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Nucleic Acid Probes in Microbiology

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ABSTRACT

Single-stranded nucleic acid molecules can interact under certain conditions with other nucleic acids in the regions of base complementarity to form double-stranded hybrid molecules. From measurement of kinetics of the hybridisation and the extent of hybrid formation, information on nucleotide sequence heterogeneity of nucleic acid fractions, sequence properties of purified classes of molecules or the degree of homology between different samples of nucleic acids can be obtained. A specific deoxyribonucleic acid or ribonucleic acid sequence with an identifiable marker can be used as a nucleic acid probe. This paper reviews the use of nucleic acid probes for classifying the microorganisms and their detection in clinical or environmental samples.

1. INTRODUCTION

The nucleic acid probes are used widely in research and now with the rapid development of new techniques they are becoming more and more popular for diagnosis in medicine, forensic science, plant breeding and other fields[1-3]. Deoxyribonucleic acid (DNA) is a polymer of nucleotides. A deoxyribose sugar, a phosphate group and a nitrogenous base in turn are components of each nucleotide where sugar phosphate linkages form the backbone of DNA. The four nitrogenous bases are adenine (A), guanine (G) (i.e., purines), cytosine (C), and thymine (T) (i.e., pyrimidines). Adenine always forms hydrogen bonds with thymine (A=T) and

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guanine with cytosine (G≡C) making a stable double helix. Each strand in the helix can act as a template for a complementary strand. Ability of complementary strands of a DNA molecule to recognise each other and anneal under optimum conditions is the basic principle underlying the usage of DNA probes. Use of DNA probes for detection of specific sequences was successfully demonstrated by Southern[4] in 1975.

A probe is a pure or partially pure nucleic acid sequence which can be labelled and used to search out and detect complementary sequences in the presence of a large amount of non-complementary DNA. The probe may be a partially pure messenger ribonucleic acid (mRNA), a chemically synthesised oligonucleotide or a related gene which could be labelled using radioactive[5,6] or non-radioactive substance[7,8]. The denatured probe hybridises to the complementary sequences and detection of such hybrids can be done either by autoradiography[9,10], liquid scintillation counting, colourimetry[8,11], fluorometry[12] or using chemiluminescent assays[13]. The direct linking of enzyme labels to probe DNA has also been reported[14]. In the present review, the use of nucleic acid probes in microbiology is reviewed which includes the techniques involved, the advantages and disadvantages of these techniques.

2. BASIC PRINCIPLES

DNA probes constitute a very useful tool for finding genetic relatedness amongst several members of a genus or different genera[15,16], for identifying species of a genus[17], for detecting pathogenic microorganisms in biological samples[18-22], genetically-engineered bacteria in environment[23] and non-culturable mycoplasma[24]. First a suitable DNA fraction to be used as probe has to be identified. This fragment is then cloned in a suitable vector, which can enable simple and rapid enrichment and harvest of the cloned fragment. This fragment is obtained by digestion or cutting of the cloning vehicle by restriction endonucleases. A particular oligonucleotide sequence can also be chemically synthesised[25] to be used as a probe. Various chemicals are used either as direct or indirect labels to tag such sequences. A list of such labels is given in Table 1. More and more efforts are being focused on finding alternatives to radioisotopic labels. An ideal label for a probe should be (i) easily attachable to DNA, (ii) detectable at very low concentrations, (iii) should produce a readable signal when the labelled probe hybridised with its target, and (iv) stable at elevated temperatures.

Two main types of labelling methods have been evolved, (i) direct labelling in which a label is attached directly to the nucleic acid by covalent linkage, and (ii) indirect labelling in which either a hapten or a ligand is attached to the DNA and detected by using a labelled protein that can specifically bind to the hapten or ligand. The labelled nucleic acid fragment obtained is used as a probe which could hybridise to the complementary target. Several hybridisation methodologies have evolved with time, wherein different solid supports are used for immobilising the target or the probe. Apart from hybridisation on solid supports, solution hybridisation which is a faster method, is also in vogue.

Table 1. Labels used in DNA probe detection

Direct labels	Indirect labels	Secondary labelling systems
Phosphorus-32	Biotin	AP-avidin/streptavidin[56]
Sulphur-35		
Tritium		β-galactosidase-avidin/streptavidin[29]
Iodine-125		Fluorescein-avidin[33]
AP[14]		HRP-avidin/streptavidin
HRP[14]		Ferritin-avidin
Bacterial luciferase		Colloidal gold-streptavidin
Firefly luciferase		Anti-biotin antibody-AP[7]
		Protein A-colloidal gold
	Digoxigenin	Anti-digoxigenin-Ab-AP
	Sulphone	Mouse monoclonal antibody to sulphonated DNA, antimouse *IgG*-AP
	2-AAF	AAF-Ab-AP[65]

AAF : Acetylaminofluorene; AP : Alkaline phosphatase; Ab Antibody
HRP : Horse radish peroxidase.

2.1 Southern Blotting

To meet the requirement of mapping sequences or knowing the transcribed sequences in a DNA restriction fragment, Southern[4] described an elegant method. The restriction fragments of DNA in the gel are denatured by alkali treatment and the gel is kept on top of buffer saturated filter paper. The upper surface of the gel is covered with a nitrocellulose paper and overlaid with dry filter paper towels. Buffer passes through the gel by capillary forces of dry filter paper carrying the single stranded DNA with it which binds,with nitrocellulose. For permanently fixing DNA on to the nitrocellulose paper, the nitrocellulose sheet is baked at 80 °C under vacuum. This paper is then placed in a solution containing denatured, radioactive labelled DNA probe for allowing hybridisation to occur. Non-specific binding of DNA is removed by stringent washes after hybridisation. The regions of hybridisation are detected using autoradiography by placing the nitrocellulose paper in close contact with photographic film. This is an extremely sensitive technique and can be used to map restriction sites around a single copy gene sequence in a complex genome.

Transfer of DNA from the agarose gel to nitrocellulose paper can be carried out as per the technique described by Southern where the DNA blotting is achieved by simple diffusion forces. But blotting of nucleic acid fragments from acrylamide or acrylamide-agarose composite gels is very inefficient by this method. Hence they can

be substituted by higher percentage of agarose gel for efficient transfer by Southern blotting. Alternatively, electrophoretic transfer is reported to give a high yield from acrylamide gels. This technique when extended to RNA blotting is called Northern blotting[26]. This extremely useful technique described by Southern is used with some modifications in many ways in diagnosis, taxonomy, genetic engineering, forensic science, etc.

2.2 Hybridisation on Immobilised Matrix

Different solid supports are used for immobilising the target, nitrocellulose filters being the most popular. Whatman 541 paper[27], nylon membranes[28] and diazobenzyloxy methyl paper[26] have also been used. Nucleic acid samples have been fixed on wells of a polystyrene microtitre plate by illumination with UV light in presence of Mg^{++} ions[12,29]. Alternative solid supports are indicated in Table 2. The probe can also be immobilised on a solid support to make an activated matrix that is used to gather the target sequence. Then the target is detected using a labelled second probe. This is known as sandwich hybridisation which is well suited to quantify specific nucleic acid sequences as in the case for diagnosis of *Salmonella*[30]. A capture probe immobilised on *CNBr*-activated Sepharose or a polypropylene surface has also been used.

Table 2. Some solid supports used in hybridisation[35]

S.No.	Solid support	Binding method
	Nitrocellulose	Baking at 80 °C under vacuum dipping membrane into dry butan-2-ol[4]
2.	Whatman 541 paper	Heat[27]
3.	Nylon membranes	Baking at 80 °C under vacuum and heat[28]
4.	Microtitre plate (polystyrene)	UV and Mg^{++} ions[12]
5	Modified polystyrene	Mercurated DNA attached via introduced sulfhydryl group
6.	Polypropylene	Non-covalent[29]
7.	Acrylic	Via reactive epoxide groups
8.	Oligo (dT)-cellulose	Hydrogen bonding of poly A DNA to oligo (dT) using Klenow fragment of DNA polymerase
9.	Sephacryl S-500	Diazotisation
	Cellulose/magnetic beads	Diazotisation
	Aminobenzyloxymethyl paper	Diazotisation[26]

2.3 Solution Hybridisation

Solution hybridisation allows target and probe to hybridise in solution and the hybrids are then separated from non-hybridised DNA by binding to hydroxyapatite. It has already been used to differentiate between *Legionella* species and non-*Legionella*

species[31]. Two non-interacting probes that may hybridise to the same target DNA may also be used. One probe is labelled with quantifiable label and the other with a ligand (for example, biotin). If complementary sequence occurs in the target, then the target will bring the two probes together and when the ligand is bound to a solid support, the quantifiable label will also be bound. In the case of biotin as a ligand, streptavidin-coated solid support can be used to bind the hybrids after separating the hybrids on hydroxyapatite columns.

After hybridisation the next step is detection of the hybrids. Depending on different labels described in Table 1, different detection methods are employed. Leary, *et al*[8] described a colourimetric method for visualising biotin-labelled DNA probes hybridised to DNA or RNA immobilised on nitrocellulose. Alkaline phosphatase was polymerised and then biotinylated. This biotinylated alkaline phosphatase polymer (BAP) was complexed with avidin. Since avidin or streptavidin have a very high affinity for biotin[32] ($Kd \approx 10^{-15}$ M), it is extensively used in conjugation with different enzymes for detection of biotinylated probes. Avidin linked with fluorescein can also be used for fluorometric assay[33]. When quantitative results are required, i.e., number of specific DNA molecules in a sample is to be estimated, then time resolved fluorometry provides a simple detection system[34], which involves a hapten-labelled probe which was detected in a two-step immunological reaction. Second antibody was labelled with Europium (Eu^{3+}). When radioisotopes are used as labels, detection is done using autoradiography or liquid scintillation counting.

3. AREAS OF APPLICATION

Early detection of microorganisms causing infectious diseases is very essential to control the spread of such diseases to larger areas. The plant, animal and human pathogens can be correctly identified as well as classified by the use of nucleic acid probes. Outbreaks associated with various modes of transmission of infectious agents can be monitored using DNA probes. Nucleic acid hybridisation is one of the important techniques to study the dispersal pattern of such transmission and their biological effects. Two main areas where nucleic acid probes are used namely, taxonomy (basic research) and rapid detection of microorganisms (applied research), are discussed in this review.

4. TAXONOMY

In recent years, the techniques of molecular genetics have introduced new criteria for determining the degree of evolutionary relationship among different bacteria which is the basic principle of classification. Molar percentage of guanine (G) plus cytosine (C) in the total DNA, the G + C content, of two closely related organisms is nearly similar although it is not a proof of genetic relatedness. Within a well-defined, closely related group such as aerobic spore-forming bacilli, much higher degree of genetic relatedness can be recognised by the relative abilities of heat denatured DNAs from different strains to anneal with each other during slow cooling, which reflects that

two sequences coming together are homologous. The third most reliable technique, which has recently been extensively used, is based on base sequence homologies in ribosomal RNA (rRNA). The 16S rRNA is digested to short oligonucleotides, sequenced and used as a probe to define phylogenetic relatedness since rRNA sequences have been highly conserved during evolution.

DNA relatedness between serogroups and serovars in the family *Leptospiraceae*[36] was established by DNA hybridisation methods and seven new *Leptospira* species were proposed based on these studies. Biochemical characteristics supplemented with hybridisation data make classification easier. While studying lactose positive *Bacillus subtilis* strains and *Bacillus amyloliquefaciens*[37], it was found that out of 130 strains classified as *B. subtilis*, 60 strains fermented lactose and utilised gluconate slowly. High DNA relatedness values of 70 to 100 per cent to the type strain (NRRL B-14393) of *B. amyloliquefaciens* indicated these organisms to be strains of that species. The 70 remaining strains did not ferment lactose, utilised gluconate strongly and were highly related genetically to the type strain (NRRL NRS-744) of *B. subtilis*. DNA relatedness values of 25-37 per cent established that neither group was related to *B. pumilis, B. coagulans, B. firmus* or *B. licheniformis*. Thus the results indicate that lactose fermentation and gluconate utilisation are characteristics that can differentiate between *B. subtilis* and *B. amyloliquefaciens*.

