

## Expression in *E. coli* of the cloned cDNA for the major antigen of foot and mouth disease virus Asia 1 63/72

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MS received 24 February 1986

**Abstract.** Double stranded cDNA for the foot and mouth disease virus was prepared, restricted with *Bam*H1 or ligated to linkers with *Bam*H1 sticky ends and cloned in *Bam*H1 site in the expression vector, pUR222. The cDNA was also cloned at the *Pst*I site in the same vector by the dC/dG tailing method. They were transferred into *E. coli* to give colourless colonies in the presence of the dye, X-gal. Many of them showed positive signal on hybridization with <sup>32</sup>P-labelled viral RNA. The middle *Bam*H1 fragment of the cDNA is known to carry the gene for the major antigen and some non-structural proteins. The clones carrying the recombinant DNA produced proteins which cross-reacted with the antibodies generated against the structural proteins of the virus in an enzyme linked immunosorbent assay, indicating that the cDNA of the major antigen is expressed in the cloned cell.

**Keywords.** Foot and mouth disease virus; cDNA; cloning; antigen; expression.

### 1. Introduction

Foot and mouth disease is a contagious viral disease, affecting primarily cloven footed animals, causing great economic loss to our country. The virus is composed of a single strand RNA of 8 kb and 4 structural proteins of 60 molecules each forming the icosahedral capsid (Sanger 1979). The viral RNA has a poly A tail of length 8–40 at the 3' end, with small stretches of As in RNA (Grubman *et al* 1979a). Near the 5' end, there is a stretch of poly C of length 100–150 just after which the initiation of translation takes place. The RNA is covalently linked to the viral VPg of size 12 kd. The RNA is translated in the infected cells into a polypeptide of 250 kd, which is cleaved into precursor proteins, P20, P88, P52 and P100. The four structural proteins in the precursor P88 are arranged in the order H<sub>2</sub>N-VP<sub>4</sub>-VP<sub>2</sub>-VP<sub>3</sub>-VP<sub>1</sub>-COOH. The precursor proteins are further cleaved by viral specific protease to yield VPg, polymerase, protease, structural proteins and other non-characterized proteins (Grubman and Baxt 1982). The structural protein VP<sub>1</sub> is the main immunizing antigen and the variations in the amino acid sequences in this protein are shown to cause the strain differences (Bachrach *et al* 1975) resulting in 7 serotypes and over 60 subtypes distributed throughout the world (Beck *et al* 1983). Vaccine produced by inactivating the virus grown in tissue culture may have the chance of causing the disease in addition to the risk of disseminating the live virus during bulk handling. In order to produce the VP<sub>1</sub> in large quantities and to study the viral genome and their products, a double stranded cDNA for the RNA of Type Asia 1 virus was prepared and cloned in the expression vector, pUR222 at *Bam*H1 and *Pst*I sites by following three different methods. The expression of the VP<sub>1</sub> protein was

followed by an enzyme linked immunosorbent assay (ELISA) (Suryanarayana *et al* 1985).

## 2. Materials and methods

### 2.1 Materials

Foot and mouth disease virus (FMDV) Type Asia 1 63/72 (Prasad 1976), Mukteswar isolate, is maintained as a stock vaccine strain at the Indian Veterinary Research Institute, Bangalore.

Plasmid pUR222 and bacteria *E. coli* RR1 TΔ5 were obtained from Boehringer Mannheim, Germany.

### 2.2 Chemicals

Amino acids, vitamins, agarose, bovine serum albumin (BSA), horse raddish peroxidase (HRP), isopropyl- $\beta$ -thio-galactoside (IPTG), orthodianisidine dihydrochloride, dithio-threitol (DTT), sodium dodecyl sulphate (SDS) and polyvinylpyrrolidone (PVP) from Sigma Chemical Co., St. Louis, USA, RNase free sucrose, reverse transcriptase, DNA molecular weight markers (Hind III digested  $\lambda$  DNA) from Bethesda Research Laboratories, Gaithersburg, USA, RNasein, bacterial alkaline phosphatase from Enzo Biochem, USA, T<sub>4</sub> ligase, calf thymus terminal transferase, linkers,  $\gamma$ -<sup>32</sup>P-ATP, (specific activity 3000 Ci/mmol) <sup>3</sup>H-dCTP (50 Ci/mmol) from Amersham, England, restriction enzymes and oligo(dT) cellulose from Pharmacia, PL Biochemicals, Uppsala, Sweden, oligo(dT)<sub>12-18</sub>, proteinase K and T<sub>4</sub> polynucleotide kinase from Boehringer Mannheim, Germany, nitrocellulose filters, BA85 from Schleicher and Schuell Inc., Dassel, Germany, were used. All other chemicals used are of analytical grade.

