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Rapid methodology for antigenic profiling of FMDV field strains and for the control of identity, purity and viral integrity in commercial virus vaccines using monoclonal antibodies

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ABSTRACT

Monoclonal antibodies (MAbs) developed against different foot-and-mouth disease virus (FMDV) vaccine strains were extensively used to study any possible antigenic variations during vaccine production in Argentine facilities. Additionally, a typing ELISA using strain specific MAbs was developed to detect potential cross contaminations among FMDV strains in master and working seeds with high specificity and sensitivity and to confirm strains identity in formulated vaccines. This assay was carried out for the South American strains currently in use in production facilities in Argentina (A24/Cruzeiro, A/Argentina/01, O1/Campos and C3/Indaial) and for the strain O/Taiwan, produced only for export to Asia. These non-cross reactive MAbs were also used to analyze the integrity of viral particles belonging to each one of the individual strains, following isolation of 140S virions by means of sucrose density gradients from the aqueous phase of commercial polyvalent vaccines. Antigenic profiles were defined for FMDV reference strains using panels of MAbs, and a coefficient of correlation of reactivity with these panels was calculated to establish consistent identity upon serial passages of master and production seeds. A comparison of vaccine and field strain antigenic profiles performed using coefficients of correlation allowed the rapid identification of two main groups of serotype A viruses collected during the last FMD epidemic in Argentina, whose reactivity matched closely to A/Argentina/2000 and A/Argentina/2001 strains.

1. Introduction

Foot-and-mouth disease virus (FMDV), a member of the genus Aphthovirus, is probably the most detrimental virus for cloven-hoofed mammals and is responsible for large economic losses for both cattle breeders and meat industry (reviewed by Grubman and Baxt, 2004). FMDV presents large antigenic variability due to the high mutation rate of its genomic RNA that takes place during its replication cycle (Diez et al., 1989; Domingo et al., 2003; Gebauer et al., 1988). In endemic areas, this phenomenon is responsible for the periodical appearance of FMDV field strains with novel antigenic characteristics, which demands precise and fast monitoring of viral isolates in order to allow efficient epidemiological studies leading to the adequate vaccine updating (Bergmann et al., 1988; Costa Giomi et al., 1982; Mattion et al., 2004). It has also been suggested that host cells themselves play a significant role in the selection of FMDV variants in culture (Giraudo et al., 1987). This could be particularly relevant during vaccine production where different cell systems, such as BHK-21 cells (Capstick et al., 1962) or bovine tongue tissue (BTT) cultures (Frenkel, 1951) are used for large scale virus replication.

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The identification and typing of prototype and new emerging FMDV field strains had been performed by complement fixation (CF; Davie, 1964) and seroneutralization (SN; Rweyemamu et al., 1978) tests. The introduction of ELISA (Kitching et al., 1988) in combination with FMDV MAbs have provided very fast and more sensitive methodology for the characterization of field and vaccine strains (Crowther, 1993; Crowther et al., 1995; Grubman and Morgen, 1986; Mc Cullough et al., 1987; Thomas et al., 1988; Van Maanen and Terpstra, 1990).

In countries where vaccination is practiced, vaccine production requires facilities complying with Good Manufacturing Practices (GMP) and demands appropriate specific tools for quality control processes. Different assays have been developed for in-process and final vaccine batch control, which proved to be very useful to help meet quality standards. The evaluation of potential cross contaminations among viral strains at early stages of the production process, in master seeds, subcultures or in vaccine batch formulations, together with the control of viral particles integrity is of great importance to assure vaccine quality. In this regard, degradation of viral particles during the antigen concentration processes was reported as one of the main causes of vaccine failure, when using either polyethilenglycol (PEG) precipitation or ultrafiltration methods (Doel and Collen, 1982, 1983; Doel and David, 1984).

In 1999, when vaccination was stopped in Argentina, FMDV vaccines were formulated with the strains A/Argentina/79, A/Argentina/87, C3/Argentina/85 and O1/Argentina/Caseros, all of them isolated from local FMD outbreaks. Shortly after vaccination was stopped, FMDV was reintroduced in the country. Emergency vaccination was initially implemented with tetravalent available vaccines (Mattion et al., 2004). Since January 2001, the virus strain A/Argentina/00 was incorporated into this vaccine together with A24/Cruzeiro and O1/Campos strains. Twice-yearly systematic vaccination campaigns were started again in April 2001. A tetravalent vaccine was subsequently developed including strains O1/Campos, A24/Cruzeiro and the new strains A/Argentina/00 and A/Argentina/01 (Mattion et al., 2004). In the year 2005, the strain C3/Indaial was included in the vaccine formulations, while strain A/Argentina/00 was eliminated.

For many years, our laboratory have used the technique described by Crowther (1993), using panels MAbs developed against local and South American FMDV strains to analyze the antigenic profiles of samples provided by vaccine producers and by the National Animal Health Service (SENASA). Using the criteria of Samuel et al. (1991), no significant differences could be detected among the antigenic profiles of master and production seeds belonging to the same strain. This technique became very valuable, because once the antigenic profile of each strain was established, it was possible to follow its evolution across the different steps of vaccine production (unpublished data).

In order to complement this analysis, a typing assay was developed using strain specific MAbs (only reacting with the virus to which the MAbs were originally raised) to detect cross contaminations during the vaccine manufacturing process. Both techniques, determination of antigenic profiles and strain typing, had been important tools to rapidly assess the identity and purity of the vaccine antigens.

In this work, we report several validation steps of the typing assay for the local and regional vaccine strains manufactured in Argentina at present (A24/Cruzeiro, A/Argentina/01, O1/Campos and C3/Indaial and for the Asian strain O/Taiwan) and the analysis of the integrity of viral particles in intermediate vaccine bulks and in final polyvalent formulations. We also report the use of MAb antigenic profiles for the rapid analysis and comparison of field isolates with reference strains.

2. Materials and methods

2.1. Viral strains and vaccine antigens

Local FMDV vaccine strains and field isolates were provided by SENASA: O1/Caseros/Argentina/68 (O1/Caseros), A/Argentina/79 (A/Arg/79), A/Argentina/87 (A/Arg/87), A/Argentina/2001 (A/Arg/01), A/Argentina/2000 (A/Arg/00) and C3/Argentina/85 (C3/Arg/85). South American strains A24/Cruzeiro/Brazil/55 (A24/Cruzeiro), C3/Indaial/Brazil/71 (C3/Indaial), C3/Resende/Brazil/55 and O1/Campos/Brazil/58 (O1/Campos) were supplied by the Pan American Foot-and-mouth Disease Center (PANAFTOSA, Brazil). O/Taiwan/97 (O/Taiwan) strain was imported by SENASA. Viruses were grown on BHK-21 clone 3 (BHK-21c3) cell cultures, harvested, frozen, thawed and clarified by centrifugation for 10 min at 2000 × *g*. The supernatants were titrated and stored at −20 °C in 50% glycerol. The identity of viral strains used for production of MAbs was originally controlled by RNAse T1 maps (Auge de Mello et al., 1986; La Torre et al., 1982) and later by sequencing (König et al., 2001; Mattion et al., 2004).

Inactivated vaccine antigens were provided either by vaccine manufacturers or by SENASA. Viral particles from oil adjuvanted vaccines were recovered from the aqueous phases after the rupture of the water-in-oil emulsions with chloroform. Briefly, one volume of vaccine was extracted overnight with equal volume of chloroform at 4 °C, followed by centrifugation at 10,000 rpm for 15 min.

2.2. Polyclonal serum and monoclonal antibodies

Specific mouse and rabbit antisera for A24/Cruzeiro, A/Arg/79, A/Arg/87, A/Arg/01, O1/Campos, C3/Indaial and C3/Arg/85 viral strains were prepared by subcutaneous inoculations of 50 μg of 140S FMDV particles purified in sucrose density gradients, and emulsified in complete or incomplete Freund's adjuvant (Periolo et al., 1993). The first inoculation was accomplished with 50 μg of purified particles in complete Freund adjuvant. Subsequent inoculations within 20 days intervals were prepared in incomplete Freund adjuvant. Antibody titers were measured by ELISA. Animals were bled out when titers were higher than 1:8000. In all cases, animal handling and management was performed following institutional welfare established procedures.