Based on genetic data some of the organisms are also reclassified. *Xanthomonas ampelina* panagopolous 1969 has been transferred to a new genus *Xylophilus* gen.nov., as *Xylophilus ampelina* panagopolous 1969 comb. nov[38]. They determined generic and suprageneric relatedness by hybridisation between 23S ^{14}C-labelled RNA from *Xanthomonas ampelina* (NCPP 2717^{r}) and DNA from eight *Xanthomonas ampelina* strains. *Xanthomonas campestris* (NCPPB 528^{T}) and the type strains of 16 possibly related strains. Hybridisation of *Lactobacillus* isolates[39] from different sources with DNA probe specific for *L. curvatis* suggested that these isolates classified by conventional techniques might have been incorrectly classified. A DNA probe has been described for taxonomic and diagnostic identification of the North American cattle pathogen *Leptospira interrogans* genotype *hardjo bovis*[16]. Nine strains of four species of *Campylobacter, (C. jejuni, C. fetus, C. coli, C. lardis)* were studied by genomic Southern hybridisation of restriction digests of chromosomal DNA with an oligonucleotide probe for 16S rRNA[17].

The DNA probes are more specific for identification of strains which cannot be biochemically identified. Chromosomal DNA probes have been used for identification of *Bacteroides* species[40]. Twenty-two *Bacteroides* species were compared by DNA-DNA homology studies using S1 endonuclease method. None of the currently defined species shared more than 30 per cent DNA homology with any other species examined with the exception of *B. buccae* and *B. capillus* along with *B. pentosaceus* now considered single species shared 86 per cent of their DNA sequences. Two clusters showed weak genetic relationships with DNA homology more than 10 per cent. The DNA probes correctly identified 94 per cent of the clinical strains. DNA probe and

biochemical identification was 100 per cent for two of the five species. In contrast only 86 per cent of the strains biochemically identified as *B. intermedius* were identified by the DNA probe. The DNA probes gave a species identification to seven strains which could not be identified using biochemical characteristics.

Commercially available probes are now being used for identification and classification; one such probe for *Mycobacterium avium* complex has been evaluated by a Japanese group[41].

5. DETECTION OF MICROORGANISMS

Nucleic acid probes are being used extensively in the detection of microorganisms in biological and environmental samples. The genetically-engineered bacteria when released in environment fail to grow at the same rate as natural population of that particular area. Thus, more sensitive methods are required to detect microorganisms in small numbers. Also, in case of non-culturable microorganisms, the conventional microbiological methods cannot be used. The nucleic acid probes provide a specific and sensitive test for detecting such microorganisms. In recent years these have become more popular in diagnosis of pathogenic organisms, i.e., bacteria, viruses and parasites.

5.1 Detection of Bacteria

Bacteria have been implicated in a number of human and animal diseases. Diarrhoeal diseases are one of the leading causes of death in children under five years of age living in developing countries and are a significant cause of adult morbidity in these areas[42]. *E. coli* was implicated in nursery epidemics in mid 1940s[43-46] for the first time. With the availability of DNA probes, there is quantitative information now available to suggest that different types of *E. coli* are relatively common cause of diarrhoea. Four major categories of *E. coli* are recognised as causes[47] of diarrhoeal disease, including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC). The nucleotide sequence coding for heat labile enterotoxin (LT) of ETEC[48,49] and heat stable enterotoxin (ST) have been determined[50] and cloned in plasmids[51] and used as probes to detect ETEC.

Cloned ^{32}P-labelled gene probes for ETEC have been used in epidemiological studies[52-55]. Limited access to facilities for ^{32}P isotope work, the short functional half-life of ^{32}P-DNA probes, the expense and radioisotope disposal problems, however limit the use of this technique for routine application in clinical settings. Recently, several methods for non-isotope labelling of nucleic acids were developed[7,8,11-14,56]. A dot-blot hybridisation test was developed for the detection of ETEC without the use of radioisotopes[57]. Three biotin-labelled DNA (Bio-DNA) probes corresponding to structural genes specifying heat labile and heat stable enterotoxins of porcine and human origin were prepared and reacted with protein and RNA-free DNA preparation in a dot-blot hybridisation assay. Bio-DNA probes were highly specific and could readily detect 160 pg of target DNA. A colony hybridisation method for visual detection[58] of heat labile enterotoxin producing *E. coli* was also developed.

