Optimization of Serological Diagnostic Methods for Rapid Field Detection of Foot-and-Mouth Disease Virus Antigen and Antibodies in Natural Infected Bovine Specimens

GSG Zeedan1*, AM Abdalhamed1, TK Farag1, MK El-Bayoumy1, AH Mahmoud2 and KA Abd EL-Razik3

1Parasitology and Animals Diseases Department, National Research Centre, 33 Bohouth St., Dokki, Giza, P. O. box 12622, Egypt; 2Biotechnology and Food hygiene department, Animal Health Institute, Dokki Giza; 3Reproduction diseases department, National Research Centre, 33 Bohouth St., Dokki, Giza, P. O. box 12622, Egypt

*Corresponding author: gamilzee@yahoo.com; gam.zee.123@hotmail.com

ABSTRACT

Foot-and-mouth disease (FMD) is an economically important highly contagious transboundary animal’s disease, infecting cloven-hoofed species. It is endemic in Egypt where three different virus serotypes are present (O, A, and SAT 2). Detection of FMD virus antigens and antibodies is an essential cornerstone, for controlling and preventing recurrent FMD. Developing of diagnostic tools for rapid, easily performed in the field for FMDV detection is important for controlling a FMD outbreak and containing its spread. The present study aimed to determine the FMD virus in clinical field samples collected from bovine during FMD outbreak in 2017 in Egypt by improving and developing a rapid field test for detection FMD antigen or antibodies. The study includes detection of FMD virus in 46 tongue epithelium biopsies and vesicles fluid collected from naturally infected cattle and buffaloes. 180 serum samples were collected from cattle, buffaloes, sheep, and goat during FMD outbreaks in 2017. Tongue epithelium, saliva and infected cell culture fluid used for developing rapid slide immune-agglutination test (RSIAT), 3ABC tube immune-chromogenic (TIC-3ABC) test and Latex agglutination test (LAT) compared with commercial 3ABC-ELISA. FMD antigen serotypes were detected in 18(39.1%) and 22(47.8%) tissue biopsy, saliva and fluid vesicles examined by LAT and ELISA tests. However, 18 (39.1%) positive samples by RSIAT and only 16 (34.7%) samples positive by LAT, all those tests used specific FMD virus type’s antisera mono and polyclonal. RSIAT showed sensitivity 81.8% and specificity 87.5% with accuracy 91.3% and LAT test showed sensitivity 95.4% and specificity 95.8% with accuracy 97.3% while, TIC showed sensitivity 72.7% and specificity 65.2% with accuracy 86.9% comparing S-ELISA sensitivity 99.9% and specificity 96.4% with accuracy 97.8%. The results of RSIAT using staphylococcal agglutination test (SPA) and TIC-3ABC-test were similar to those obtained with 3ABC-ELISA. RSIAT, LAT, and TIC-3ABC revealed nearly the same results of TIC-3ABC and commercial 3ABC-ELISA in a parallel manner to confirm each other. Detecting FMD antibodies by 3ABC-ELISA were found to be consumed long time (8 hours), while the RSIAT test needs a very short time (1-5 minutes). Also, TIC-3ABC showed titers similar to those obtained by 3ABC-ELISA for detection of FMDV antibody. It was noticed that the highest antigen or antibody titers showed strong agglutination while weak agglutination was noticed with lower titers. It is concluded that RSIAT is rapid; sensitive and accurate field test for detection FMDV antigen and antibody.

Key words: FMD, Rapid slide immune-agglutination test, Latex agglutination test, ELISA, 3ABC-ELISA, Bovine, Cattle, TICT

INTRODUCTION

Foot and mouth disease (FMD) is a single-stranded positive-sense RNA of approximately 8 kb and it belongs to genus Aphthovirus, family Picornaviridae (Rueckert 1996; El-Khabaz and Al-Hosary, 2017). FMDV has seven serotypes of FMD virus O, A, C, Asia 1, SAT1, SAT 2, and SAT-3 (Biswal et al., 2012). The disease affects cattle, sheep, goats, wild ruminants and water buffaloes (Chepkwony et al., 2015; Kitching, 2019).

FMDV spreading from an infected animal, among contact susceptible animals and aerosol (Chepkwony et al., 2015; Paton et al., 2018). FMD clinical signs appear within 2 to 3 days after exposure to FMDV from direct or indirect infected animals and last for 7 to 15 days. It is cussing great economic losses among cloven-hoofed animals. Economic losses included the trade of animals hindering on a national and international level where major epizootics have developed in many parts of the world every year included the Middle East despite (Leforban, 1999; Kronovet and Skern, 2002). In Egypt, different serotypes and prototype of FMDV has been detected FMDV serotype A and SAT 2 caused outbreaks 1953, 1958 and 1960 (Zahran, 1961). FMDV serotype O was prevalence from 1960, FMDV serotype A reintroduced in 2006 in Egypt producing several disease episodes. In 2012, FMDV serotype SAT2 was found in Egyptian cattle (Mandour et al., 2014; Khodary et al., 2016; Diab et al., 2019). In 2018, FMDV outbreaks occurred in different governorates in cattle and buffaloes (Al-Hosary et al., 2018); rapid detection and serotyping of FMDV is a keystone and essential for control of FMD disease. FMDV recovered from the oesophageal-pharyngeal fluid of infected cattle during the convalescent phase; carrier animals isolated at 28 days, or later, after infection, of cattle carrier last long 3.5 years, and in sheep and goats (Van Bekkum et al., 1959; Condy et al., 1985; Chitraray, 2018). African buffalo have been reported to carry the live virus for up to 5 years (Ferris and Dawson, 1988; Kitching, 2019). Rapid diagnosis is necessary to control foot and mouth disease. Several diagnostic tests such as complement fixation test (CFT), virus neutralization test, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) are available for diagnosis of FMDV but these tests are required skilled manpower, more time, sophisticated labs and high cost (Muhammad et al., 2013; Khana et al., 2015; Sobhy et al., 2018). The advantage of the agglutination test is that it is simple, sensitive and rapid (Kessler, 1981; Dong et al., 2019). RT-PCR is specific, fast and sensitive procedures used for diagnosis (King et al., 2006). Although several techniques are currently available to diagnose foot and mouth disease virus (FMDV), a highly sensitive, highly specific field test is needed for specific and rapid diagnosis of the disease. The present study was aimed to optimize serological tests using a staphylococcal agglutination test (SPA) for rapid and accurate field test production to detect FMD antigens and antibodies.

MATERIALS AND METHODS

Samples

46 tongue epithelium biopsies were obtained from the naturally infected animals showing suspected FMD clinical signs that include high body temperature, vesicles, and erosions in the mouth with characteristic salivation, smacking and lameness. 2g of tongue biopsies were mixed with 2 mL of PBS with antibiotics were ground well with sterile sand. The mixture was frozen and ground for successive three times. It centrifuged at 4000 rpm for 15 min/4°C. One hundred and eighty serum and uncoagulated blood samples were randomly collected from different animal’s cattle, buffaloes, sheep, and goats (80, 30, 50 and 20) from different governorates (Beni-Suef, Giza and El-Fayoum) in Egypt in 2017 from vaccination or non-vaccinated animals male or female respectively.

Latex beads (polystyrene)

Particle diameter 0.31 µ Sigma Aldrich USA, was labeled with Different FMD antigen serotypes A, O and SAT-2 as well as different antibody mono or poly hyperimmune serum against different FMDV serotypes for antibodies and antigens.

Reference anti-FMD sera

Anti FMD virus type O, A, and SAT2 anti-sera were kindly supplied by Veterinary Serum and Vaccine Research Institute, Abbassia (VSVRI) and used as positive controls in the applied serological tests.

Bacterial strain

Staphylococcus aureus was kindly supplied from Microbiology and Immunology Department National Research Centre, Dokki, Egypt, used for the preparation of SPA (Kessler, 1975).

Culturing and processing of S. aureus

Staphylococcus aureus was cultured in brain heart infusion broth. Catalase and coagulase tests were performed for S. aureus and grown on Mannitol Salt Agar (MSA) using the standard procedures (Gilliam and Field, 1993) S. aureus was cultivated in 199 medium supplemented with 0.5% yeast extract. The inoculated medium was dispended in a culture bottle was incubated at 37oC for 24hrs with gentle shaking every 1hrs. Growing bacteria were collected by centrifugation at 2000 rpm for 15minutes and washed twice with PBS at PH 7.2 containing 0.05% (w/v) sodium azide (PBS azide). Bacteria were resuspended to 10% (w/v) concentration in PBS- azide. The suspended bacteria were killed by heating at 80oC with rapid swirling in a water bath for 5 min followed by rapid cooling in PBS azide. Inactivated S. aureus was washed 3 times with PBS by centrifugation. S. aureus was diluted 10% in 0.02% PBS containing sodium azide and kept at 4°C, it was stable for at least 4 months. Before using the SPA suspension should be treated with NP-40 (0.5%) in Net Buffer PH 8.0; for 20 min at room temperature. The treated SPA suspension was then washed once with 0.05% NP-40 in a Net buffer and finally re-suspend to the original concentration (10% suspension) in the later buffer. The Optical Density (OD) value of S. aureus suspension was determined at 525nm by spectrophotometer.