### 2.3 Isolation of viral RNA

Single plaque of FMDV Asia 1 63/72 was grown in baby hamster kidney (BHK, clone 13) monolayers in 5 l Provitsky bottles at 37°C for 10–12 hr. The virus was precipitated with polyethylene glycol-6000 (PEG-6000) and purified by centrifuging through 17 ml of 10–50% linear sucrose gradient in 50 mM potassium phosphate buffer, pH 7.5, containing 0.2 M KCl and 10 mM EDTA at 46,600 g for 16 hr at 4°C (Wagner *et al* 1970). Fractions of 0.5 ml were collected and the three fractions at the A<sub>260 nm</sub> peak were pooled, diluted with the same buffer and the virus pelleted at 197,000 g. The pellet was suspended in 10 mM Tris-HCl, pH 7.4, containing 0.1 M NaCl and 1.5 mM MgCl<sub>2</sub>. The yield of the virus was 180  $\mu$ g/l culture. The RNA from the virus was extracted using the Proteinase-K method and purified by centrifuging through 15–30% (w/w) sucrose gradient in NETS buffer (100 mM NaCl, 1 mM EDTA, 100 mM Tris-HCl, pH 7.2, and 0.5% SDS) at 57,000 g, 17 hr at 23°C (Grubman *et al* 1979b). Fractions of 0.5 ml were collected and 5 of the A<sub>260 nm</sub> peak fractions were pooled, adjusted to 0.2 M with NaCl and the RNA precipitated with 2.5 vol of ethanol at –70°C. The viral RNA was further purified by oligo(dT) cellulose chromatography. The yield of the poly A<sup>+</sup> RNA was 40  $\mu$ g from 1 mg of virus.

## 2.4 Isolation of plasmid DNA

Plasmid pUR222 with or without the insert was isolated from the overnight culture in L. broth (1 l) of *E. coli* RR1 TΔ5 harbouring the plasmid grown in the presence of ampicillin (200 µg/ml) by alkali lysis method (Birnboim and Doly 1979) with modifications. The cells were pelleted by centrifugating at 4,000 g at 4°C for 5 min. The pellet was washed once with 200 ml ice-cold 0.1 M NaCl, 10 mM Tris-HCl, pH 7.8 and 1 mM EDTA, suspended in 20 ml of 50 mM glucose, 25 mM Tris-HCl, pH 8, 10 mM EDTA containing 100 mg of hen egg-white lysozyme and kept at 25°C for 5 min. Then, 40 ml of freshly prepared 0.2 N NaOH-1 % SDS were added, and the mixture kept on ice for 10 min. An ice-cold solution of 5 M potassium acetate (30 ml), pH 5.0, was added, spun at 12,000 g for 20 min and the crude plasmid DNA in the supernatant solution was precipitated with 0.6 vol of isopropanol at 25°C. The precipitate was washed once with ethanol (70 %), dried in vacuum and dissolved in 16 ml of 10 mM Tris-HCl, pH 8.0, containing 1 mM EDTA (TE) and extracted once with phenol saturated with 3 % NaCl and then once with chloroform. The aqueous phase was treated with RNase A Type II Sigma (5 µg/ml) for 1 hr at 25°C. The RNase A was previously kept at 85°C for 10 min. The solution was then adjusted to 0.5 % with SDS and treated with Proteinase K (200 µg/ml) at 37°C for 1 hr and extracted once with phenol and then with chloroform, adjusted to 0.3 M with respect to sodium acetate, pH 5.2, and the DNA precipitated with 2 vol of ethanol at -20°C overnight. The DNA was collected by centrifugation at 20,000 g for 30 min, washed with 70 % ethanol, dried in vacuum and dissolved in 0.6 ml TE. The plasmid DNA was further purified by gel filtration through Sepharose 4B column, using TE containing 0.6 M NaCl. The  $A_{260\text{ nm}}$  peak fractions in the void volume were pooled and the DNA was precipitated with ethanol, washed, dried and dissolved in 200 µl TE. The yield was 600 µg/l of the culture.

