with Pulmonary Tuberculosis*. N. Kennedy, S. H. Gillespie, A. O. S. Saruni, G. Kisyombe, R. McNerney, F.I. Ngowi, and S. Wilson; 1998	10 cases	PCR with colorimetric detection system was compared i.e. microscopy and culture for assessing treatment response in TB cases. Relapse cases were detected earlier by PCR than by culture.	Fair
HIV				
1	*HIV viral load markers in clinical practice.* M.S. Saag, M.Holodniy, D.R. Kuritzkes, W.A. O'Brien, R. Coombs, M.E. Poscher, D.M. Jacobsen, G.M. Shaw, D.D. Richman & P.A. Volberding; Nature Medicine, Vol. 2, No. 6, June 1996.		Plasma HIV RNA determinations are an important prognostic marker of disease progression.	Fair
2	*Changes in plasma HIV 1 RNA and CD4+ Lymphocyte counts and the risk of progression to AIDS.* O'Brien, W.A. et al; N. Engl, J. Med.334, 425-431 1996.		Plasma HIV RNA measured by PCR is used to indicate risk of progression to AIDS.	Fair
3	*Association of plasma human immunodeficiency virus type-1 RNA level with risk of clinical progression in patients with advanced infection.* Coombs, R.W. et al.; J. Infect Dis. 1997.		Association and HIV RNA level with ask of clinical progression in patients with advanced infection was determined by PCR.	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
4	*Multicenter evaluation of quantification methods for plasma human immunodeficiency virus types 1 RNA.* Lin, HJ, et al.; J. Infect. Dis. 1970, 553-562.		PCR was used as quantitation methods for plasma HIV type 1 RNA.	Fair
5	*Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion.* Mellors, J.W et al.; Ann Intern. Med. 123, 573-579 1995.		HIV-1 RNA quantitation by PCR showed prediction outcome after seroconversion.	Fair
6	*Prognosis value of viraemia in patients with long-standing human immunodeficiency virus infection.* Galetto-Lacour and others; The Journal of Infectious Disease 173:1388-1393 (Swiss). June 1996.		HIV RNA level was determined by PCR on prognostic value.	Fair
7	*Identification of levels of maternal HIV-1 RNA associated with risk of prenatal transmission: effect of maternal treatment on viral load.* Dickover RE and others; Journal of the American Academy of Medicine 275:599-605.1996.		HIV-1 RNA level was used to determined effect of maternal treatment.	Fair
8	*Quantitation of human immunodeficiency virus in vertically infected infants and children.* Alimenti A and others; Journal of Pediatrics 119(2): 225-229. August 1991.		Significance viral load in vertical transmission was determined in infected infant and children.	Fair
9	*Quantitation of human immunodeficiency virus type*	19 mothers	Relationship of viral load to vertical	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	1 during pregnancy: relationship of viral titer to mother-to-child transmission and stability of viral load. Weiser B and others; Proceedings of the National Academy of Sciences USA 91(9): 8,037-8,041.1994.		transmission was studied and showed 5 children were infected. All mothers with RNA > 80000 copies/ml had vertical transmission.	
HEPATITIS B				
1.	*PCR for HBV, HCV and HIV-1 experiences and first results from a routine screening program in a large blood transfusion service.* Schottstedt V et al. Intero-virology. 1998; 41(1): 24-34.	428896 donated blood samples, which were serologically negative, were tested. (Cohort)	PCR on 428896 pooled blood samples (average 418 blood donations pooled), detected 2 hepatitis-B positive blood which were serologically negative. The cost was 15DM per donation for all 3 viruses including logistics, developments and investments.	Fair
2.	*Frequent chronic hepatitis B virus infection in HIV-infected patients positive for antibody to hepatitis B core antigen only. Swiss HIV Cohort Study*. Hofer M et al. Eur J Clin Microbiol Infect Dis. 1998 Jan; 17(1): 6-13.	A cohort of 57 HIV - infected patients who were positive only for anti-HBcAg were retested for HBV markers, including HBV DNA	PCR to detect DNA for HBV core and surface genes was positive in 62.4% and 59.9% of all samples, respectively over 31 months. Over time, HBV DNA was detected at least once in 89.5% while HBsAg only in 24.6%.	Fair
3.	*A randomised, controlled trial of interferon alfa-2b*	Randomised controlled	In this study HBV DNA level before	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	alone and after prednislone withdrawal for the treatment of chronic hepatitis B. Perrillo R. et al. N Eng J Med 1990; 323:295-301.	trial of 169 patients (126 patients with 43 controls), 4 months of treatment and 6 months of follow-up after completion of treatment.	treatment was found to be the most important independent predictor of response. < 7% of patients with circulating HBV DNA levels > 200 pg/ml responded to treatment with 5MU of alpha-IFN. In contrast 53% of patients with low levels of HBV DNA (< 100 pg/ml) responded to the same treatment regimen. ($p < 0.0001$).	
4.	*Demonstration of HBV DNA by PCR in the serum and the liver after spontaneous or therapeutically induced HBeAg to Anti-HBe or HBsAg to Anti-HBs seroconversion in patients with chronic hepatitis B*. Loriot M-A et al. Hepatology 1992; 15:32-36	25 patients were included in the study. PCR was performed on serum 6 and 12 months after HbeAg to anti-HBe seroconversion in 12 patients and 2,6 and 12 months after HBsAg to anti-HBs seroconversion in 13 patients.	There is a persistence of HBV replication, at a lower level in 83% patients 6 or 12 months after HBeAg seroconversion and might be predictive of reactivation. In contrast, HBV replication progressively disappears in most patients with HbsAg seroconversion.	Fair
5.	*Individuals with antibodies against hepatitis B core antigen as the only serological marker for hepatitis B infection: high percentage of carriers of hepatitis*	164 individuals who were positive for anti-HBc alone were tested	Using PCR with primer pairs from 3	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	B and C virus. Jlg W et al. J Hepatol. 1995 Jul; 23(1): 14-20.	for HBV DNA as well as for HIV and HCV. (cohort)	different regions of the hepatitis B genome, 54(32.9%) with anti-HBc alone were found positive HBV. The study of individuals positive for anti-HBc alone revealed a high number of carriers of HBV and HCV.	
6.	*Assay of HBV DNA by PCR and its relationship to pre-S and S-encoded viral surface antigens.* Gerken G et al.Hepatology 1991; 13:158-166	The PCR was evaluated as a diagnostic tool in 72 CHB virus carriers	Assay of HBV DNA in the serum by PCR is more proficient than by dot blot and it cannot be replaced by serological assays of HBeAg or pre-S antigen. 107 patients were studied. HBV DNA by PCR was detected in 93% of patients with CHB but none in patients who had recovered form acute hepatitis B and healthy blood donors. PCR was also	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
			detected in 57% of so-called asymptomatic "healthy" carriers, thus identifying infectious subjects, contrary to what is normally assumed.	
7.	*Evidence for hepatitis B virus infection in patients with chronic hepatitis C with and without serological markers of hepatitis B.* Villa E et al. Dig Dis Sci. 1995 Jan;40(1):8-13.	A case control study comparing 19 HBsAg-positive, anti-HCV-positive patients with 38 HBsAg-negative, anti-HCV-positive patients	HBV and HCV infections were investigated by standard serology and PCR. HBV DNA sequences were found, in 71.4% of CAH and in 83.3% of cirrhotics in the HBsAg-positive subjects. In the HBsAg-negative ones, only 10% of CAH but 77.7% of cirrhotics had demonstrable HBV DNA sequences. Conventional serology gives partial information on the true occurrence of HBV infection in	Poor

