Journal of Virological Methods 278 (2020) 113835

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Inactivation of foot-and-mouth disease virus A/IRN/8/2015 with commercially available lysis buffers

Britta A. Wood[a,*], Valérie Mioulet[a], Elisabeth Henry[a], Ashley Gray[a], Mehreen Azhar[a], Barsha Thapa[a], Sandra Diederich[b], Bernd Hoffmann[c], Martin Beer[c], Donald P. King[a], Michael Eschbaumer[c]

[a] The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF, United Kingdom
[b] Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany
[c] Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany

ARTICLE INFO

Keywords:
Foot-and-mouth disease virus
Lysis buffer
Virus inactivation

ABSTRACT

Laboratories working with foot-and-mouth disease virus (FMDV) must maintain a high level of biocontainment. However, if infectious virus is reliably inactivated during sample processing, molecular and serological testing can be performed at a lower level of containment. In this study, three commercial lysis buffers (AL, AVL, and MagMAX CORE) were tested in two laboratories for their ability to inactivate FMDV A/IRN/8/2015 in different sample matrices (cell culture supernatant, epithelial tissue suspension and milk). Residual infectivity after the addition of lysis buffer was evaluated by inoculating susceptible cell cultures. No cytopathic effect was observed for all three lysis buffers, indicating that the buffers are capable of reducing viral infectivity (estimated range 3.1 to > 5.1 Log_{10}). These results highlight the capacity of lysis buffers to decrease FMDV infectivity; however, additional validation experiments should be conducted, particularly if different sample matrices and/or lysis buffers are used.

High-level biocontainment laboratories (e.g., SAPO4 in the United Kingdom, BSL-3-Ag in the United States or t4/S4 in Germany) play a crucial role in infectious disease research and preparedness, but given the financial and logistic challenges of their operation, these facilities are limited in number and capacity. In an outbreak situation, when sample submissions are high and turn-around times are critical, high-containment laboratories may need assistance from other laboratories where procedures that do not require the handling of 'live' pathogens (e.g., molecular or serological assays) can be adopted. Alternatively, within a facility, limited space in high-containment might force all non-infectious pathogen work to be conducted in lower containment areas. Regardless of the situation, high-consequence pathogens in biological matrices must be reliably inactivated before removal and handling at a lower level of containment.

It is commonly assumed that the lysis buffers supplied in commercial nucleic acid extraction kits are capable of inactivating viruses present in biological samples. However, many factors can influence the effectiveness of a lysis buffer, including the virus titer, nature of sample matrix, contact time and reaction temperature, virus structure (enveloped or non-enveloped particles), and concentration/composition of the lysis buffer (denaturing agent and/or detergent, pH). Inactivation experiments have been conducted for viruses from a variety of genera, including *Alphavirus*, *Betainfluenzavirus, Ebolavirus, Enterovirus, Flavivirus, Marburgvirus, Mastadenovirus, Orthobunyavirus, Orthopoxvirus, Phlebovirus* and *Simplexvirus* (Blow et al., 2004; Burton et al., 2017; Colavita et al., 2017; Haddock et al., 2016; Ngo et al., 2017; Rosenstierne et al., 2016; Smither et al., 2015; Vinner and Fomsgaard, 2007). The results of these studies varied from complete to incomplete inactivation for the virus/lysis buffer combinations tested; however, where residual infectivity was observed, this was usually eliminated after an additional inactivation step, such as heating (Ngo et al., 2017; Smither et al., 2015) or adding a detergent (Burton et al., 2017). This variation highlights the need to perform lysis buffer validation experiments for each virus and sample matrix handled in a high-containment laboratory.

Foot-and-mouth disease (FMD) is a livestock disease of high economic consequence. The causative agent, foot-and-mouth disease virus (FMDV; family *Picornaviridae*, genus *Aphthovirus*), exists in seven distinct serotypes (O, A, C, Asia 1, Southern African Territories (SAT) 1, SAT 2 and SAT 3) (OIE, 2017), with multiple variants occurring within

* Corresponding author.
E-mail address: britta.wood@pirbright.ac.uk (B.A. Wood).

https://doi.org/10.1016/j.jviromet.2020.113835
Received 9 September 2019; Received in revised form 3 February 2020; Accepted 4 February 2020
Available online 05 February 2020

each serotype (Knowles and Samuel, 2003). The disease affects domestic cloven-hooved animals (e.g., cattle, pigs, sheep and goats) and at least 70 species of wild animals in endemic regions throughout Africa, Asia and the Middle East (reviewed in Alexandersen and Mowat, 2005). Clinical signs include vesicular lesions on the tongue, oral cavity, feet, snout and teats (in lactating cows), as well as fever and lameness (Grubman and Baxt, 2004). Although the mortality rate is often low, FMD can have a significant impact on herds and farmers (e.g., through decreased milk production, restrictions on the sale of animals or their products), as well as nation-wide effects (e.g., costs for diagnostic testing, vaccination and control; animal import/export restrictions; decreased tourism revenue) (Knight-Jones and Rushton, 2013). Given that FMDV is highly contagious, its introduction into a naïve population often results in a high number of clinical cases and many samples submitted for diagnostic testing. The handling of diagnostic samples containing infectious FMDV is generally limited to laboratories with high-containment facilities. However, if the virus can be inactivated, molecular and serological testing can be safely performed in regular diagnostic laboratories (EuFMD, 2013).

This two-site study was motivated by differing FMDV inactivation results generated independently at the German National Reference Laboratory for FMD, Friedrich-Loeffler-Institut (FLI), Greifswald, Germany, and the World Reference Laboratory for FMD, The Pirbright Institute (TPI), Pirbright, United Kingdom. Given that there were substantial differences in the methods used between laboratories (e.g., different lysis buffers, virus titres and passage history, sample matrices, cell lines, etc.), clear conclusions as to the effectiveness of each lysis buffer could not be made. However, these data indicated that lysis buffer effectiveness can vary, particularly for more complex biological matrices (e.g., blood, milk, probang fluid and serum), but did not appear to be FMDV serotype-specific. