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Rapid concentration and detection of hepatitis A virus from lettuce and strawberries

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Abstract

Immunomagnetic beads-PCR (IM-PCR), positively-charged virosorb filters (F), or a combination of both methods (F-IM-PCR) were used to capture, concentrate and rapidly detect hepatitis A virus (HAV) in samples of lettuce and strawberries experimentally contaminated. Direct reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of the collected HAV-beads complex showed a detection limit of 0.5 plaque forming units (PFU) of the virus present in 1-ml of wash solution from the produce, which was several hundred-fold more sensitive than that demonstrated by RT-PCR. In separate trials, virus-containing wash solutions from the produce were passed through the filters and the captured virus was eluted with 10 ml volumes of 1% beef extract. Of the 62% filter-captured HAV, an average of 34.8% was eluted by the 1% beef extract. PCR amplification of 2 μl from this eluate failed to produce a clear positive band signal. As little as 10 PFU, present on each piece of the lettuce or strawberry, was detectable by the F-IM-PCR, which was almost 20 times less sensitive than the detection limit of 0.5 PFU by the IM-PCR. However, considering the large volumes (≤ 50 ml) used in the F-IM-PCR, the sensitivity of detection could be much greater than that of the IM-PCR, which was restricted to ≤ 20 ml volumes. These data indicate that the F-IM-PCR method provides the potential for a greater sensitivity of detection than the IM-PCR, since low levels of virus could be detected from large volumes of sample than possible by the IM-PCR method. Although positively-charged filters captured a greater amount of virus than both the IM-PCR and F-IM-PCR methods, direct PCR amplification from beef extract eluates was not successful in detecting HAV from produce.

Keywords: Hepatitis A virus; Concentration; Immunocapture; Detection; Food

1. Introduction

Hepatitis A virus (HAV) is a 27 nm, non-enveloped picornavirus, with four capsid proteins (VP1–4) encompassing a highly conserved positive polarity single-stranded RNA genome

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(Hollinger and Ticehurst, 1990). New clinical isolates of HAV are very difficult to grow in cell culture and do not produce a cytopathic effect. Consequently, there is no recommended cell line to propagate HAV at present. Virus transmission occurs mostly through the fecal–oral route, by person-to-person contact, or through the ingestion of fecally contaminated foods which have been associated with many foodborne hepatitis A outbreaks (Cliver, 1985; Cliver et al., 1992; Anon, 1993). The recent outbreaks due to the consumption of contaminated gourmet foods prepared by a foodhandler and frozen strawberries (Dalton et al., 1996, Anon, 1997) re-enforced the need to develop rapid methods to detect HAV in foods, such as produce.

Traditional methods to concentrate and detect HAV in environmental and food samples are lengthy, cumbersome and suffer from low sensitivity and the possibility of virus inactivation or loss during the various steps (De Leon and Sobsey, 1990; Romalde, 1996; Lopez-Sabater et al., 1997). Although the RT-PCR is rapid and sensitive in detecting low levels of the virus, it has some drawbacks, i.e. its inhibition by various substances (Ijzerman et al., 1997) and its inability to distinguish between infectious and non-infectious virus particles (Harris and Griffiths, 1992; Cook and Myint, 1995; Richards, 1999). HAV and other enteric viruses may be found in large numbers in stool ($\geq 10^6$ virus particles per g), but they are usually present in low numbers in foods, e.g. 0.2–224 particles per 100 g shellfish meat (Williams and Fout, 1992). Therefore, various methods have been developed to capture and/or concentrate these viruses prior to their detection. These applications, however, have been limited to the capture and detection of the virus from clinical (Jansen et al., 1985; Robertson et al., 1992; Muir et al., 1993), environmental (Sobsey et al., 1985; Graff et al., 1993; Prevot et al., 1993; Deng et al., 1994; Schwab et al., 1995, 1996; Jothikumar et al., 1998), meat (Colman et al., 1995) and shellfish samples (Zhou et al., 1991; Jaykus et al., 1993, 1996; Le Guyade et al., 1994; Atmar et al., 1995).