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
			HBsAg-negative patients, while PCR defines HBV status more accurately.	
8.	*Detection and quantitation of hepatitis B virus DNA: comparison of two commercial hybridization assays with polymerase chain reaction.* Aspinall S et al. J Viral Hepat. 1995;2 (2):107-11.	2 hybridization assays:Digene and Abbot were compared in 42 patients with various HBV serological marker profiles.	A quantitative measurement of serum HBV DNA was carried out. In the case of Digene and PCR there was a 97.6% correspondence, whereas in Abbot and PCR this was only 69%. The McNemar test of symmetry showed no statistically significant difference between Digene and PCR whereas there was a significant difference ($P<0.01$) in Abbot and PCR. For low positive HBV DNA values between 1.5 and 20 pg ml-1 the Abbot assay yields inconclusive results.	Fair
9.	*Correlation between hepatitis B viraemia and the clinical and histological activity of chronic delta hepatitis*. Lozano JL et al. Med Microbiol Immunol (Berl). 1994 Jul;183(3):159-67.	HBV-DNA was analyzed by dot-blot and PCR in 49 patients with CDH.	HBV DNA was positive by dot-blot in 28.5% of chronic delta hepatitis patients, and by PCR in 87.7% patients.	Fair
10.	*Assessment of former and newly developed HBV*	Hybridization and PCR	Enzyme-immune assays to determine	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	assays in a Third World setting. Zabaleta ME et al. J Med Virol. 1992 Dec;38(4):240-5.	were used to detect HBV-DNA in 30 HBsAg chronic carriers and a reference group of 10 subjects whose only HBV marker was anti-HBc.	the presence of pre-S1 Ag and anti-pre-S2 and 2 conventional hybridization with PCR to detect HBV-DNA were used. The presence of HBV-DNA in the group with anti-HBc only was detected solely by PCR	
11.	*Perinatal hepatitis B virus transmission*. Grathwohl J et al . 1992 Jun;140(6):366-8. German.	PCR was used to examine the perinatal route of HBV transmission in 109 mother-child pairs	HBV-DNA was detected in 25(23%) of the mothers' sera from which only 5 were positive for HBsAg. At the age of 6 months only 1 baby had become positive for HBV-DNA, HBsAg and HBeAg.	Fair
12.	*Advantage of PCR for detecting low amounts of HBV DNA in patients' sera*. Bartlet V et al. Res Virol. 1991 Sep- Oct;142(5): 373-9.	54 patient with or without HBV serological markers were studied for low - value HBV-DNA serum samples using PCR and hybridization.	Sensitivity and specificity of using Genostics (standardized solution hybridization), was compared with PCR for low value HBV-DNA serum samples. 38% of patients considered negative in the quantitative assay (<1.5pg/ml) were found positive for HBV-DNA in serum after PCR	Fair
13.	*Serum hepatitis B virus DNA in healthy HBsAg-negative Chinese adults evaluated by polymerase chain reaction.* Shih LN et al. J Med Virol. 1990 Dec;32(4):257-60.	Serum HBV-DNA was assayed using PCR in 107 HBsAg-negative normal Chinese subjects.	8 (7.3%) had HBV-DNA. In 20 subjects negative for all hepatitis B serological markers, 2 (10%) were found to have HBV-DNA.	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
14.	*Polymerase Chain Reaction to detect hepatitis B virus DNA and RNA sequences in primary liver cancer from patients negative for hepatitis B surface antigen*. Paterlini P et al. New Eng J Med 1990; 323: 80-85	PCR was used to evaluate 28 patients with liver cancer who were negative for HBsAg, for DNA and RNA sequences of the virus. 22 of these patients had cirrhosis	HBV DNA sequences were detected in 60.7% of patients. This study shows the importance of PCR in identifying the cause for liver cancer, especially if HBs Ag is negative.	Fair
15.	*Persistent HBV infection of mononuclear blood cells without concomitant liver infection*. C. Feray et al. Transplantation 1990; 49: 1155-8.	The recurrence of HBV in 30 patients after liver transplantation and given high doses of anti-HBs	The PCR assay identified HBV DNA sequences in the peripheral blood mononuclear cells (PBMC) of 64% of subjects who were serum HBsAg negative and HBV DNA negative by PCR. Therefore the application of the sensitive PCR assay demonstrates persistent infection of PBMC in the absence of liver HBV.	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
		immunoglobulin were studied.		
16.	*Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection.* Carman WF et al. Lancet 1989; 588-90.	Landmark study that first alerted hepatologists regarding the pre-core mutant HBV. Sera were taken from 21 HbsAg positive patients.	Seroconverison from HBeAg to anti-HBe was thought to imply loss of viraemia but by using PCR, HBV DNA was found in some anti-HBe positive patients (5-60%). This finding alerted hepatologists regarding the presence of a mutant virus other than the wild-type and is made possible by using PCR.	Fair
17.	*A randomized controlled trial of lymphoblastoid Interferon-alpha in patients with chronic hepatitis B lacking HBeAg* . Fattovich G et al. Hepatology 1992; 15: 584-89.	A randomized controlled trial of lymphoblastoid Interferon-alpha in 60 patients who were	42% responded to treatment. Of these, 87% of patients relapsed 7-12 months after withdrawal of Interferon treatment.	Good

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
		HBeAg negative (pre-core mutant) for 6 months and followed up for a year.		
18.	*Natural course and response to Interferon of chronic hepatitis B accompanied by antibody to hepatitis B e Antigen.* Brunetto MR et al. Hepatology1989;2:198-202	30 consecutive patients with HBV DNA-positive and HBeAg-negative CHB patients were studied over a 2-year period. After 2 years of observation,24 were allocated randomly to one of 2 groups: 12 served as untreated controls and 12 were treated with Interferon.	HBV DNA negativity and normalisation of liver enzymes in 53% of treated patients and in 17% of controls ($p < 0.01$). 27% relapsed. In another study (Brunetto MR et al, Hepatology 1989; 10: 198-202), the relapse rate was 87%.	Fair
HEPATITIS C				
1.	*A long-term study of hepatitis C virus replication in Non-A non-B hepatitis.* Farci P et al. NEJM 1991;325: 98-104	One of the earliest landmark studies to show that HCV RNA is the only diagnostic marker of infection. Study of 5 patients with post-transfusion NANB	The study showed that anti-HCV do not correlate well with chronicity of disease. The absence of HCV RNA correlated with disease resolution and its continued presence (persistent viraemia) predicted patients progressing to chronic hepatitis.	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
		hepatitis followed up for an average of 12.8 years after transfusion.		
2.	*Systematic review of role of PCR in defining infectiousness among people infected with hepatitis C virus*. Gregory JD et al. BMJ 1997;315: 333-37	29 published studies of hepatitis C transmission were reviewed. Various modes of transmission and their relative risks for HCV infection were studied in 2022 people found positive to anti-HCV.	Negative results by PCR indicate an extremely low probability of transmission of hepatitis C from a person with antibody to it.	Good
3.	*Prevalence of Hepatitis C virus infection in heamodialysis patients: A longitudinal study comparing the results of RNA and Antibody assays.* Chan TM et al. Hepatology 1993;1:5-8	Longitudinal study of 51 patients from 2 haemodialysis centres to determine HCV prevalence in heamodialysis patients. The study was over 19 months.	The first generation anti-HCV is unreliable. Although there is good correlation between 2nd generation anti-HCV ELISA assay and HCV RNA, the latter test detected disease much earlier (frequently > 1 year). Diagnosis is therefore quicker with PCR.	Good.
4.	*Hepatitis C in asymptomatic blood donors.* Alter HJ et al.Hepatology 1997; 26: 29S-33S	A prospective study of 280 HCV RNA-positive asymptomatic blood donors to assess disease activity using liver enzymes and histological grading as markers of activity.	Only 17% had normal liver enzymes and all donors who had HCV RNA in serum had histological evidence of chronic hepatitis although only 13% had severe hepatitis or cirrhosis.	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
5.	*Therapy of Hepatitis C*: Overview. Lindsay KL Hepatology 1997; 71S-77S	A meta-analysis of 536 patients in 9 Interferon trials to study the prediction of treatment response by HCV RNA.	68.4 % of those with HCV RNA levels $< 1 \times 10^6$ genomes/ml achieved end-of –treatment response (ETR) compared with 51.1% of those with levels greater than this value. The level of viraemia was also associated with long term (sustained) response. Of the 652 patients in 11 reviewed studies, sustained response (SR) was more frequent in those pretreatment HCV RNA levels $< 1 X 10^6$ genomes/ml than in subjects with higher levels (50.5% vs 17.3%)	Good
6.	*Factors predictive of a beneficial response to therapy of chronic hepatitis C*. Gary L Davis et al. Hepatology 1997; 122S-127S	A review of 10 interferon treatment trials to study the prediction of treatment response by viral genotype (390 patients with genotype 1 and	HCV has at least 6 distinct genotypes and numerous subtypes of HCV identified throughout the world. HCV genotype has also been associated with treatment response. Patients with genotypes 1a and 1b respond poorly to treatment compared with those with	Good.

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
		223 patients with genotypes other than 1a or 1b).	genotypes 2 and 3. In a review of 10 interferon treatment trials, the ETR in 390 patients with genotype 1 was 41.2%, whereas it was 57.8% in the 223 patients infected with genotypes other than 1a and 1b. In 15 studies reporting long term results after short courses of IFN treatment , SR was achieved in only 18.1% of 536 patients with genotype 1 compared to 54.9% of the 288 patients infected with other genotypes .	
HLA TYPING				
1.	*Application of an HLA-B PCR-SSOP typing method to a bone marrow donor registry.* William F; Mawhininnery H; Middleton D. Bone Marrow Transplant 1997 Feb; 19(3) 205-8.	Large sample size RCT	PCR method is more specific than serology method and able to detect HLA-B even in the presence of a second HLA-B allele.	Good
2.	*HLA genotyping by PCR sequence - specific primers (PCR-SSP) a streamline method for rapid routine investigations*. G.Cavanagh et al. Transfusion medicine (1997) 7, 41 -45.	RCT	The PCR method is faster, more accurate, needs smaller sample and does not rely on human typing sera as compared to serology.	Good
3.	*Analysis of HLA-A and B serologic typing of bone marrow registry donors using PCR with sequence-*	Large RCT	DNA- based typing techniques for characterization of donor Class I types	Good

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	specific oligonucleotide probes and DNA sequencing. Santasath DM, Bei M, Steiner N, NgJ Alosco JD et al.Tissue Antigens 1997 Oct 50 (4): 336 -71.		should allow a more consistent definition of types and should speed the donor selection process.	
4.	*Comprehensive method for the typing of HLA-A, B and C alleles by direct sequencing of PCR products obtained from genomic DNA.* Bettinotti MP, Mitsuishi Y, Bibee K, Lau M, Terasaki PI. J. Immunother 1997 Nov: 20(6) : 425-30.	Cohort	PCR allows higher resolution to identify sequence/alleles, which give significant functional implications in HLA typing suitable for large scale handling of samples for clinical use.	Good-Fair
5.	*Rapid HLA-DR genotyping by PCR -amplification with sequence- specific primers.* Tan J, Xie T, Xu D, WandX. China Med J (Eng) 1996 Sept(9): 720- 3.	Small RCT (112)	To establish a rapid genotyping for HLA-DR alleles by polymerase chain reaction with sequence- specific primers.	Good-Fair
6.	*Comprehensive, serologically equivalent DNA typing for HLA- B by PCR using sequence- specific primers (PCR-SSP).* Bunce M, Fanning GC, Welsh KI. Tissue Antigen 1995 Feb; 45(2) : 81-90	160 control individuals.	PCR -SSP system for HLA Class I and II allows to do one step PCR-SSP for all relevant HLA loci in under 3 hours in a system suitable for the typing of cadaver donors.	Good-Fair
7.	*HLA-Class I A and B typing in the clinical laboratory using DNA based techniques.* Hemmatpour SK, Evans PR, McQuilkin S, Sage DA, Howell WM. Transplant Int 1996; 9 Suppl 1: S356-63.	210	Non urgent clinical HLA-A and B typing can be performed by PCR-SSOP with resolution at least equal to that of serology	Good
8.	*Comparison of HLA A antigen typing serology with two polymerase chain reaction based DNA typing*	Small	PCR-SSP and SSOP methods can be used in routine HLA A typing of	Good