MAbs were produced using standard protocols (Earley and Rener, 1985). Briefly, Balb/C mice were inoculated intraperitoneally with 10–100 μg of sucrose gradient purified 140S particles emulsified in complete Freund's adjuvant. Subsequent inoculations with virus emulsified in incomplete Freund's adjuvant were performed at 20 days intervals. Mice were boosted 3 days before fusion. Spleen cell pools from two or more mice were fused with myeloma Sp2/0 cells. Supernatants of hybridomas were selected by ELISA. Neutralizing capacity of the supernatants with homologous virus was tested on BHK-21c3 cell cultures. Titers higher than 1:8 were considered as positives. Reactivity with reference strains and field isolates were performed by ELISA as described (Crowther, 1993; Mc Cullough et al., 1987; Samuel et al., 1991). Reactivity with intact virions (140S) and 12S virus subunits was analyzed with a sandwich ELISA, using MAbs for antigen capture and specific guinea pig polyclonal sera as detectors (data not shown). The reactivity of MAbs with linear trypsin sensitive and non-linear epitopes was studied by Western blot under denaturing conditions and isotypes were determined using a commercial kit (Bio-Rad, Hercules, CA). Selected hybridomas were cloned and MAbs stored at −20 °C in small volumes before being used in the profiling assays.

The MAbs generated are shown in Table 1. They are grouped according to the strain from which they were originated. Some properties, such as capacity to neutralize

Table 1
Monoclonal antibodies against FMDV strains

Strain[a]	MAb	Neut	WB	Isot	A24Cr	A79	A87	A2000	A2001	C85	C3 Res	C3 Ind	O1Cas	O1Cam	O Taw
A24 Cruz	4A2	+	+VP1	IgG2a											
	2C2	+	+VP1	IgM											
	1E12	+	+VP1	IgM											
	4B4	+	-	IgM											
	3C3	+	-	IgG2a											
	3D9	+	-	IgG2b											
	2E7	+	-	IgM											
A79	407	+	+VP1	IgM											
	205	+	+VP1	IgG2a											
	211	+	+VP1	IgM											
	109	+	+VP1	IgG2a											
	212	+	+VP1	IgA											
	206	+	+	IgG2a											
	110	+	-	IgG2a											
	7	-	-	IgG1											
	13	-	-	IgM											
	112	-	-	IgM											
A87	317	+	+VP1	IgG2b											
	408	+	+VP1	IgG1											
	220	+	+VP1	IgG2b											
	108	+	+VP1	IgM											
	211	+	-	IgG2a											
	20	+	-	IgG2a											
	403	+	-	IgG2a											
	105	-	-	IgM											
	405	-	-	IgG1											
	111	-	-	IgG2b											
	204	-	-	IgG1											
A/01	2-2	nd	-	IgM											
	3-2	nd	-	IgG3											
	1-2	nd	-	IgM							nd				nd
	2-6	-	-	IgM							nd				nd
	3-3	+	-	IgG1							nd				
	2-4	nd	-	IgG1							nd				nd
	1-5	nd	-	IgG1							nd				nd
C3 Res	D7	-	-	IgG2a											
	G1	-	-	IgG1											nd
	A1	-	-	IgG1											nd
	B3	-	-	IgG1											nd
	111	-	-	IgG1											nd
	B4	-	-	nd											nd

Strain[a]	MAb	Neut	WB	Isot	A24Cr	A79	A87	A2000	A2001	C85	C3 Res	C3 Ind	O1Cas	O1Cam	O Taw
C3 Arg/85	43	+	+VP1	IgM											
	9	+	-	IgM											
	14	+	-	IgG2b											
	26	+	-	IgG2b											
	56	-	-	IgG2a											
	61	-	-	IgM											
	89	-	-	IgM											
C3 Ind	3-2E	nd	-	IgM											
	1F12	nd	-	IgG2b											
O1 Cas	8G	+	+VP1	IgG2a											
	3A	+	+VP1	IgG2a											
	13	+	-	IgG2b											
	74	+	-	IgG2a											
	2	-	-	IgG1											
	3	-	-	IgG1											
	69	-	-	IgG2a											
	1-1E	nd	-	IgM											
	2-6F	nd	-	IgG1											
	4-3C	nd	-	IgG1											
	4-12H	nd	-	IgM											
O1 Cam	17	+	+VP1	IgM											
	G8	+	+VP1	IgG2b											
	F11	-	-	IgG1											
	D3	-	-	IgG1											
O Taw	3A1	nd	-	IgG2A							nd				
	3D1	-	-	IgG1							nd				
	4B2	-	-	IgG1							nd				
	1A11	nd	-	IgG1							nd				
	2C2	-	-	IgG1							nd				
	2F8	nd	+	IgG2a							nd				
	1B3	nd	-	IgG2a							nd				
	1B9	-	-	IgG1							nd				
	2C8	-	-	IgM							nd				
	2D4	-	+	IgM							nd				
	2E8	nd	-	IgM							nd				
	3A7	nd	-	IgM							nd				
	2C9	-	-	IgG1							nd				
	3A2	-	-	IgG1							nd				
	3G10	-	-	IgG2b							nd				

a: Strain of origin; N: neutralizing; Isot: Isotype; wb: western blot; Reactivity: 76-124% 46-75% 20-45% < 20%

[a]Strain of origin; N: neutralizing; Isot: isotype; wb: Western blot (■ 76–124%, ■ 46–75%, ■ 20–45%, □ <20%).

the strain of origin, reaction in Western blots and isotypes are shown. To the right, ELISA reactivity with several strains is presented as a percentage of homologous reactivity, which has been considered as 100%. More data generated on the characterization of these MAbs is available upon request.

2.3. Trapping ELISA

Microtitre ELISA plates (Maxisorb, Nunc) were coated with strain specific rabbit hyperimmune serum (1:4000) in carbonate–bicarbonate buffer, 0.05 M, pH 9.6, overnight at 4 °C. Virus strains trapped by specific rabbit sera were reacted with MAbs and specific mouse polyclonal serum (PAb) and their reactivity was detected with a mouse antiserum conjugated with horseradish peroxidase (Jackson InmunoResearch, USA). Enzymatic activity was developed by the addition of the substrate/chromophore mixture H_2O_2/ABTS (2,2-azino-bis 3 ethyl-benzothiazoline-6 sulfonic acid diammonium salt, Sigma, USA). The colour reaction was inhibited after 30 min incubation by addition of 0.2% sodium fluoride. The optical density (OD) readings were measured using an automatic microplate reader (Bio-Rad) at a wavelength of 415 nm. Fifty μl volumes were used throughout the assay. Between each step, plates were washed five times with PBS. Except for the capture antibody, all reagents were diluted with PBS containing 2% NaCl, 1% Tween, pH 7.6 (ELISA dilution buffer).

This method was used for both typing and determination of antigenic profiles.

2.4. Typing ELISA protocol

The typing ELISA was carried out using non-cross reactive MAbs for FMDV strains A24/Cruzeiro, A/Arg/01, O1/Campos, C3/Indaial and O/Taiwan. The plate layout shown in Fig. 1 is adequate for the testing of five FMDV reference strains and nine samples. In the first step, columns 1 and 7 were coated with A24/Cruzeiro rabbit capture serum; columns 2 and 8 with A/Arg/01 capture antiserum; columns 3 and 9 with C3/Indaial capture antiserum and columns 4, 5, 10 and 11 with O1 capture antiserum. As a second step, reference viruses were incubated in the left half of the plate, as shown in the figure (row A, A24/Cruzeiro; row B, A/Arg/01; row C, C3/Indaial; row D, O1/Campos and row E, O/Taiwan). Samples were incubated as shown in Fig. 1(S1–S9), and dilution buffer with no virus was added in line H. Non-cross reactive MAbs were then added in the corresponding columns, followed by an anti mouse antiserum conjugated with horseradish peroxidase and the same substrate/chromophore mixture as in Section 2.3. The MAbs used were as follows: for A24/Cruzeiro, a pool of MAbs *3C3*, *109* and *204*; for A/Arg/01, a pool of MAbs *2-2* and *3-2*; for C3/Indaial, a pool of MAbs *3-2E* and *1F12*; for O1/Campos, MAb *3*; for O/Taiwan, a pool of MAbs *3A1* and *3D1*. MAbs were selected considering the absence of cross reactivity with the strains produced presently in Argentina and their ability to recognize highly concentrated antigens. In the case of MAbs *109* and *204*, which were raised against strains A/Arg/79 and A/Arg/87, respectively (see Table 1), they were used as "strain specific" for A24/Cruzeiro because they were non-cross reactive with of any the strains presently used in Argentina. Polyclonal O1/Campos anti-serum was used for capture of O/Taiwan because it proved to be suitable for this purpose. Of interest, heterologous protection against O/Taiwan was achieved in swine vaccinated with O1/Campos FMDV strain (Chen et al., 1998).

