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**Evaluation of a novel proximity ligation assay for the sensitive and rapid  
detection of foot-and-mouth disease virus**

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**Abstract**

A novel proximity ligation assay (PLA) using a pan-serotype reactive monoclonal antibody was developed and evaluated for the detection of foot-and-mouth disease virus (FMDV) in clinical samples collected from field cases of disease. The FMDV-specific PLA was found to be 100 times more sensitive for virus detection than the commonly used antigen capture-ELISA (AgELISA). As few as 5 TCID<sub>50</sub> were detected in individual assays, which was

26 comparable with the analytical sensitivity of real-time RT-PCR. Although this assay was  
27 capable of detecting diverse isolates from all 7 FMDV serotypes, the diagnostic sensitivity of  
28 the PLA assay was lower than real-time RT-PCR mainly due to a failure to detect some SAT  
29 1, SAT 2 and SAT 3 FMDV strains. In conclusion, this new PLA format has high analytical  
30 sensitivity for the detection of FMDV in clinical samples and may prove valuable as a rapid  
31 and simple tool for use in FMD diagnosis.

32

### 33 **Keywords**

34 Proximity ligation assay (PLA), Foot-and-mouth disease (FMD), Diagnostic test, Pathogen  
35 detection.

36

### 37 **Abbreviations**

38 Proximity Ligation assay (PLA), monoclonal antibody (mAb), Signal to noise (S/N), Tissue  
39 culture infectious dose (TCID<sub>50</sub>), Phosphate-buffered saline (PBS), Reverse Transcriptase  
40 PCR (RT-PCR), threshold cycle (Ct)

41

### 42 **Introduction**

43 Foot-and-mouth disease virus (FMDV) is a member of the family Picornaviridae and exists as  
44 seven immunologically distinct serotypes (A, O, C, SAT 1, SAT 2, SAT 3, and Asia 1). The  
45 disease caused by this virus is endemic in many regions of Africa, Asia and South America,  
46 often causing extensive epidemics in domesticated cloven-hoofed livestock. In addition, more  
47 than 70 species of wild mammals belonging to more than 20 families are susceptible to  
48 infection. The virus can also cause persistent infection of the pharynx in cattle, sheep, goats,  
49 and the other ruminants which can complicate the epidemiology and control of the disease.  
50 The highly contagious nature of FMD motivates great urgency in laboratory diagnostic

51 analysis, especially when the virus is introduced into countries previously classified as FMD-  
52 free. Such was the case in the United Kingdom in 2001 when the FMD epidemic resulted in  
53 devastating losses to the food, farming, tourism and leisure industries. (Thompson et al.,  
54 2002).

55

56 Diagnosis of FMD depends upon early recognition of the clinical signs of disease in the field,  
57 followed by confirmation of the presence and serotype-specificity of FMDV in the laboratory.  
58 Established laboratory assays for the detection of FMDV include virus isolation (VI) in cell  
59 culture (Snowden, 1966), antigen capture ELISA (Ferris and Dawson, 1988), and reverse  
60 transcription polymerase chain reaction (RT-PCR; Reid et al., 2002, 2003). VI has high  
61 sensitivity and is considered the gold standard method but it can be slow and laborious. In  
62 contrast, antigen ELISA is more rapid but has lower sensitivity and therefore cannot be  
63 reliably used to confirm negative cases. RT-PCR has recently been shown to be a rapid  
64 method with still higher diagnostic sensitivity than VI (Shaw et al., 2004; King et al., 2006).  
65 Furthermore, since FMD cannot be differentiated clinically from the other vesicular viral  
66 diseases of swine e.g. swine vesicular disease (SVD), vesicular stomatitis (VS) and vesicular  
67 exanthema of swine (VES), differential diagnosis is an important aspect of laboratory  
68 investigation.

69

70 The aim of this study was to evaluate the proximity ligation assay (PLA) for detection of  
71 FMDV in clinical samples. This is a new technique that has been used for detection of  
72 proteins and microorganisms in complex biological samples and has already been shown to be  
73 as sensitive as more established nucleic acid detection assays such as PCR (Gustafsdottir  
74 et.al., 2006). The basis of the PLA is that FMDV specific-antibodies binding target proteins  
75 are coupled to oligonucleotide strands. These oligonucleotides can be joined by ligation when

76 two or more such reagents are brought into proximity by binding to the same target molecule  
77 or target molecule complex (Figure.1). The DNA ligation products are subsequently detected  
78 by PCR amplification using fluorogenic probes to detect the amplified product.

79

## 80 **Material and Methods**

### 81 **Propagation of FMDV cell culture-grown antigen and determination of TCID<sub>50</sub>**

82 TCID<sub>50</sub> values for cell culture-grown viruses were calculated according to the method  
83 described by Kärber (1979).

84

### 85 **Monoclonal antibody (mAb) 1F10 for use as a binding ligand in the PLA**

86 The mAb 1F10 was chosen for this study due to its capability to recognise all seven FMDV  
87 serotypes in both a trapping ELISA (Samuel et al, 1991) and a sandwich ELISA (Brocchi et  
88 al. 1993). In principle, in the trapping ELISA, the mAb 1F10 reacted with each of the seven  
89 serotypes preliminarily immune-captured onto the solid phase by an homologous rabbit  
90 antiserum; in the sandwich ELISA, the mAb 1F10 was used either as antigen-capture  
91 antibody or as the second antibody conjugated with peroxidase.

92 The mAb 1F10 was obtained from a mouse immunised with the FMD virus type O, strain  
93 UK31/2001. The mAb belongs to IgG1 isotype, does not neutralise virus infectivity and is  
94 presumably directed against a conformation-dependent epitope since it does not recognise  
95 isolated viral proteins in immuno-blotting test (data not shown).