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	methods: implication for proficiency testing. Bozon MV, Delgado JC, Turbay D, Salazar M et al .Tissue Antigens 1996 Jun; 47(6):512-8.		patients and donors for transplantation with a greater precision than serology.	
9.	*Comparison of serological and molecular(PCR-SSP) techniques of HLA-DR typing in clinical laboratory routine*. Woszezek G, Borowice M, Mis M, Gorska M, Kowalski ML. Ann Transplant 1997 ;2(1): 39 -42.	Small RCT (28)	Molecular PCR typing allowed for precise antigen determination in all patients PCR-SSP is very useful in routine HLA-DR determination, especially valuable in patients where problems in serological testing are expected.	Good-Fair
10.	*Serology versus PCR_SSP in typing for HLA DR and HLA-DQ: a practical evaluation.* Otten HG, Tilanus MG, Barnstijn M, van Heugten JG, de Gast GC. Tissue Antigen 1995 Jan; 45(1);36-40	Small RCT	Serology is reliable technique only when restricted to HLA-DR 1-10 and HLA-DQ1-3 but PCR is reliable for more HLA-DR1-16 and DQ1-3.	Good-Fair
11.	*DNA polymorphism analysis of HLA_DRB1 gene using PCR-SSP among Korean Subjects*. Kyung Ok Lee, Tack-Kyu Park, Moon-ju Oh and Yoon -Jung Kim. J Biochem. Mol. Biol Vol 29, No 1 pp45-51.	Small RCT	PCR_SSP is relatively simple, fast and a practical tool for determination of HLA-DRB1 genotypes. The results and genotype frequency and heterozygosity of HLA DRB1 gene could be useful for database study before being applied to individual identification and transplantation immunity.	Good - Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
12.	*Strategies for prenatal HLA-typing for potential cord blood donor evaluation.* Pollack MS, Grant GJ. Blood Cells 1994;20(2-3): 310- 4: disc 314 -5.	NCCS Multicentre	HLA Class I and II typed by PCR method can be used for the prenatal identification of a potential identification of a potential donor for an affected fetus.	-
13.	A 25% error rate in serologic typing of HLA_B homozygotes.	Small RCT	High error rate in serologic definition of HLA-B molecules Argues for the use of rapid DNA-based techniques in HLA typing even in the setting of solid organ transplantation.	Good - Fair
14.	*Molecular techniques for typing unrelated marrow donors: potential impact of molecular typing disparity on donor selection.* Baxter_Lowe LA. Bone Marrow Transplant 1994: 14 Suppl 4: S42-50		Precise HLA typing may contribute to the success of future transplants utilizing alternative donors.	-
15.	*HLA DR typing for kidney transplants: advantage of polymerase chain reaction with sequence specific primers in a routine hospital laboratory.* Lynas C, HurlockNJ, Copplestone JA, Prentice AG, McGonigle RJ.J Clin Pathol 1994 Jul: 47 (7): 609-12.	RCT(93)	PCR-SSP provides more reliable and detailed information on HLA-DR polymorphism than serology, and does so within a routine tissue typing laboratory.	Good
16.	*DNA HLA -DR typing results of 4000 kidney*	Large RCT	The discrepancy rate between PCR	Good

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	transplants. Mytilineos J, Scherer S, Dunckley H, TrejautJ, Chapman J, Fischer G et al. Transplantation 1993 Apr: 55(4): 778 -81.		_DRB and serology HLA-DR was 25% for organ donors and 27.8% for kidney recipients.	
17.	*HLA-DR typing by PCR amplification with SSP in 2 hours: an alternative to serological DR typing in clinical practice including donor recipient matching in cadaveric transplantation*. Olerup O, Zetterquist H. Tissue Antigen 1992 May; 39(5):225-35	Small RCT	PCR-SSP is an accurate typing technique with high sensitivity, specificity and reproducibility. The method is rapid and in expensive.	-
18.	*Comparison of typing results by serology and polymerase chain reaction with SSP for HLA-Cw in 650 individuals.* Mytilineos J, Christ U, Lempert M, Opelz G. Tissue Antigen1997 Oct; 50 (4); 395-400.	Large RCT	HLA-Cw is associated with high frequency of blanks with serology method. Molecular typing allow the proper investigation of HLA-Cw matching in kidney and bone marrow transplantation.	Good
19.	*DNA amplification for DQ typing as an adjunct to serological prenatal HLA typing for identification of potential donors for umbilical cord blood transplantation.* Pollack MS, Auerbach AD, Broxmeyer HE, ZaafranA, Griffith RI, Erlich HA. Hum Immuno 1991 jan: 30 (1): 45-9.	Case studies	Fetal cell DNA amplification and hybridization for DQ typing can be an important procedure to verify serologically determined HLA types.	-

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
20.	*Selection of unrelated bone marrow donors by PCR-SSP typing and subsequent nonradioactive sequence- based typing for HLA DRB1/3/4/5, DQB1, and DPB1 alleles.*	Large RCT	PCR allows matching of bone marrow transplant pairs with the highest degree of reliability for assessment of HLA Class II identity.	Good
21.	*HLA Class I (A,B) and II (DR,DQ) gene and haplotype frequencies in blood donors from Wales. Darke C, Guttridge-MG, Thompson* J, McNamara S, StreetJ, et al Exp Clin Immunogene 1998; 15(2) 69-83.	Large RCT	PCR SSP typing suggest an accurate level of specificity of Bone marrow registry panel donors who had previously been typed by serology alone.	-
22.	*Rapid HLA-DQB typing by eight polymerase chain reaction with sequence specific primers*. Bunce M, Taylor CG , Welsh KL. Hum Immuno 1993 Aug 37(4); 201-6.	Cohort	No false positive or false negative result found in PCR-SSP method.	-
23.	*An HLA-DR typing protocol using specific PCR amplification followed by restriction enzyme digests.* Westman P, Kuismin T, Partanen J etal Eur J Immunogene 1993 Apr 20(2); 103 -9	Large RCT	The results suggest that PCR amplification gives reliable HLA-DR types and works well in the routine use.	Good
24.	*Analysis of HLA Class II polymorphism using PCR.* Erlich H, Bungawan T, Begovich A,Scharf S. Arch -Patho-Lab Med 1993 May, 117(5) 482-5	Small RCT	A simple, rapid and precise method of typing HLA class II by PCR would be valuable in the areas of disease susceptibility, tissue transplantation, individual identification and anthropological genetics.	Good-Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
25	*A rapid molecular diagnosis of post transfusion graft versus host disease by polymerase chain reaction.* Hayakawa S, Chishima F, Sakata H, Fujii K et al. Transfusion 1993 May 33(5) ; 413-7	Case study	PCR is a rapid technique for the early diagnosis of acute posttransfusion GVHD in female patients.	Fair
26.	*HLA_DPBI typing with polymerase chain reaction and restriction fragment polymorphism technique in Danes*. Hvid TV, Madsen HO, Morling N. Tissue Antigen 1992 Sept; 40(3) 140-4.	Small RCT (27)	No equivocal result in the PCR method	Good-fair
27.	*HLA-DR Band DBQ typing by PCR amplification using sequence specific primers assessment after 1 year of routine use by three laboratories.* Mercier b, al Daccak R, Samaan A, Carta QA et al. Eur J Immunogene 1994 ;21(2) 105 –23.	Multicentre study.	The experienced of three laboratories using this technique in context of organ or bone marrow transplant is encouraging.	Fair
28.	*HLA-DQBI low resolution typing by PCR amplification with sequence specific primers (PCR-SSP)* Aldener –Cannava A, Olerup O. Eur J Immuno 1994 Feb 21(1) 1-9	Small RCT (27)	This method enabled the detection of DNA polymorphism including point mutations at a variety of point in the DNA fragments of HLA-DRBI gene.	Good-Fair
29.	*Phenotype analysis of hemopoietic CD34+ cell*		The result suggests that circulating	-

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	populations derived from human umbilical cord blood using flow cytometry and cDNA polymerase chain reaction. Thoma SJ, Lamping CP, Ziegler BL. Blood 1994 Apr 15; 83(8) 2103-14		CD34+CD7CD19CB cells cannot be distinguished by flow cytometry can be detected by cDNA-PCR.	
30.	*Molecular analysis of the HLA_DRB genes in two tribes of Brazilian Indians.* Petzl Erler-MI Mc Devitt HO Hum- Immuno 1994 Nov ;41(3) 180-4.	Large RCT	HLA-DRBI-DRB3, DRB4 andDRB5 alleles of the Guarani and Kaigang Amerindians were described.	-
31.	*HLA- DR typing for kidney transplants advantage of polymerase reaction with sequence specific primers(PCR-SSP) in routine hospital laboratory.* Lynas-C Hurlock NJ, Copplestone JA, Prentice AG et al.J Clin Patho 1994 Jul 47(7) 609-12.	Large RCT	PCR-SSP can assign HLA-Dr type more accurately than serology in routine hospital laboratory.	Good
32.	HLA- B44 subtyping in Spanish population: further evidence of Caucasian population diversity. Santos_S; Vicarario- JL; Merino -JL; Balas-A. Tissue Antigen 1997 Feb; 49(2); 124-8.	Small RCT (144)	High resolution PCR for typing HLA-Class 1 will be great relevance and advantages in unrelated bone marrow transplant.	Good
33.	*Development of PCR-SSOP for the identification of HLA- 02 frequencies within different ethnic populations.* Williams-F; Middleton-D; Savage-D; Gorodezky-C; Wilson DW et al. Tissue Antigen 1997; Feb 49(2):129-33.	Large Population study	HLA-02 have been identified in Northern Ireland, Singapore Chinese, Shetland Island and Mexican.	Good