The assay was considered valid if the blank OD reading (OD_B) was <0.3, considering the fact that the mean OD reading of numerous blanks assayed plus 3 standard

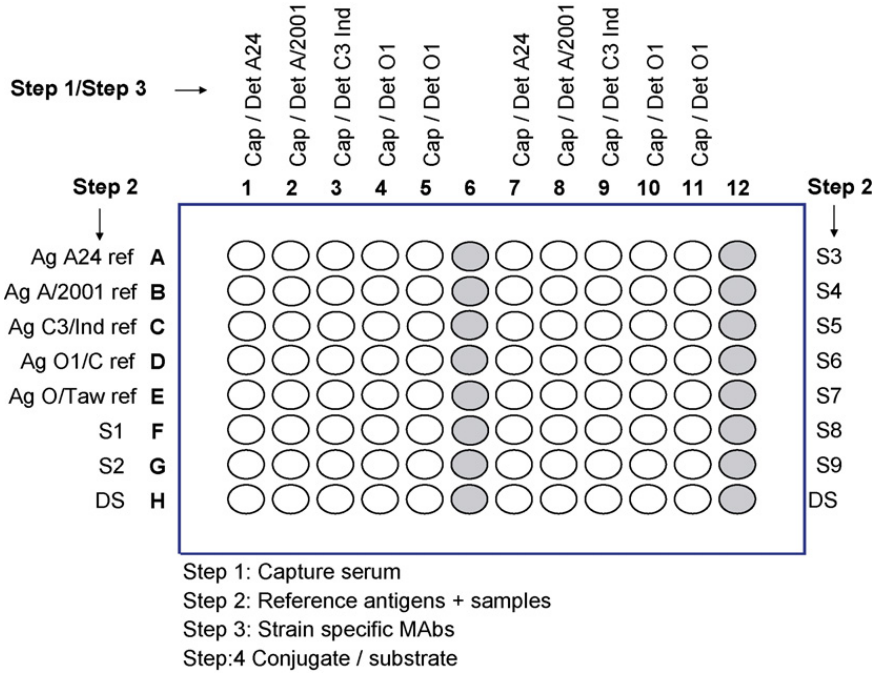


Fig. 1. Diagram depicting the layout of the plate used for typing ELISA. Cap, Capture; Det, detector; S1–9, samples; Ag: antigen; O/Taw, O1/Taiwan; O1/C, O1/Campos.

deviations (S.D.) was always lower than 0.3. The reference antigens were prepared to give an OD reading (OD_R) $>3 \times OD_B$. This value was also established as a condition for the validity of each assay. A sample X was considered as positive if $OD_X > 2 \times OD_B$ (empirical consideration).

2.5. Analysis of the integrity of 140S viral particles in sucrose density gradients

For determination of the integrity of 140S viral particles, 6 ml of the vaccine aqueous phases were treated with 0.5% (w/v) Sarkosyl (*N*-lauryl sarcosine, Sigma) in NET buffer (0.1 M NaCl, 2 mM EDTA, 50 mM Tris–HCl, pH 7.6) for 30 min at 4 °C and then analyzed by means of 20–45% (w/v) sucrose density gradient in NET buffer using a SW28 rotor in a Beckman ultracentrifuge, at 26,500 rpm for 3.5 h at 4 °C. Density gradient fractions were collected from the bottom of the tubes using a peristaltic pump and the OD at 260 nm was continuously recorded in a spectrophotometer Ultrospec 2100 *pro* (GE Healthcare) provided with a continuous flow cell. Total antigenic mass was calculated by integration of the area under the 140S peak, which is the sedimentation coefficient for intact FMD virus particles.

Ten to 16 collected density gradient fractions (1–3 ml each) were diluted 1:10 in ELISA dilution buffer and tested by typing ELISA with the MAbs described in Section 2.4 for each one of the strains present in the vaccine bulk or in the final product.

2.6. Antigenic profiles of FMDV reference vaccine strains

Antigenic profiles were determined using the same methodology as in Section 2.3 except that panels of MAbs and strain specific mouse polyclonal sera against A24/Cruzeiro, A/Arg/01, C3/Indaial and O1/Campos were used. The panels of MAbs used were as follows: for serotype C3 strains, MAbs *9*, *14*, *26*, *43*, *56*, *61*, *89*, *D7*, *A1*, *1F12* and *3-2E*; for serotype O1 strains, MAbs *8G*, *3A*, *74*, *13*, *3*, *2*, *69*, *2-6F*, *D3*, *17* and *G8*; for A24/Cruzeiro strain, MAbs *204*, *3C3*, *4A2*, *2C2*, *3D9*, *2E7*, *1E12*, *407*, *205*, *109* and *211*; for A/Arg/01, MAbs *1-2*, *1-5*, *2-2*, *2-4*, *2-6*, *3-2*, *3-3*, *403*, *108*, *43*, *2C2*; for C3/Indaial, MAbs *43*, *14*, *B3*, *56*, *61*, *D7*, *B4*, *1F12* and *3-2E*; for O/Taiwan, MAbs *3A1*, *3D1*, *4B2*, *1A11*, *2C2*, *2F8*, *1B3*, *2D4*, *1B9*, *2C8* and *2C9* (see Table 1). The plate layout was as described by Samuel et al. (1991) and Crowther (1993). Vaccine antigens (taken at different production stages) and the corresponding reference strains were reacted with the panels of MAbs. The reactivity (*R*) of each MAb in the panel with a given strain (X) compared to the mouse polyclonal serum (PAb) was calculated by subtracting the respective blanks using the formula $R_X = [(OD_{MAbX} - OD_{MAbB})/(OD_{PAbX} - OD_{PAbB})]$, in order to normalize the readings according to the amount of the different virus attached to the wells. These values were then related to those obtained with the homologous reference virus by using the formula $\%R = R_X/R_{Ref} \times 100$.

Interpretation of the results was done as described by Samuel et al. (1991): %*R* from 76% to 124% was considered a reactivity similar than the reference virus strain; %*R* from 46% to 75% and 20% to 45%, reflected lower reactivity than the reference virus strain; and %*R* < 20%, no reactivity.

2.7. Determination of coefficients of correlation (*r*) of reactivity

Mathematical calculations were applied to obtain a match factor by plotting the %*R* or absorbance values of the unknown sample against the reference strain. Linear regression was used to fit the best straight line, and the correlation coefficient *r* was calculated. If the antigenic profiles are identical, the plotted points will fall on a quasi straight line (the correlation coefficient *r* will be close to 1). If the antigenic profiles differ, the points will be widely scattered (the correlation coefficient will be close to 0 for totally dissimilar antigenic profiles).

2.8. Antigenic profiles of FMDV field isolates

Samples from field isolates were reacted with a larger panel of MAbs using the trapping ELISA as described in Section 2.4. The panel used for serotype A strains consisted of the following 29 MAbs: *4B4*, *3C3*, *4A2*, *2C2*, *3D9*, *2E7*, *1E12*, *407*, *205*, *108*, *211*, *109*, *212*, *110*, *206*, *112*, *7*, *13*, *317*, *211*, *408*, *220*, *20*, *403*, *108-1*, *105*, *111*, *204* and *405* (see Table 1).

