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APPENDIX I

Commonly used reagents, buffers and other preparations for molecular work.

Molecular biology grade chemicals were used for making reagents and solutions for molecular biology. Double glass distilled deionised water (ddH_2O) was used for all molecular tests.

Preparation of size fractionated silica

Six grams of silicon dioxide (99% Sigma) was suspended in 50 ml of sterile ddH_2O in a glass bottle and allowed to sediment for 24 hours at room temperature. The supernatant (43 ml) was removed and the sedimented silica was re-suspended to a final volume of 50 ml. A second sedimentation was allowed for the duration of 5 hours at room temperature. The supernatant (44 ml) was removed and then the pH of silica was adjusted to 2 by adding 60 µl of HCl (32% W/V). Aliquots of 240, 280, 360 and 400 µl were prepared in 1.5 eppendorf tubes and stored in dark. Fourty µl of this suspension was used per tissue cultured supernatant of the virus samples.

EDTA (1M)

An amount of 372.2 g of disodium ethylenediaminetetra-acetate·$2H_2O$ (EDTA) was added to 800 ml of water, vigorously stirred on a magnet stirrer, and the pH adjusted to 8.0 with NaOH (~20 g of NaOH pellets). The solution was dispensed into aliquots and sterilised by autoclaving.

Preparation of L6 lysis buffer

Sixty grams of Guanidine Thiocyanate (GuSCN, Promega) was dissolved in 50 ml of 0.1M Tris-HCl (pH 6.4). 11 ml of 0.2M EDTA and 1.3 g of Triton X-100 were added and kept at 50°C in water bath until they completely dissolved. The bottle was wrapped in aluminium foil and stored at room temperature.

Preparation of L2 wash buffer

L2 wash buffer was prepared in the same way as L6 lysis buffer preparation but without the addition of EDTA and Triton X-100.

Tris (1 M) preparation

Tris base (121.1 g) was dissolved in 800 ml of dH_2O, and the solution was allowed to cool to room temperature before final adjustment of pH to 7.4 with concentrated HCl. After adjusting the volume of the solution to 1 litre with dH_2O, it was dispensed into aliquots and sterilised by autoclaving.

DNA loading buffer (6x)

DNA loading buffer was made by dissolving 0.25% (w/v) bromophenol blue and 40% (w/v) sucrose in sterile distilled water (Sambrook *et al.*, 1989). The buffer was stored at – 4°C in 1 ml aliquots.

Tris acetate buffer

Tris-acetatte (TAE) was used for electrophoresis of the RT-PCR products in agarose gels. It consisted of 40 mM Tris-acetate and 1 mM EDTA. Generally a 50X solution was made by dissolving 242 g of Tris base, 57.1 ml of glacial acetic acid and 100 ml of 0.5 M EDTA (pH 8.0) in deionised distilled water to a final volume of 1l. The buffer was autoclaved, and diluted 1:50 in deionised distilled water before use.

Ethidium bromide (10 mg/ml)

One gram of ethidium bromide was dissolved in 100 ml of dH_2O. The solution was stirred on a magnetic stirrer for several hours to ensure that the dye was completely dissolved. The container was wrapped in aluminium foil and stored at – 4°C. This dye was used to track PCR products in agarose gel electrophoresis. Because of the powerful mutagen and moderately toxic property of ethidium bromide, all the material that has been in contact with the reagent when working with solutions had been disposed in hazard bags, and necessary precautions were taken to ensure maximum safety and avoid skin or any other contact with the reagent.

Deoxyribonucleoside triphosphates (dNTPs)

Free dNTPs are required for DNA synthesis. Equimolar amounts of the four dNTPs (dATP, dGTP dCTP dTTP) were used in the reaction mixture to minimize misincorporation errors. All DNA polymerase enzymes add deoxynucleotides (dNTP) in a 5' to 3' direction to a primer. From stock solutions of each dNTP commercially available (Roch), dilutions were made to obtain final concentrations of

10pmol/µl of working solutions and each were stored separately at minus 20°C in small aliquots.

PCR Master Mix per reaction

The PCR was performed in a 50 µl volume in the presence of 3 µl of cDNA were constituted as follows:

ddH_2O	X µl
Sense primer (Inqba Biotech)	2.5 µl (stock = 10 pmol, final conc = 25 pmol)
Antisense primer (Inqba Biotech)	2.5 µl (stock = 10 pmol, final conc = 25 pmol)
dNTPs (Roche)	1 µl (stock = 10 mM, final conc = 200 µM)
Taq buffer (Promega)	1 µl (stock = 10X, final conc = 1X)
Taq (Roche)	0.5 µl (stock = 5U, final conc = 2,5 U)
cDNA template	3 µl
Total	50 µl

Detail information of the sense and Antisense primers used for PCR against each serotype of FMDV are described in their respective chapter.

Summary of reactivity of serum samples from buffalo tested by the Lpb ELISA and 3ABC ELISA

Serum samples ident. No.	SAT-			A	O	C	3ABC ELISA
	1	2	3				
152	2.003	1.687	-	-	-	-	0.838
153	2.005	2.055	1.559	-	-	-	-
154	-	-	-	-	-	-	0.705
155	-	-	-	-	-	-	0.678
156	1.742	-	-	-	-	-	0.995
157	-	2.086	-	-	-	-	-
158	1.617		-	-	-	-	0.671
159	2.238	2.108	1.600	-	-	-	-
160	1.680	-	-	-	-	-	1.256
161	2.005	1.735	-	-	-	-	0.911
162	2.355	2.239	1.626	-	-	-	0.863
163	1.886	1.874	-	1.702	-	-	-
164	2.355	2.355	1.800	-	-	-	-
165	2.172	2.255	-	-	-	-	1.124
166	2.199	2.164	1.600	-	-	-	0.782
167	2.055	2.267	-	1.772	-	-	0.958
168	1.982	1.953	-	-	-	-	-
169	2.013	1.942	-	-	-	-	1.063
170	1.927	1.681	-	-	-	-	1.079
171	2.089	2.104	-	-	-	-	-
172	2.165	2.102	-	-	-	-	1.055
173	-	2.055	-	1.910	-	-	0.661
178	-	1.760	-	-	-	-	0.880
179	-	-	-	-	-	-	-
180	-	-	-	-	-	-	-

Total serum samples from Buffalo = 25 (152 – 180) (Origin Omo national park).
- indicates non-reactive sera

Lpb ELISA: Sera with titres > 1/40 that showed an OD of > 1.6 were considered as reactive sera (non-reactive sera ≤ 1.600) (Section 6.2.3.1). Results indicated under column SAT-3 were considered as negative.

3ABC ELISA: an index values of > 0.5 accepted as reactive sera and those results below this value considered as non reactive sera (Section 6.2.3.2).