In this study, immunomagnetic bead and positively-charged virosorb filters were used, either separately or combined, to capture and concentrate HAV from lettuce and strawberries. This was followed by its rapid detection by a one-tube RT-PCR method.

2. Material and methods

2.1. Cells and viruses

FRhK-4 cells and HAV (strain HM-175) were kindly provided by Dr M.D. Sobsey, University of North Carolina, Chapel Hill, N.C. Sabin vaccine strain of poliovirus (PV1) was received courtesy of D. McLeod, Health Canada, Ottawa. Cultivation and maintenance of the cells and preparation of virus pools have been described previously (Mbithi et al., 1991, 1992).

2.2. Inoculation of fruits and vegetables

Locally purchased romaine lettuce and strawberries were selected as representative of a vegetable and fruit, respectively. Individual lettuce leaves were cut into pieces of $\approx 6 \times 7$ cm, washed with a mild detergent (Ivory liquid soap™, Proctor and Gamble, Toronto, Ont.), thoroughly rinsed in water for 2 min and allowed to dry for 20–30 min in a laminar flow hood. Intact individual strawberries were also washed and dried in the same manner. Each side (front and back) of the lettuce pieces and the strawberries was then exposed to UV light (30 W) for 1 min to reduce and/or eliminate contaminating microorganisms that might have interfered with the plaque assay. Each piece of lettuce was placed in a clean and UV-disinfected weighing boat, whereas individual strawberries were placed in a sterile glass beaker. A portion (10 μl) of a known plaque forming unit (PFU) (10^3 for the magnetic bead experiments, or 10^4–10^5 for the positive filters) of HAV was spread evenly over a demarcated area of nearly 2×1 cm along the length of the lettuce midrib and over an $\approx$1-cm diameter area on each strawberry and were allowed to dry in a laminar flow hood for 20–30 min. The boats and the beakers were then covered with UV-sterilized Parafilm™ and incubated at 4°C overnight.

2.3. Virus recovery

After transferring each inoculated strawberry to a UV-sterilized weighing boat, the inoculated virus on the lettuce or strawberries was recovered by repeated pipetting/washing (> 25 times) of the demarcated area with 1 ml of phosphate-buffered saline (PBS), pH 7.6, through the fine end of a sterile 1 ml-capacity tip fitted onto a 1000P Gilson pipettor. The virus-containing wash solution was then collected from the boat, using the same tip and was used to investigate the capture, concentration, detection and quantitation of the virus as described below.

2.4. Plaque assay

HAV and PV1 titres were determined by the plaque assay in a Costar™ 12-well cluster plates (Fisher Scientific, Ottawa, Ont.). Each of three wells of an overnight FRhK-4 cell mono-layers was inoculated with 100 μl of each virus dilution. After incubation at 37°C for 90 min, each well was overlayed with 2 ml of an agarose-medium mixture (Mbithi et al., 1991). The plates were incubated at 37°C for 8 days/5% CO_2 (for HAV) and 2 days (for PV1). The mono-layers were fixed, stained and the plaques counted as described previously (Sattar et al., 1989).

2.5. RNA extraction

The QIAamp viral RNA extraction kit (Qiagen, Mississauga, Ont.) was used to extract RNA from the HAV stock culture, according to manufacturer's instructions. Extracted viral RNA was diluted serially and subjected to RT-PCR to determine the sensitivity of detection.