In the case of field isolates, specific polyclonal sera were not available for normalization of the results. In this situation, low reactivity with the available reference polyclonal sera might introduce some distortion of the %*R* values. For this reason, OD values obtained with each MAb after subtracting their corresponding blank values were plotted and used for calculation of *r* for each sample instead of %*R*. The 90% confidence interval (90%CI) was calculated for comparison of the OD antigenic profile of each isolate with those of the strains A/Arg/79, A/Arg/87, A24/Cruzeiro, A/Arg/00 and A/Arg/01. Although antigenic profiles are usually shown as bars, in this case we plotted linear antigenic profiles which allowed a better comparison of different samples in the same graph.

3. Results

3.1. Assessment of potential cross contaminations among virus strains during vaccine manufacturing processes

Specificity studies of the MAbs obtained against different FMDV strains were performed using A24/Cruzeiro, A/Arg/01, O1/Campos, C3/Indaial and O/Taiwan vaccine strains after two passages in BHK-21c3 cells. The virus concentration used was adjusted between 3 and 5 μg/ml. This amount of virus was saturating for the respective MAb, giving an OD value higher than three times the OD_B. OD values $>3 \times OD_B$ were required for validation of the test positive controls.

For repeatability studies the five FMDV strains mentioned above were assayed 18–20 times with the specific MAb or pool of MAbs described in Section 2.4. Results including the respective OD values, S.D. and 95%CI for each of the five FMDV strains mentioned above, assayed with homologous or heterologous MAbs are shown in Table 2. No significant differences were found in the values obtained with heterologous MAbs when compared to the values of the blank reactions carried out in the absence

Table 2
Repeatability of the trapping ELISA using non-cross reactive MAbs

FMDV strains	Monoclonal Ab[a]				
	A24/Cruz	A/Arg/01	C3/Indaial	O1/Campos	O/Taiwan
A24/Cruz	**2.023 ± 0.281**[b] **(1.900–2.146)**[c] **n = 20**[d]	0.124 ± 0.020 (0.115–133) *n* = 19	0.124 ± 0.022 (0.114–0.134) *n* = 19	0.123 ± 0.021 (0.114–0.132) *n* = 20	0.147 ± 0.05 (0.123–0.171) *n* = 16
A/Arg/01	0.155 ± 0.030 (0.142–0.168) *n* = 19	**1.555 ± 0.235 (1.446–1.664) n = 18**	0.140 ± 0.025 (0.128–0.152) *n* = 18	0.117 ± 0.019 0.108–0.126) *n* = 18	0.119 ± 0.02 (0.110–0.128) *n* = 19
C3/Indaial	0.132 ± 0.026 (0.121–0.143) *n* = 20	0.111 ± 0.016 (0.104–0.118) *n* = 18	**1.203 ± 0.292 (1.075–1.331) n = 20**	0.115 ± 0.017 (0.108–0.122) *n* = 20	0.119 ± 0.017 (0.111–0.127) *n* = 19
O1/Campos	0.128 ± 0.027 (0.116–0.140) *n* = 20	0.110 ± 0.027 (0.098–0.122) *n* = 19	0.124 ± 0.037 (0.108–0.140) *n* = 20	**1.429 ± 0.270 (1.311–1.547) n = 20**	0.120 ± 0.020 (0.111–0.129) *n* = 19
O/Taiwan	0.127 ± 0.025 (0.116–0.138) *n* = 19	0.114 ± 0.017 (0.106–0.122) *n* = 18	0.121 ± 0.017 (0.113–0.129) *n* = 19	0.124 ± 0.018 (0.116–0.132) *n* = 19	**1.043 ± 0.304 (0.906–1.180) n = 19**
Blank w/o virus	0.132 ± 0.029 (0.119–0.145) *n* = 19	0.128 ± 0.037 (0.111–0.145) *n* = 19	0.123 ± 0.024 (0.112–0.134) *n* = 20	0.132 ± 0.024 (0.121–0.143) *n* = 20	0.126 ± 0.020 (0.117–0.135) *n* = 19

[a] The MAbs used are described in Section 2.4. Reactivity with the homologous virus is shown in bold.
[b] OD readings at 415 nm. Mean ± S.D.
[c] Associated 95% confidence interval.
[d] Number of repetitions.

of virus, suggesting a high level of specificity. Moreover, closely related strains, such as O1/Campos and O/Taiwan showed a total lack of cross reactivity. The upper limits of the 95%CI of the OD readings with heterologous MAbs as well as in the blank reactions were always lower than 0.3.

Virus suspensions of known concentration (antigenic mass) were diluted up to an $OD_X = 2 \times OD_B$, and the detection limits were found to be as follows: for A24/Cruzeiro, 5–30 ng/ml; for A/Arg/01, 5–20 ng/ml; for C3/Indaial, 5–30 ng/ml; for O1/Campos, 5–30 ng/ml; for O/Taiwan, 5–15 ng/ml.

Table 3 shows several examples of the application of this method for the analysis of purity of commercial concentrated monovalent viral suspensions and master seeds (MB), as well as for analysis of strain identity in polyvalent vaccine aqueous phases, either before formulation (AP) or after rupture of the emulsions in the final products (V). Tetravalent formulations are produced to be used in Argentina, whereas bivalent (A24/Cruzeiro and O1/Campos) and trivalent (A24/Cruzeiro, O1/Campos and C3/Indaial) are for exportation to South American countries. The samples were provided by Argentine FMD vaccine manufacturers. The assay was found to be highly reproducible, even with highly concentrated ($\geq 40\times$) antigen suspensions, and with different production methods (BHK-21 cells or Frenkel).

3.2. Assessment of viral particles integrity in polyvalent FMD vaccines

Mono or polyvalent viral suspensions obtained at different steps of vaccine production or recovered from the aqueous phase of commercial oil adjuvanted vaccines were prepared and analyzed on sucrose density gradients. The position of the intact 140S viral particles was established by its OD absorbance at 260 nm. One to 3 ml fractions of each sucrose density gradient were collected and analyzed by ELISA using the MAbs described in Section 2.4. Using this method, it was possible to confirm the presence of intact FMDV particles in the $OD_{260\ nm}$ peak of each one of the strains included in polyvalent vaccines. The typing ELISA also allowed the detection of other viral antigens present along the density gradient, such as 75S empty particles and the 12S capsomers (Fig. 2), which are breakdown products of 140S virions or incomplete or unassembled components generated during virus replication in vitro (Vásquez et al., 1979).

In order to illustrate the potential use of this technology for vaccine control, three different examples are presented (Fig. 2).

Case 1: Trivalent commercial vaccine obtained from the market one year after approval. The antigenic 140S components of this vaccine formulated with viruses A24/Cruzeiro, O1/Campos and C3/Indaial with a total antigenic load of 15 μg/ml could be clearly distinguished (Fig. 2A). In this case, strain O1/Campos showed an unusually high 12S component, but still showing a large amount of 140S viral particles. Interestingly, strain A24/Cruzeiro presented an additional component banding at the 75S region of the density gradient, corresponding to empty capsids. Neither O1/Campos nor C3/Indaial strains presented a detectable reactivity at the 75S particles density region.
Case 2: Tetravalent vaccine submitted to official control. This vaccine was formulated with viruses O1/Campos, A/Arg/01, A24/Cruzeiro and C3/Indaial, with a total antigenic load of 15.6 μg/ml of 140S particles. The

Table 3
Assessment of potential cross contamination among FMD virus strains in monovalent or polyvalent concentrated vaccine antigens

