Determination of Single Hybridization Temperature for Serotyping of Human Group A Rotavirus with Oligonucleotide Probes

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Abstract: The possibility of use of a single hybridization temperature instead of different temperature for different probes for detection of human group A rotavirus (HRV) was explored. The sensitivity and specificity of the 5 oligonucleotide probes were assessed using cell culture adapted rotavirus (RV) strains by carrying out hybridization at a fixed temperature of 50℃. The results obtained indicate that a single hybridization temperature may be used for all the probes with virtually no decrease in specificity and sensitivity.

Key words: Rotavirus, Hybridization temperature, Oligonucleotide probes, Serotype, Sensitivity and Specificity

INTRODUCTION

Human group A rotavirus (HRV), members of the family Reoviridae, genus *Rotavirus*, is the most important pathogen of acute diarrhoea in infants and young children on a global scale (Kapikian *et al.*, 1985). The virus possess a double stranded RNA (dsRNA) segments which are enclosed within a double capsid protein shell. The outer layer contains two independent serotype-specific neutralization proteins, VP7 and VP4 (Hoshino *et al.*, 1985, Offit and Blavat, 1986). Protein VP7 is encoded by segments 7, 8 or 9, depending on the strains, and defines the VP7 or G-serotype and producing major neutralizing antibody whereas the VP4 protein is encoded by segment 4 and defines the VP4 type or P-type and producing minor neutralizing antibody (Estes and Cohen, 1989). In human and animal rotaviruses, 14 VP7 serotypes have been identified so far of which at least 7 (serotype 1-4, 8, 9 and 12) are responsible for human disease (Kapikian and Chanock, 1990, Urasawa *et al.*, 1990). Based on the information of nucleotide sequences of VP7 genes, Flores *et al* (1989) developed a dot hybridization assay for distinguishing of RV serotypes 1 to 4 with cDNA probes. Recently a

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method has been reported for serotyping of HRV with synthetic 25 bases of oligonucleotide probes (Sethabutr *et al.*, 1990, Bingnan *et al.*, 1991, Ali *et al.*, 1993). These probes are designated as HuG1Ac, HuG2Ac, HuG3Ac, HuG4Ac and HuG8Ac and three different temperatures allowed hybridization of them. However use of different temperatures for different probes is often inconvenient.

In this report, we present our findings that a single hybridization temperature can be used for 5 different oligonucleotide probes for serotyping of HRV.

Materials and Methods

Cell and Viruses

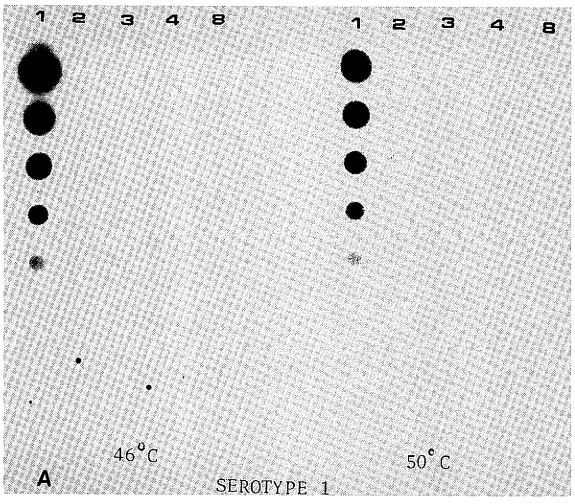
The rhesus monkey kidney cell line, MA104 (Microbiological Associates, Bethesda, Maryland), was used to cultivate the rotavirus strains. MA104 cells were grown in the Dulbecco's modified medium (GIBCO Laboratories, New York) supplimented with 10% foetal calf serum (GIBCO Laboratories, New York), 100 units/ml penicillin and 100 μg/ml streptomycin. Rotavirus strains Wa (serotype 1), RV5 (serotype 2), RV3 (serotype 3), ST3 (serotype 4) and B37 (serotype 8) were used in this experiment. All the virus strains were grown following the method of Albert *et al.* (1987).