It is convenient to prepare oligonucleotide gene probes in large quantities for use in clinical laboratories. They are also highly specific. A disadvantage however is that the sensitivity of short oligonucleotides may be affected by minor nucleotide changes in target sequences. However, in case of ETEC three relatively long oligonucleotide probes have been used in colony hybridisation[59] and found to be equally efficient as cloned polynucleotide probes. The oligonucleotide gene probes have been used to detect ETEC directly in fecal smears[60].

Non-isotopically-labelled oligonucleotide probes have also been used in detection of ETEC. Alkaline phosphatase conjugated oligonucleotide probes for ETEC heat labile toxin and heat stable toxin gene were compared with conventional Y1 adrenal cell and suckling mouse assays[61] for their ability to identify ETEC and found to be highly specific. A 26 bp oligonucleotide DNA probe conjugated to alkaline phosphatase[62] was compared with the same probe labelled with ^{32}P for identification of ETEC and found to be equally efficient. In piggeries in Victoria, ETEC has been detected by DNA hybridisation using K88, K99 fimbrial antigens LT, ST1 and ST2 probes[63] and found to be more efficient than ELISA or slide agglutination methods.

Other enteric pathogens detected using DNA probes are *Campylobacter. spp.*, *Shigella dysenteriae* and *Salmonella typhi.* Synthetic oligonucleotide probe labelled with alkaline phosphatase for *C. jejuni* [64] has been used for its detection. Non-radioactively labelled whole DNA with 2-acetylaminofluorene[65] has been used to identify *Campylobacter spp.* after a short primary culture. *In situ* DNA hybridisation using biotin-labelled bacterial DNA has been used[66] to detect *C. jejuni* in feces.

A set of DNA probes has been developed to study genes for Shiga like toxin[67]. Screening of different gram negative bacteria with toxin probes revealed that only two species carry related sequences, i.e., enterohemorrhagic *E. coli* and *Shigella dysenteriae I.S. typhi,* causative agent of typhoid fever can also be diagnosed using 8.6 Kb Eco RI fragment[68] highly specific for the via *B* gene region as hybridisation probe. Studies undertaken to assess the specificity and sensitivity to this probe[69] indicate that possibility of false-negative results are unlikely.

DNA probes have been successfully used to detect leptospires in biological fluids[70]. DNA isolated from *L. interrogans* serovar *pomona* was labelled with ^{32}P by nick translation and used as genomic probe to detect leptospira DNA in serum and urine. Neither serum nor urine interfered with assay system. Another report has described DNA probe for diagnosis of North American cattle pathogens *L. interrogans* genotype *hardjo bovis*[16].

Whole chromosomal DNA probes were used to identify clinical isolates of *Mycobacterium tuberculosis, M. avium* complex and *M. gordonae*[71]. Solution hybridisation of two ^{125}I-labelled cDNA probes has been used to identify *M. avium* complex isolates[72] where one probe is complementary to RNA from *M. avium* and other is complementary to RNA from *M. intercellulare.* Usefulness of Gen-probe for identification and classification[41] has also been studied.

A fragment of about 500 bp of the *a*-hemolysin gene from *Listeria monocytogenes*[73] was used to screen different bacterial strains by DNA colony hybridisation. Intestinal *Bacteroides. spp.*[74] have been identified using chromosomal DNA probes by whole cell dot-blot hybridisation. *Legionella spp.*[75] and *Neisseria gonorrhoea*[76] have also been detected using DNA probes.

5.2 Detection of Mycoplasma

DNA probes specific for *Mycoplasma pneumoniae* and *Mycoplasma genitalium* were selected[77] from genomic libraries prepared in pUC13. A comparison of both isotopic and non-isotopic labelling showed that ^{32}P-labelled probes could detect, by dot-blot hybridisation down to about 0.1 ng of specific mycoplasma DNA or 10^5 CFU, biotinylation of probe decreased the sensitivity of detection and produced non-specific background reaction with non-homologous DNAs. Sulphonation of probe yielded a similar level of sensitivity with less background. An 800 bp long species specific DNA probe[78] has been proved to be specific and sensitive in selective detection of *Mycoplasma gallisepticeum*. The DNA probe for *Mycoplasma genitalium* has been used in clinical diagnosis of men[79] with urethritis, attending a sexually transmitted diseases clinic in USA.