Type of sample		Monoclonal antibody[a]				
		A24/Cruz	A/Arg/01	C3/Ind	O1/Cam	O/Taw
Monovalent[b] A24/Cruz	40X	**2.183**[c]	0.091	0.124	0.086	0.089
	40X	**2.247**	0.107	0.114	0.104	0.110
	40X	**2.262**	0.117	0.126	0.125	0.119
	40X	**2.039**	0.111	0.123	0.120	0.118
Monovalent A/Arg/01	40X	0.132	**1.762**	0.163	0.113	0.109
	40X[d]	0.163	**1.894**	0.163	0.134	0.137
	40X	0.169	**1.906**	0.163	0.129	0.123
	MB	0.163	**1.782**	0.161	0.114	0.110
Monovalent O1/Cam	40X	0.085	0.082	0.093	**2.028**	0.092
	40X	0.156	0.110	0.106	**1.916**	0.116
	40X	0.112	0.123	0.121	**2.107**	0.150
	MB	0.077	0.071	0.089	**1.410**	0.081
Monovalent C3/Ind	40X[d]	0.129	0.119	**1.604**	0.134	0.171
Monovalent O/Taw	40X	0.146	0.124	0.130	0.128	**1.593**
	40X	0.165	0.133	0.140	0.128	**1.449**
	40X	0.237	0.186	0.187	0.200	**2.348**
Bivalent	AP	**1.794**	0.081	0.110	**1.695**	0.093
	AP	**1.794**	0.081	0.110	**1.695**	0.093
	V	**1.870**	0.082	0.114	**1.823**	0.094
Trivalent	AP	**1.754**	0.082	**1.291**	**1.719**	0.091
	AP	**2.040**	0.096	**1.213**	**1.585**	0.111
	V	**1.654**	0.082	**1.010**	**1.552**	0.094
	V	**2.322**	0.139	**1.038**	**1.899**	0.152
Tetravalent	V[d]	**1.767**	**1.439**	**1.541**	**1.617**	0.077
	V[d]	**1.789**	**1.456**	**1.469**	**1.562**	0.086
	V	**1.650**	**1.498**	**1.496**	**1.628**	0.093
	V	**1.620**	**1.584**	**1.441**	**1.650**	0.091

[a] The MAbs used are described in Section 2.4. Reactivity with the homologous virus is shown in bold.
[b] Each line represents a different sample.
[c] OD reading at 415 nm.
[d] Antigen produced in bovine tongue tissue (Frenkel). 40X, Viral suspensions concentrated 40 times; MB, Master Seed Bank; Taw, Taiwan. AP, Vaccine aqueous phase; V, vaccine aqueous phase recovered from oil formulations.

MAbs reactivity with A/Arg/01 strain was absent at the 140S region and could be detected mainly at the top of the density gradient, suggesting breakdown of the 140S particles into 12S viral subunits and soluble proteins (Fig. 2B). The poor performance of this strain in the indirect potency test (rejected vaccine, data not shown) confirmed previous observations that intact 140S particles are the main antigenic components of FMDV vaccines (Vazquez et al., 1979). A24/Cruzeiro strain showed well defined peaks at 140S, 75S and at 12S, while O1/Campos presented peaks at 140S and 12S and C3/Indaial strain presented mostly 140S particles.

Case 3: Tetravalent commercial vaccine rejected in a PPG challenge trial. This vaccine was manufactured before the year 1999 with viruses A/Arg/79, A/Arg/87, O1/Caseros and C3/Arg/85 with a total antigenic load of 15 μg/ml. In this case, no reactivity was detected at the 140S peak of the sucrose gradient for the two serotype A strains, whereas a high 140S peak was seen for serotypes O1 and C3 (Fig. 2C). This vaccine was capable to protect animals challenged with O1/Caseros and C3/Arg/85 virus strains whereas it failed to protect animals challenged with A/Arg/87 strain (challenge with A/Arg/79 strain was not carried out). Analysis of sera from these vaccinated animals by liquid-phase blocking ELISA (lpbELISA) used for quantification of protective antibodies (Hamblin et al., 1986; Robiolo et al., 1995) showed an excellent performance for viruses O1/Caseros and C3/Arg/85**,** whereas the antibody response to A/Arg/87 and A/Arg/79 was below the pass mark level of approval established in Argentina (data not shown).

It should be pointed out that the typing ELISA values shown are not quantitative, and they only indicate the presence of particular antigens at different regions of the sucrose density gradient. In this regard, it is clear that the detection of the main antigenic component (140S particles) is relevant to predict vaccine behaviour in potency tests.

3.3. Analysis of antigenic profiles of FMDV vaccine strains with panels of MAbs

Panels of MAbs for viral serotypes A, O and C were selected based on the characterization data of more than 80 MAbs (Table 1). The panels used for each serotype are described in Section 2.6. *%R* with each MAb was plotted either as an histogram or as a continuous line, defining an antigenic profile for each vaccine strain (Fig. 3). A

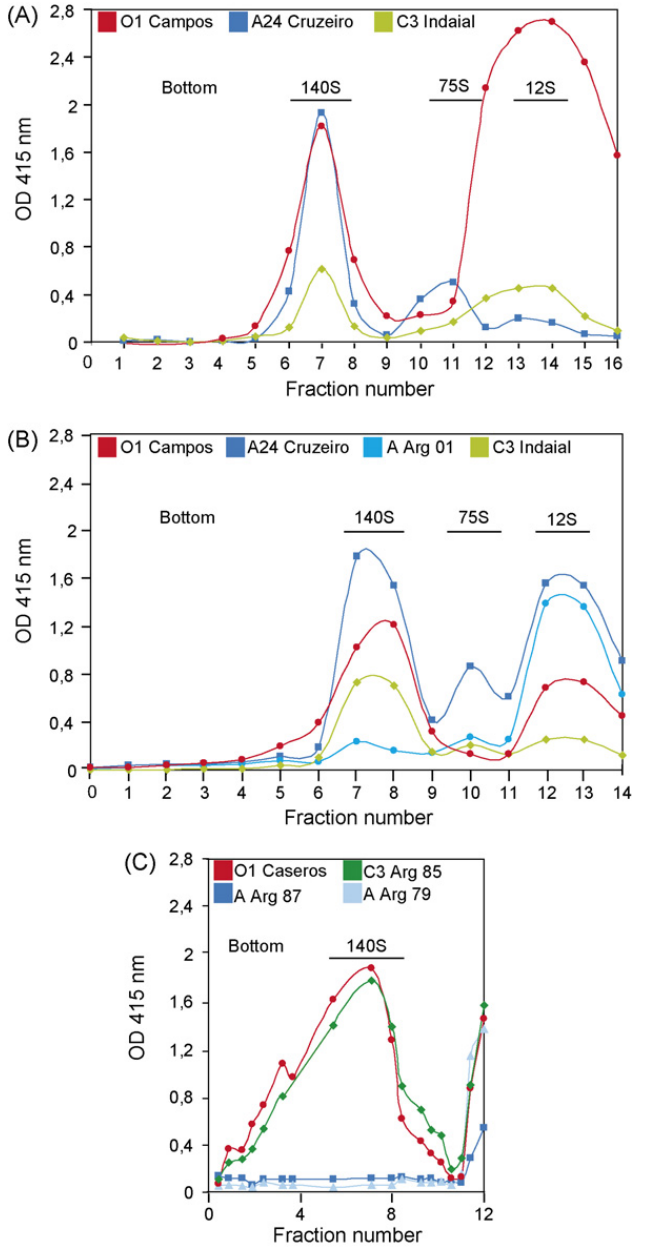


Fig. 2. Assessment of viral particle integrity in FMD polyvalent vaccines. Analysis of the aqueous phase of oil adjuvanted commercial trivalent (panel A) and tetravalent (panels B and C) vaccines on 20–45% sucrose density gradients. The independent absorbance values at 415 nm obtained when the fractions were tested with specific MAbs are shown in colour. The composition of the vaccines is shown in each panel. The position of the intact 140S viral particles was established by its OD absorbance at 260 nm. The density gradient regions corresponding to 75S empty particles and 12S capsomers are also indicated. Degradation 140S viral particles for both serotype A antigenic fractions is evident in panel (C).

continuous line linking the %*R* values of each MAb is also shown for C3/Indaial (Fig. 3A) and C3/Arg/85 (Fig. 3B) strains. We postulate that, once the characteristic antigenic profile of a reference strain is obtained, the antigenic profile of an given strain can easily and rapidly be compared with this standard, as it is shown in Fig. 3C and D, where a serotype C unknown viral seed was compared to C3/Indaial and C3/Arg/85 references. In this case, the *r* values obtained for the unknown seed were 0.94 (close matching with C3/Indaial) and −0.22 (poor matching with C3/Arg/85).