96

### 97 **Biotinylation of mAb and preparation of proximity ligation probes**

98 Biotinylation was performed according to the manufacturer's instructions (Roche Diagnostics  
99 Corp, Germany). Briefly, D-biotin-N-hydroxysuccinimide ester was mixed with the antibody  
100 in a 10-fold molar excess and with a volume ratio of 1:10. The solution was incubated for 4

101 hours at room temperature with continuous agitation. The biotinylated mAbs were then  
102 dialysed thoroughly in phosphate buffered saline (PBS, pH 7.4) to remove unbound biotin.  
103 Proximity ligation probes were constructed by combining the free 3' or 5' ends of  
104 streptavidin-oligonucleotide conjugates (a gift from Olink AB, Uppsala, Sweden) with  
105 biotinylated antibodies or biotinylated peptide according to the instructions, as previously  
106 described (Gullberg et al., 2004).

107

108

### 109 **Functionality test of the biotinylated mAb**

110 The biotinylated mAb was tested by the in-house FMDV AgELISA (Svanova Biotech) by  
111 substituting it with the HRP-conjugated mAb in the assay. To visualize the binding of the  
112 biotinylated mAb, HRP-labeled streptavidin (Dakopatts, Denmark) was used at a  
113 concentration of 0.08µg/ml. Absorbance in the wells was measured with a spectrophotometer  
114 (Flow Laboratories, UK) set at 450 nm.

115

### 116 **Clinical samples**

117 Table 1 lists the samples ( $n = 54$ ) used to evaluate the diagnostic sensitivity of the PLA. These  
118 mainly comprised epithelial suspensions (ES) prepared in phosphate buffer upon sample  
119 receipt (Ferris and Dawson, 1988) which had been stored in the interim between -50 and -  
120 90°C. Twelve of these samples were FMDV negative (including samples positive for SVDV,  
121 VESV and VSV) while the remaining 42 were FMDV positive; having previously been  
122 serotyped following inoculation onto cell culture and by antigen ELISA (Ferris and Dawson,  
123 1988).

124

125

**126 Detection of FMDV by homogenous phase PLA**

127 Before being used in the different test systems, the ES to be tested were diluted 1 in 5 in PBS.  
128 In the homogenous PLA, 1  $\mu$ l of each sample was incubated for 1 hr at 37°C with 4  $\mu$ l of  
129 solution containing both proximity probes (each diluted to a concentration of 24 pM in probe  
130 dilution buffer) in optical PCR tubes (Applied Biosystems, US). The ligation and TaqMan  
131 PCR mix (50  $\mu$ l) (Gullberg et al., 2004) was then added and after ligation for 5 min at room  
132 temperature the tubes were transferred to a real-time PCR instrument for temperature cycling  
133 at 95 °C for 2 min, followed by 95 °C for 15 s and 60 °C for 60 s, repeated 40 times (Applied  
134 Biosystems PRISM 7000 and MX4000, Stratagene, Amsterdam, The Netherlands). The  
135 results were presented either as  $C_t$  values or as signal to noise (S/N) values where the number  
136 of ligations of proximity probe pairs that occurred in the sample was divided by the number of  
137 ligations in the negative control.

138

**139 Detection limit of FMDV in clinical samples by PLA**

140 In order to determine the analytical sensitivity of the PLA and to ascertain a cut-off value for  
141 the assay, a clinical sample, with known TCID<sub>50</sub> titer was serially diluted in a negative field  
142 sample. The assay cut-off value was set at two standard deviations (SD) over the background  
143 signal (i.e. S/N 6.5). Test and control samples with values below this threshold were  
144 considered to be negative while samples with values higher than the threshold were positive.

145

**146 Real-time RT-PCR**

147 All clinical samples were tested by two real-time RT-PCR assays (cut-off 32 Ct) targeting  
148 conserved and independent regions of the FMDV genome, namely the 5' untranslated region  
149 (5' UTR) and the 3D (RNA polymerase). Briefly, total nucleic acid was extracted from all  
150 samples by an automated procedure on a MagNA Pure LC as described previously (Shaw et



151 al., 2007). RT-PCR reaction mix containing a primers/probe set against the 5' UTR (Reid et  
152 al., 2002) was prepared as described previously (Shaw et al., 2007). An automated  
153 programme on the MagNA Pure LC then added 20  $\mu$ l of the reaction mix to 5  $\mu$ l of template  
154 in an optical reaction plate. The same programme also added 5  $\mu$ l of template to 20  $\mu$ l of a  
155 second reaction mix which was the same as the first one except that a primers/probe set  
156 targeting the 3D region (Callahan et al., 2002) instead of the 5' UTR was included. RT-PCR  
157 amplification was performed in an Mx4000 Multiplex Quantitative PCR System (Stratagene,  
158 Amsterdam, The Netherlands) as described previously (Shaw et al., 2007).

159

#### 160 **In-house antigen capture-ELISA**

161 To perform the AgELISA, plates (Nunc Maxisorp) were coated overnight at 4°C with 100 $\mu$ l  
162 of purified mAb 1F10 at a protein concentration of 3  $\mu$ g/ml in 50mM carbonate buffer, pH  
163 9.6. These were subsequently blocked with 2% bovine serum albumin (BSA) in PBS for 1 hr  
164 at room temperature. Inactivated cell cultures were serially diluted in PBS and added in 100 $\mu$ l  
165 volumes to the wells for 1hr at room temperature. Biotinylated mAb 1F10 was diluted in PBS  
166 containing 0.05% Tween (PBST) and 100 $\mu$ l were added per well and incubated for 1 hr at  
167 room temperature. HRP-streptavidin (Dakopatts, Denmark) was diluted in PBST, was then  
168 added (100 $\mu$ l per well) and incubated for 1 hr at room temperature. Substrate, 50 $\mu$ l/well, was  
169 3,5,3'-tetramethylbenzidine and 3.5mM H<sub>2</sub>O<sub>2</sub> in substrate buffer (0.1 M sodium acetate, pH  
170 6.0). The enzyme reaction was stopped after 10 min by adding 50 $\mu$ l of 2M H<sub>2</sub>SO<sub>4</sub>, and the  
171 optical density (OD) was read at 450 nm in a spectrophotometer (Flow laboratories, UK).