5.3 Detection of Viruses

The use of recombinant DNA technology has made it possible to produce probes in almost unlimited quantities. Several organisms, mentioned and not mentioned here are detected using nucleic acid probes, where nucleic acids bound on to a solid phase are detected by labelled DNA probes. The method is specific, sensitive and relatively rapid and it also enables the detection of latent infections. Several viral DNA probes are available of which a few are discussed here.

Enteric adenoviruses were implicated in the etiology of gastroenteritis in infants but their clinical significance was unclear because a rapid test to distinguish these agents from other agents was not available. A dot-blot hybridisation assay developed using a cloned DNA fragment specific for enteric adenoviruses[80] was found to be 91 per cent sensitive and 71 per cent specific for detecting enteric adenoviruses. The disadvantage of the hybridisation assays for routine diagnostic use is need for radioisotopes which have short half-lives and are difficult to handle. The presence of adenovirus DNA in nasopharyngeal mucus aspirate specimens[81] collected from children with acute respiratory infections were assayed using both radioactive and non-radioactive methods and results indicated that both radioactive and biotin-labelled probes can be used for the diagnosis. Although non-radioactive labels are less sensitive than radioactive probes, more and more refinements and use of highly specific reagents are forthcoming to improve their sensitivity to match the radiolabelled probe detections. In both radioactive and non-radioactive methods quantification of DNA detected is difficult which is important to determine the number of specific DNA molecules in a sample. Time-resolved fluorometry was used to detect quantitatively

biotinylated DNA probes for adenovirus type-2 bound to microtitre wells[12]. Adenovirus type-2 DNA in stool and nasopharyngeal specimens[82] were detected to a sensitivity of 100 pg using time-resolved fluorometry.

Hepatitis *A* virus (HAV) is endemic in developing countries and is usually spread via feco-oral route, replicates in liver and is excreted into feces via the biliary system. Viremia has been documented prior to or during acute hepatitis. Detection of HAV RNA would be useful for laboratory studies and clinical studies. Molecular hybridisation with cDNA probes was used to detect HAV[83] in seeded estuarine samples. In clinical samples, HAV was detected[84] using combined immunoaffinity cDNA-RNA hybridisation assay. Hybridisation of HAV RNA extracted from cell culture, serum, liver and feces with HAV cDNA[85] was found to be more than 10 times more sensitive than conventional immune electron microscopy or radioimmunoassay and less sensitive than HAV infectivity in primates or in cell culture.

Hepatitis *B* virus (HBV) causes 7 to 13 per cent cases of post transfusion hepatitis[86,87] because some individuals with persistant HBV infection may not always have detectable surface antigen (HBsAg) in their sera. Therefore, need for a sensitive and direct laboratory test to identify these potentially infectious units of blood was felt. DNA hybridisation was the only answer and a molecular hybridisation for detection of HBV DNA was developed[88,89]. The blood of healthy donors was screened[90] for HBV DNA and the results suggested that the absence of HBsAg in otherwise apparently healthy individuals may not be enough to ensure lack of circulating HBV.

Rotavirus is a major cause of gastroenteritis in children throughout the world[91]. In addition, rotavirus causes a common nasocomial infection on pediatric wards in hospitals[92,93]. Recently, outbreaks of rotavirus infection has also been identical in elderly patients[94]. Detection of rotavirus using DNA probes is both specific and rapid. A hybridisation assay for detection of rotaviruses[95] has been developed. Several atypical rotavirus strains have been isolated from calves, pigs, lambs, birds and humans and described as antigenically distinct rotaviruses (ADRVs)[96] which are evidenced as etiologic agents of epidemics of diarrhoea. It is thus important to determine genetic and antigenic relatedness of human and animal strains of ADRVs. Nucleic acid hybridisation has been used to assay it. The dengue virus complex (*Flaviviridae*) consists of four serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). Dengue virion possess a single-stranded RNA genome of positive polarity approximately 11 kb in length. The increasing incidence of dengue virus infections throughout tropics has prompted increased efforts to develop rapid and reliable diagnostic techniques. Nucleic acid hybridisation has both these advantages over conventional surveillance involving isolation of virus from mosquitoes and amplification in a bioassay and serological identification. cDNA and systemic oligonucleotide probes have been successfully used in detection of purified dengue virus RNA and viral RNA in dengue virus infected, vero cells and C6/36 cells[97-99]. A molecular hybridisation technique with radiolabelled, strand-specific RNA was developed to detect dengue virus type 2 RNA in pools of infected *Aedes albopictus* mosquitoes[100].