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
34.	*HLA-B typing by allele separation followed by direct sequencing.* Eberle-M; Knapp-LA; Iwanaga-KK; Domanico-MJ; Aiyer-K et al . Tissue Antigen 1997 Apr; 49 (4) 365- 75.	Small RCT (57)	PCR technique able to determined the allele of the individual, previously difficult to identify by serology.	Good
35.	*Report from the HLA class II typing by PCR Multicentre Study.* Zetterqiust -H; Bengston-M; Backstrom-g; Eagle-Jansson I et. Eur- J- Immunogenet 1997Jun; 24(3): 191-9.	Large RCT (360)	Through PCR method, the success rate of correctly identified HLA-DR and DQ alleles of 91-98% could be maintained., even under sub-optimal typing condition.	Good
36	*High resolution HLA-C typing by PCR-SSP : identification of allelic frequencies and linkage disequilibrium in 604 unrelated random UK Caucasoids and a comparison with serology .* Bunce-M; Barnardo-MC; Proter-J Marsh SG; Vilches-C; Welsh-KI . Tissue Antigen 1997 Jul; 50(1):100-11.	Large RCT (604)	PCR-SSP has proved a reliable, accurate and rapid method for high-resolution HLA-C alleles typing in most heterozygous combinations.	Good
37.	*DNA typing of HLA B27 by polymerase chain reaction.* Lucotte-G Burckel-A. Mol-Cell- Probes 1997 Aug;11(4):313-5	Large RCT (100)	PCR and flow cytometric method PCR method were compared.	Good
38.	*HLA Class II DRBI, DQAI and DQBI polymorphisms in Polish population from*	Large RCT	PCR was able to determine the HLA polymorphism reliably.	-

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	Wielkopolska. Jungerman-M; Sanchez-Mazas_A; Fichna -P ;Ivanova-R et al. Tissue Antigens 1997 Jun : 49(6); 624-8.			
39	*Rapid HLA-DQB typing by eight polymerase chain reaction amplifications with sequence specific primers PCR-SSP.*	Small RCT (138)	PCR-SSP methods were compared with PCR-SSO method. PCR-SSP has proved reliable and rapid method for typing HLA-DRB1. The total time taken for PCR-SSP only 130 minutes from start to finish.	Good
40	*HLA class II 'typing" direct sequencing of DRB,DQB and DQA genes.* Santamaria-P Boyce-Jacino-MT; Lindstrom AL; BarbosaJJ et al. Hum Immunol 1992 Feb; 69-81.	Small RCT	Serology methods have many drawback as compare to PCR method.	Good-Fair
HPA				
1.	*Human platelet antigen frequencies of platelet donors in the French population determined by PCR with sequence-specific primers.* Merieux Y. et al. Pathol Biol (Paris) 1997 Nov:45(9):679-700	Large sample RCT	Reports the human platelet antigen frequencies of four platelet-specific alloantigen systems in the French population. This study confirms the need to type donors and recipients in the HPA-1 system at least, in case of post-	Good

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
			transfusion purpura and platelet refractoriness to platelet transfusion therapy.	
2.	*Flow cytometry immunophenotyping and PCR –SSP genotyping for HPA-1 alloantigens in an Italian population*. Tazzari PL. et al Vox Sang. 1998: 74(1) 42	Large sample RCT	Flow cytometry was unable to distinguish heterozygous from homozygous subjects for large scale immunophenotyping of platelet alloantigen which PCR-SSP method was able to do. Therefore PCR is the assay of choice for zygosity testing, antenatal diagnosis and thrombocytopenic alloimmunized patient.	Good
3.	*Use of a rapid method for genotyping human platelet antigen systems in neonatal alloimmune thrombocytopenia.* Porreti L. et al. Haematologica 1997 Sep-Oct : 82(5) : 600	Cohort	Allow identification of newborn which are incompatible with their respective mothers in cases of neonatal alloimmune thrombocytopenia. Serology gave inconclusive results.	Good -Fair
4.	*Genotyping of the human platelet antigen-1 by ELISA detection of allele-specific amplicons.* Muller TH, et al. Vox Sang. 1997 : 73(3) : 185	Small sample RCT 100	To establish a valid and efficient method for genotyping of the human platelet antigen in large sample numbers -efficient HPA genotyping in large numbers of donors and patients.	Good -Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
5.	*Genetic typing of human platelet antigen 1 by oligonucleotide ligation assay in a specific and reliable semi-automated system*. Zotz RB et al. Br. Journal Haematology 1997 Jan :96(1) 198	Comparative study	The genotypes determined with this method assay were in 100% concordance with the results obtained by conventional allele-specific restriction enzyme site analysis and PCR amplification with SSP. The automated oligonucleotide ligation assay provides rapid, reliable, nonisotopic method to genotype human platelet antigens that can rapidly be applied to large population screening.	Good -Fair
6.	*Frequency and functional relevance of genetic threonine 145/methionine145 dimorphism in plateletglycoprotein !b alpha in an Italian population*. Mazzucato M, et al Transfusion 1996 Sep;36(10):891	Large sample RCT 379	Threonine 145/methionine145 dimorphism in platelet glycoprotein !b alpha defines the human platelet antigen (HPA-2) system implicated in refractoriness to HLA-matched platelet transfusion and in neonatal immune thrombocytopenic purprura. Genotypic and phenotypic frequencies in the HPA-2 system here are consistent with those reported among whites.	Good
7.	*Genotyping of the human platelet antigen systems 1 through 5 by multiplex PCR and ligation based assay*. Legler TJ, et al Transfusion 1996 May: 36(5): 426	Small sample RCT 110	A new genotyping, Multipex PCR in combination with ligation – based typing allows fast typing of large numbers.	Good -Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
8.	*Rapid typing for Human Platelet Antigen system-1,-2,-3 and –5 by PCR amplification with sequence specific primers.* Kluter H, et al. Vox Sang. 1996:71(2): 121	Blind quality control study 112	The method is comparable to PCR-RFLP technique but PCR-SSP results are available within 3 hours	Good -Fair
9.	*Human Platelet Antigen-2 and –3 genotyping by PCR-SSP*. Metcalfe P et al Transfusion Medicine 1995 Dec:5(4):285	To evaluate methodology	HPA-2 and –3 genotype can be determined by PCR-SSP and the results are in concordance other conventional with other genotyping using PCR followed by restriction enzyme digestion (PCR-ASRA)	Good
10.	*Platelet alloantigens HPA-1,-2,-3,-5 and –6 in Finns* Kekomaki S, et al. Transfusion Med. 1995 Sept;5(3): 193	RCT 200	PCR-ASRA Vs MAIPA (serology) -no discrepancies observed between the results obtained with the PCR-method and those obtained serologically.	Good
11.	*HPA gene frequencies in Austrian population* Holensteiner A,et al Haemostasis 1995 May-June;25(3); 133	Population study 900	Serological and genotyping (PCR) showed no discrepancies.	Good -Fair
12.	*DNA-based typing of HPA systems by PCR-single strand conformation polymorphism method.* Fujiwara K, et al Vox Sang. 1995:69(4) 347	Comparative study between serological and PCR-SSCP	Good agreement between the 2 methods. PCR-SSCP is a simple and sensitive method for determining HPA genotype and identifying unknown polymorphism.	Good -Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
13.	*Molecular genetics of HPA*. Simsek S, et al Infusion & other Transfusions Med 1994 Nov:21 Suppl 3:29-33	Review	To review the molecular genetics of HPA, the application of molecular biological techniques to detect mutations underlying polymorphism and the importance of these techniques for diagnosis of immune mediated platelet destruction.	Good
THALASSAEMIAS (HAEMOGLOBINOPATHIES)				
1	*Molecular diagnosis and carrier screening for β thalassaemia.* Antonio Coa et al. JAMA October 1997 Vol 28 No 15 :1273-12777	Review of molecular diagnosis	Mutation detection of β thalassaemia is carried out by a number of PCR–based procedures. e.g. reverse dot blot analysis, primer specific amplification followed by DGGE and direct sequencing if not detected by the above techniques	Fair
2	*Reverse dot blot probes for the screening of β thalassaemia in Asians and American Blacks* Shi-ping et al. Human Mutation 3:59-63 (1994).	Cases from Asian population	The reverse dot blot assay is a simple and rapid system to type an individual for potential mutations. The panel used for Asian mutations covers 98% of Malaysian population	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
3	Single cell detection of β thalassaemia mutations using silver stained SSCP analysis: an application for pre-implantation diagnosis. El Hashimite et al. Mol Hum Reprod 1997 Aug;3(8):693-8	294 single somatic cells examined on the third day of *in vitro* fertilisation	Using SSCP particularly for pre-implantation diagnosis provides a simple means of assessing allele-specific amplification failure. Combination of nested PCR and automated silver stained SSCP analysis offers exceptional resolution accuracy and speed which are essential for pre-implantation.	Fair
4	*Detection of β thalassaemia mutation insertion ATCT at codon 47/48 by ARMS technique for screening and prenatal diagnosis* Saxena R et al. Indian J Med Res1997 Jun;105:275-7	Offered to five couples at risk for this mutation	Allele specific primers by PCR based amplification refractory mutation system (ARMS) useful in cases which remained uncharacterized after screening for the common and rare population. Assay provides an easy non isotopic method and will be useful for screening and prenatal diagnosis of couples at risk	Poor
5	*Prenatal diagnosis of the thalassaemia syndromes by rapid DNA analytical methods* Kanavakis et al. Mol Hum Reprod 1997 Jun:3(6): 523-8	147 pregnancies (150 fetal samples) at risk of wide range of haemoglobinopathy	Using simple PCR protocols and a few techniques – DGGE, ARMS, restriction endonuclease analysis of PCR fragments, oligonucleotides hybridization and gap PCR for detection of deletions -18 different mutations in	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
			the alpha and beta globin clusters were identified .Advantages of each PCR based protocol were then assessed.	
6	*Misdiagnosis of homozygous α thalassaemia 1 may occur if polymerase chain reaction alone is used in prenatal diagnosis* Ko TM. Prenat Diag1997 Jun; 17(6):505-9	180 fetus at risk of Homozygous SEA deletion of alpha globin chain genes	Both the PCR and Southern Hybridization (SH) was performed. 3 were misdiagnosed by PCR as heterozygote, 2 as normal and 2 as affected. Misdiagnosis of normal and affected was due to maternal DNA contamination while misdiagnosis of heterozygotes was probably due to failed PCR	Fair
7	*Reverse dot blot detection of Thai β thalassaemia mutations.* Pranee Sutcharitchan et al. British Journal of Hematology 1995 90: 809-819	Case studies	Reverse dot blot enables screening of several mutation with a single hybridization reaction, but has to be targeted to common mutations of particular ethnic groups – in this case that accounts for 96% of thalassaemia in Thailand and 6 less common mutation. The second strip precludes the need of more challenging techniques.	Poor
8	*Rapid detection and prenatal diagnosis of β thalassaemia: studies in Indian and Cypriot population in the UK* JM Old et al Lancet 1990;336:834-37	100 first trimester patients	Application of the amplification refractory mutation detection system (ARMS) to individuals of β thalassaemia mutations in heterozygotes parents and at risk fetuses Results : Detection of 17 different mutations. This techniques allows the	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
			determination of mutations in both the parental and fetal DNA on the same day	
9.	*Denaturing gradient gel electrophoresis and direct sequencing of PCR amplified genomic DNA: a rapid and reliable diagnostic approach to β thalassaemia* M. Losekoot British et al. Journal of Haematology 1990.76,269-274.	Case studies	Combinations of DGGE and direct sequencing determination of PCR amplified genomic DNA represents valid alternative to the ASO probes approach	Fair
10	*A PCR based strategy to detect the common severe determinants of α thalassaemia*. Bowden DK et al British Journal of Haematology 1992, 81, 104-108	Case studies	A rapid and inexpensive PCR based strategy is used to detect the three common severe α thalassaemia determinants observed in South East Asia	Poor
11	*Detection of α thalassaemia –1 (Southeast Asian type)and its application for prenatal diagnosis* Winichagoon P et al. Clin Gent 1995;:47:318-320	Case studies	A simple non radioactive method based on the polymerase chain reaction was used to detect the Southeast Asian type of α Thalassaemia	Poor
12	*Prenatal diagnosis for thalassaemia in a multicultural society* Trent RJ et al. Prenat Diagn 1998Jun;18(6):591-8	Case studies	Using PCR approximately 12 % of β thalassaemia and 9% of α Thalassaemia cases require further study. Capillary electrophoresis has proven helpful since a DNA scanning approach such as SSCP can be automated to identify the region of DNA to be sequenced	Poor