Production of hyper-immune sera in rabbit

Monovalent and trivalent FMD vaccines were prepared by using 70% mountainside oil and 30% FMD intramuscularly with 2mL of FMDV 7log10 TCID50/ml serotypes, monovalent serotypes O, A, and SAT-2 were inoculated subcutaneous and intramuscularly in rabbits for each, Also, the trivalent vaccine was injected in rabbit, and kept one rabbit un-inoculated control negative. Antibodies in the sera were detected by using indirect ELISA (Sting et al., 1990). Briefly, it was prepared according to (Sting et al., 1990) 5 rabbit of average
weight from 2 kg, 4 rabbits were injected subcutaneously and intramuscularly with 2 mL of FMDV serotypes O, A, SAT-2, and 3 serotypes 70% mountainside oil and keep one rabbit as control negative as 4 successive doses as follows: 1st dose at 0 day, 2nd dose at 14 days, 3rd dose at 28 days and 4th dose at 35 days. After seven days of the last dose, the rabbits were slaughtered and blood was collected, then centrifuged at 3000 rpm for 10 minutes and the collected serum was stored in sterile screw-capped vials and kept at -20°C until used.

Latex agglutination test (LAT)
Sensitization of latex beads with the locally prepared FMDV antibodies for detection of FMDV antigen in field samples, 0.25 g of latex beads were washed in carbonate-bicarbonate buffer and centrifuged at 8000 rpm for 2 minutes discarded the supernatant and repeated washing steps, and the pellet was suspended in 2.5 mL of carbonate-bicarbonate buffer (0.5M, pH 9.6) preparing 1% suspension. 2.5 ml of diluted FMDV antibodies for anti-FMD mono A, O, SAT-2 and poly A, O and SAT-2 or 2.5 ml of diluted were mixed with 2.5 ml of 1% suspended latex beads. The mixture was shaken on a shaker incubator for 6 hours and then centrifuged for 3 minutes at 8000 rpm and the pellet was collected. The pellet was suspended in 2.5 mL PBS containing 0.5% FCS and the mixture was incubated overnight at 37°C. After centrifugation at 8000 rpm for 3 minutes, the pellet was collected and suspended in 1.5 mL PBS containing 0.5% fetal calf serum (FCS) + 1% sodium azide.

Preparation and Optimization of SPA conjugated with rabbit antisera
125 μl from each of the monovalent rabbit anti-O, anti-SAT-2, anti-A, and trivalent (anti-O, anti-SAT-2, and anti-A) and Control negative or diluted antigen was mixed with 2ml of Staphylococcus aureus suspensions (1/5, 1/10 and 1/100 dilutions, respectively) and kept in an incubator at 37°C for 2 h then centrifuged at 3000 rpm at 15min. Pellet was washed twice and centrifugation with 0.02% sodium azide containing PBS at pH 7.2. Then conjugates were suspended in 2ml PBS with 0.02% Sodium azide and 0.25% Tween 20 were tested against FMD O, SAT-2 and O viruses. Also, control negative healthy animal saliva and PBS at Ph7.2.

Rapid Slide Immune Agglutination test – SPA (RSIAT-SPA)
Forty- six of clarified sample suspensions from naturally infected cattle were mixed with an equal conjugated anti-FMD with the amount of SPA particles (prepared by mixing staphylococci suspension with specific anti-FMDV monovalent or trivalent antiseraum against FMD virus on a glass slide surface and the mixture were gently rotated for up to 5-10 min. at room temperature. Each sample was tested in triplicated with the control and the anti- FMD type O, A and SAT-2 conjugates. Serum samples which collected from cattle during outbreak examined with FMDV virus non-structural polypeptide (NSP). NSP antigen coated slide was used for differentiation between vaccinated and infected samples by detection antibody against NSP by developing RSIAT-SPA-3ABC-FMDV. The agglutination pattern was read microscopically. Sensitivity was determined by the formula: number of true positives results divided by the number of true positive and false negative results (Sørensen et al., 1998; Morsy et al., 1992).