172

173

174

175

176 **Results**

177 **FMDV pan-reactivity of mAb 1F10**

178 The reactivity profile of mAb 1F10 with the seven FMDV serotypes is shown in figure 2a  
179 (trapping ELISA) and 2b (sandwich ELISA). Serial dilutions of one or more representative  
180 strains for each of the seven serotypes, grown in cell culture, were used as source of antigen  
181 in the sandwich ELISA, while serial dilutions of mAb (as hybridoma supernatant) were  
182 reacted with a fixed virus concentration in the trapping ELISA.

183 Dose-response curves obtained by trapping ELISA provided evidence that mAb 1F10 cross-  
184 reacts with the seven serotypes, although with slightly different efficiency: FMD viruses type  
185 O, A, C and Asia 1 were recognised with similar strength and more efficiently than the three  
186 SAT types (figure 2a).

187 Results of the sandwich ELISA confirmed previous observations, with the four serotypes O,  
188 A, C and Asia 1 more strongly reactive compared to the SAT types; furthermore, they proved  
189 the accessibility of the target epitope, repeatedly exposed in the viral antigens, to the multiple  
190 binding with mAb 1F10.

191

192 **Functionality test of biotinylated mAb**

193 A positive result was obtained when the biotinylated mAb was diluted up to 100,000-fold  
194 (corresponding to an antibody concentration of 0.03 $\mu$ g/ml) in the in-house AgELISA (data  
195 not shown).

196

197 **Analytical Specificity of the PLA**

198 The specificity of the test system was determined by preparing serial dilutions of cell culture-  
199 grown FMDV of the 7 serotypes as well as cell culture supernatants of SVDV and VSV. The  
200 resulting titration curves confirmed the ability of the mAb to specifically detect all 7 serotypes

201 while no reaction was obtained with representative strains of SVDV and VSV cell culture-  
202 grown viruses (data not shown).

203

#### 204 **Detection limit of the PLA in comparison with RT-PCR and ELISA**

205 The detection limits of the PLA and both RT-PCR assays were compared by testing each  
206 system on a serial dilution of an FMDV sample with a known TCID<sub>50</sub> titre. Figure 3a shows  
207 that as few as 5 TCID<sub>50</sub> could readily be detected in 1µl sample by PLA. The RT-PCRs used  
208 for detection of a genomic sequence showed slightly higher sensitivity in detecting 1 to 5  
209 TCID<sub>50</sub> which is visualized in Figure 3a for the 5'UTR RT-PCR. The cut-off for the PLA, set  
210 as 2 x SD above the C<sub>t</sub> value of the background, gave a figure of 1-5 TCID<sub>50</sub> or S/N 6.5. In  
211 the RT-PCR a C<sub>t</sub> value above 32 is considered negative. The detection limit of the PLA was  
212 compared to that of the antigen capture ELISA using a dilution series of inactivated virus  
213 (serotype Asia 1). The PLA was found to be 100 times more sensitive (Figure 3b).

214

#### 215 **Analysis of clinical samples by PLA and RT-PCR**

216 The fifty four samples were analysed with the PLA and by the two comparative RT-PCR  
217 tests. These samples had previously been classified and serotyped as either positive ( $n = 42$ )  
218 or negative ( $n = 12$ ) by the combination of VI and antigen capture AgELISA. All except one  
219 of the samples were also subjected to the RT-PCR. All of the samples typed as serotype O, A,  
220 C and Asia 1 scored positive by the PLA except for one type O strain but several SAT 1 and  
221 SAT 2 viruses scored negative and none of the SAT 3 viruses were detected (Table 1 and  
222 Figure 4). All but three of the negative samples scored correctly negative. Results for the real-  
223 time RT-PCR (using a cut off of 32 cycles; Shaw et al., 2007) showed that all samples were  
224 scored correctly positive or negative in both tests, except for 2 SAT 2 viruses which were not

225 detected by the 5' UTR assay (positive by the 3D assay). Lower diagnostic sensitivity of this  
226 assay for SAT serotypes viruses has been previously documented (King et al., 2006).

227

## 228 **Discussion**

229 Established laboratory assays for detection of viral proteins such as VI and antigen detection  
230 ELISA can be laborious or lacking or low in sensitivity. In this evaluation we have shown  
231 that the PLA has an analytical sensitivity close to that of real-time RT-PCR; suggesting this  
232 new methodology could be a quick and reliable tool for diagnosis of FMD and an alternative  
233 to VI and antigen-detection ELISA. The PLA assay could detect as few as 5 FMDV TCID<sub>50</sub>  
234 which was similar to the analytical sensitivity of the real-time RT-PCR assays used for  
235 comparison but superior to that of the capture ELISA. It was 100-fold more sensitive than the  
236 in-house AgELISA which employed the same mAb and antigen components.

237

238 The diagnostic sensitivity, specificity and robustness of the PLA depend heavily upon the  
239 suitability of the binding ligand i.e. the mAb used in the assay. Our choice was one that had  
240 been shown to be pan-reactive by detecting all 7 serotypes of FMDV. Initial experiments  
241 using inactivated viral cell cultures showed that the mAb could detect all 7 serotypes although  
242 there was a lower reactivity against SAT 1, 2 and 3 serotypes. Factors that may influence this  
243 variation of reactivity could be minor differences in the target epitope configuration between  
244 the different serotypes that in turn are reflected as a difference in binding affinity. Also the  
245 rabbit antisera used as trapping antibody could have different capacity binding the different  
246 serotypes of FMDV.