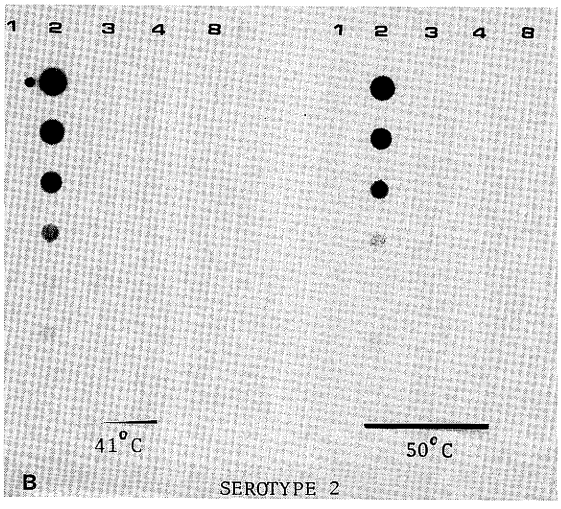
Extraction of RNA from tissue culture supernatant

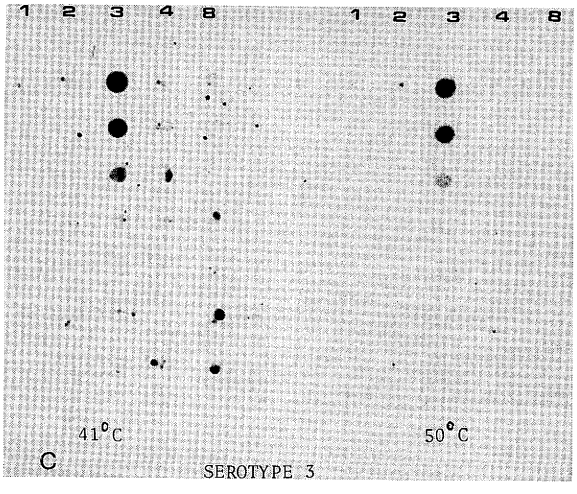
Virus particles from tissue culture supernatants was concentrated by 20% polyethylene glycol (MW 4000)/2.5 M NaCl. RNA extraction from concentrated virus preparation was carried out following the method of Herring *et al.* (1982). Briefly, a 450 μl volume of concentrated virus preparation in phosphate buffered saline (PBS), pH 7.2, was mixed with 50 μl of 1 M sodium acetate with 1% sodium dodecyl sulphate, pH 5.0, and then mixed with 500 μl phenol: chloroform: isoamyl alcohol (25:24:1). After 1 min of vortexing, the mixture was centrifuged at 10,000 rpm for 4 min at room temparature. The supernatant was harvested and extracted once more with equal volume of phenol: chloroform: isoamyl alcohol. The extracted RNA was pelleted down with ethanol and purity of the ds RNA preparation was dertermined by spectrophotometer from the ratio of ODs at 260/280nm (Maniatis *et al.*, 1982).

Dot hybridization

The purified dsRNA was denatured with equal volume of 6.15 M formaldehyde−10×SSC (1×SSC=150 mM sodium chloride plus 15 mM sodium citrate) for 15 min at 65℃ and serially diluted dsRNA from 5 rotavirus serotypes were spotted onto 5 separate pieces of 0.45 μm pore nitrocellulose filter (Bio-Rad Laboratories, Richmond, CA). Filters were air dried and baked at 80℃ for 2 hours. The five synthetic oligonucleotide probes (serotype 1-4, and 8) were kindly provided by Dr. Peter Escheverria of the Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. The 5′ end labelling with T4 polynucleotide kinase (Bethesda Research Laboratories, USA) and [γ-^{32}P]-ATP (Amersham, UK) was carried out as previously described (Sethabutr *et al.*, 1990). The dotted membranes were hybridized with the serotype specific oligonucleotide probes at hybridization temparatures 46, 41, 41,