2.6. RT-PCR and hybridization

The Titan™ one tube RT-PCR system kit (Boehringer-Mannheim, Laval, Quebec) was used according to the manufacturer's instructions. The same primers (upstream 5′-GTTTTGCTCCTC-TTTACCATGCTATG-3′ and downstream 5′-G-GAAATGTCTCAGGTACTTTCTTTG-3′; producing a 247 bp amplicon) and thermocycling conditions as described previously by Deng et al. (1994), were used. Briefly, 2 μl portions of either extracted RNA or virus suspension (which had been subjected to 95°C for 5 min) was added to 18 μl of the reaction mixture. The tubes were incubated in a thermocycler model 9600 (Perkin-Elmer, Montreal, Quebec) set to run the following programs consecutively: 42°C for 30 min, 99°C for 5 min, 5°C for 5 min and then 35 cycles of denaturation at 95°C for 1 min and annealing and extension at 60°C for 1 min, with an additional 7 min extension at 60°C. PCR amplicons were resolved on a 2% agarose gel, visualized by ethidium bromide and photographed. The PCR bands were then transferred onto a positively-charged nylon membrane (Boehringer-Mannheim) for hybridization. A specific internal probe (5′-TCAA-CAACAGTTTCTACAGA-3′) located between nucleotides 2232 and 2251 (Deng et al., 1994) was labelled with digoxigenin-dUTP by using terminal transferase, according to the instructions in the DIG oligonucleotide 3′-end labelling kit (Boehringer-Mannheim). Membranes were pre-hybridized at 42°C overnight and then hybridized with the DIG-labelled probe at the same temperature in a hybridization oven (Robins, Model 400, VWR, Montreal, Quebec). The hybridization signal was obtained by immunological colour detection according to the instructions in the DIG Nucleic Acid Detection kit (Boehringer-Mannheim).

2.7. Sensitivity of detection of RT-PCR

Quantities of 2 μl containing 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03 and 0.015 PFU of HAV were amplified by PCR. The highest virus dilution which demonstrated a positive band signal, confirmed by the DIG-labelled oligoprobe, was considered as the limit of detection.

2.8. Antigen-capture by magnetic beads (IM-PCR method)

Magnetic beads M-280 (Dynal, Great Neck, NY) were coated with anti-HAV K3-2F2 monoclonal antibodies (MAb) (Serum Commonwealth Labs.,Victoria, Australia) according to the manu-

facturer's instructions. The coated beads were resuspended in 1% BSA (Sigma, Oakville, Ont.), incubated for 30 min at room temperature on a roller and then separated, washed four times and resuspended in PBS-BSA. The beads were stored at 4°C and used within 2–3 weeks.

Optimal MAb to beads ratio was determined by cross-titration: 1 mg of beads was coated with 7.5, 10, 12.5, 15, 17.5 and 20 μg MAb. Portions (25 μl) of the coated beads were then incubated for 90 min/37°C with 0.1, 0.5, 1, 5, 10^1, 10^2, 10^3, 10^4 and 10^5 PFU of HAV/ml on a rotating roller. The beads (with captured virus) were collected, washed and resuspended in either 1 ml PBS (for plaque assay) or in 3 μl PCR buffer from the Titan™ kit for the RT-PCR (Fig. 1). Non-specific binding of HAV to the beads was investigated by incubating 25 μl portions of uncoated beads (10 mg/ml) with 1-ml volumes containing 10^1, 10^2, 10^3, 10^4 or 10^5 PFU of HAV. The beads were separated and the supernatant decanted into a clean tube. The beads were washed four times each with 500 μl PBS-BSA and resuspended in 3 μl PBS, while the wash solutions (2 ml in total) were added to the 1 ml supernatant. The beads were run through an RT-PCR, whereas the combined wash and supernatant solution was plaque-assayed. The same procedure was used to determine the percentage of virus captured specifically by the antibody-coated beads, except that 25 μl of MAb-coated beads was incubated with 1 ml containing 10^3 PFU of HAV.

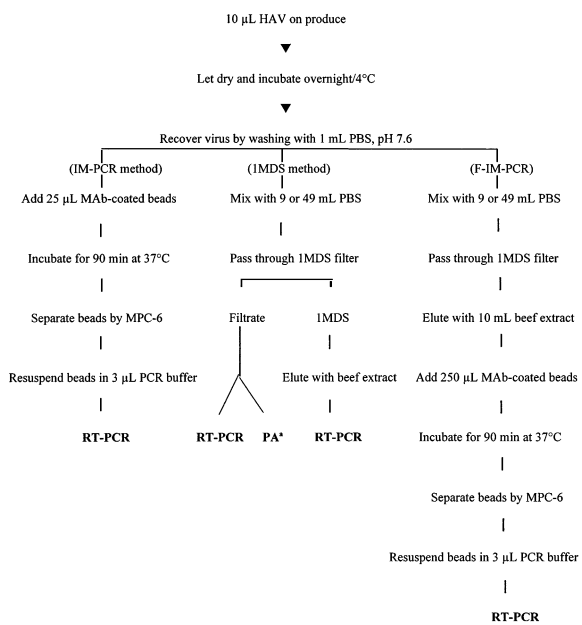

Fig. 1. Schematic illustration of the immunomagnetic-polymerase chain reaction (IM-PCR), the virosorb 1 MDS filtration (F) and the combined filtration-immunomagnetic (F-IM-PCR) methods used to concentrate and detect hepatitis A virus (HAV) from produce.