247

248 Evaluation of the PLA, carried out on clinical samples that had previously been classified as  
249 either positive or negative by a combination of VI and AgELISA, showed that the assay could

250 detect most of the FMDVs typed as O, A, C and Asia 1 while the SAT 1, 2 and 3 serotypes  
251 were detected less well. A number of factors may have contributed to the lack of signal with  
252 these samples including a lower amount of virus in these samples or the reduced affinity of  
253 the mAb for the SAT serotypes. It might also due in part by the poor condition of these  
254 samples since the mAb/antigen binding can easily be blocked if the epitope is degraded and  
255 assay signal can be reduced by the presence of inhibitors of ligation or PCR in these samples.  
256 Problems with inhibitors can be avoided by performing the PLA assay with help of a solid  
257 support where virus particles are first trapped and washed before addition of the PLA reagents  
258 (Gustafsdottir et.al., 2006). Furthermore, another factor influencing the poor signal with these  
259 samples may be that only 1µl of an already highly-diluted sample is used as the actual starting  
260 volume for the PLA which is extremely small.

261 All but three of the negative samples were correctly scored as negative. Two samples scored  
262 weakly positive with values just above the estimated cut-off level. The cut-off value assigned  
263 in this study is preliminary and may require adjustment in the future after examining a larger  
264 amount of samples with the PLA. The reason that a third sample expected to be negative  
265 scored as definitively positive is unclear.

266

267 The performance of the PLA was compared to real-time RT-PCR used for the laboratory  
268 diagnosis of FMD. The two RT-PCR assays cover all seven serotypes when used in  
269 combination. Whilst the RT-PCR demonstrated higher diagnostic sensitivity, the PLA is  
270 much simpler to perform. It does not require a sample preparation step as only a pre-dilution  
271 of the original sample is performed before 1µl is added to a mix of the proximity probes. We  
272 suggest that the higher diagnostic sensitivity of the PCR might be due to the purity of the  
273 sample (purification of nucleic acid prior to analysis).

274

275 The PLA assay always give a certain amount of back ground noise due to the non-specific  
276 ligation of proximity probes. This could certainly be a limiting factor for the assay especially  
277 if analyzing samples containing very low amount of viral particles where a positive answer  
278 could be difficult to interpret in combination with an existing background noise. We have  
279 minimised chance of this non specific background by using very low concentrations of the  
280 antibody reagents minimising the chance of self ligation of the proximity probes. The high  
281 concentration of the connector may also contribute to a lower background by hybridizing to  
282 all proximity probes that have not bound to target molecules, thereby inhibiting the target-  
283 independent formation of new ligation substrates

284

285 Established laboratory assays for detection of viral proteins have draw-backs such as being  
286 laborious or lacking sensitivity. With an analytical sensitivity close to that of RT- PCR the  
287 PLA is a quick and reliable tool for diagnosis of FMDV and could be an alternative to VI and  
288 antigen ELISA.

289

290 Future possibilities for the PLA in the veterinary field could be to improve quantification of  
291 virus particles in the sample. For example, if the actual viral load in a vaccine or the amount  
292 of a certain protective protein is known, this could easily be correlated to the level of  
293 protection of a vaccinated animal. In such a study the actual amount of the proteins eliciting  
294 protective antibodies are more relevant for the study than the amount of RNA detected by RT-  
295 PCR.

296

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302

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352

**353 Figure 1, PLA performed in three steps**

354 Clinical samples were analyzed by proximity ligation on intact FMDV particles (left side of  
355 cartoon) and reverse transcriptase PCR on FMDV-RNA (right side of cartoon). For the  
356 proximity ligation analysis, 1 uL of sample was incubated with proximity probes (antibodies  
357 extended with single-stranded oligonucleotides) directed against FMDV surface proteins. In  
358 the next step, free ends of the oligonucleotide extensions on the probes -bound to the same  
359 viral particle were ligated. The newly formed DNA sequence was amplified and detected by  
360 real-time PCR. FMDV-RNA was extracted from the samples for the reverse transcriptase  
361 PCR amplification and detection that was performed in real-time.

362

**363 Figure 2. Evaluation of the reactivity of mAb 1F10 with the seven FMDV serotypes by****364 ELISA assays**

365 A), Trapping ELISA

366 B), Sandwich ELISA

367 Details of the virus strains used are shown in figure legend

368

**369 Figure 3. Detection of FMDV**

370 A), measurement of dilutions of FMDV by PLA (blue) and RT-PCR against the 5' UTR  
371 (purple). The x-axis displays the mean values of the total number of viral infectious units  
372 (TCID<sub>50</sub>) present in each analysis. The y-axis displays the Ct values obtained in each test  
373 system.