1 2 3 4 8
1 2 3 4 8
46°C
50°C
A
SEROTYPE 1


1 2 3 4 8
1 2 3 4 8
41°C
50°C
B
SEROTYPE 2


1 2 3 4 8
1 2 3 4 8
41°C
50°C
C
SEROTYPE 3

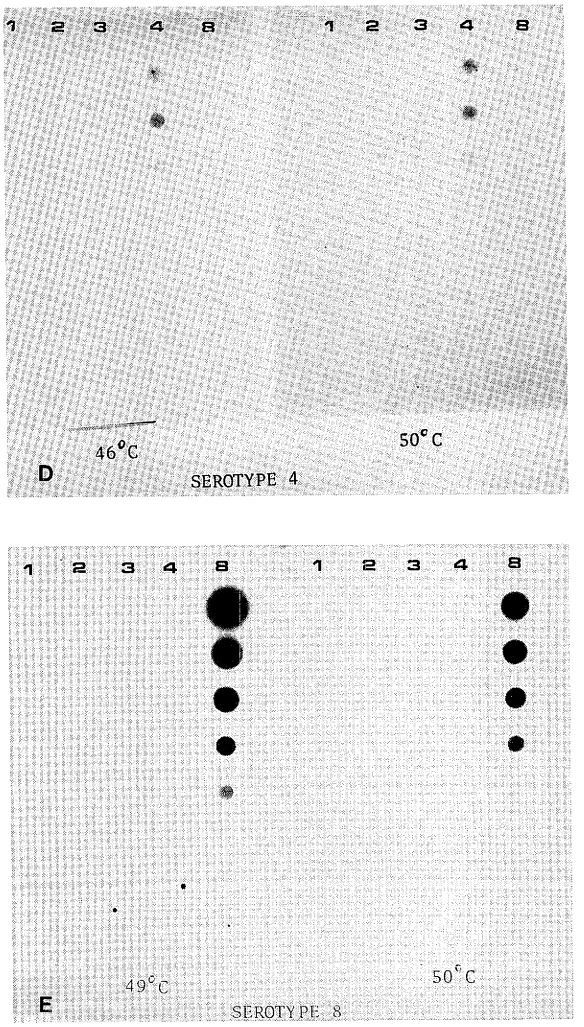


Fig. Dot hybridization assay of five reference rotavirus dsRNA (A) Wa (serotype 1), (B) RV5 (serotype 2), (C) RV3 (serotype 3), (D) ST3 (serotype 4) and (E) B37 (serotype 8). Two-fold dilution of dsRNA spotted onto nitrocellulose filters and hybridized with serotype 1-, 2-, 3-, 4-, and 8-specific probes at 46, 41, 41, 46, and 49℃ for HuG1Ac, HuG2Ac, HuG3Ac, HuG4Ac and HuG8Ac, respecytively and 50℃ for all the probes. Serotype of strains are indicated at the top of the figure.

46, and 49℃ for HuG1Ac, HuG2Ac, HuG3Ac, HuG4Ac and HuG8Ac (Bingnan *et al.*, 1991, Ali *et al.*, 1993) and at 50℃ for all the probes.

Results and Discussion

The result of hybridizations carried out at two temperatures (those used by Bingnan *et al.*, 1991, Ali *et al.*, 1993 and 50℃ proposed by us) for all five probes are shown in the figure. At a hybridization temperature of 50℃, it was observed that at this temperature cross-reaction was satisfactorily removed and similar sensitivity to that of temperatures used by Bingnan *et al.* (1991) and Ali *et al.* (1993) was observed. The probes did not show cross-reactivity with concentrated RNA preparations from other serotypes with the exception that at 41℃, HuG3Ac (see figure C). However the cross-reactivity signal was significantly weaker than the positive signal. But when the hybridization was performed at 50℃, the probes did not show any cross-reactivity. In addition, partial cross-reactivity exhibited by HuG3Ac at 41℃ was also eliminated when hybridization was carried out at our proposed common temperature i,e. 50℃. When hybridization was performed with the extracts from *Shigella* spp, *Escherichia coli*, isolates from five different diarrhoeagenic categories, *Giardia* spp and *Entamoeaba histolytica*, and it was observed that at both temperatures, probes did not react with either of the extract (data not shown).

The sensitivity of each probe was determined at two hybridization temperatures and compared. At hybridization temperature of 50℃, it was observed that at this temperature the sensitivity of the probes were also unaltered with the exception of HuG1Ac and HuG8Ac (see figure A and E). At 50℃, HuG1Ac and HuG8Ac showed slightly weaker signal than at 46 and 49℃, although the difference between the two signals does not affect the identity of the serotype. Therefore, 50℃ can be chosen as an appropriate temperature for hybridization of all probes as opposed to the use of a range of temperatures for different probes by Bingnan *et al.* (1991) and Ali *et al* (1993).

Although the salt concentration of the hybridization buffer can adversely affect the specificity of the probe but we did not experiment with different salt concentration of the hybridization buffer because we used the standard salt concentration described elsewhere.

This study was thus shown that a single temperature can be used for serotyping of HRV using synthetic oligonucleotide probes. This might prove to be an useful technical advantage in handling large number of samples.

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