Tube immune-chromogenic test (TIC) for Detection of FMDV
Tongue tissues biopsy and saliva samples were added to micro-immune-tube previously coated with specific anti FMDV, serotype A, O, SAT-2 antibody incubated for 2h at 37°C after incubation discard suspension and washed 3 successive times, anti-species conjugated with horse radish peroxide was added and incubated for 15 min at RT in dark place. Then added TMB and stopped the reaction after 5 min. The test is considered positive when the color changed compared to negative tube. Also, non-structural polypeptide (NSP) of FMD antigen were used to differentiate between vaccinated and infected samples by the detection antibody against NSP.

Detection of FMD NSP antibodies
3ABC-Enzyme-Linked Immunosorbsent Assay (ELISA)
Serum samples were examined by 3ABC-ELISA kit (IDEXX FMD 3ABC Bo-Ov) to detect antibody against non-structural polypeptide (NSP) of FMD antigen followed the manufacturer instructions.

Sandwich-ELISA (S-ELISA)
For detection of FMD virus serotypes present in infected cattle and buffaloes biopsies samples; tongue tissues biopsies, vesicles, and saliva. Sn-ELISA, (Brescia, Italy and Pirbright, UK) was performed according to the method described by (Mathur et al., 1993), with slight modification (Sørensen et al., 1998).

RESULTS
Screening hyper immune-serum collected from inoculated experiment rabbits by indirect ELISA found positive and un-inoculated control rabbit found negative against different FMD antigens A, O, SAT-2 serotype.
RSIAT coated antigen and ELISA was able to detect the mono and polyvalent - FMDV type O; A and SAT2 hyper immune serum prepared in rabbits with titer ranged from ≥log10 within 1-6 minutes while ELISA needs 6 hours.

FMD virus detection
Sensitivity = [TP / (TP+FN)] X 100 where TP = true-positive and FN = false-negative
Specificity= [TN / (TN+FP)] X 100, where TN = true negative, FP = false positive result.
Accuracy = True positive /Total number of samples X 100

Table 2 showed clearly that the different FMD antigen serotypes were detected in 18(39.1%) and 22(47.8%) tissue biopsy, saliva and fluid vesicles examined by LAT and ELISA tests. However, 18 (39.1%) positive samples by RSIAT and only 16 (34.7%) samples positive by TIC, all those tests used specific FMD virus type’s antisera mono and polyclonal. RSIAT showed sensitivity 81.8% and specificity 87.5% with accuracy.
Results indicated that firming each other for Kang (2013) comparing S. velvetin in the initial diagnosis of FMDV (Rahman et al., 2002) or serotypes is done by using rapid developing tests and specificity 96.4% with accuracy 97.8%. Differentiated accuracy 86.9 showed sensitivity 72.7% and specificity 65.2% with specificity 95.8% with accuracy 97.3% while, TIC 91.3% and LAT test showed sensitivity 95.4% and specificity 95.8% with accuracy 97.3% while, TIC showed sensitivity 72.7% and specificity 65.2% with accuracy 97.8%. Differentiated between FMD of vaccinated and infected animals. Each of FMD antibodies vaccinated or non-vaccinated animals gave positive results con-vaccinated animals naturally infected animals revealed that both FMDV in Egypt is serotyping O. RSAT (Muhammad et al., 2010; El Wahed 2016) and typing of FMDV depending on its simplicity; rapidity tool in both quantitative and qualitative for the detection FMDV which provides rapid, sensitive and specific tools for identifying and characterizing FMDV strains in clinical samples (Brochichi et al., 2006; Ludi et al., 2017).

In the present study, RSAT-SPA standardized for detection of FMDV in vesicular fluid and tongue epithelium tissue samples using SPA conjugated immune sera against FMD virus produced in rabbits react well with rabbits-IgG (De Diego et al., 1997; Rémont et al., 2002). A specific and accurate diagnosis of FMV is important for the rapid detection of the virus antigens or antibodies (De Diego et al., 1997; Mackay et al., 1998). Preparation of SPA for (RSAT-SPA) on infected tongue epithelium and/or FMDV antigen in comparison with S-ELISA showed their abilities to detect of FMDV serotype O; A and SAT2 and faster results within 2-6 minutes while S-ELISA needs 6 hr. RSIAT coated antigen and ELISA was able to detect the mono and polyvalent - FMDV type O; A and SAT2 hyper immune serum prepared in rabbits with titer ranged from ≤log10 within 1-6 minutes while ELISA needs 6 hours. In addition, RSIAT-SPA examined serum samples obtained from vaccinated and non-vaccinated animals gave positive results similar to those obtained by ELISA as in Table 1 & 2 and those results are agreed with (De Diego et al., 1997) noticed that the strength of agglutination was strong with high titers of virus antigen or antibodies and weak with lower titers.