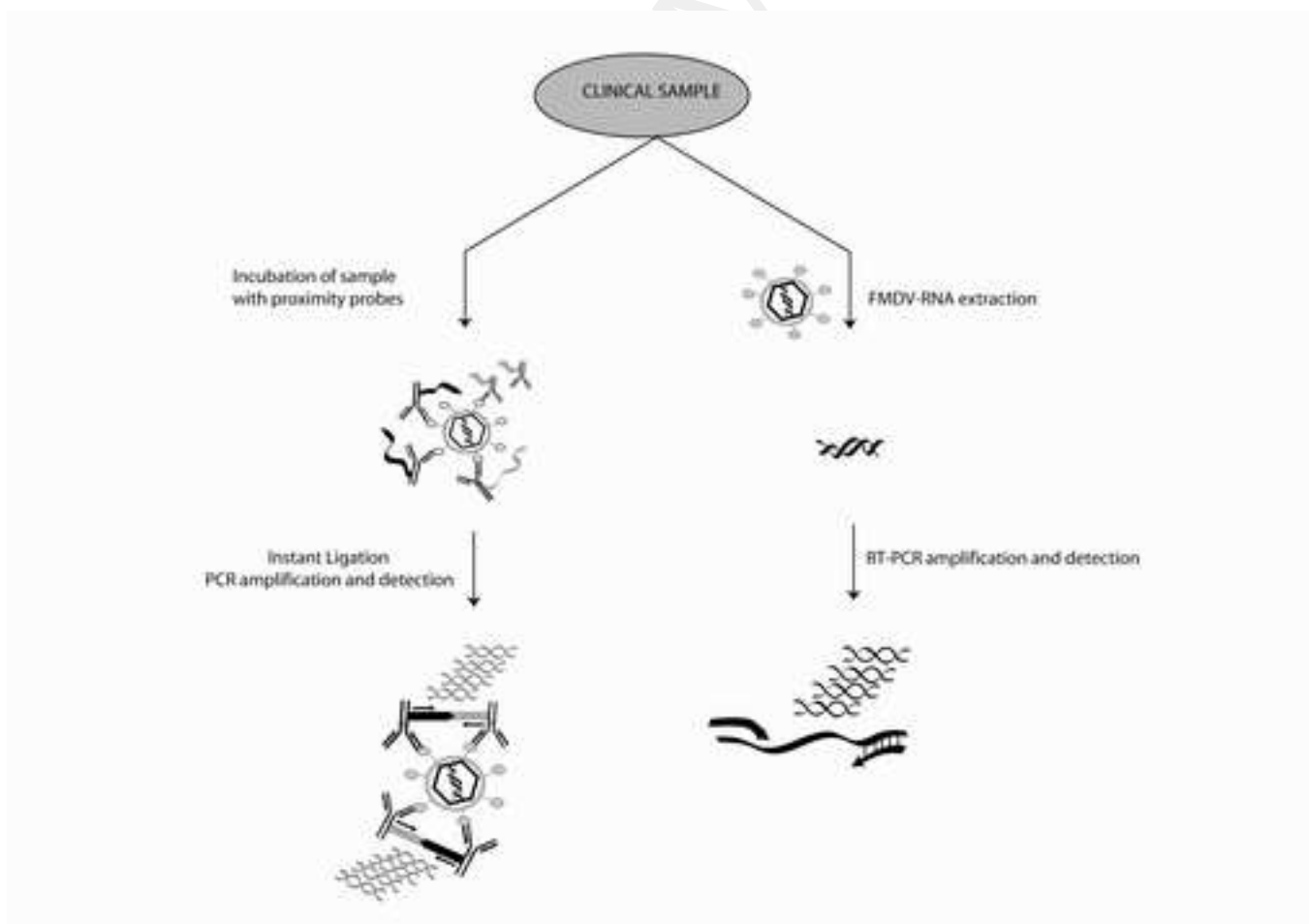
374 B), measurement of dilutions of FMDV (serotype Asia 1) by PLA (blue) and antigen capture-  
375 ELISA (red). The x-axis displays the serial dilution factor of the inactivated FMDV

376 suspension. The left hand Y axis displays the absorbance at 450nm for the ELISA and the  
377 right hand y-axis shows the Ct values of the PLA.

378

379 **Figure 4. Detection of FMDV in clinical samples**

380 Detection of FMDV in field samples by PLA (blue bars), PCR and AgELISA. The X axis  
381 displays the obtained results in each assay. The Y axis shows the S/N ratio for the PLA. The  
382 red line displays the cut-off value of the PLA assay. N.t = not tested. ± = different results in  
383 the two PCR assays.



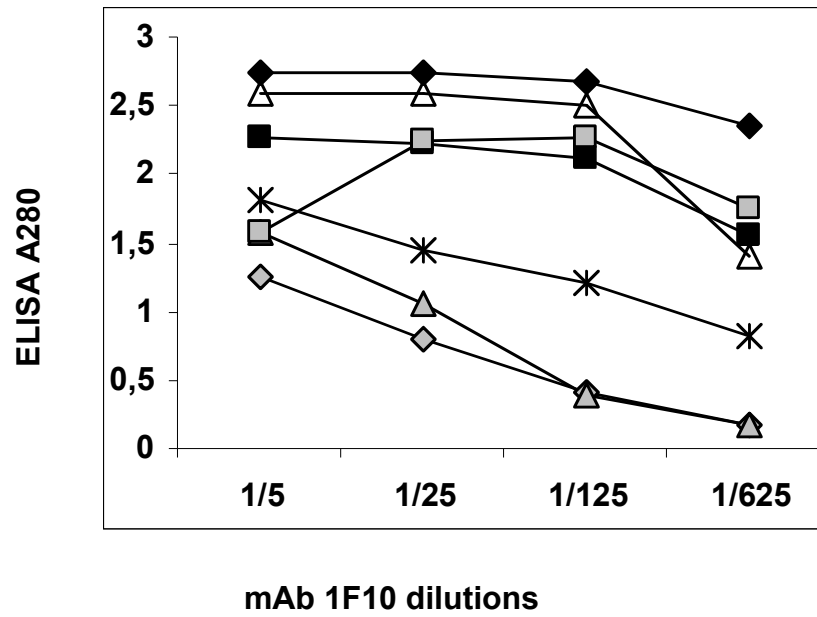
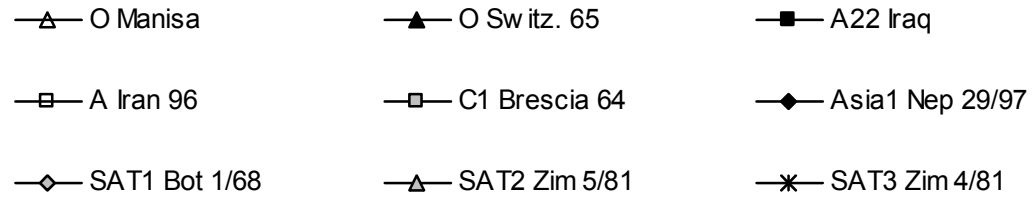


Figure 2a

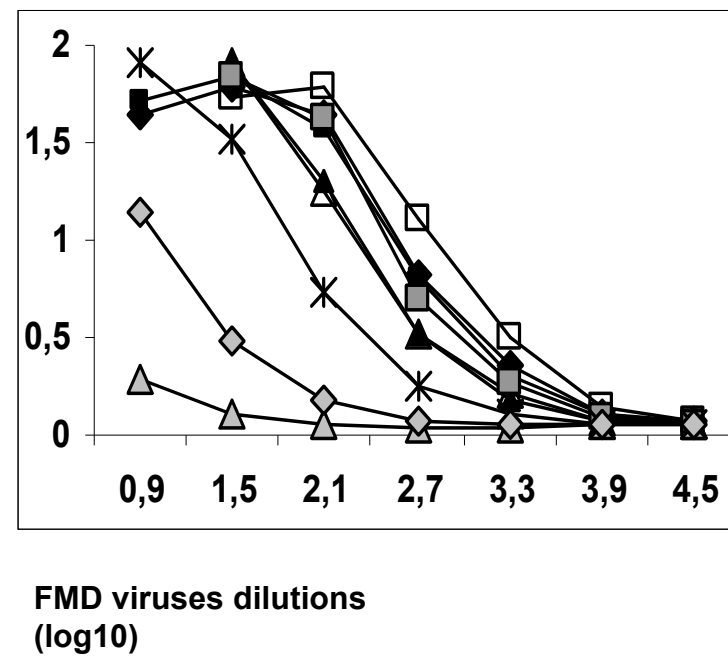


Figure 2b