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
13	*Early prenatal diagnosis of β thalassaemia in Singapore* Ng IS. Ann Acad Med Singapore 1996 Nov;25(6):779-82	Case studies on 16 couples at risk for β thalassaemia	Chorionic villous sampling (CVS) taken at first trimester and direct gene analysis on the foetal sample using various molecular techniques.	Poor
14	*Molecular genetics of β thalassaemia in Pakistan: a basis for prenatal diagnosis*. Ahmed S et al. Br J Haematology 1996Sept;94(3):476-82.	Cohort	Characterized 1216 β thalassaemia alleles from five major ethnic groups.	Fair
15	*Comparison of the Hb H inclusion test and a PCR test in screening for alpha thalassaemia in Hong Kong* Chan AY. J Clin Pathol 1996 May;49(5):411-3	Cohort	Comparison between Hb H inclusion test with PCR test in routine screening for α Thalassaemia. Hb H inclusion tests were positive in 78 (79%) patients, 73 (93.7%) of whom carried the (--SEA) deletion on analysis of their DNA by PCR Hb H inclusion test can be replaced by PCR as the investigation of choice.	Fair
16.	*Prenatal diagnosis of β thalassaemia mutations using the reverse dot blot techniques.* Muralitharan S et al. Nat Med J India 1996 Mar-Apr;9(2):70-1.	Case studies	CVS sampling on 12th week old foetus and was amplified by specific primers using PCR and analysed by the reverse dot blot test.	Poor

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17	*Prenatal diagnosis of β thalassaemia and sickle cell anemia in Turkey*; Tuzmen S et al. Prenat Diagn 1996 Mar;16(3):252-8	Case studies on 70 parents	Molecular screening was carried out by allele specific probes to the 12 common mutations in Turkey. DNA sequencing was done to detect three other rare mutation. All diagnoses were successfully accomplished and there were no misdiagnoses.	Fair
18	*Molecular heterogenicity of β thalssaemia in Malaysia :a practical approach.* Thong ML et al .Ann Acad Med Singapore 1996 Jan ;25 (1): 79-83	20 children	Patients with β thalassaemia were characterised by using multi modal approach consisting of a dot blot assay hybridisation, reverse dot blot ARMS and genomic sequencing. This strategy detected 100% mutation in the Chinese sub-population in Malaysia	Poor
19	*Rapid diagnosis of β thalassaemia by mutagenically separated PCR (MS-PCR) and its application to prenatal diagnosis* Chang JG et al. Br J Haematology 1995 Nov; 91(3):602-7	Case studies 14 prenatal samples	MS –PCR Simple handling and provides a within assay quality control for the exclusion of false negative results	Poor
20	*The amplification refractory mutation system (ARMS):a rapid and direct prenatal diagnostic technique for β thalassaemia in Singapore* Tan JA et al. Prenat Diagn 1994 Nov;14(11):1077-82	Case studies	ARMS technique was evaluated as a routine test for prenatal diagnosis of β major Six mutation along the gene are studied and offered to 90% of Chinese patients and 54.6% of Malay couples at risk This techniques provides an	Poor

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			accurate rapid and simple method for a β thalassaemia prenatal diagnosis programme.	
21	*Identification of Hb Constant Spring :Using a PCR Based Technique.* Aliza MY et al Malaysian Journal of Medical Sciences Vol 5 No1 Jan1998 (42-44).	Case studies`	DNA extracted from 2 HBH CS patient This technique is simple and can be easily established in the laboratory	Poor
22	*Prenatal diagnosis of HB Barts hydrops fetalis in West Malaysia the identification of the alpha thal 1 defect by PCR based strategies* George E et al. Singapore Med J 1996Oct;37(5):501-4.	Case studies	Fetal blood samples were used for detection of complete absence of α globin genes by PCR and non-radioactive primers. Results were made available within 1-3 days as compared to using restriction enzymes and hybridization to radio labeled probes which took 2 weeks	Poor
23	*Molecular characterization of β globin gene mutations in Malay patients with HbE β thalassaemia and thalassaemia major*. Yang KG et al Br J Haematology	27 patients	Molecular characterization by reverse dot blot able to characterise the mutation	Poor
24	*Selective enzymatic amplification of α 2 globin DNA for the detection of the hemoglobin Constant Spring mutation* Kropp GL et al. Blood 1989 May15;73(7):1987-92.	Case studies	Selective enzymatic amplification of α 2 globin DNA allows unambiguous diagnosis to be made using the allele specific hybridization	Poor

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
25	*Haemoglobin constant spring in Bangkok : molecular screening by enzymatic amplification of the α 2 globin* Thonglairoam V. Am J Haematology 1991Dec;38(4): 277-80	Case studies	Selective enzymatic amplification of α 2 globin DNA and allele specific hybridization for the HbCS gene provide accurate diagnosis of Hb Constant Spring. The gene frequency calculated in Thailand for α constant spring from a total of 406 cord blood was found to be 0.008	Poor
26	*Detection of β thalassaemia mutation using DNA heteroduplex generator molecules* Savage DA et al. Br J Haematology Jul;90(3):564-71	Case studies	A rapid PCR based method for the detection of β thalassaemia mutation. Three DNA heteroduplex generator molecules were evaluated for the detection of mutations. Three molecules are capable of detecting approximately 95%of the mutations found in the Singaporean population	Poor
DUCHENNE MUSCULAR DYSTROPHY				
1	*Deletion patterns in Argentine patients with Duchenne and Becker muscular dystrophy*. Baranzini SE et al. Neurol Res. 1998 Jul;20(5):409-414.	DNA from 75 patients with DMD/BMD was analyzed by multiptex PCR. (cohort)	Deletions were detected in 24(32%) and were mainly clustered in 2 areas of the dystrophin gene; the 5'end (exons 3-12) and the central part (exons 44-53).	Fair
2	*Deletion screening of the Duchenne/Becker muscular dystrophy gene in Croatian population.* Sertic J et al.	53 DMD and 21 BMD male were tested for dystrophin gene	The overall percentage of deletion cases observed was 50%; 61%(53/32) for DMD and 38%(21/8) for BMD. The	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	Coll Antropol. 1997 Jun;21(1):151-6.	deletion by using multiplex PCR. (cohort)	number of deleted exons was variable, but generally DMD deletions involving single-exon and larger exon were more frequent.	
3	*Are there ethnic differences in deletions in the dystrophin gene?* Banerjee M et al. Am J Med Genet. 1997 Jan 20;68(2):152-154.	160 cases of DMD from all parts of India were studied using multiplex PCR of 27 exons.(cohort)	103(64.4%) showed intragenic deletion. Most (69.7%) of the deletions involved exons 45-51.It was concluded that there is likely to be no ethnic difference with respect to deletions in the DMD gene.	Fair
4	*Molecular deletion patterns in Turkish Duchenne and Becker muscular dystrophy patients*. Dincer P et al. Brain Dev. 1996 Mar-Apr;18(2):91-4.	57 DMD, 7 BMD and 1 DMD-BMD intermediate were investigated for dystrophin gene deletion patterns. (case series)	Deletions, analyzed by multiplex amplification of selected exons, were observed in 58%(38) of the patients. The application of PCR to deletion analysis in D/BMD was found to be very useful in delineating the extent of the deletion in most of the cases (82%).	Poor
5	*Carrier detection and microsatellite analysis of Duchenne and Becker muscular dystrophy in Spanish families.* Kruyer H et al. Prenat Diagn. 1994 Feb;14(2):123-	44 D/BMD families with living affected members were tested by using multiplex PCR to determine the carrier status.	Deletions were detected in 20(45.5%) out of 44D/BMD families with living affected members, more often in sporadic cases of DMD than in familial ones.	Poor