Fig. 1: Showed that the TIC -3ABC-FMDV was compared with 3ABC-ELISA used the non-structural protein of FMDV (FMDV-NSP) on serum samples of vaccinated or non-vaccinated animals naturally infected animals revealed that both tests were nearly had the same results confirming each other for each of FMD antibodies vaccinated or non-vaccinated infected animals.

Table 1: Rapid side immune agglutination test-SPA (RSAT-SPA) and Sandwich ELISA used for detection mono and polyantibodies.

<table>
<thead>
<tr>
<th>Anti FMDV serotype</th>
<th>Prepared rabbit sera</th>
<th>FMDV different serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAT-2</td>
<td>S-ELISA</td>
</tr>
<tr>
<td>Monovalent anti SAT-2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Monovalent anti O</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Monovalent anti A</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trivalent anti SAT-2, O</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Control negative</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
+ Positive biopsy samples: %: Percent of positive biopsy samples.

Table 2: Detection of FMDV by different serological test in naturally infected animals’ biopsy samples.

<table>
<thead>
<tr>
<th>Prepared rabbit antisera</th>
<th>C &amp; B</th>
<th>RSAT-SPA</th>
<th>S-ELISA</th>
<th>LAT</th>
<th>TIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono FMD- S-A</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Mono FMD- S-O</td>
<td>8</td>
<td>22</td>
<td>10</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Mono FMD- S-SAT-2</td>
<td>6</td>
<td>13</td>
<td>6</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Mixed infection</td>
<td>2</td>
<td>4.3</td>
<td>6.5</td>
<td>2</td>
<td>4.3</td>
</tr>
<tr>
<td>Poly FMD S-A, O, and Sat-2</td>
<td>18</td>
<td>39.1</td>
<td>47.8</td>
<td>21</td>
<td>45.6</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The rapid and accurate diagnosis of foot-and-mouth disease (FMD) is cornerstone for are very important and essential for control of disease outbreaks and implementation strategy for vaccination of infected animals. Most of laboratory-based methods can provide objective results within a few hours or few days of sample receipt. Therefore, rapid and easy-to-perform tests, which can be used in the field (on-site on place) in case of a suspected disease outbreak, would be a valuable tool for veterinarians in the initial diagnosis of FMDV (Kang et al., 2017; Jain et al., 2018). The present study of FMDV and serotypes is done by using rapid developing tests (RSIAT, LAT, and TIC) for lab and field for diagnosis and can replace the Sandwich-ELISA and complement fixation test (CFT) as the routine method of diagnosis.
are similar to S-ELISA for detection of FMDV in tongue epithelium but more sensitive and reliable test than TICT. These results are parallel to obtained by (Lewis-Rogers et al., 2008). The present work showed that the differences FMDV antigen serotypes were detected in 18(39.1%) and 22(47.8%) tissue biopsy, saliva, and fluid vesicles examined by LAT and ELISA tests respectively. However, 18 (39.1%) positive samples by RSIAT-SPA and only 16 (34.7%) samples positive by TICT, all those
tests used specific FMD virus type’s antiserum mono and
colpogenic. RSAT showed sensitivity 81.8% and
specificity 87.5% with accuracy 91.3% and LAT test showed
sensitivity 95.4% and specificity 95.8% with accuracy 97.3% while, TICT showed sensitivity 72.7% and
specificity 65.2% with accuracy 86.9% comparing S-
ELISA sensitivity 99.9% and specificity 96.4% with
accuracy 97.8%. The test system described is sensitive and
specific as in Table 2 obtained results are in agreed
with (Ali et al., 2017). The assay system is easily prepared
and therefore can be applied to both clinical as well as
research screening for staphylococcal protein A. The
correlation between coagulase production and protein A
eexpression as judged by this test system allows
investigators another parameter by which to screen S.
aureus isolates (Yang et al., 2013). TIC-3ABC-FMDV
using nonstructural protein of FMDV on serum samples
of vaccinated or non-vaccinated animals naturally infected
animals nearly the same results of 3ABC-ELISA as in
Figure-1 and those results are in agreed with (King et al.,
2006; Khana et al., 2015). It could be concluded that latex
and SPA attached to immune-globulin molecules of
various species are rapid and simplicity for the rapid
diagnosis of FMDV in different livestock.