## 2.5 Kinasing the DNA and RNA

The RNA (1 µg) was treated with bacterial alkaline phosphatase (1 unit) in a total volume of 50 µl of 10 mM Tris-HCl, pH 8.0 and incubated at 65°C for 30 min, deproteinized with phenol-chloroform (1:1 v/v) and the RNA was precipitated in 0.2 M NaCl with 2.5 vol of ethanol at -70°C. The RNA was dissolved in 10 µl water, heated at 72°C for 3 min, chilled in ice, and incubated with 30 µCi of  $\gamma$ -<sup>32</sup>P-ATP in 50 µl of 70 mM Tris-HCl, pH 7.6, 10 mM MgCl<sub>2</sub>, 5 mM DTT and 2 units of T<sub>4</sub> polynucleotide kinase for 30 min at 37°C (Richardson 1971). It was then diluted with 100 µl of water and passed through Sephadex G 25 to remove the ATP and salts. The radioactive peak fractions at the void volume were pooled and concentrated to 200 µl by extraction with *n*-butanol, thrice.

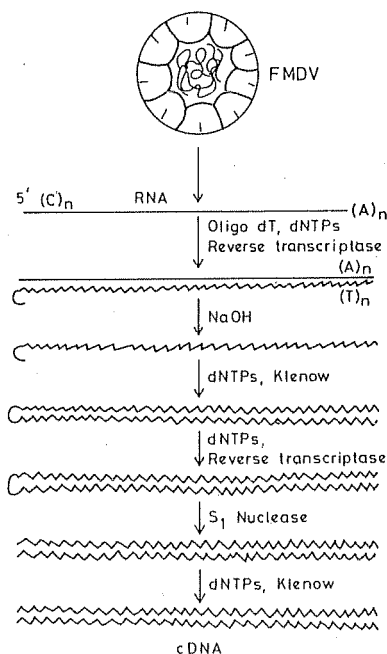
The cDNA was kinased by the exchange reaction (Beckner and Folk 1977) at 37°C for 1 hr with 30 µCi of  $\gamma$ -<sup>32</sup>P-ATP, 1 mM ADP and T<sub>4</sub> polynucleotide kinase (2 units) in a reaction volume of 50 µl of 50 mM imidazole-HCl, pH 6.6, 10 mM MgCl<sub>2</sub>, 5 mM DTT, 0.1 mM spermidine and 0.1 mM EDTA. The DNA was deproteinized and absorbed on DEAE Sephacel (1.8 ml column) and eluted with 0.6 M NaCl, 10 mM Tris-HCl, pH 8.0, containing 1 mM EDTA. The fractions of peak  $A_{260\text{ nm}}$  were pooled and the DNA was precipitated with two volumes of ethanol at -20°C.

The deoxynucleotide (1 µg) was kinased by the forward reaction (Maniatis *et al* 1978)

by incubating with 2 units of  $T_4$  polynucleotide kinase at  $37^\circ\text{C}$  for 1 hr in a total volume of  $10\ \mu\text{l}$ , containing 66 mM Tris-HCl, pH 7.6, 1 mM ATP, 1 mM spermidine, 10 mM  $\text{MgCl}_2$ , 15 mM DTT and 200  $\mu\text{g}/\text{ml}$  BSA.

## 2.6 Preparation of cDNA

The cDNA was prepared according to the procedure of Maniatis *et al* (1982) with some modifications (figure 1). The purified viral RNA ( $10\ \mu\text{g}$  in  $10\ \mu\text{l}$  of water) was heated at  $70^\circ\text{C}$  for 3 min, cooled in ice, and then mixed with  $5\ \mu\text{g}$  of oligo(dT) as primer, 40  $\mu\text{g}$  BSA, 50  $\mu\text{Ci}$  of  $^3\text{H}$ -dCTP (specific activity 50 Ci/mmol) and 100 units of AMV reverse transcriptase, 25 units of RNasein in the presence of 1 mM deoxynucleoside triphosphate in a total volume of  $50\ \mu\text{l}$  of 100 mM Tris-HCl, pH 8.3, 10 mM  $\text{MgCl}_2$  and 50 mM KCl, and incubated at  $42^\circ\text{C}$  for 90 min. The reaction was stopped by adding  $2\ \mu\text{l}$  of 0.5 M EDTA, pH 8.0, followed by  $25\ \mu\text{l}$  of 150 mM NaOH and incubated at  $65^\circ\text{C}$  for 1 hr to hydrolyse the RNA template. The DNA was extracted once with an equal volume of phenol-chloroform (1:1 v/v), passed through a Sephadex G-150 column, the radioactive peak fractions in the void volume pooled, an equal volume of 4 M ammonium acetate added and the DNA precipitated with 2.5 vol of ethanol at  $-70^\circ\text{C}$ . The precipitate was washed with 70% ethanol, dried in vacuum and dissolved in  $50\ \mu\text{l}$  of water. It was converted into double stranded cDNA by incubating with 25 units of Klenow fragment of *E. coli* DNA polymerase I for 24 hr at  $15^\circ\text{C}$ . The reaction was stopped by adding  $2\ \mu\text{l}$  of 0.5 M EDTA and the cDNA was extracted once with phenol-

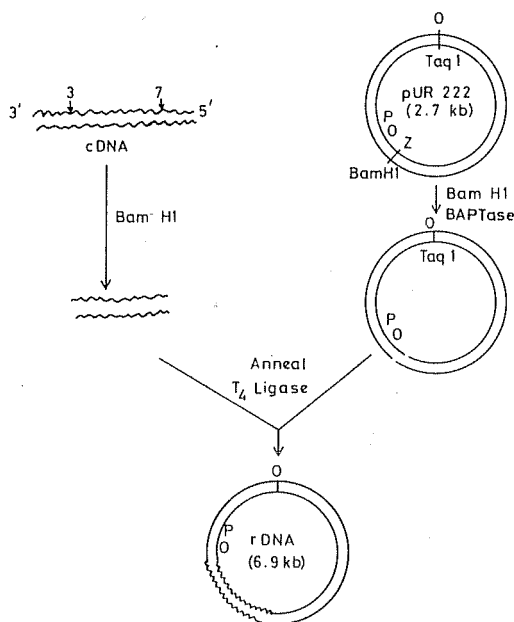


**Figure 1.** Various steps in the preparation of cDNA: The double stranded cDNA was prepared by using reverse transcriptase and DNA polymerase detailed under §2 (materials and methods).

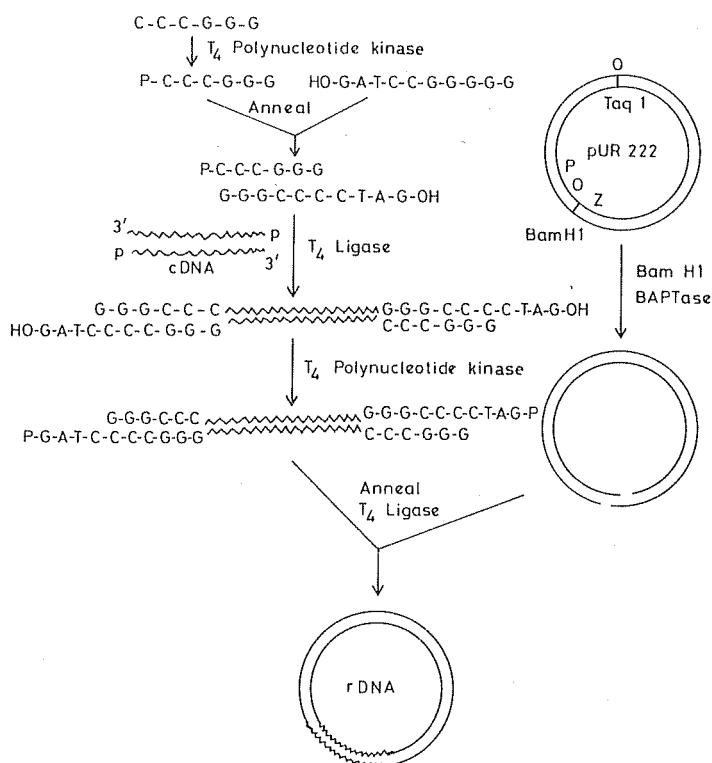
chloroform (1:1 v/v) and passed through the Sephadex G-150. Fractions of 0.5 ml were collected, the 5 fractions at the void volume having maximum radioactivity pooled and the cDNA precipitated with ethanol, washed, dried and dissolved in water. This was incubated further at 37°C with reverse transcriptase as described before in the absence of oligo (dT) primer, RNasein, for filling up the gaps.

## 2.7 Preparation of recombinant DNA

The double strand cDNA preparation was divided into three equal portions. The cDNA in one portion was restricted with *Bam*H1 (1 unit), deproteinased and ligated to the *Bam*H1 dephosphorylated pUR222 DNA (1 µg) as followed by Thomas *et al* (1983) (figure 2). The *Hpa*II linker, 5'-dCCCGGG-3' (1 µg) was kinased with ATP and *T*<sub>4</sub> polynucleotide kinase and annealed to 5'-dGATCCCCGGG-3' (1 µg) in 10 µl of 100 mM NaCl, 10 mM Tris-HCl, pH 7.8, 0.1 mM EDTA by incubating at 65°C for 5 min, 50°C for 1 hr, and then at room temperature (Bahl *et al* 1976). The preadappter thus obtained was then ligated to the cDNA by incubating at 4°C for 16 hr with *T*<sub>4</sub> ligase (10 units) in a reaction volume of 40 µl containing 25 mM Tris-HCl, pH 7.4, 5 mM MgCl<sub>2</sub>, 5 mM DTT, 0.25 mM spermidine, 1 mM ATP, 1.25 mM hexamine cobalt chloride and 10 µg/ml BSA. It was then kinased by the forward reaction and ligated to the *Bam*H1 restricted, dephosphorylated pUR222 DNA to get covalently circular recombinant molecules (figure 3). The cDNA in the third fraction was tailed with (dC) residues using calf thymus terminal transferase (5 units) at 37°C for 90 s in a reaction mixture of 40 µl containing 100 mM potassium cacodylate, pH 7.2, 2 mM CaCl<sub>2</sub>,