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
6	*Deletion detection for diagnosis of Duchenne muscular dystrophy in the Japanese population--comparison between the polymerase chain reaction and the Southern blot analysis*. Katayama S et al. Jpn J Hum Genet. 1993 Jun;38(2):177-84.	30 males (27 Japanese families) with DMD were studied by the multiplex PCR, and 24 of them were also investigated by southern blot.	Deletions were detected in 14(52%) out of 27DMD families by the PCR. Southern blot detected deletions in 14(64%) out of 22 families. 13(93%) of the 14 DMD families with deletions detected by Southern blotting were also confirmed by the multiplex PCR. Provided care is taken in cases where the deletion is limited to a single exon, the multiplex PCR appears to be an efficient and useful alternative to conventional southern blot analysis for detecting deletions during the prenatal and postnatal diagnosis of DMD.	Poor
7	*Screening of deletions and RFLP analysis in Turkish DMD/BMD families by PCR.* Gokgoz N et al. Clin Genet 1993 May;43(5):261-6.	76 DMD and 5 BMD screened for deletions using 2 multiplex gene amplication systems.	The use of both systems revealed deletions in 52% of the cases in the Turkish population. The majority of these deletions (33/37) were found to be localized within the central region of the dystrophin gene.	Fair
8	*Dystrophin gene analysis and prenatal diagnosis of Duchenne muscular dystrophy in Russia*. Baranov VS et al. Prenat Diagn. 1993 May;13(5):323-33.	119 families with at least 1 affected child or an affected close male relative of women at	By means of multiplex PCR with different sets of oligoprimer providing amplication of 10-11 different exons, 49 dystrophin gene deletions were detected	Poor

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		risk of being a DMD carrier.	(41%). Carrier status was ascertained in 20 and rejected in 32 female relatives in 40 DMD families.	
9	*Analysis of dystrophin gene deletions in patients from the Mexican population with Duchenne/Becker muscular dystrophy*. Coral-Vazquez R et al. Arch Med Res. 1993 Spring; 24(1):1-6.	40 unrelated Mexican patients with D/BMD were analyzed for DMD gene deletions.	Using the multiplex amplification of 15 deletion-prone exons described by Chamberlain et al. and Beggs et al., it was found that the percentage of deletions was 52.2% and the majority of them (86.3%) were located at the hot spot deletion region .	Poor
10	*Molecular genetic analysis of 67 patients with Duchenne/Becker muscular dystrophy*. Nieman-Seyde S et al. Hum Genet. 1992 Sep-Oct;90(1-2):65-70.	56 DMD and 11 BMD patients were involved.	The patients were analyzed by extended multiplex amplification of the D/BMD gene and deletions were found in 60% of these patients.	Poor
11	*Diagnosis of Duchenne and Becker muscular dystrophies by polymerase chain reaction. A* Jeffrey SC et al. JAMA. 1992 May 20;267(19):2609-15.	A multicenter study. Patients with D/BMD were screened for deletion mutations using multiplex PCR, and the results were compared with Southern blot	The multiplex PCR detected 82% of those deletions detected by Southern analysis method. In 1 of 745 analyses, the multiplex method suggested a single exon deletion, which was not confirmed by Southern blot, thus representing a false-positive rate of 0.013%. Multiplex PCR represents a sensitive and accurate method for deletion detection of 46% of all cases of D/BMD. The method requires 1 day for analysis, is easy to perform and does not use radioactive	Poor

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			tracers. It can represent an efficient and rapid method for pre/postnatal diagnosis of D/BMD.	
12	*Molecular deletion patterns in families from southern France with Duchenne/Becker muscular dystrophies.* Claustres M et al. Hum Genet. 1991Dec;88(2):179-84.	38 unrelated patients (Southern France) with D/BMD were studied for the gene deletion.	Both multiplex amplification of selected exons and cDNA were used. Of the 26(68%) patients found to have deletions, 24(92%) were detected by multiplex PCR.	Poor
13	*A screening for dystrophin gene deletions in Japanese patients with Duchenne/Becker muscular dystrophy by the multiplex polymerase chain reaction.* Sakubara H et al. Brain Dev. 1991 Sep;13(5):339-42.	92 unrelated Japanese patients were tested by multiplex PCR	Dystrophin gene deletions were found in 33% of the patients.	Poor
14	*Rapid detection of deletions in the Duchenne muscular dystrophy gene by PCR amplification of deletion-prone exon sequences.* Hentemann M et al. Hum Genet 1990 Feb;84(3):228-32	The DNA of 42 patients with D/BMD were screened by using PCR for deletions within the DMD gene. (case-control)	2 regions within putative deletion "hot spots" of the DMD gene were tested, and deletions were found in 16.6% of patients.	Poor
15	*Molecular diagnosis of Duchenne muscular dystrophy in Singapore.* Low et al. Ann Acad Med Singapore, 1996. Jan; 25 (1): 84-89.	Case series	Partial deletions accounted for 58.3% of the patients and were detected by multiplex PCR. Direct demonstration of the gene defect gave 100% assurance of the accuracy and specificity of the diagnosis. Linkage analysis was useful for prenatal diagnosis and carrier	Poor

