with the same primer binding sites but a deletion of about 36 bp in the middle of the amplified region. This yielded a 350 bp product using the outer primer and a 290 bp product with the innner primer. The sensitivity of the PCR assay was evaluated by using serial dilutions of *Hind*III-digested cloned VZV DNA (*Kpn*I fragment of the VZV genome cloned into vector pT7/T3a-19). PCR products were detected by agarose gel electrophoresis followed by ethidium bromide staining. Southern blot hybridization using a Digoxigenin-dUTP labelled probe, and enzyme-linked immunoassay. The probe was generated by digestion of the *Kpn* I s fragment with *Mlu*I. A 392 bp fragment, which overlaps with 290 bp of the amplified region, was isolated and DIG labelled.

Results and conclusion. The sensitivity of the PCR assay was determined with cloned VZV DNA. About 200 copies of the target sequence were necessary for detectable amplification by standard PCR and less than 20 copies by nested PCR. Out of 24 human trigeminal ganglia five tested positive for VZV DNA by standard PCR (21%), in eleven cases VZV DNA was detectable using nested PCR (46%). Sequences specific for VZV could be detected in PMBC from children with acute varicella up to six days after the onset of rash by standard (one child) or nested (three children) PCR. This confirms that at the time of haematogenous spread before and during the rash viral DNA can be found within the mononuclear cells. Thus the use of nested primers enhances the sensitivity of the assay and allows the detection of only a few genomic copies of viruses in human tissues.

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Influence of DNA preparation and application on specificity of a 3′-end labelled oligonucleotide probe in a dot blot assay

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The aim of our studies was to establish a method that could detect orthopoxvirus- (OPV) DNA with a oligonucleotide probe within one day.

Methods and results. Purified DNA of five different species of the genus OPV was used. The negative control was parapoxvirus DNA. We chose a nucleotide sequence as probe that contained a conserved region of the OPV species[1,2]: 5′CTTACTTTTTCTTTCTC3′.

The Oligonucleotide Tailing Kit (Boehringer, Mannheim) was used to label the nucleotide sequence[3]. This kit was used to attach a poly-A tail comprising approximately 50 nt to the end 3′-end by means of terminal transferase. Prehybridization (20 min) and hybridization were carried out at 35 °C (Tm 9 °C), as were the stringency washes. Detection was carried out colorimetrically following the standard procedure. For the DNA preparation and application two different procedures were compared:

A) The DNA to be investigated was heated for 10 min in 0.4 N NaOH at 94 °C then cooled on ice. The DNA was applied to a nylonfilter under vacuum with the aid of Hybridot Manifold apparatus (Gibco/BRL) and was then rinsed and simultaneously fixed with NaOH.

B) The DNA was diluted in TE 10/1 buffer (pH 8.0) and denatured by boiling for 10 min prior to application with a pipette onto the membrane. Fixation was carried out by incubation for 20 min at 120 °C.

Method A showed nonspecific reactions especially with NaOH, although an optimization was attempted. Furthermore, the intensity of the signals was very variable. This observation led to the conclusion that NaOH and the powerful suction of the Hybridot Manifold apparatus change the surface of the membrane and therefore the labelled oligonucleotides bind unspecifically. Method B on the other hand yielded reproducible results without background reactions.

Of the three tested nylon membranes, Hybond N, Hybond N+ (both Amersham) and 'Membrane, positively charged' (Boehringer, Mannheim), the latter appeared to be the most suitable with regard to signal development, sensitivity and background. The optimal probe concentration for labelling was found to be 200 ng oligonucleotide per ml hybridization liquid.

After varying the duration of hybridization (0.5 h, 2 h, 6 h), 2 h appeared to be sufficient. In this system it was possible to detect the presence of 100 pg OPV DNA within 8 h. Attempts to improve the sensitivity by using a combination of various labelled oligonucleotides were unsuccessful.

Conclusions. Optimization of the parameters of sample preparation, sample application, probe concentration and also membrane suitability have a considerable influence on the specificity of 3′-end labelled oligonucleotides.

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