Cytomegalovirus (CMV) infection accounts for significant morbidity and mortality in newborns and in patients with impaired immunity[101,102]. Rapid diagnosis is becoming increasingly important with advent of new treatment protocols. Biotinylated DNA probes have been used for rapid detection of CMV in clinical specimen by hybridisation assay[103]. DNA isolated from paraffin embedded tissues has been used to detect CMV by dot hybridisation[104]. *In situ* hybridisation has also been used to detect CMV in bronchoalveolar lavage[105]. CMV pneumonia has been diagnosed[106] using *in situ* hybridisation. An *in situ* biotinylated DNA probe assay was evaluated as an adjunct to anti-CMV early nuclear antigen indirect immunofluorescence and cytopathic effect on CMV-infected monolayers in shell vial cultures. Viral infection was detected by early nuclear antigen indirect immunofluorescence at 24 h.and by DNA probe assay and shell vial CPE at 5 days[107].

Human immunodeficiency virus (HIV) has been associated with AIDS and related disorders. Assay to detect antibodies to HIV proteins have been developed and used to screen sera for the identification of individuals who have been exposed to the virus. Although these serological tests have significant sensitivity and specificity for detecting exposure to the virus, they do not provide direct identification of HIV. *In situ* hybridisation using nucleic acid probe[108] showed that less than 1 in 10,000 cells of the lymph node of infected individuals contain viral nucleic acid sequences. Such low copy number HIV-I sequence have also been detected using a capture DNA sequence[109] bound covalently to microtitre wells through linker arms. Bio-DNA fragment derived from sequences adjacent to capture sequences is used as detection probe.

Nucleic acid probes are also available for herpes simplex virus[110], enteroviruses[111] and varicella-zoster virus[112] and for many others not covered here.

5.4 Detection of Parasites

Chlamydia trachomatis infection in urinogenital tract[114] of women has been diagnosed by DNA hybridisation analysis. Purified DNA fragments that hybridise to repetitive DNA elements have been used as probes for the detection of various parasites including those causing malaria, i.e., *Plasmodium spp.*[115-118], leishmaniasis[119,120], filariasis[121], and trypanosomiasis[122,123]

6. POLYMERASE CHAIN REACTION

This *in vitro* DNA amplification procedure[123] has made the identificaiton of a very low copy number sequences also possible which may not be possible using conventional hybridisation methods. A pair of primers and thermostable DNA polymerase called as Taq polymerase are used to amplify a particular sequence. The primers anneal to their complementary sequences in a similar manner as DNA probes and target sequence undergoes a number of replication cycles to make multiple copies. It has been used successfully to detect HIV[124-127] type-I, human *T* cell lymphotropic virus[128] type-I, HBV[129], human papilloma virus[130], human cytomegalo virus[131], toxigenic *E. coli*[132,133], and *Legionella pneumophila*[134].

7. CONCLUSIONS

Nucleic acid probes have already made substantial contribution towards the development of fundamental concepts both in microbiology and diagnostic microbiology. Technical advances made in the research laboratory will be transferred rapidly to clinical laboratories leading to simple, rapid and more accurate diagnosis. Non-radioactive labels have advantages in extending nucleic acid hybridisation to clinical laboratories or field use in remote areas, thereby eliminating the hazards of handling radioactive materials. At the same time major drawbacks with non-radioactive probes are their lower sensitivity of detection of the target over radioactive probes and appearance of background grains of colour. Recent advances in the development of more sensitive non-radioactive methods include polymerase chain reaction. The development of non-isotopic methods will simplify the application of nucleic acid probes making them a tool for routine field testing.

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