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
			detection for the remaining 41.7%.	
16	*A quantitative polymerase chain reaction (PCR) assay completely discriminates between Duchenne and Becker muscular dystrophy deletion carriers and normal* females. Pastore et al Mol Cell Probes, 1996	Case series of deletion carriers	Quantitative PCR could completely discriminate deletion carriers from normal females.	Poor
17	*Accurate diagnosis of carriers of deletions and duplications in Duchenne/Becker muscular dystrophy by fluorescent dosage analysis.* Yau et al. J Med Genet., 1996. Jul: 33(7):550-8	Randomised control trial (blinded), 150 samples from female carriers	Flourescent multiplex PCR to detect 98% reported deletions and 90% duplications. 63 deletion carriers, 2 duplication carriers and 84 normal females were all correctly identified. Detects over 70% of all mutations and is partially automated.	Poor
FRAGILE X SYNDROME				
1.	Elbaz A, Suedois J, Duquesnoy M, Beldjord C et al. Prevalence of fragile-X syndrome and FRAXE among children with intellectual disability in a Caribbean island, Guadeloupe, French West Indies. J.Intellect Disabil Res 1998 Feb;42(Pt 1):81-89	163 boys and 85 girls with mod. to severe intellectual disability (prior unknown origin).	6.7% and 0% prevalence among boys and girls, respectively, yielding a minimum FXS incidence of 0.42 per 1000 male births / year.	Fair
2.	Arvio M, Peippo M, Simola KO. Applicability of a checklist for clinical screening of the fragile X syndrome. Clin Genet 1997 Oct;52(4): 211-215	A population study of 340,000 in Southern Hame Finland with 541 intellectually	Of these, 197 already had a confirmed etiological diagnosis, with 20 having the fragile X syndrome. Of the other 344 males were screened, 6 new fragile	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
		disabled adult males.	X were found. The minimum prevalence of the fragile X syndrome in the district was calculated to be 1:4400.	
3.	*A five-year experience with fragile X testing. Setting laboratory standards of practice and a cost-effective protocol*. Marini T, Pflueger S, Jackson A, Naber S, Karpells S, Naeem R Baystate Medical Center, Springfield, MA 01199, USA Diagn Mol Pathol 1997 Oct;6(5):304	652 pts. tested for fragile X syndrome using cytogenetic analysis alone (Protocol 1) or a combination of DNA analysis and routine karyotyping (protocol 2).	The overall positive rate for fragile X was 3.1% with an incidence of other chromosomal abnormalities (OCAs) of 3.2%. A fragile X testing protocol combining routine karyotyping with definitive molecular technology represents the most cost-effective diagnostic approach to this clinically challenging patient population.	Fair
4.	*Combined molecular and cytogenetic analysis for the rapid diagnosis of fragile X syndrome* Bussani Mastellone C, Giovannucci Uzielli ML, Grasso M, Chiurazzi P, Neri G, Wang Q. Acta Genet Med Gemellol (Roma) 1996;45(1-2):165-8	A protocol for screening programme and prevention studies in families at risk. cytogenetic testing.	Molecular techniques enable the detection of the mutation and also of the exact length of this DNA sequence, allowing the classification of the tested subjects as normal, carrier or affected. A protocol of analysis that combines a method of non-radioactive PCR, Southern blotting and cytogenetics is proposed.	Fair
5.	*Fragile-X syndrome in east Finland: molecular approach to genetic & prenatal diagnosis* Ryynanen M, Pulkkinen L, Kirkinen P, Saarikoski S. Am J Med Genet 1994 Jul 15;51(4):463-465	Fragile X syndrome verified in 51 families of the population of 900,000 in East-Finland during 1989-92.	93 relatives with full mutation, 127 healthy carriers with the premutation, and 28 decreased males were obligate carriers of the fragile X syndrome were diagnosed. Systematic genetic counseling and carrier screening in these families is essential for	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
			understanding of the occurrence of fragile X syndrome and for better prevention of this syndrome in future generations.	
6.	*DNA testing for fragile X syndrome in schools for learning difficulties. Dept. of Medical Genetics, Churchill Hospital, Oxford.* Slaney SF, Wilkie AO, Hirst MC, Charlton R, McKinley M, Pointon J, Christodoulou Z, Huson SM, Davies KE Arch Dis Child 1995 Jan;72(1):33-37	Clinical and molecular studies to screen for fragile X syndrome in 154 children with moderate and severe learning difficulties of previously unknown origin.	Southern blot analysis of peripheral blood showed the characteristic abnormally large (CGG)n repeat sequence associated with fragile X syndrome in 4 of the 154 children. The findings were confirmed by cytogenetic observation of the fragile site and by further molecular studies. The families of the affected children were offered genetic counseling and DNA tests to determine their carrier status.	Fair
7.	*Direct DNA testing for fragile X syndrome.* Ramos FJ, Eunpu DL, Finucane B, Pfendner EG. Department of Pediatrics, Albert Einstein Medical Center, Philadelphia, PA. Am J Dis Child 1993 Nov;147(11):1231-1235	396 patients with no family history of fragile X syndrome or mental retardation for whom the risks of fragile X syndrome are unclear. and 35 normal controls are studied.	39 cytogenetically positive affected males and 6 females had full mutation, 16 normal obligate carrier females bore the premutation,. Of 124 patients with a family history of fragile X syndrome, 5 (8%) males and 25 (40%) females had the premutation. 5 (2.2%) of the 231 mentally impaired patients with no confirmed family history of fragile X syndrome were found to have the full mutation. 12 (33%) of 36 mentally impaired males and 1 (20%) of 5 females with unknown family history were found to carry an amplified CGG	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
			repeat.	
8.	*A statewide public and professional education program on fragile X syndrome* Keenan J, Kastner T, Nathanson R, Richardson N, Hinton J, Cress DA. Developmental Disabilities Center, Morristown Memorial Hospital, NJ 07960. Ment Retard 1992 Dec;30(6):355-361	The project was developed to increase the public and professional awareness of fragile X syndrome in the state of New Jersey.	Fragile X syndrome is the most common inherited cause of mental retardation, but the majority of affected individuals are undiagnosed. As a result of this project there were increased efforts at diagnostic screening, provision of client and family support services, and prevention. This educational program proved to be a cost-effective method for increasing community awareness of a genetic disease on a statewide level.	Fair
9.	*Fragile X syndrome: a public health concern.* Trusler S, Beatty-DeSana J. Am J Public Health 1985 Jul;75(7):771-772	Tested for fragile X syndrome in institutionalized male patients with nonspecific mental retardation, referral patients, and relatives of fragile (X) patients.	Thirty-one of 91 (34.0 per cent) subjects tested were positive for the fragile (X) chromosome; relatives had the largest percentage of positives. The data reflect the value of this test for detecting families having potential carriers who can benefit from genetic counseling.	Fair
MALIGNANT TUMOURS				
1.	*The value of PCR in the diagnosis of cutaneous T-cell infiltrates.;* Ashton-Key M. et al.	Observational	PCR demonstrated clonality of the T-cell infiltrates in 50% of borderline biopsies which later	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	American Journal of Surgery; 1997.		developed mycoises fungoides.	
2.	*Inverted papillomas of the paranasal sinuses and the nasal cavity : DNA indices and HPV infection.* Bernauer HS et al. ;American Journal of Rhinology; 1997.	Case series - 7	The identification of specific HPV sequences of types 16 and 18 by PCR in 7 cases was used to predict biological behaviour and the possibility of malignant transformation.	Fair
3.	*HPV analysis as a prognostic marker following colonization of the cervix uteri.* Chua KL et al. Gynecol. Oncol. 1997.	Case control.	HPV-DNA testing use to discriminate patients who developed recurrent CIN-3 from those who did not. HPV prevalence was shown in 96% of the recurrent group and none in the control group.	Fair
4.	*Identification of CEA-producing cells circulating in blood of patients with colo-rectal carcinoma by RT-PCR.*	53 samples.	RT-PCR recognition of the tumour marker CEA in patients with colorectal liver metastasis	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	Jonas s. et al. Gut. 1996.		showed positive expression in 26 out of 31 cases, compared with controls(5/22).	
5.	*Detection of liver cells in peripheral blood of patients with advanced-stage HCC.* Kar S et al. Hepatology. 1995.	5 samples	Using RT-PCR to measure mRNA of human albumin gene, a liver cell marker, small numbers of circulating tumour cells were detected in 16 out of 17 cases of advanced HCC but not in the 43 control samples of normal, cirrhotic or other tumour metastases to the liver.	Fair
6.	*Efficacy of RT-PCR screening for micrometastatic disease in axillary LNs breast cancer patients.* Lockett MA et al. Am Surg. 1998.	25 samples	In pathologically-negative axillary lymph nodes from 35 breast cancer patients, RT-PCR identified micrometastases in 14 cases.	Good to fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
7.	*Is the PCR or cure of Helicobacter pylori infection of help in the differential diagnosis of early gastric MALT lymphoma ?* Rudolph B. et al. J. Clin. Oncol. 1997.	20 patients	In 4 out of 20 patients with suspected low-grade gastric MALT lymphoma, PCR detected B-cell monoclonality before histology showed lymphoma.The other 16 cases were confirmed histologically to have gastritis only.	Good to fair
8.	*Present evidence on the value of HPV testing for cervical cancer : a model-based exploration of the cost-effectiveness.* van Ballagooijen et al. Br.J of Cancer.1997.	Not applicable.	In one model, calculated mortality reduction from HPV screening was higher and more cost-effective than Pap smear screening. Another model showed the opposite outcome.	Poor
9	*Attitudes to predictive DNA testing in familial adenomatous polyposis.* Whitelaw S et al. J Med Genet 1996.	n=62.	Attitudes to predictive prenatal DNA testing for familial adenomatous polyposis and termination of pregnancy were documented. 93% stated they would like their children tested by DNA analysis at birth or in infancy. 10 % who had refrained from having children for fear of passing on the polyposis gene felt that DNA testing would enable them to consider planning a family.	Good.

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
10	*A collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene.Implications for presymptomatic testing and screening.* Shattuck-Eidens D et Al. JAMA 1995.	n= 1086.	1086 women with either breast or ovarian cancer were tested for BRCA1 mutations. Although BRCA1 can be used for a simple screening test, more data needs to be accumulated to address the sensitivity and specificity.	Good to fair
11.	*BRCA1 mutations and breast cancer in the general population: analyses in women before age 45 years with first-degree family history*. Malone KE et al. JAMA 1998.	n=401	Young women with breast cancer were analysed for germline BRCA1 mutations – concluded that it may be difficult to develop screening criteria among women with modest family history profiles.	Fair
12.	*Genetic counselling and gene mutation analysis in FAP in Western Australia* Walpole IR et al. . Med J Aust 1995.	n=200.	Accurate genetic counselling for families with FAP was possible with DNA analysis, thus improving management in asymptomatic individuals.	Fair
13	*Detection of circulating tumour cells and nodal metastases by RT-PCR technique* Wong LS et al.	n= 38	A RT-PCR assay used to detect occult metastases in lymph nodes or circulating tumour cells was successful - could be useful in	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	Br J Surg 1997.		early diagnosis, assessment of prognosis, and detection of residual disease after treatment.	
14	*Detection of circulating tumour cells in colo-rectal cancer by immunobead PCR is a sensitive prognostic marker for relapse of disease*. Hardingham JE et al. Mol Med 1995.	n=27	Circulating tumour cells detected in 30 %, using immunobead PC. 7 patients died due to recurrent disease. Tumour cells not detected in the remaining 18 patients who remained disease free (median follow-up16 months).	Fair
15	*BRCA1,BRCA2, and HNPCC mutations in an unselected ovarian cancer population: relationship to family history and implications for genetic screening*. Rubin SC et al. Am J Obstet and Gynecol 1998.	n=116.	The majority (90%) of ovarian cancer patients tested negative for genetic mutations known to predispose to the disease. The relationship between mutation status and suggestive family history was unreliable for screening patients with ovarian cancer.	Fair.

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
16	*BRCA1 mutations in women attending clinics that evaluate the risk of breast cancer*. Fergus et.al. N Engl J Med. 1997	Case series 169 patients with familial breast cancer	27 BRCA1 mutations were identified.	Poor
17	*BRCA1 sequence variations in 160 individuals referred to a breast/ ovarian family cancer clinic.* Dominique et.al. Am. J. Hum. Genet, 1997	Case series 160 women with breast/ ovarian cancer family history	38 mutations were identified in 38 of the patients.	Poor
18	*A high proportion of Novel mutations in BRCA1 with strong founder effects among Dutch and Belgian hereditary breast and ovarian cancer families*. Peelen et.al. Am. J. Hum. Genet. 1997	Cross sectional 643 Dutch and 23 Belgian breast and ovarian cancer families	79 mutations detected in 79 families. One recurrent mutation in 19 families.	Fair
19	*BRCA1 and BRCA2 mutation analysis in 86 early onset breast/ ovarian cancer patients.*	Case series 86 patients < 40 years	6 BRCA1 mutations and 3 BRCA2 mutations in 9 patients.	Poor

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	Alex et.al. J Med. Genet. 1997	of age		
20	*Multiplex mutation screening of the BRCA1 gene in 1000 Japanese breast cancers* Emi M et. al. .Jpn J Cancer Res 1998	Case series 1000 patients .	Screening was done in exon 11 only. 8 mutations were identified , 5 of them with patients with bilateral breast cancer.	Fair
21	*BRCA1 mutations in women attending clinics that evaluate the risk of breast cancer.* Couch FJ et al. N Eng J Med 1997.	Level 4 n= 263	In a referral clinic specializing in screening women from high-risk families, less than 45 % of tests for BRCA1 mutations will be negative and therefore uninformative.	Good.