The same criteria was used to establish consistent identity (absence of cross contamination) upon serial passages of O1/Campos production seeds during subcultures compared to the reference O1/Campos strain and to another serotype O strain, O1/Arg/Caseros, manipulated in the facility (Fig. 4). The panel of MAbs against serotype O strains used was described in Section 2.6. The *r* values calculated for both serotype O strains are shown in the same figure. The results indicated that the seed strains clearly matched with the strain O1/Campos (*r* values $\geq$0.83) and very poorly with the strain O1/Caseros (*r* values $\leq$0.31).

3.4. Antigenic profile of field isolates from FMD the 2000–2001 outbreaks in Argentina

Conventional serological tests (CF, ELISA) typed most of the FMDV isolates from the 2000–2001 outbreaks in Argentina serotype A. Only a few isolates were characterized as serotype O, and they rapidly disappeared from the field with vaccination (Mattion et al., 2004). A rapid analysis involving many field isolates was performed by ELISA using the serotype A panel of 29 MAbs described in Section 2.8. FMDV strains A/Arg/79 and A/Arg/87 (present in Argentine vaccines before 1999) and the South American strain A24/Cruzeiro were also included in this analysis. Through the study of the antigenic profiles (Fig. 5) and the individual *r* values (Table 4), it was possible to detect the emergence of two main groups of strains: Group 1 included A/Arg/00-like isolates whereas Group 2 included A/Arg/01-like isolates. Using a simplified approach similar to the previously described by Samuel et al. (1991), we found the mentioned strains within groups of high range of reactivity (above 75%) with the mentioned strains. The 90%CI calculated ($n = 29$) for $r = 0.75$ was [0.57–0.86]. Based on these values, we found that all the field isolates were in a range of low homology with A/Arg/79, A/Arg/87 and A24/Cruzeiro vaccine strains. Interestingly, three isolates clearly belonging to Group 2 (San Martin, Las Heras, and Conelo), shared also some lower homology with A/Arg/00 strain. This isolates were collected in March 2001, with the first A/Arg/01-like strains. One of the field strains that was included in Group 1 (GVillegas00B) showed a lower reactivity ($r = 0.71$). Nevertheless, this isolate was included in this group because it presented very low homology with all the other strains analyzed, and because according to genetic data, the phylogenetic analysis clearly grouped it with the A/00-like strains (König et al., 2007). Other two minor variants were also detected which differ from the two main groups and from each other, but their presence in the field was transient and not significant in number (data not shown).

The graphic analysis of the antigenic profile of field isolates shown in Table 4 (Fig. 5), belonging to Groups 1 (Fig. 5D) or 2 (Fig. 5E), and of the antigenic profile of the strains incorporated into vaccines [A24/Cruzeiro (Fig. 5A), A/Arg/00 (Fig. 5B) and A/Arg/01 (Fig. 5C)] was also carried out.

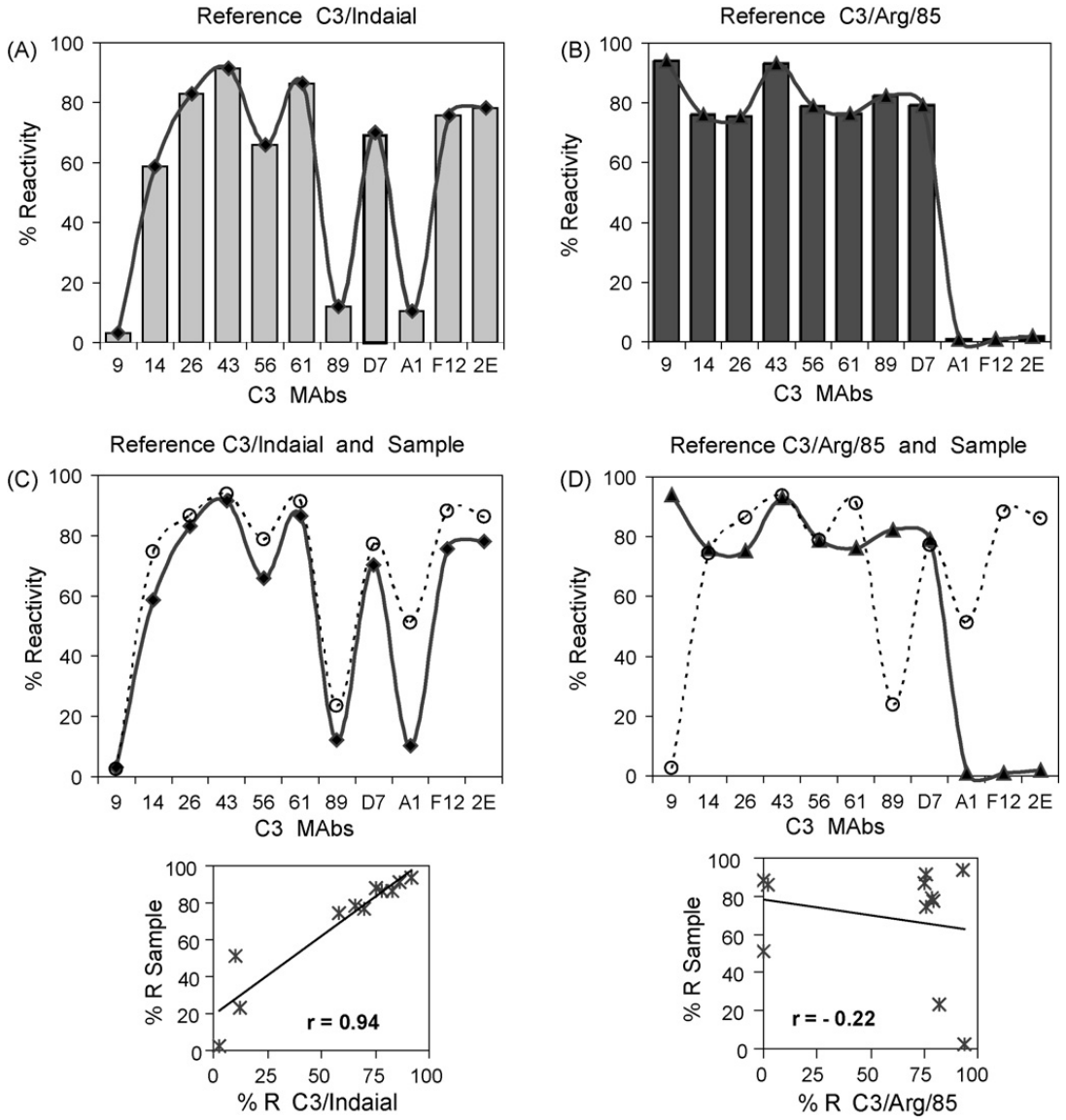
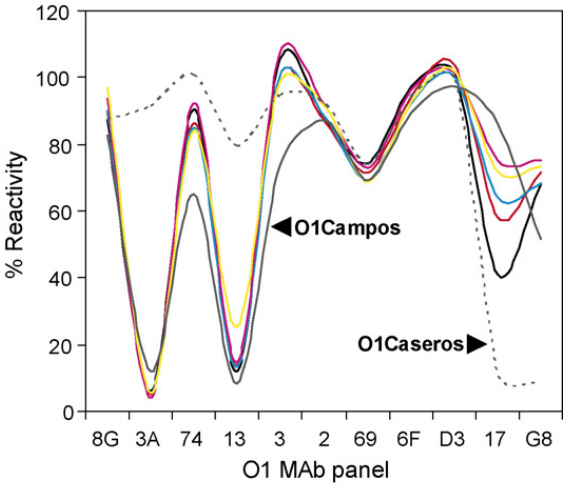


Fig. 3. Antigenic profile of vaccine strains determined by ELISA using specific MAb panels, as indicated on the *x*-axis. Antigenic profiles of C3/Indaial (A) and C3/Arg/85 (B) vaccine strains are shown individually. The antigenic profile of a serotype C3 sample is compared to similar (C) or dissimilar (D) C3 reference strains. Regression curves using %reactivity of samples and reference strains are shown at the side of each figure. Reactivity correlation coefficients (r) for similar ($r = 0.94$) or dissimilar ($r = -22$) profiles are shown.