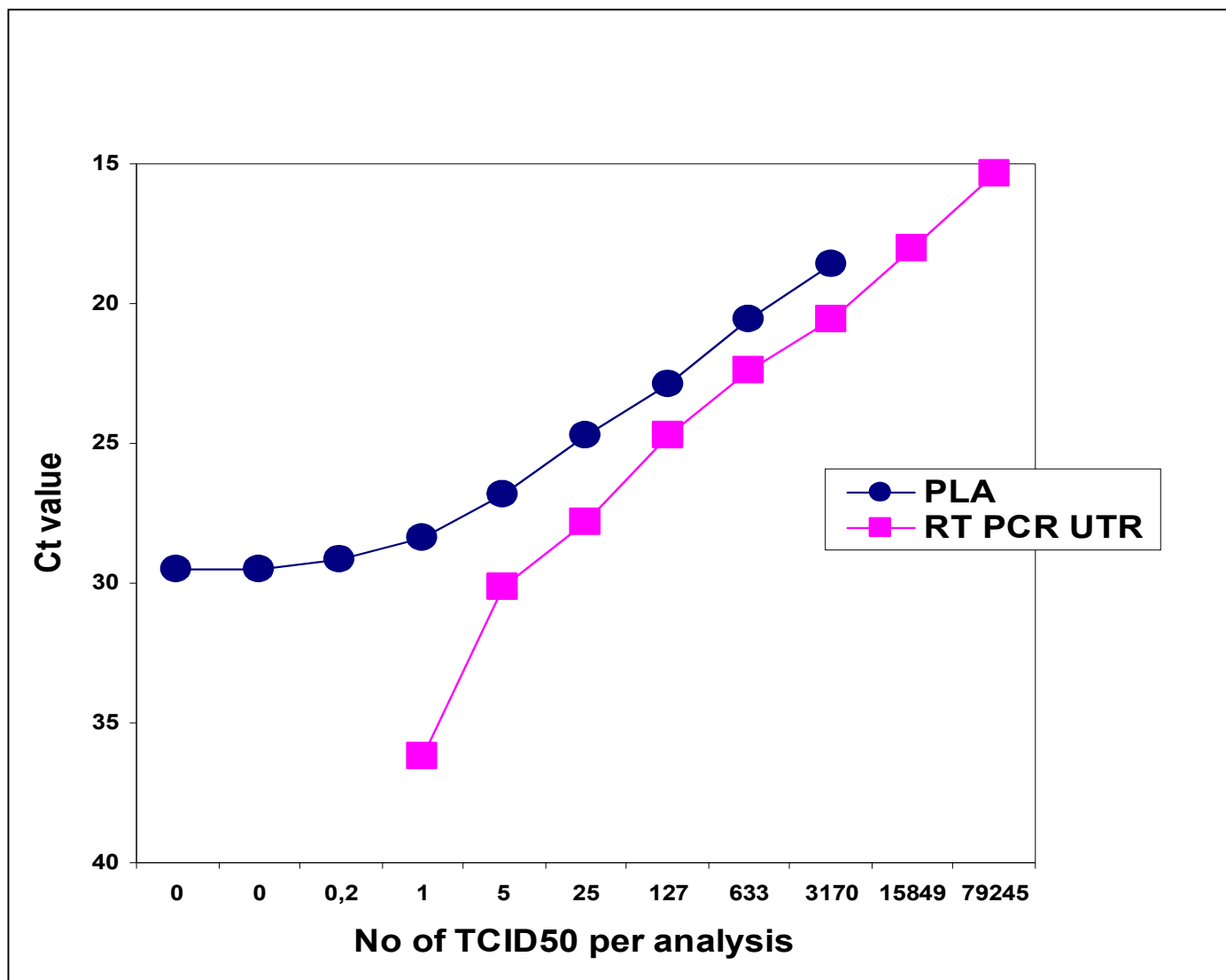
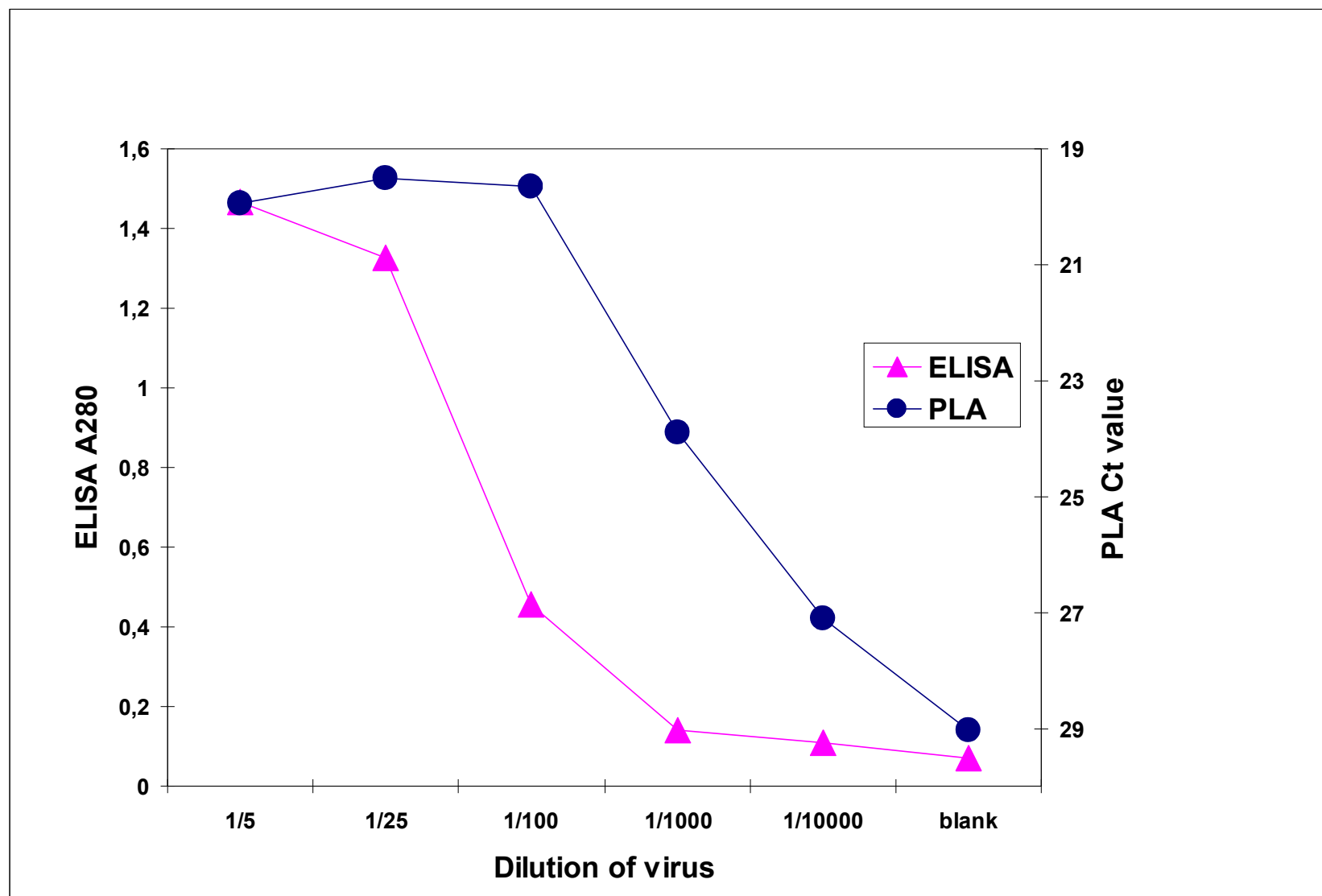


Figure 3a.