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
22	*In a resource-poor country, mutation identification has the potential to reduce the cost of family management for HNPCC.* Goldberg PA et al; Dis Colon Rectum 1998.	n= 150	The impact of mutation analysis on the management of a family from a resource-poor country (poor colonoscope facilities, public transport, and sophisticated medical facilities) with more than 150 members at risk for HNPCC , was proved to simplify management, surveillance, counseling and prophylactic colectomy.	Fair
23	*Molecular approaches for colorectal* Jen J et al. Eur J Gastroenterol Hepatol 1998.		The development of a PCR assay for detection of K-ras mutation from stool of patients with colorectal cancer has the potential to be a sensitive, specific and cost-effective screening tool.	
24	*The impact of molecular diagnosis on familial colorectal cancer.* Bennett G et al. Clin Chem Lab Med 1998.	n=23	In a family with a strong history of colorectal cancer, predictive testing for DNA mutation clarified the genetic status of 11 individuals , enabling them to make informed choices regarding disease management, the future, removing the anxiety associated with the unknown.	Fair

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
25	*A novel method for the direct quantification of gene transfer into cells using PCR in- situ.* Catzavelos C. et al. Gene Ther 1998.	Level 5.	Gene-modified haemotopoietic cells were successfully identified in a trial of retrovirus mediated gene transfer into blood forming stem cells.	Fair.
26	*Clinical value of gene diagnosis in breast* cancer. Iwase R. Rinsho Byori 1998.	Not applicable	Telomerase activity is a sensitive and definitive analysis for diagnosing certain cancers and the reverse-transcriptase PCR method can detect a small number of metastatic cancer cells in the axillary lymph nodes.	Not applicable.
PATERNITY TESTING				
1	Kratzer A et al. Forensic hematology genetics—paternity testing. Ther Umsch. 1997.		Using PCR-based DNA systems, exclusion or probability for paternity exceeds 99.8%, the required value for proof of paternity.	Poor;

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
2	Mingjun L et al. Application of DNA profiling to paternity testing during early pregnancy. Hum Hered.1993.	n= 30.	DNA profiles of 8-to 10-week-old chorionic samples and their respective parents were analysed. The results showed strict Mendelian inheritance as well as exceedance of the required standards for cumulative paternity index and probability of paternity. DNA testing can resolve cases of paternity during very early pregnancy.	Poor.
3	Huckenbeck W et al. Serological findings and efficiency of DNA profiling in transfused patients and their significance for identity and paternity tests. Int J Legal Med. 1994.	n=13 .	DNA systems were investigated in 13 multipli-transfused individuals; it was demonstrated that DNA patterns were not affected by the transfusions and the possibility of paternity reached 99.9%. Testing of the classical blood group systems showed transfusion changes.	Good.

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
4.	Pakkala S et al. Paternity after bone-marrow transplantation following cinditioning with total body irradiation(TBI). Bone Marrow Transplant,1994.	n=1.	A 28-year-old man with CML was treated with bone marrow transplant,chemotherapy and TBI. 4 years later,his wife gave birth to ahealthy child. The patient was proved azospermic. DNA fingerprinting gave unequivoval results showing that the patient was the father. This is the first case where DNA methogoly was used to prove paternity after TBI.	Poor.
FORENSIC IDENTIFICATION				
1	*Validation of multiplex polymorphic STR amplification sets developed for personal identification.* Micka KA et al. J Forensic Science.1996		Two STR triplex systems and their corresponding mono- plexes evaluated for sensitivity of detection, and amplification properties. Both systems were successful with as little as 0.25 ng of DNA template; success rate 95.6% to 100%.	Fair
2	*Human identification by genotyping of personal articles.* Sasaki M.		Using STR , identification of 3 corpses in varying stages of decomposition was successful	Poor

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	Forensic Science Int. 1997.		using personal articles from the respective individuals.	
3	*Personal identification using DNA polymorphism - the identification of forensic biologic materials.* Shino H. Nippon Hoigaku Zasshi. 1996.		The ABO blood group genotypes were determined in all 29 blood and 14 fingerprint samples using PCR-RLFP , and successfully identified suspects in sexual assaults .	Fair
4	*STR typing of bodies from a mass disaster.* Whitaker JP et al. Biotechnique 1995 Apr;18(4):670-7	Level 4.	In a mass disaster, using a PCR-based DNA-typing method 66% of severely degraded tissue samples exposed to extreme thermal, physical, and chemical insult were successfully typed.	Good to Fair.
5	*Validation of mitochondrial DNA sequencing for forensic casework analysis.* Wilson MR.et al. Int. J of Legal Med. 1995		2 sets of studies were performed to evaluate the utility of sequencing mDNA and amplified by PCR , on a variety of human samples. This system is a valid and reliable means of forensic identification.	-

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
6.	*Allele frequency distributions of 13 PCR-based systems in a population from North-East Spain.* Crespillo M et al. Int. J of Legal Med. 1997.	n= 129 to 292	Using a combination of 13 PCR-based polymorphic systems the genotype distributions were found to be in accordance with the Hardy-Weinberg expectations. PCR provides a high power of discrimination and power of exclusion for use in forensic casework and paternity testing	Good to Fair.
7.	*Ethical and legal issues raised by DNA fingerprinting.* Mangin P. Medical Law 1996.		In France, DNA fingerprinting aroused major concerns on use of data by the police for identifying and investigating potential criminal suspects, and the widespread use without safeguards for private investigation of paternity and for insurance purposes. In 1994,the French Parliament decreed that DNA investigation should be strictly	-

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
			limited to judicial use and be performed by accredited laboratories.	
8.	*Application of forensic identity testing in a clinical setting. Specimen identification*. Tsongalis GJ et al. Diagn Mol Pathol 1997.	n=1	In a case of questionable mix-up of surgical specimens, DNA typing was utilised to match specimen and patient.	.Poor.
9	*The applicability of formalin-fixed paraffin embedded tissues in forensic DNA analysis.* Romero RL et al. J of Forensic Sci 1997.		Formalin-fixed paraffin embedded tissues is a useful source of DNA using 12 PCR systems.	Poor .
10	*HLA-DQ alpha genotype and allele frequencies in Malays, Chinese, and Indians in the Malaysian population* Koh CL et al Hum Hered 1994.	n=392.	Using a commercial DNA amplification and typing kit, allele frequencies in the 3 ethnic groups were found to be distinct, such that it can be used as a marker in forensic identity testing in Malaysia.	Good.

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
11	*DNA typing of azoospermic semen at the DIS80 locus.* Skinker DM et al. J Forensic Sci 1997.	n=6	PCR analysis is used in the evaluation of variably degraded ,small quantities of DNA in mixed samples of postcoital vaginal samples and azoospermic semen donors.	Poor.
12.	*Rapid and efficient resolution of parentage by amplification of short tandem repeats.* Alford RL et al. Am J Hum Genet 1994.	n=50	Parentage testing was conducted using 9 STR loci in 50 paternity cases. In the 37 positive cases ,the probability of paternity calculated was >99%, validating the use of DNA typing with STR loci for parentage testing.	Good to Fair.

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
13	*AmpliType PM and HLA DQ alpha typing* from pap smears, semen smears, and postcoital slides. Roy R et al. J Forensic Sci 1995.		DNA samples extracted from the smears and slides, and amplified by PCR were typed for the AmpliType PM and HLA DQ alpha allelles. These provided a valuable source of material to the determine the genetic profile of the sample donor, for sexual assault or missing person case.	Poor.
14	*Validation studies for the genetic typing of the D1S80 locus for implementation into forensic casework.* Gross A et al. J Forensic Sci 1007	n=> 600.	A series of validation experiments designed to evaluate the utility of the D1S80 typing system, in various simulated casework type mixtures - proven to be suitable.	Good to Fair.
15	*D1S80 VNTR locus genotypes in population of south Poland: meta-analysis pointer to genetic disequilibrium of human populations.* Turowska B et al. Forensic Sci Int 1995.	n= 133.	A database of local population was established using the locus D1S80, amplified by PCR. A meta-analysis of the genotype frequencies showed that interpretation could be biased by inter population differences.	Good.
16	*Consistency and reproducibility of AmpliType PM results between seven laboratories: field*		7 forensic laboratories used commercial kits and showed 98.2% and 95. Positive results	Fair.

No.	Title, Author, Journal, Year	Type of Study, Sample size	Characteristics & Outcome	Comments, Grade of evidence
	trial results. Fildes N et al. J Forensic Sci 1995.		respectively for the PM and DQA1 loci. This system can be easily used for forensic casework analysis.	
17.	*TWGDAM validation of the AmpFISTR blue PCR amplification kit for forensic casework analysis*. Wallin JM et al J Forensic Sci 1998.		Studies were performed to validate the kit for forensic casework analysis and was shown to reproducibly yield specific and sensitive results	.

Appendix

LEVELS OF EVIDENCE SCALE

Level	Strength of Evidence	Study Design
1	Good	Meta-analysis of RCT, Systematic reviews.
2	Good	Large sample of RCT
3	Good to fair	Small sample of RCT
4		Non-randomised controlled prospective trial
5	Fair	Non-randomised controlled prospective trial with historical control
6	Fair	Cohort studies
7	Poor	Case-control studies
8	Poor	Non-controlled clinical series, descriptive studies multi-centre
9	Poor	Expert committees, consensus, case reports, anecdotes

SOURCE: ADAPTED FROM CATALONIAN AGENCY FOR HEALTH TECHNOLOGY ASSESSMENT (CAHTA), SPAIN

THE FOLLOWING HTA REPORTS ARE AVAILABLE ON REQUEST:

REPORT	**YEAR**
1. LOW TEMPERATURE STERILISATION	1998
2. DRY CHEMISTRY	1998
3. DRY LASER IMAGE PROCESSING	1998
4. ROUTINE SKULL RADIOGRAPHS IN HEAD INJURY PATIENTS	2002
5. STROKE REHABILITATION	2002
6. MEDICAL MANAGEMENT OF SYMPTOMATIC BENIGN PROSTATIC HYPERPLASIA	2002
7. CHILDHOOD IMMUNISATION	2002
8. ROUTINE NEONATAL VITAMIN K ADMINISTRATION AT BIRTH	2002
9. USE OF POLYMERASE CHAIN REACTION IN LABORATORY TESTING	2002