**Figure 2.** Insertion of the *Bam*H1 fragment of cDNA at the *Bam*H1 site: The *Bam*H1 restricted cDNA was annealed and ligated with the *Bam*H1 restricted and dephosphorylated pUR222 DNA as described in the text.



**Figure 3.** Insertion of full length cDNA using *Bam*H1 adaptor: The *Bam*H1 adaptors were ligated to both ends of the cDNA, annealed and ligated to the *Bam*H1 sticky ends of the plasmid pUR222 DNA as described under §2.

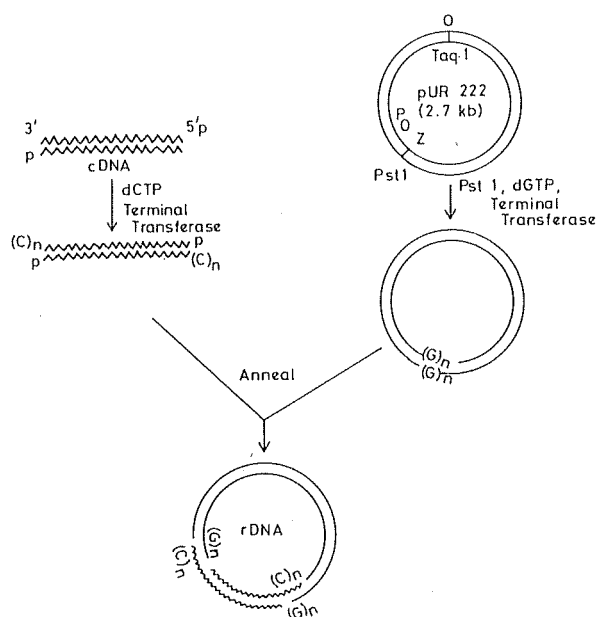
0.2 mM DTT and 1 mM dCTP (Choudhary *et al* 1976). The dC tailed cDNA was deproteinased and annealed to the *Pst*I dG tailed pUR222 plasmid DNA (1 µg) to get recombinant DNA molecules (figure 4).

## 2.8 Transformation

The recombinant DNA molecules were transferred into the *E. coli* RR1 TA5 according to the method of Mandel and Higa (1970) as followed by Thomas *et al* (1983) with some modification. The recombinant DNA was mixed with sensitized *E. coli* cells (0.1 ml, 0.02 *A*<sub>650 nm</sub>), kept on ice for 30 min and then at 37°C for 2 min, diluted to 1 ml with L. broth, and grown at 37°C for 1 hr. It was diluted ten fold with L. broth and 0.1 ml was plated and incubated at 37°C for 16–18 hr on a L. agar plate containing ampicillin (200 µg/ml), IPTG (0.2 mM) and X-gal (40 µg/ml).

## 2.9 Identification of colonies

The colourless colonies were screened by colony hybridization according to Grunstein and Hogness (1975) with some modification. The recombinant clones were transferred



**Figure 4.** Insertion of the full length cDNA by dC/dG tailing: The cDNA was tailed with dC using calf thymus terminal transferase and the *Pst*I restricted pUR222 DNA was tailed with dG and annealed as described under §2.

