Use Of Molecular Technology In Laboratory Diagnosis

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Genetic diagnosis and genetic characterization of polymorphism have been used by laboratory clinicians in the diagnosis of disorders such as:
Infectious diseases;
Cancers;
Inherited disorder e.g. Deamutase deficiency;
Haemostatic disorders, Haemoglobin disorders;
Immune System disorders.

The technique is also used to determine certain forms of therapy such as bone marrow transplantation and to detect engraftment of transplanted tissue. This is essential so that reemergence of residual tissue or a new disease can be detected hence treatment intervention can be instituted.
A number of molecular techniques have found widespread application in the biomedical sciences. Sequencing is a method to determine the sequence of a stretch of DNA based on its differential cleavage pattern in the presence of different chemical exposures. A nucleic acid chain can be cleaved following G, A, C or C and T by exposure of ^{32}P-labeled DNA to neutral dimethylsulphate acid, hydrazine NaCl piperidine or hydrazine piperidine. There is also the term "Sanger Sequencing This method relies on the random incorporation of dideoxynucleotides into a growing enzyme catalysed DNA chain.
A family of DNA fragments is generated and this can be visualized on a Polyacrylamide gel. It is the most commonly used method to determine the sequence of DNA.

PCR

Polymerase Chain Reaction.
This technique finds use in several laboratory techniques in recombinant DNA technology. It is based on the ability of primers to hybridize to a cloned DNA of interest.
Following extension from the primers on the cDNA template by DNA polymerase, the reaction is heat denatured and allowed to anneal with the primers again. Another round of extension leads to a multiplicative increase in DNA products.
By this method, a minute amount of cDNA can be amplified in an exponential fashion to give enough DNA to use in laboratory diagnosis.
Controls have to be in place. Clinical examples of the use of PCR include
Detection of diagnostic chnomosomal rearrangement.
Haemoglobinopathies e.g Sickle cell disease
Haemostatic diseases
Factor V Leiden
Haemophilia.

Southern Blotting

This technique is used to detect specific sequence in an admixture of DNA. DNA is fractionated in to sizes and transferred unto nitro cellulose acetate or a suitable membrane.
The nitrocellulose replica of the original gel electrophoresis is allowed to hybridize with a cloned DNA.
Any specific DNA present on the blot will combine with the labeled probe and is detected auto radiographically.
Co-electrophoresing DNA fragments of known molecular weight and size can assist in detecting the DNA in question.

Northern Blotting

This is a modification of a Southern blot. This is used in detecting specific RNA.

Western Blotting

This techniques detects specific proteins present in a heterogeneous sample. Proteins are denatured, size fractionated by polyacrylamide gel electrophoresis, and then probed with an antibody to the protein of interest.
Immune complexes present on the blot are then detected using a labeled second antibody.
The size of the target protein can be determined
Techniques used in laboratory diagnosis

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Polymerase chain reaction (PCR)
This technique has permitted the production of large amounts of DNA identical to the DNA of interest. It can detect polymorphysm or DNA, of residual disease. It can also be used to produce DNA for DNA sequencing. This is achieved by

1. Changing the temperature,
2. Using a thermal-stable DNA polymerase
3. Dinucleotides
   Each cycle doubles the amount of segments of DNA

Restriction fragment length polymorphism This alteration can alter the recognition sequence of a specific restriction enzyme. In the presence or absence of the cut site,and this is used as an indicator of the altered DNA sequence. This helps to make diagnosis using other full length DNA amplified by PCR..

WHAT THE NEW MOLECULAR BIOLOGY INVOLVES

DNA Cloning: The desired fragment is selectively amplified and thereafter the structure function can be comprehensively studied. This techniques can be either: Cell-based cloning or cell-free cloning.

Molecular Hybridization: The desired fragment is specifically detected by using labeled DNA, RNA or oligonucleotide target probes against native DNA complex.

Dot-blot or slot blot techniques: Which are convenient for identifying nucleic acid sequence in unfractionated nucleic acid sample.

Allele-specific oligonucleotide hybridization (ASO) which can discriminate between alleles differing at a single nucleotide position. ASO probes are typically 15-20 nucleotides long and are normally employed under hybridization condition at which DNA duplex between probes and target are stable and are of perfect base complementarity.

Southern and Northern blot hybridization: This can detect DNA or RNA fragments that have been fractionated by gel electrophoresis. Target DNA is digested by restriction endonuclease, size fractionated by agarose gel electrophoresis, denatured and transferred to nitrocellulose or nylon membrane for hybridization.

In situ hybridization: usually involves the hybridizing of a nucleic acid probe to the denatured DNA of a chromosome preparation or the DNA or RNA of a tissue section on glass slide.

MOLECULAR PATHOLOGY

This has propelled a shift from phenotypic to genotypic diagnosis
The success of the molecular biology is dependent on proper collection and storage of both the original and / or processed (usually nucleic acid) specimens.
Examples of common source of specimen are:
Blood a widely used source of DNA
Mouth wash and buccal scrapes
Chronic villi biopsy sample
Hair, semen etc. for criminal investigation
Archival pathologic specimen

Clinical symptoms are often the end result of a long chain of causation and effects, and all too often they are not predictable or even readily comprehensible with our present state of knowledge.