Conclusions

FMD is still important contagious viral disease
threatened the livestock population in Egypt, Detection of
FMDV antigens and antibodies using nonstructural
polypeptide lead to differentiate between vaccinated and
non-vaccinated. The RSIAT, LAT and TIC are safe; easy
to be used, high stable, highly sensitive, low cost; not
requires special laboratory equipment so they are
considered a field diagnostic test for FMDV antigens and
antibodies.

Acknowledgments

I would like to express my sincere thanks to the
Department of microbiology and Immunology, National
Research Centre, Cairo, Egypt. I would also like to thank
Veterinary Research Division at the National Research
Centre, Cairo, Egypt for their support through this work.

REFERENCES

with different serotypes of FMDV in vaccinated cattle in
of indirect ELISA and serum plate agglutination (SPA) test
for the detection of Mycoplasma gallisepticum in chicken.
Armsom B, Walsh C, Morant N, et al., 2019. The development of
two field-ready reverse transcription loop-mediated
isothermal amplification assays for the rapid detection of

Ayebazibwe C, Mwini FN, Tjomnehi J, et al., 2010. The role
of African buffalos (Syncerus caffer) in the maintenance of
Biswal JK, Sanayal A, Rodriguez LL, et al., 2012. Foot-and-
mouth disease: global status and Indian perspective. Indian
Brocchi E, Bergmann IE, Dekker A, et al., 2006. Comparative
evaluation of six ELISAs for the detection of antibodies to
the non-structural proteins of foot-and-mouth disease virus.
Vaccine, 24: 6966–6979.
detection of Foot and Mouth Disease Virus (FMDV)
vaccines and diagnostics through structural design
(Doctoral dissertation, University of Pretoria).
Condy JB, Hedger RS, Hamblin C, et al., 1985. The duration of
the foot-and-mouth disease virus carrier state in African
buffalo in the individual animal and (ii) in a free-living
De Diego, Brocchi M, Mackay E, et al., 1997. The non-
structural polypeptide 3ABCV of foot-and-mouth disease
virus as a diagnostic antigen in ELISA to differentiate
infected from vaccinated cattle. Arch Virol, 140: 2021–
2033.
outbreaks in Egypt during 2013-2014: Molecular
caracterization of serotypes AO and SAT2. Vet World, 12:
190-197.
Dong XM, Jing TAO, Li TT, et al., 2019. A rapid, simple and
sensitive immunooagglutination assay with silica
nanoparticles for serotype identification of Pseudomonas
aeruginosa and Acinetobacter baumannii. J Appl Microbiol, 20:
Eble PL, Bouma A, Weerdmeester K, et al., 2007. Serological
and mucosal immune responses after vaccination and
reverse transcription recombinase polymerase amplification
assay for rapid detection of foot-and-mouth disease virus.
El-Khabaz KAS and Al-Hosary AAT, 2017 Detection and
identification of Foot and Mouth disease virus serotypes in
Ferris NP and Dawson M, 1988 Routine application of enzyme-
linked immunosorbent assay in comparison with
complement fixation for the diagnosis of foot-and-mouth
Gilliam SE and Field HJ, 1993. The effect of (S)-1-(3-hydroxy-
2-phosphonyl-methoxypropyl) cytosine (HPMPC)
on bovine herpesvirus-1 (BVH-1) infection and reactivation in
test for detection of foot-and-mouth disease virus specific
antibodies using gold nanoparticles Virus Dis, 29: 192-198.
15/283, 723.
Kessler SW, 1975. Rapid isolation of antigens from cells with a
staphylococcal protein A-antibody adsorbent: parameters of
the interaction of antibody-antigen complexes with protein
Kessler SW, 1981. Use of protein A bearing staphylococci for the
immunoprecipitation and isolation of antigens from cells.
of Foot and Mouth Disease Virus (FMDV) through
Khodary MG and El-Deeb AH, 2018. Molecular characterization
of foot and mouth disease virus (O-EA3) isolated during
King DP, Ferris NP and Shaw AE, 2006. Detection of foot-and-
mouth disease virus: comparative diagnostic sensitivity of

Mackay DKJ, Forsyth MA, Davies PR, et al., 1998. Differentiating infection from vaccination in foot-and-mouth disease using a panel of recombinant, non-structural proteins in ELISA. Vaccine, 16: 446–459