The sensitivity of detection of the IM-PCR was determined by incubating 25 μl portions of MAb-coated beads with 1 ml quantities of HAV dilutions containing 80, 40, 20, 10, 5, 2.5, 1, 0.5, 0.25, 0.1 and 0.05 PFU, and the beads were then amplified by the RT-PCR.

To investigate the capture and detection of HAV from lettuce and strawberries by the IM-PCR, the demarcated areas on the produce were inoculated with 10 μl portions containing 80, 40, 20, 10, 5, 2.5, 1, 0.5, 0.25, 0.1 or 0.05 PFU, allowed to air-dry and were then recovered with 1 ml PBS, as described above. MAb-coated beads were then incubated with 1 ml of the virus recovery solution, after which the beads were separated and assayed by the Titan™ one-tube RT-PCR. To determine if the presence of other viruses could affect the IM-PCR, 10 μl of HAV (10^3 PFU) and 10 μl of PV1 (10^5 PFU) were inoculated onto the same demarcated areas on the produce and were then recovered, captured and detected by the IM-PCR, as described above.

2.9. Antigen-capture by positively-charged virosorb filters

Positively-charged, 47 mm diameter, 0.2 μm, virosorb filters (Cuno Inc., Meriden, CT) were used to capture HAV from inoculated lettuce and strawberries. Experiments were designed to investigate the following: (1) the efficiency of virus recovery rates from filters, as well as PCR compatibility, using 10 ml of 1% beef extract eluents; (2) the capture and concentration of HAV from beef extract eluates by magnetic

beads; and (3) HAV recovery and detection from lettuce and strawberries using a combined filtration and immunobead procedure. In initial trials, 10-ml volumes of PBS seeded with 10^5 PFU of HAV were passed through the filters and the filtrates were collected and tested by both RT-PCR and the plaque assay. The filter-adsorbed virus was eluted by passing 10 ml of 1% beef extract twice through the filter, by a drop-by-drop process (Borrego et al., 1991). A cordless pipettor was used to apply suction through the pressure relief valve at the filter holder to drain off as much as possible of the eluate from the filter. The virus-containing eluates were then collected and assayed by both the PCR and plaque procedures.

In a separate trial to simulate the use of larger volumes of wash solution for virus recovery from the produce, 1 ml containing 10^5 PFU of HAV was mixed with 49 ml of PBS and the entire solution (50 ml) was passed through the filter. The filtrates, as well as the virus eluted from the filters by the beef extract, were assessed by the plaque assay (Fig. 1).

A combined filtration and immunomagnetic beads procedure (F-IM-PCR) was used to further concentrate the eluted virus, as well as to remove the inhibitory effect of beef extract on the RT-PCR. This was done by incubating MAb-coated beads with the virus-containing 10 ml beef extract eluates and the beads were then collected, resuspended in 3 μl PBS and tested by the PCR, as described for the IM-PCR. In this procedure, two sets (each consisting of duplicates) each of lettuce and strawberries were inoculated with 10 μl volumes containing 1000, 100, 50, 25, 10, 1 or 0.1 PFU of HAV and allowed to dry. The virus was then recovered with 1 ml PBS from each of the samples, mixed with either 9 or 49 ml of PBS and passed through the filters. The filtrates were saved and HAV was eluted from the filters with 10-ml beef extract. One set of the eluates was processed by the RT-PCR directly, whereas to the second set, MAb-coated beads were added to capture the virus, which was then subjected to RT-PCR (Fig. 1).