on to nitracellulose filters kept on moist L. agar plates containing ampicillin (200 µg/ml), grown at 37°C till they reached 3–4 mm in size and lysed, and the DNA denatured with 0.5 M NaOH containing 1.5 M NaCl. Then, the alkali was neutralised with 1 M Tris-HCl, pH 7.4 and the filter transferred to fresh layers of Whatman 3 MM filter paper soaked in 0.5 M Tris-HCl, pH 7.4, containing 1.5 M NaCl. The filter was air dried, baked at 80°C for 3 hr under vacuum, soaked in a pre-hybridization mixture containing 6 × SSC (150 mM NaCl and 15 mM Na<sub>3</sub> citrate, pH 7.0), 5 × Denhardt's solution (1.0 g Ficoll, 1.0 g PVP and 1.0 g BSA in 1 l of 3 × SSC) and 0.1 % SDS, incubated for 8 hr at 48°C. The filter was then placed in hybridization mixture containing 6 × SSC, 5 × Denhardt's solution, 10 mM Tris-HCl, pH 7.5, 5 mM EDTA, 0.5 % SDS, 50 % deionized formamide and <sup>32</sup>P-labelled FMDV RNA (15 × 10<sup>6</sup> cpm) and hybridized at 40°C for 48 hr in a sealed Petri dish. The filter was washed thoroughly (four times, 15 min each time) with 2 × SSC containing 0.1 % SDS and subjected to autoradiography using Indu X-ray film at –70°C for 12 hr.

## 2.10 Preparation of cell extract

The bacteria harbouring the plasmid with or without the cDNA was grown overnight in 250 ml of L. broth containing 1 mM of IPTG at 37°C and treated with 2 % CHCl<sub>3</sub> for 5 min at 37°C and centrifuged at 4,000 g for 10 min. The pellet was washed once with phosphate buffered saline (PBS) (containing 137 mM NaCl, 3 mM KCl, 6 mM Na<sub>2</sub>HPO<sub>4</sub> and 1 mM KH<sub>2</sub>PO<sub>4</sub>), pH 7.2, and then suspended in 5 ml PBS, kept on ice

and sonicated twice with a 30 s pulse. The lysate was centrifuged at 30,000 g for 20 min and the supernatant fraction collected.

### 2.11 Preparation and purification of antibody

Antiserum in hamsters against FMDV Asia I was raised by injecting the purified and inactivated virus (25 µg) subcutaneously using complete Freund's adjuvant. Hyperimmune serum in guinea pigs against Asia I virus was raised by repeated injections at 21, 50 and 80 days after the first injection of the guinea pig passaged live virus (0.25 ml of the 1% of the foot pad extract) according to the method of Brooksby (1952). The IgG from both preparations were precipitated with 40% ammonium sulphate at room temperature, dissolved in PBS, and dialysed against the same buffer overnight at 4°C. The antibodies (5 mg protein/ml) were purified by affinity chromatography using the extracts of the *E. coli* harbouring the plasmid pUR222 immobilized to CNBr activated Sepharose (10 mg/ml) (Erlach *et al* 1979). Anti-guinea pig rabbit antibodies were prepared by injecting guinea pig IgG (200 µg protein/ml) in Freund's incomplete adjuvant (1 ml) into rabbits at weekly intervals, after 21 days of the first injection. After the sixth injection, the rabbits were bled and the serum was collected and the anti-guinea pig rabbit antibodies purified by immunoaffinity column using the normal guinea pig IgG coupled to the CNBr activated Sepharose 4B. The bound anti-guinea pig rabbit IgG was eluted with 0.1 M glycine-HCl, pH 2.5, containing 0.5 M NaCl and fractions of 1 ml were collected on 20 mg of solid Tris. The  $A_{280\text{ nm}}$  peak fractions were pooled and concentrated by PEG. It was conjugated to HRP (Type VI) as described by Wilson and Nakane (1978) with modifications. Horse raddish peroxidase (5 mg) in 1 ml of 1 mM sodium acetate, pH 4.2, and 0.1 M NaCl, was treated with 0.2 ml of 0.1 M NaIO<sub>4</sub> in the same buffer, stirred for 20 min at room temperature and dialyzed against acetate buffer overnight at 4°C. The pH of the solution was raised to 9–9.5 by the addition of 40 µl of 0.2 M sodium carbonate buffer, pH 9.5, containing 0.1 M NaCl and treated with rabbit IgG (10 mg protein/ml) in 0.10 M sodium carbonate buffer, pH 9.5, containing 0.1 M NaCl. The reaction mixture was stirred for 2 hr at room temperature, 0.1 ml of freshly prepared sodium borohydride (4 mg/ml water) was added and the mixture was kept overnight at 4°C, and gel filtered through on Sephadex G-200 using PBS. Fractions (4 ml) were collected and the fractions having a maximal  $A_{403\text{ nm}}/A_{280\text{ nm}}$  ratio of 0.3–0.6 were pooled and made 1% with respect to BSA, aliquots of 0.2 ml were frozen and stored at –20°C. The conjugation of the anti-guinea pig rabbit IgG with HRP was almost 95%.

