CHAPTER 35
Nucleic Acid Probes to Detect Viral Diseases

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The presence of a virus infection can be documented either by isolation of the virus by culture or by detection of a virus-specific component. These viral components include structural proteins (which are usually identified on the basis of their antigenicity), virus-induced enzymes, and viral nucleic acids.

There are several features of nucleic acid hybridization that have prompted investigation of this technology for the detection of viral diseases. First, nucleotide sequences can be identified as unique for the genome of specific agents and therefore can constitute probes of very high specificity. Second, the high avidity of complementary nucleic strands for each other should permit highly sensitive hybridization assays. Finally molecular cloning and oligonucleotide synthesis make possible the availability of virtually unlimited amounts of standardized reagent for the detection of any organism.

Procedures

Nucleic acid from infected tissue can be detected either in situ to permit histologic and cytologic localization, or after extraction of the nucleic acid from tissues. The theory and practice of nucleic hybridization are detailed elsewhere [1–3], and in situ hybridization is discussed by Haase (Chapter 36, this volume). Although kinetic liquid phase hybridization can best be applied to the quantitative determination of concentration, complexity, and homology of nucleic acid preparations [2], fixation of nucleic acid to a solid filter support permits much less cumbersome assays which are semiquantitative and amenable to the examination of a large number of samples [3].

DNA or RNA is placed on a nitrocellulose or nylon filter either directly by spotting [4,5] or by filtration through a vacuum manifold [3]. The filter
is then baked [3] or irradiated [6] to ensure bonding of the nucleic acid to the solid support, or in some procedures the filter may be used directly [7]. First, DNA must be partially purified before placement on the solid support. The protein and lipid contaminants in samples must be removed prior to immobilization. If filtration is used, contaminants will clog the filter. More importantly, contaminants can compete with either target or probe binding to reduce specific reactions; also, protein coimmobilization with target sequences can lead to spuriously high backgrounds. Techniques for the extraction of RNA for assay on filters have also been developed [7,8]. In general, less than 10 μg of total nucleic acid or the contents of 10^5 to 10^6 cells are applied on a surface area of approximately 5 mm². Larger quantities of material will not bind with 100% efficiency on such a small area and can lead to high background hybridization signals.

In addition to the examination of partially purified DNA with the dot-blot format, DNA may be digested with restriction endonucleases, fractionated by agarose gel electrophoresis, and then transferred to filters (Southern blot) [1,3,9]. Similarly, electrophoretic fractionation of RNA and transfer to filters may be performed ("Northern" blot) [1,3]. These blotting techniques combine the high resolution of gel electrophoresis with the high sensitivity and specificity of nucleic acid hybridization. Such blots may increase the specificity of hybridization by eliminating or diluting nonspecific reactions. Analysis of size-fractionated nucleic acid by these methods also permits the characterization of restriction endonuclease fragments of DNA, grouping of the size classes and genome mapping of RNA transcripts, determination of the relative abundance of genes and subgenomic fragments or of transcripts, and the possibility of integration of viral genes in host DNA (see below).

The specificity of a DNA probe may vary greatly with the portion of the genome comprising the probe. Several subgenomic fragments of both herpes simplex virus (HSV) and cytomegalovirus (CMV) have been shown to hybridize with mammalian DNA [10–12]. Whether this cross-reactivity proves to be due to true sequence homology or to guanosine–cytosine rich (GC) sequences will not alter the fact that such fragments are undesirable as probes for viral nucleic acid in the presence of human cells. The virus specificity of a probe can also depend on the fragment selected. The 3' end of enterovirus genomes can be used to detect most enteroviruses [13,14], whereas a poliovirus probe consisting only of the first 220 5'-nucleotides reacts only with poliovirus RNA [14].

The BamH1-A fragment of HSV type 1 is only two- to fourfold less sensitive for detecting HSV type 1 DNA as compared to HSV type 2 DNA [15]. Fragments from the junction regions of HSV-1 and HSV-2 yield absolutely type-specific probes under stringent hybridization and washing conditions [16]. These probes can then be used for the detection, identification, and typing of HSV DNA in specimens from a number of epidemiologically unrelated patients.

Synthetic oligonucleotide probes which are usually 14–30 nucleotides in