- Figure 3b

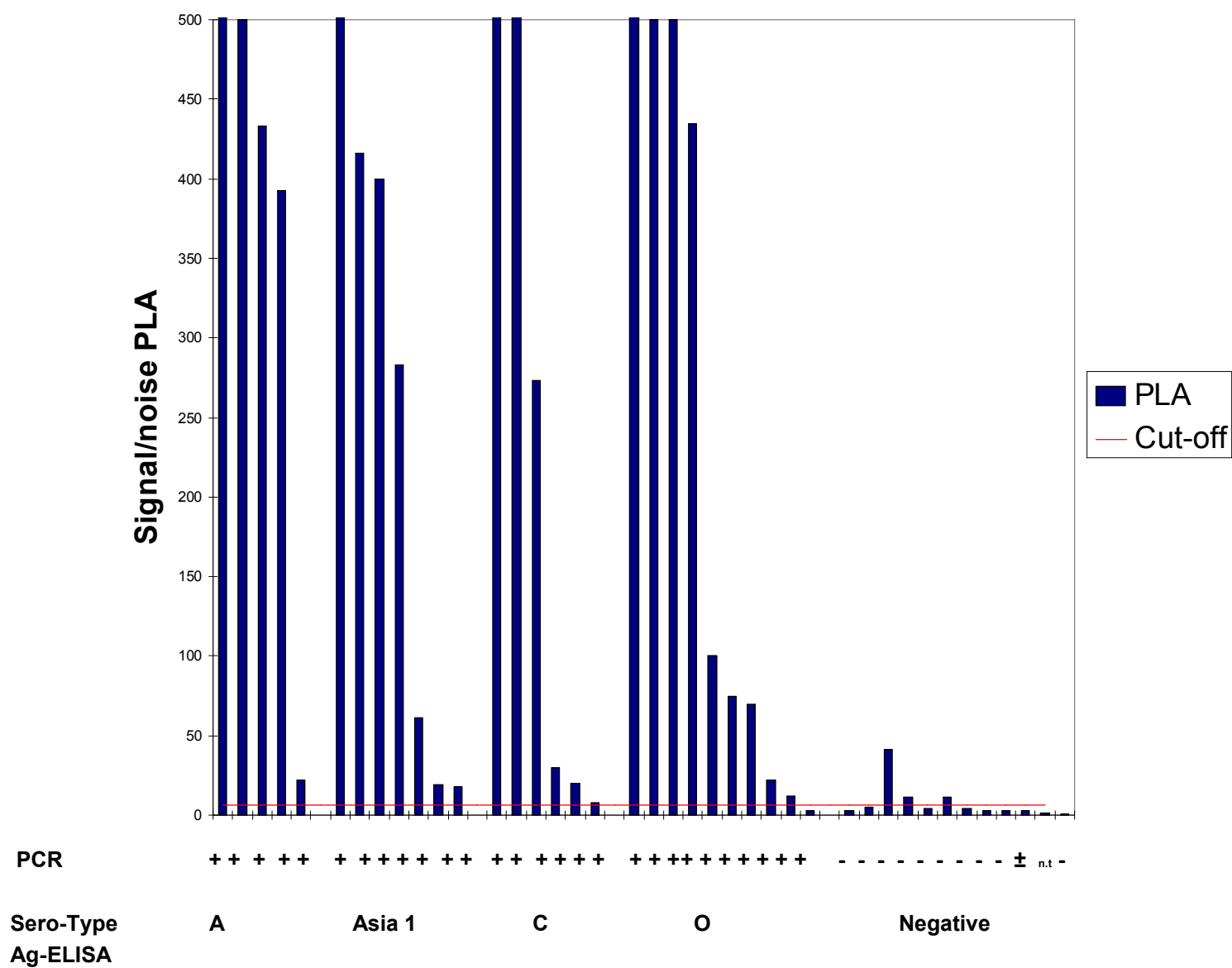


Figure 4.



**Table 1****Detailed list of samples used for evaluation of the diagnostic sensitivity of the PLA**

<b>Sample ID (origin)</b>	<b>Serotype</b>	<b>PLA signal/noise</b>	<b>PCR 5'UTR Ct</b>	<b>PCR 3D Ct</b>
MAY 4/2003	A	650,93	17,55	19,28
TAI 4/2003	A	500,00	18,51	18,84
IRN 10/2005	A	433,00	13,32	13,94
ERI 3/98	A	392,68	24,97	20,07
IRN 7/2004	A	21,79	18,96	18,35
IND 5/89	Asia 1	903,82	15,32	15,72
PAK 1/2004	Asia 1	416,08	14,2	13,64
AFG 3/2001	Asia 1	400,00	21,9	14,49
GRE 2/2000	Asia 1	283,34	12,73	12,53
HKN 8/2005	Asia 1	61,39	15,02	14,93
BHU 27/2002	Asia 1	18,96	17,11	16,48
IRN 30/2004	Asia 1	18,00	13,44	13,38
ANG 2/73	C	517,83	16,81	16,28
SRL 4/78	C	504,57	18,67	25,54
USS 4/74	C	273,36	18,63	18,28
KUW 9/82	C	30,00	22,19	22,3
SAU 14/84	C	19,79	16,35	15,27
PHI 3/94	C	7,56	20,53	23,28
UKG 312/2001	O	739,41	19,13	18,27
SAU 1/2002	O	500,00	15,32	15,07
MAI 2/2005	O	500,00	22,82	17,24
PHI 3/2005	O	434,85	15,88	14,47
TAW 84/94	O	100,00	19,18	16,49
BUN 6/2003	O	75,00	24,8	14,29
ISA 9/74	O	70,00	13,79	12,43
ISA 1/74	O	21,69	15,22	14,09
O1 BFS 1860 Pig SE24	O	12,39	16,05	13,82
MAY 1/2002	O	3,00	19,08	18
Negative bovine	Negative	3,19	no ct	no ct
UKG 36/94	NVD*	4,79	no ct	no ct
UKG 37/94	NVD*	41,27	no ct	no ct
Negative bovine	Negative	11,53	no ct	no ct
Negative bovine	Negative	4,28	no ct	no ct
Negative porcine	Negative	11,53	no ct	no ct

Negative porcine	Negative	4,41	no ct	49,75
VE B1-34 4/7/53	VESV	3,00	no ct	no ct
UKG 39/2002	NVD*	3,00	no ct	no ct
ITL 4/77	SVDV	3,00	no ct	42,75
VJEP-0037	ERAV	1,75	not tested	not tested
HKN 5/91	SVDV	0,89	no ct	no ct
RHO 5/66	SAT 1	100,00	17,9	14,53
RV 11/37 15/9/37	SAT 1	117,46	21,08	20,14
KEN 1/2005	SAT 1	1,35	20,2	15,14
UGA 3/99	SAT 1	207,80	25,36	20,39
KEN 7/2004	SAT 2	0,77	19,91	14,77
ZIM 2/97	SAT 2	6,57	20,03	14,28
MAI 7/91	SAT 2	1,38	27,17	18,66
BOT 1/2005	SAT 2	1,06	14,19	12,52
SAU 4/2000	SAT 2	500,00	no ct	15,79
CAR 117/2005	SAT 2	0,95	no ct	24,02
BEC 2/65	SAT 3	0,67	20,01	17,65
MAL 3/76	SAT 3	1,10	29,5	21,76
RHO 2/74	SAT 3	1,45	23	18,4
ZIM 6/91	SAT 3	4,61	27,26	17,42

\*NVD = no viral detection