Molecular pathology involves the study of tissue changes, both structural and functional, at the molecular level in various disease conditions. This combines biochemistry with genetics and translates basic science to clinical medicine.

MOLECULAR PATHOLOGY ATTEMPTS TO EXPLAIN

Why a genetic disorder results in a particular phenotype
Effect of mutation on the quality, quantity and function of the gene products
Why the changes are or not peculiar to any particular cell or stage of development.

The impact of molecular biology is more marked in the improvement being made in the actual process of diagnosis.

Though conventional morphological diagnosis is still the hallmark, new powerful marker and techniques such as PCR, ISH for DNA and RNA and flow cytometry have provided opportunities for earlier and more accurate diagnosis on smaller fragment of materials.

CHROMOSOMAL STUDIES AND GENETIC TESTING

This involves identification of clinically relevant chromosomal abnormalities with greater precision in chromosomal preparations. These molecular techniques include:

Fluorescene in-situ hybridization (FISH) uses fluorescently labeled probes for visualization of DNA sequences on metaphase spread or interphone nuclei . Both numerical and structural aberrations can be determined. Can also be performed on tissue sections.

Whole chromosme specific painting (WCP)
Comparative genomic hybridization (CGH) This technique is useful in the study of solid tumours. The entire genome can be studied for gains and losses of genetic material in a single experiment and is essentially a modified ISH. Differentially labeled test (green) and reference (red) DNA are co-hybridized to normal metaphase spread. Gains of genetic material in the test DNA are seen as an increase in the green: red fluorescence ratio and losses are seen as a decrease in the ratio. The fluorescence ratio are quantified on digital image analysis.

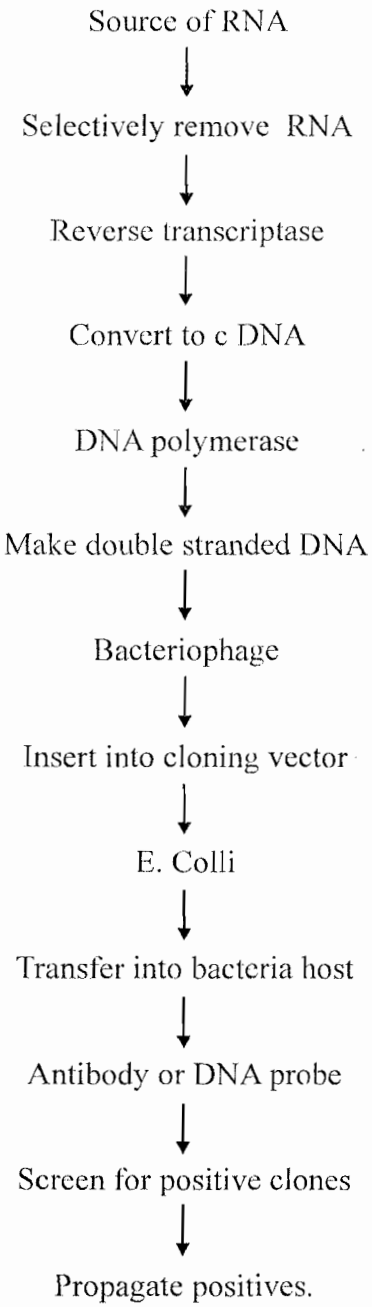
Spectral karyotyping (Sky) uses a combination of fluorochomes which label all chromosomes in different colours enabling visualization of every chromosome in a single experiment. Cryptic rearrangements and marker chromosomes are easily detected.
All these DNA can be visualized in chromosomal preparations.

WCP has helped in identifying chromosomal rearrangement not easily detectable in cases with less that optimum quality of metaphase or those in which the origin of the material present in a maker chromosome cannot be determined. Analysis of previously unknown genetic alteration prsnet in some cancer and in cells with low mitotic index has been possible through the use of CGH.

DNA profiling: used to establish relationship or identity
DNA finger printing determine reliability of the zygosity of twin, disproved or establish paternity. Powerful tool for forensic investigation.

CONCLUSION
In order to determine if the benefits of molecular biology its inherent and its cost, the new procedure must be integrated into the total assessment of disease to fully observe the effects on patient care.
Once any gene/disease gene has been characterized, molecular biology can be used to dissect gene function, can be used to design novel intervention therapy and manipulations or molecular genetic based therapeutic approaches molecular

General Scheme for cloning



biologic/gene technologies have devised a variety of novel therapeutic approaches based on the ability to clone individual types of genes.
Transfer the genes into recipient cells and express than.
The ability to redesign protein.
The ability to inhibit or enhance the expression of a specific predetermined gene.
Thus genetic engineering approaches have resulted into the cloning of normal gene products, genetically engineered antibodies and genetically engineered vaccines

REFERENCE
1. Hematology 2002 American Society of Hematology, Education program book. Pages 490-508