3. Results and discussion

3.1. Virus recovery

Plaque assays showed that an average of 84 and 81% of HAV were recovered from the lettuce and strawberries, respectively. These rates were used as the baseline virus titre in subsequent experiments. The weighing boats provided a very smooth and non-stick surface which allowed the retrieval of $\approx 970–980$ μl of the 1 ml of wash solution.

3.2. RT-PCR

Comparable positive PCR signals were obtained when 2 μl quantities of either the extracted RNA or intact viruses were amplified (Fig. 2a). The identity of the 247 bp band was confirmed by hybridization using the Dig-labeled oligoprobe (Fig. 2b). Amplification of HAV dilutions showed that the sensitivity of detection varied between 0.5 and 2 PFU (results not shown), which was within the range reported by other investigators (Tsai et al., 1993; Monceyron and Grinde, 1994; Grinde et al., 1995). It appears that this variation is inherent in the RT-PCR itself rather than the Titan™ one-tube RT-PCR system, which demonstrated a greater sensitivity and reproducibility than the conventional RT-PCR assays, which incorporated only the Taq DNA polymerase. The increased sensitivity could be due to the higher fidelity of the enzymes used in the Titan™ RT-PCR system (i.e. Expand™ which includes both Taq and Pwo DNA polymerases) that allow for better proofreading of the template and minimize the effect of any interference by secondary RNA structures (Frey and Suppmann, 1995).

3.3. Virus capture and detection by magnetic beads and RT-PCR (IM-PCR)

3.3.1. Effect of magnetic beads on FRhK-4 cells and PCR performance

FRhK-4 cell monolayers inoculated with 2 mg of magnetic beads for 14 days did not show any noticeable cytopathic or toxic effect. The presence of 3 μl of beads (300 μg) in the RT-PCR

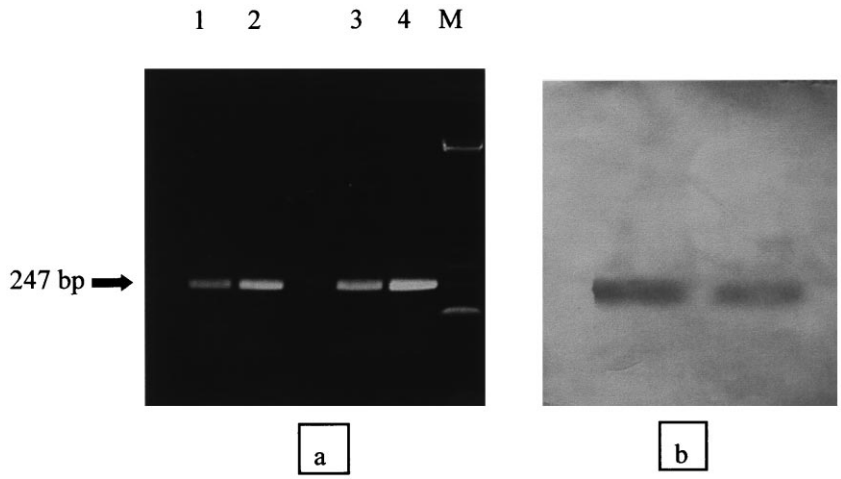

Fig. 2. (a) Amplification by the Titan™ one-tube RT-PCR system of extracted RNA from hepatitis A virus (HAV) and from intact whole HAV. Lanes 1 and 2: extracted RNA; Lane M, DNA Marker ladder 123 bp; Lanes 3 and 4 containing 4×10^4 PFU of HAV. (b) Southern hybridization with Dig-labeled oligoprobe.

reaction mix, although occasionally producing a weaker band intensity, did not inhibit the reaction nor affect the sensitivity of detection (results not shown). Monceyron and Grinde (1994) also observed a decrease in band intensity when similar type of beads were present in the PCR reaction and therefore, only the cDNA was transferred to the PCR mix, without the beads. However, Hedrum et al. (1992) using the same type of beads, did not notice any differences and thus, left the beads in the PCR reaction mix. Therefore, in this study, the bead-virus complex was transferred to the single-tube RT-PCR reaction tube, where the entire amplification process was completed.