### 2.12 Enzyme linked immunosorbent assay

The antigenic protein was detected by following sandwich ELISA (Abu Elzein and Growther 1978). Dyantech Immulon Remova Polystyrene wells of 0.3 ml were coated with anti-Asia I hamster IgG by keeping the IgG (2 µg protein) in 0.2 ml of 0.1 M carbonate buffer, pH 9.5, overnight at 4°C. The wells were washed thrice, for 3 min each time, with PBS containing 0.05% Tween-20 and the leftover sites in the wells were saturated with BSA by keeping 3% BSA in PBS overnight at 4°C. The bacterial extract (100 µl, 800 mg protein) in PBS-Tween-20 containing 1.5% BSA was added and



incubated at 37°C for 3 hr. The wells were washed thrice, 100  $\mu$ l of anti-Asia I guinea pig IgG (1  $\mu$ g protein), pre-titrated in an ELISA test and diluted to 1:600 with PBS-Tween-containing 3% BSA, was added and incubated at 37°C for 1 hr. The solution in the wells was decanted, the wells were washed with PBS-Tween and incubated with 0.2 ml of 0.02%, 3,3' orthodianisidine dihydrochloride in 50 mM sodium acetate buffer, pH 5.0, containing 0.02% H<sub>2</sub>O<sub>2</sub> for 1 hr at 25°C. The reaction was stopped by adding 5 N HCl (25  $\mu$ l) to a final concentration of around 0.5 M. The colour developed was followed by  $A_{400\text{ nm}}$ .

### 3. Results and discussion

#### 3.1 Poly A<sup>+</sup> RNA

On sucrose density gradient centrifugation of the FMDV preparation, a symmetrical peak at a 146 S value was obtained showing that the viral preparation is homogeneous. The profile of the isolated RNA on sucrose density gradient showed a major as well as a minor peak (figure 5). The RNA in the major peak was highly infective in BHK cells, while the one in the minor peak was non-infective like the RNA from other strains (Grubman *et al* 1979b). The RNA (140  $\mu$ g) from the major peak was dissolved in the binding buffer (10 mM Tris-HCl, pH 7.2, 0.5 M NaCl and 0.5% SDS) and the poly A<sup>-</sup> and poly A<sup>+</sup> RNA were separated by oligo(dT) cellulose column chromatography (figure 6). The poly A<sup>-</sup> RNA containing RNA less than 10 A residues was about 75% and the poly A<sup>+</sup> RNA containing more than 10 A residues was about 25%. The poly A at the 3' end has no effect on infectivity (Baxt *et al* 1979). The poly A<sup>+</sup> RNA was precipitated in 0.2 M NaCl with alcohol, washed, dried and dissolved in water.

#### 3.2 Size of the cDNA

Analysis of the <sup>32</sup>P-labelled cDNA by electrophoresis on 0.6% agarose using *Hind*III  $\lambda$  DNA fragments as molecular weight markers and subsequent autoradiography showed

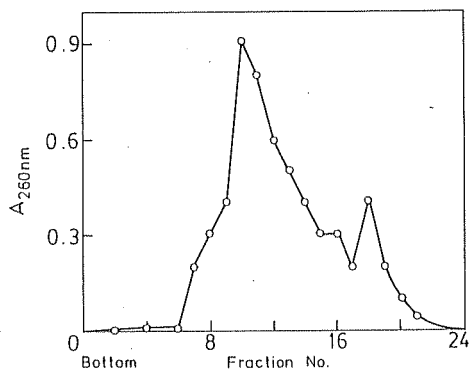
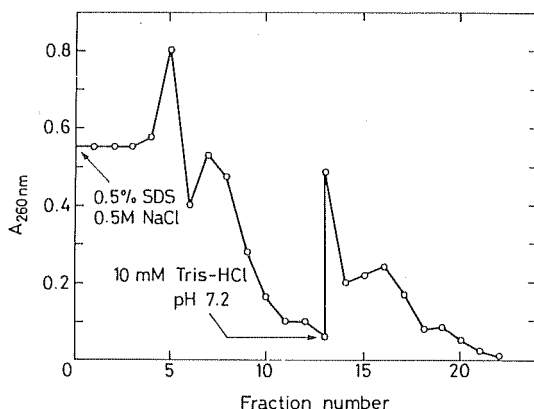


Figure 5. Profile of the FMDV RNA on sucrose density gradient centrifugation: The isolated RNA was purified by centrifuging through 15–30% (w/w) sucrose density gradient. Fractions of 0.5 ml were collected and the  $A_{260\text{ nm}}$  was followed.



