Application of Molecular Epidemiology Methods in viral detection

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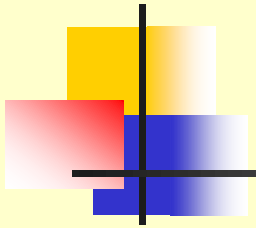


- General Epidemiology - Identifying the viral subtypes at different times and geographical locations, one can detect major changes in the epidemiological patterns of infection e.g. HIV and HCV.
- Molecular epidemiology- use the genetic material as an indicator for pathogen detection.
- Subtyping - in some viruses, different subtypes are associated with different clinical manifestations e.g. enteroviruses, adenoviruses, and human papillomaviruses.
- Investigation of Outbreaks - to support or disprove a link between the symptoms and pathogen.

Methods Use - Complete or Partial genome?

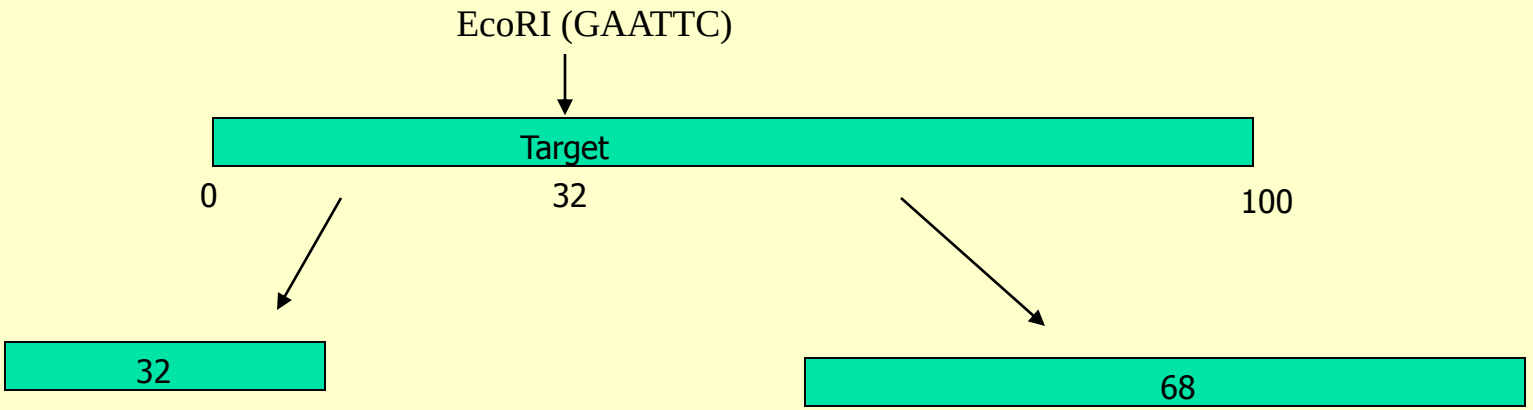
- For greatest degree of accuracy, the complete genome should be used for the purpose of detection.
- viral genomes ranges from 3500 bp to over 200,000 bp, it would be highly impractical to sequence the whole genome.
- Certain simple methods are still used for the detection of complete genomes e.g. restriction fragment lenth polymorphism(RFLP) for CMV, HSV, and Adenoviruses.
- Nowadays in practice, a small part of the genome is amplified first by PCR and the product investigated by sequencing or other methods.

Strategies for identification of the PCR Product (Commonly used methods)

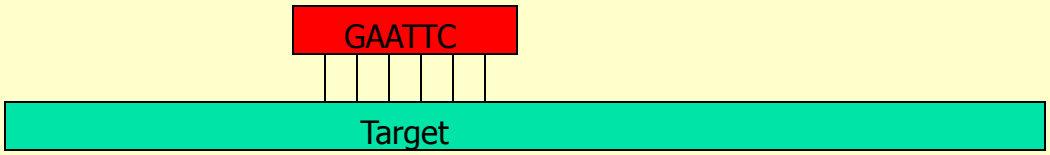
- Sequencing of the PCR product
  - the gold strategy but expensive and not widely available.
  - PCR product may be sequenced directly or cloned before sequencing.
  - Sequencing can be used to confirm results of other molecular epidemiological assays. As a matter of fact, all other assays can be considered as simpler screening assays.
- Restriction Fragment Length Polymorphism (RFLP) - very simple, rapid and economical technique but the result may be difficult to read.
- Hybridization with a specific oligonucleotide probe - A wide variety of formats is available e.g. dot-blot, Southern blot, reverse hybridization, DNA enzyme immunoassay etc.

Principles behind Restriction Enzyme Analysis and Hybridization Probes

RFLP



Hybridization Probes



PCR-RFLP (PRA)

- The target gene must be present in all viral strains.
- It is amplified with primers directed against conserved areas in the target gene so that all subtypes can be amplified.
- The PCR product is then digested with one or more restriction enzymes and on an agarose or polyacrylamide gel.
- The species or genotype is identified from the restriction patterns seen.
- Therefore PRA can be considered as probably the simplest DNA fingerprinting technique.
- The principle of PRA is similar to that of RFLP of whole viral genomes and pulse field gel electrophoresis.
- It is quick, simple and cheap and this is why it is preferred by many molecular biologists.
- Examples include HCV genotyping and identification of mycobacteria.

Specific Oligonucleotide Probe

- Simple to carry out, particularly suitable for large scale testing
- Results are usually easier to read than REA and requires less skill to interpret
- Preferred strategy by commercial companies e.g. INNO-LIPA HCV, Sorin DEIA, Roche Amplicor and Taqman.
- Can be made into a highly automated closed system e.g. Roche-Amplicor.
- Therefore more attractive than PRA for the routine laboratory but the costs could be prohibitive.
- Specific nucleic acid probe assays are available where the specimen is tested directly without amplification. However the sensitivity is much lower.

Summary

- A wide variety of molecular epidemiological methods are available, of which DNA sequencing is the gold standard.
- It is now usual to analyze a small part of the genome rather than the complete genome. The target fragment is first amplfied by PCR before analysis.
- The most widely used screening methods involve either restriction enzyme analysis or hybridization with specific nucleic acid probes, or a combination of the two.
- Other screening methods such as SSCP and other heteroduplex analysis techniques are rarely used outside a research setting because they often suffer from poor laboratory instrument .
- The choice of the genomic region to use is critical: it is often advisable to use more than one genomic region.
- It is important to remember that all molecular epidemiological methods available for viruses can be applied to bacteria but not vice-versa.

Points to Consider

- Molecular epidemiology techniques are can be used with good effect to disprove or improve a link between disease and pathogen .
- The probability of a link depends on many factors including the prevalence of that particular genotype and the methods used.
- Where the outbreak carries huge medical-legal implications e.g. HIV transmitted through blood so it need for high level of safety program for worker.
- It is important to remember that molecular epidemiological investigation does not replace a good basic epidemiological investigation but as an additional method for investigation.