3.3.2. Optimization of the ratio of MAb to magnetic beads

Both 10 and 12.5 μg of MAb per 1000 μg beads produced similar PCR band intensities (results not shown). Subsequently, the beads were coated with 12.5 μg of MAb to ensure the presence of sufficient antibody to capture the virus and to reduce non-specific binding by coating more of the beads surface area with the specific antibody. The 12.5 μg of MAb falls within the suggested range of 10–20 μg MAb per 1 mg of beads (Luk and Lindberg, 1991; Monceyron and Grinde, 1994; Grinde et al., 1995).

3.3.3. Non-specific binding of HAV to uncoated beads

PCR amplification bands were not visible at HAV concentrations of 10^1 and 10^2 PFU, a faint band was sometimes seen at 10^3 PFU and slightly stronger signals were seen at 10^4 and 10^5 PFU. Plaque assay results demonstrated a wide variation in non-specific binding, ranging between 0.6 and 2.6% (average 1.5%) (Table 1). Pre-treatment of beads with 1% BSA reduced the non-specific binding to nearly 0.93%, where only an occasional faint band was seen at 10^3 PFU. Non-specific binding was also observed by Monceyron and Grinde (1994), but actual percentages were not indicated.

3.3.4. Specific binding of HAV to MAb-coated beads

Plaque assays using HAV-MAb-bead suspensions resulted in frequent inconsistencies in plaque counts, possibly due to partial neutralization of the virus by MAb, which did not allow for reliable determination of the rate of specific binding. However, plaque results from the supernatant were more consistent and showed that an average of 58.4% of HAV remained unadsorbed in the supernatant, indicating that nearly 41.6% of the virus was bound to the MAb-coated beads (Table

1). Based upon an average of 1% non-specific binding, it was estimated that almost 40.6% of the virus bound specifically to the beads. This was around 42 times greater than the rate of non-specific binding and was less than the estimated 100-fold difference between the specific and non-specific HAV binding rates reported by Monceyron and Grinde (1994). Vigorous washing of the beads did not produce a noticeable reduction in the specific binding of HAV to the beads, confirming previous observations by other authors (Monceyron and Grinde, 1994).

3.3.5. IM-PCR sensitivity of detection

When MAb-coated beads were incubated with various HAV dilutions, the PCR bands obtained demonstrated a detection limit ranging between 0.5 and 10 PFU (Fig. 3a). Although similar to the

Table 1
Specific and non-specific binding of hepatitis A virus (HAV) by magnetic beads

HAV PFU/ml (PBS)	Trial	Percent (±S.E.) specific binding of virus to MAb-coated beads			Percent (±S.E.) non-specific binding of HAV to uncoated beads		
		Plaque assay[a]		Beads RT-PCR	Plaque assay[a]		Beads RT-PCR
		Beads	Supernatant		Beads	Supernatant	
10^3	1	29	71	+[c]	0.6	99.4	−[d]
	2	48	52	+	1.4	98.6	−
	3	57	43	+	2.6	97.4	±[e]
	4	34	66	+	ND[f]	ND	ND
	5	40	60	+	ND	ND	ND
Average		41.6 ± 4.99[b]	58.4 ± 4.99[b]		1.5 ± 0.58[b]	98.46 ± 0.58[b]	

[a] The percentage of specific binding of HAV to beads was derived by subtracting the amount (%) of the unbound HAV in the supernatant from 100% which is the initial amount of input virus.
[b] The S.E. of the mean for the specific and non–specific binding by the beads is similar to the S.E. for the supernatant, since the (%) values reflect the difference from 100%.
[c] Positive PCR amplification signal.
[d] No PCR amplification signal.
[e] An occasional faint PCR amplification signal.
[f] Not done.