**Figure 6.** Separation of poly A<sup>+</sup> RNA by oligo(dT) cellulose chromatography: The poly A<sup>+</sup> and poly A<sup>-</sup> RNA from the purified FMDV RNA were separated by oligo (dT) cellulose chromatography. The poly A<sup>-</sup> RNA was eluted with 10 mM Tris-HCl, pH 7.2, 0.5 M NaCl, 0.5% SDS and the bound poly A<sup>+</sup> RNA was eluted with 10 mM Tris-HCl pH 7.2.

a dark band corresponding to about 7 kb size. The smaller fragments might have been removed by the Sephadex G-150 gel filtration of the cDNA preparation. The RNA of FMDV strain O<sub>1</sub>K has a length of 8 kb including the poly C and the poly A tracts (Grubman *et al* 1979a). The length of 7 kb indicates the near full length cDNA of the FMDV that was obtained in the reverse transcript reaction.

### 3.3 Transformation

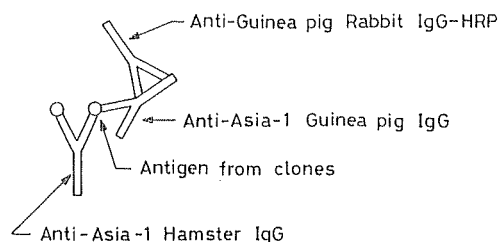
The recombinant DNA molecules obtained by insertion of *Bam*H1 fragments of the cDNA at the *Bam*H1 site, the linker ligated cDNA annealed to *Bam*H1 sticky ends and the dC/dG tailed cDNA at *Pst*1 site were transferred into the *E. coli* RR1 TA5 host according to the method of Mandel and Higa (1970) as followed by Thomas *et al* (1983). Since pUR222 is an expression vector with the  $\beta$ -galactosidase gene and its promoter, any recombinant will generate colourless colonies on X-gal plates (Miller 1982). Seventy two and 27 colourless colonies were obtained from the insert DNA at the *Bam*H1 and *Pst*1 sites respectively. There were a few blue colonies showing the absence or rejection of the foreign DNA in the plasmid vector.

### 3.4 Identification of colonies

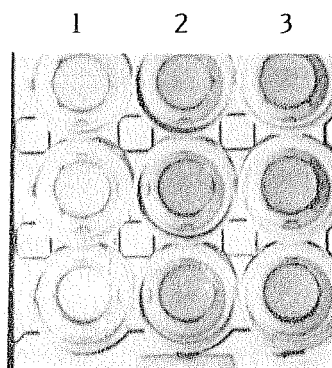
The clones containing the recombinant DNA were grown on a nitrocellulose filter and hybridized to 5' labelled <sup>32</sup>P-FMDV RNA. There were 13 colonies showing strong signals on hybridization, 2, 5 and 6 colonies containing *Bam*H1 restricted cDNA fragments, *Bam*H1 adapter ligated cDNA and tailed cDNA, respectively.

### 3.5 Expression of the antigen in clones carrying cDNA

The antigenic protein was detected by the sandwich ELISA. The polystyrene wells were coated with anti-Asia I hamster IgG which was reacted with the protein extracts of



**Figure 7.** Schematic representation of the ELISA: The polystyrene wells were coated with anti-Asia I hamster IgG which was reacted with the antigenic protein produced by the clones, followed by the reaction with anti-Asia I guinea pig IgG and rabbit anti-guinea pig IgG conjugated with HRP.



**Figure 8.** ELISA for the antigenic protein: The bound HRP in the ELISA reaction (figure 7) was assayed by the reaction with orthodiansidine dihydrochloride and  $H_2O_2$  and the  $A_{400nm}$  followed. The colour developed was photographed as described under §2.

*E. coli* cells harbouring the plasmid with or without the insert (figure 7). It was further reacted with the purified and diluted anti-Asia I guinea pig IgG and then treated with rabbit anti-guinea pig IgG, conjugated with HRP. The bound enzyme was assayed by the reaction with orthodiansidine dihydrochloride and  $H_2O_2$ . The reaction was stopped by the addition of HCl and the reddish brown colour developed was followed by  $A_{400nm}$  and photographed (figure 8). The extracts from the clones carrying the cDNA gave  $A_{400nm}$  of about 0.6 (rows 2 and 3), while the controls, the clones carrying pUR222 without any cDNA insert as well as Type C and O viruses which do not cross-react with the antibodies raised against the Asia 1 virus gave the  $A_{400nm}$  of 0.05 (row 1). Thus the *Bam*H1 inserts as well as the complete cDNA clones were transcribed and translated into the antigenic protein which gave immunoreactivity in the ELISA.

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