4. Discussion

In this work, a typing ELISA applied to quality control of FMDV vaccines at different steps of the manufacturing process is described. In this ELISA, non-cross reactive MAbs developed against the FMDV vaccine strains used in South America and O/Taiwan have been used. In addition, a rapid and simple method to compare antigenic profiles obtained with panels of MAbs is proposed.

Assessment of identity and purity of master and working viral seeds is a must in vaccine production under GMP conditions. Although this can be carried out by methods, such as sequencing or reverse transcription PCR, this is not practical to follow up potential cross contamination events during the production process. On the other hand, immunological techniques using polyclonal antibodies are not sensitive and specific enough to allow the unequivocal distinction among strains belonging to the same serotype, or the detection of low concentrations of a contaminant strain in the presence of high mass of the desirable strain of the same serotype. The detection of tiny amounts (approximately 15–20 ng/ml) of virus can be rapidly assessed by ELISA with strain specific MAbs. These MAbs did not show cross reaction with other strains of the same or different serotype, even in highly concentrated polyvalent antigen suspensions or in the final products (Table 3). It can also be applied for virus production on both cells or Frenkel substrates. Preliminary spiking experiments with known amounts of contaminant virus were also carried out with similar results (data not shown). The determination of antigenic profiles using panels of MAbs has been of big help in the past for the distinction between the two closely related strains O1/Campos and O1/Caseros (Fig. 4), that cannot be unequivocally differentiated by classical serological methods, including seroneutralization (unpublished results). Using non-cross reactive MAbs



r O1	Caseros	Campos
····	1	0,04
—	0,04	1
■	0,31	0,83
■	0,19	0,89
■	0,17	0,91
■	0,10	0,91
■	0,11	0,92

Fig. 4. Consistency of FMDV production seeds. Several cell culture passages of O1/Campos viral seeds performed during vaccine production were analyzed with a panel of MAbs (described in Section 2.6). Reactivity correlation coefficients (r) values for O1/Campos and O1/Caseros FMDV vaccine strains are shown in table.

within the validated parameters, it is possible to rapidly detect cross contaminations of strain O1/Campos with O1/Taiwan, or strain A24/Cruzeiro with A/Arg/01, and among different serotype C3 strains during the vaccine production process. As shown in Table 3, the identity of the strains present in each monovalent, bivalent, trivalent or tetravalent vaccine can be clearly established even in the aqueous phases extracted from the final oil vaccines. Recently, this assay has been validated for an Argentine manufacturer exporting vaccines, because although the different vaccine antigens are strictly segregated during production (campaign production), national sanitary authorities strictly control vaccines that might generate antibodies to exotic strains in the vaccinated animals.

During vaccine manufacturing, virus strains are subjected to different manipulations, such as subculture, inactivation, concentration, purification, etc., where antigens are exposed to cellular or bacterial proteases and selective pressures, which are capable of inducing changes in the viral capsids, where the main antigenic sites are located (Doel and Collen, 1983). Although the integrity of viral particles in monovalent vaccine formulations may be monitored by analysis of the 140S peak ($OD_{260\ nm}$) in sucrose density gradients, this is not the case for polyvalent formulations, in which the analysis of integrity by means of quantification of 140S particles of each of the strains was, up to date, not possible. Data presented in this work show the value of using non-cross reactive MAbs in the monitoring of strain specific 140S particle content in polyvalent vaccines, which is a critical parameter associated with their performance in the potency tests and in their field efficacy. This is particularly important considering the well known difficulty of obtaining strain specific MAbs with exclusive binding to 140S particles (Crowther et al., 1995; Samuel et al., 1991).

The technique presented here is a powerful tool to predict the eventual potency failure of specific antigenic fractions of polyvalent vaccines. Although this type of assay is not quantitative, the ratio between 140S/12S could be a good indicator for predicting vaccine performance. Our laboratory is currently working on the analysis of this ratio in Argentine commercial vaccines with the idea of establishing a relationship between 140S particles breakdown and the concomitant increment in 12S capsomers, with vaccine performance in direct or indirect potency tests. In this regard, it is shown that vaccines with degraded 140S antigens (Fig. 2C) were unable to protect vaccinated animals in challenge trials and in indirect potency tests. From the manufacturers' point of view, it allows discarding or reprocessing eventual defective vaccine bulks before being bottled or sent for official control.

Correlation coefficients of reactivity were used for the comparison of antigenic profiles of vaccine strains during production. The comparison was carried out with the %R of the MAb panels with respect to the corresponding polyclonal sera. This comparison proved very useful to establish consistent identity upon serial passages of master and production seeds and also in vaccine bulks or bottled products.

The assay was successful in the profiling of FMDV isolates for general field screening purposes. In this regard, Crowther (1993) had proposed the use of a MAb trapping ELISA to compare field isolates and prototype strains and suggested that this could be an appropriate procedure to distinguish a new variant from a laboratory escape, and also to predict either the failure or success of a vaccine in the area where the outbreak takes place. This author compared the reactivity of the samples with the reactivity of the homologous strains with the same MAbs and defined different levels of antigenic reactivity. García-Barreno et al. (1986), studied the antigenic variability of African swine fever virus and they classified 23 field virus isolates into six antigenic homology groups, according to a mathematical formula based on percent reactivity with MAbs. Using the same procedure, they showed the existence in a single naturally infected pig of a collection of viruses with different antigenic properties. Recently, Mahapatra et al. (2008) studied the reactivity of serotype O FMDV strains with a well characterized panel of MAbs using a similar mathematical formula. The authors tried to correlate this reactivity data with micro neutralization data with polyclonal sera, which is considered the gold standard for vaccine matching purposes. They were unable to find a correlation, which shows that it is still difficult to predict performance of vaccines based only on MAbs profiling. In our case, we did not measured reactivity in terms of protection, or with the purpose of correlation with cross protection tests. For the particular purposes of our work, we found that the screening of field FMDV strains from the 2000–2001 outbreaks in Argentina based on correlation

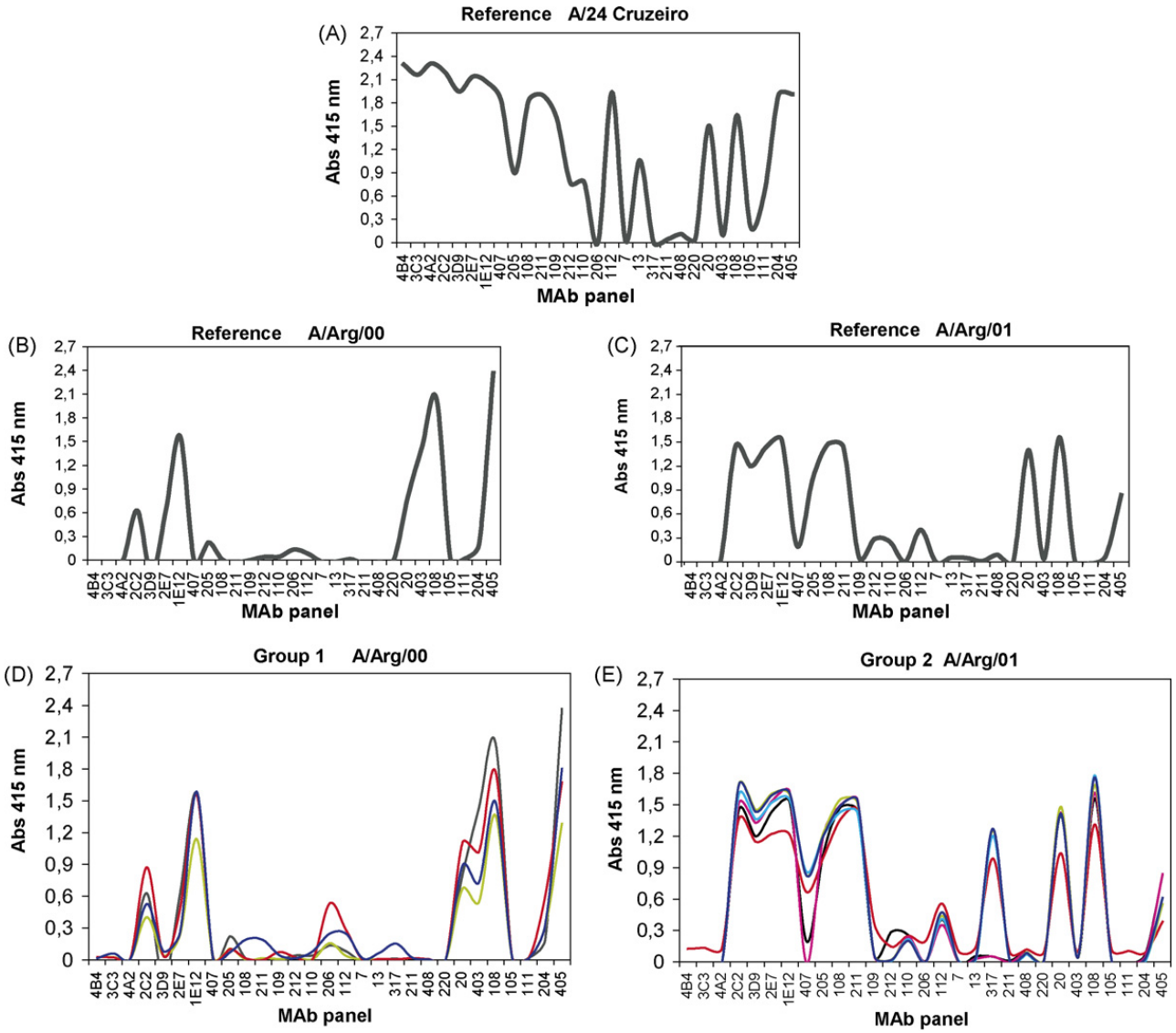


Fig. 5. Antigenic profiles of field isolates from the 2000–2001 outbreaks in Argentina. Field samples were analyzed by ELISA using a panel of 29 Mabs for serotype A strains (described in Section 2.8). The antigenic profiles of individual serotype A vaccine strains are depicted in panels (A) A24/Cruzeiro, (B) A/Arg/00 and (C) A/Arg/01. The antigenic profiles corresponding to the two major groups of isolates whose prototype are A/Arg/00 and A/Arg/01, are shown in panels (D) and (E), respectively. The corresponding reactivity correlation coefficients (*r*) values for these strains are shown in Table 4.

coefficients was capable of easily identifying two main groups of FMDV isolates and the transient presence of other minor variants. Despite the degree of diversity shown, serotype A strains were controlled in a short time by emergency vaccination and revaccination with A24/Cruzeiro, A/Arg/00 and A/Arg/01 strains, as judged by their disappearance from the field.

The same two main groups of isolates were later identified based on genetic data analysis. The phylogenetic tree generated with the VP1 gene nucleotide sequence of 43 isolates allowed the classification of type A Argentinean isolates into two major groups (A/Arg/00 and A/Arg/01). The isolates appeared as separated groups with a common ancestor, undoubtedly different from each other (König et al., 2007). The A/Arg/00 related viruses were genetically similar with sequence differences ranging from 0.16% to 1.1%. The deduced VP1 amino acid sequence of this group of isolates was similar (96% similarity) with only eight substitutions, two of them located in the 137–173 region. The VP1 nucleotide sequence of this group differed from A/Arg/01 and from the vaccine strains A24/Cruzeiro, A/Arg/79 and A/Arg/87 by 12.8%, 17.7%, 18.3% and 12.3%, respectively. This group of viruses was not related to any virus strain previously reported in the region (König et al., 2007). On the contrary, a well supported relationship was found between the A/Arg/01 isolates and Brazilian isolates from outbreaks that occurred in 1993–1994. Closely related isolates were found throughout Uruguay from April to August 2001 and in South Brazil from May to July. An interesting possibility would be to complement the genetic analysis of König et al. (2007) of isolates of Brazil and Uruguay, with the MAb analysis performed in this work. Finally, cross challenge trials using A/Ag/00, A/Arg/01 and A24/Cruzeiro strains showed the absence of cross protection between these three strains in primo vaccinated animals (Goris et al., 2008; Mattion et al., 2004; Robiolo et al., 2006).

Table 4
Reactivity correlation coefficients (r) for FMDV field strains as determined by ELISA using a panel of MAbs

Field isolates[b]	Correlation coefficient of reactivity[a]				
	A/79	A/87	A/24	A/00	A/01
A/Arg/00[c]	0.15	0.37	0.20	**1.00**	0.47
RSegundo00[c]	0.18	0.33	0.25	**0.94**	0.52
SJusto00[c]	0.17	0.29	0.20	**0.89**	0.39
Chapaleufú00A[c]	0.16	0.34	0.27	**0.97**	0.53
CTejedor00[c]	0.17	0.33	0.26	**0.95**	0.52
Tapalque01	0.22	0.31	0.28	**0.96**	0.55
Laprida01	0.23	0.34	0.28	**0.96**	0.57
GVillegas00B[c]	0.11	0.40	0.03	**0.71**	0.07
A/Arg/01[c]	0.31	−0.12	0.51	0.47	**1.00**
Capaleufú01[c]	0.30	−0.03	0.48	0.48	**0.92**
GLópez01[c]	0.34	0.07	0.49	0.52	**0.87**
Atreuco01[c]	0.31	−0.05	0.47	0.50	**0.91**
Tlauquen01	0.41	−0.07	0.52	0.41	**0.89**
Chivilcoy01[c]	0.27	0.03	0.44	0.54	**0.87**
Daireux401[c]	0.24	−0.07	0.43	0.40	**0.92**
Diamante01[c]	0.33	−0.08	0.42	0.39	**0.89**
TLauquen301[c]	0.31	0.02	0.47	0.50	**0.91**
TLauquen401A[c]	0.31	−0.12	0.51	0.47	**1.00**
TLauquen401B[c]	0.37	−0.17	0.55	0.44	**0.98**
Guamini01	0.20	−0.13	0.48	0.36	**0.91**
CSuárez01[c]	0.27	−0.11	0.49	0.46	**0.99**
25 de Mayo01[c]	0.24	−0.08	0.43	0.38	**0.92**
Guatreche01[c]	0.23	−0.09	0.43	0.36	**0.92**
Junin01[c]	0.36	0.01	0.52	0.51	**0.91**
JuninII01[c]	0.38	0.00	0.51	0.46	**0.90**
Daireaux501[c]	0.38	−0.05	0.46	0.51	**0.81**
Daireaux01	0.37	−0.03	0.42	0.52	**0.78**
3Lomas00[c]	0.30	−0.17	0.46	0.29	**0.92**
Lincoln01	0.34	−0.06	0.46	0.44	**0.85**
San Martin01	0.34	0.07	0.45	0.61	**0.83**
Las Heras01	0.32	0.13	0.48	0.62	**0.84**
Chacabuco01	0.30	0.06	0.48	0.57	**0.90**
Chacabuco201	0.31	0.15	0.45	0.56	**0.78**
T.Lauquen01	0.29	0.05	0.43	0.57	**0.90**
Conelo01	0.36	0.07	0.50	0.59	**0.83**
3Lomas01[c]	0.28	0.11	0.47	0.57	**0.89**

[a] r values for the panel of 29 MAbs described in Section 2.8.
[b] The names of field strains refer to the place of isolation.
[c] Isolates whose VP1 protein encoding gene was sequenced (König et al., 2007). r values above 0.75 are shown in bold.

Rapid characterization of new field isolates, as well as systematic control of the quality of the antigens present in commercial FMDV vaccines have been of great impact and importance in the FMD eradication campaigns in Argentina. These assays have been systematically performed in our laboratory for SENASA, for South American official and private sanitary institutions, for the Argentine antigen and vaccines bank and for private vaccine manufacturers.

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