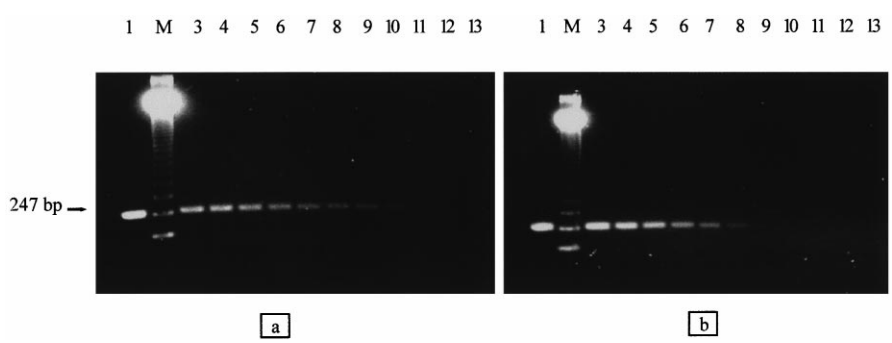

Fig. 3. (a) Sensitivity of detection of hepatitis A virus (HAV) from phosphate-buffered-saline by the immunomagnetic-PCR (IM-PCR). Lane 1, HAV positive control; Lane M, DNA Marker ladder 123 bp; Lanes 3–13, contain HAV concentrations of 80, 40, 20, 10, 5, 2.5, 1, 0.5, 0.25, 0.1 and 0.05 PFU, respectively. (b) Sensitivity of detection of HAV from lettuce or strawberries by the immunomagnetic-PCR (IM-PCR). Lane 1, HAV positive control; Lane M, DNA Marker ladder 123 bp; Lanes 3–13 contain HAV at concentrations of 80, 40, 20, 10, 5, 2.5, 1, 0.5, 0.25, 0.1 and 0.05 PFU, respectively.

Table 2
Rates of hepatitis A virus recovery from positively-charged virosorb filters by 1% beef extract

Recovery rates of hepatitis A virus[a]		
Trial	1% BE[a]	1% BE[b]
	10 ml eluate	50 ml eluate
1	32[c]	24
2	41	39
3	37	35
4	29	27
Average	34.8% ± 2.66[d]	31.3% ± 3.47

[a] Virus recovery rate when 10 ml virus-containing PBS was filtered through 1 MDS and then recovered with 10 ml of 1% BE, pH 9.5.
[b] Virus recovery rate when 50 ml virus-containing PBS was filtered through 1 MDS and then recovered with 10 ml of 1% BE, pH 9.5.
[c] Percent recovery rate.
[d] S.E.M.

detection limit of the RT-PCR, the IM-PCR was >100 times more sensitive, since it detected the virus in a 1 ml volume as compared to the same amount of virus present in the 2 μl used in the RT-PCR reaction.

3.3.6. Detection limits of IM-PCR for produce

Although the sensitivity of detection ranged frequently between 5 and 10 PFU of HAV, as low as 2.5 PFU was occasionally detectable from lettuce and strawberries. This 5- to 10-fold decrease in sensitivity, compared to the 0.5 PFU detected by the IM-PCR from PBS, may have resulted from virus loss and/or variation in virus recovery from produce (average of 82%) (Fig. 3b). Taking these factors into consideration, as well as the 42% capture efficiency by the immunobeads, it is suspected that only half or less (1.6 and 3.3) of the 5 and 10 PFU present, respectively, were actually detected. Applying the 79:1 ratio of HAV particles to infectious units (Deng et al., 1994) to the IM-PCR findings would indicate a detection limit of 5–10 PFU, corresponding to nearly 395 and 790 virus particles, respectively. Considering an average of 82% virus recovery rate from the produce (of which 42% was captured by the beads) would indicate that 126–252 virus particles were needed to be present on the produce to result in visible detection by the IM-PCR. This is at least 2- to 30-fold higher than the six to 60 (Prevot et al., 1993) and four (Deng et al., 1994) virus particles detected in spiked water and effluent environmental samples. Variation in the detection limits by the immunocapture-PCR method have also been reported by other investigators, i.e. 1 PFU/ml of HAV from water (Prevot et al., 1993), 10 PFU/ml of rotavirus from seawater (Grinde et al., 1995), 0.047 PFU from 80 μl of HAV from seeded fecal samples (Jansen et al., 1990) and 0.04 PFU from 1 ml environmental samples when using a pre-concentration step followed by an immunocapture-PCR (Jothikumar et al., 1998). These variations indicate that the immunocapture-PCR method can be influenced by a number of factors: (1) the type of immunoaffinity solid phase used (i.e. different types of beads, polypropylene surfaces); (2) the types of immunoaffinity capture system used (i.e. antibody-coated beads or streptavidin beads coated with biotinylated antibodies or oligoprobes); (3) the type of virus; and (4) the type and source of sample (clinical, environmental, shellfish, etc.).

4. Virus capture and detection by combined filters and RT-PCR (F-IM-PCR)

Elution with 10 ml of 1% beef extract resulted in an average of 35% recovery of HAV from the virosorb filters, indicating that nearly 21% of the virus remained adsorbed (Table 2). Other investigators have reported a broad range of virus recovery rates from filters ranging between 2 and $\geq 90\%$. These differences could be attributed to many factors, such as the type of virus being eluted, the type and thickness of filters used, different types and concentrations of eluents, the pH of the sample, the type of sample, as well as additional steps that might have been incorporated in the concentration procedure. Overall, the virus recovery rates in this study were comparable to those reported by other investigators who used beef extract to recover virus from the virosorb filters (Sobsey and Glass, 1980; Sobsey et al.,

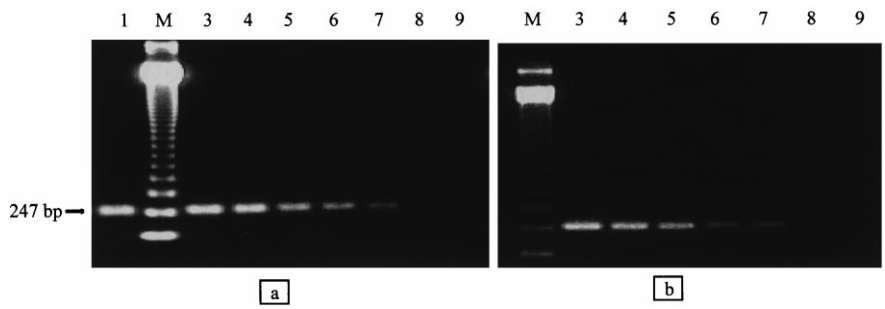

Fig. 4. Sensitivity of detection of hepatitis A virus (HAV) from lettuce and strawberries by the combined filtration-immunomagnetic-PCR (F-IM-PCR). For lettuce (a) Lane 1, HAV positive control; Lane M, DNA Marker ladder 123 bp; Lanes 3–9 contain HAV at concentrations of 1000, 100, 50, 25, 10, 1 and 0.1 PFU, respectively. For strawberries (b) Lane M, DNA Marker ladder 123 bp; Lanes 1–7, contain HAV at concentrations of 1000, 100, 50, 25, 10, 1 and 0.1 PFU, respectively.

1985; Borrego et al., 1991; Sobsey, 1993; Schwab et al., 1995). Even though a lower concentration (1 vs. 3%) of beef extract was used as eluent, direct PCR amplification of 2 μl volumes from these eluates failed to produce a clear PCR band, reflecting the inhibitory effect of beef extract on the PCR reaction, even at lower concentrations.

Amplification of the HAV-MAb-bead complex, which was added to and then separated from the virus-containing 10 ml beef extract eluates, produced a positive PCR signal (results not shown). Thus, the immunobeads, besides capturing and concentrating HAV, successfully separated the virus from the inhibitory effect of beef extract and enabled its direct detection by the PCR. Further application of F-IM-PCR procedure to capture, concentrate and detect HAV recovered from inoculated produce, demonstrated that 10–50 PFU were needed to be present on the lettuce and/or the strawberries in order to produce a positive PCR signal (Fig. 4). Trials with filtering 50 ml of virus-seeded PBS (1 ml virus plus 49 ml PBS) through the virosorb filter and virus elution with 10 ml of 1% beef extract resulted in a 31.3% virus recovery rate, which was not significantly ($P > 0.05$) different from the rate obtained when 10 ml volumes of PBS were used (Table 2). Therefore, comparable rates of virus capture by and elution from the virosorb filters would be expected if larger volumes (e.g. ≥ 50 ml) were used as washing solutions to recover the virus from larger amounts of produce.

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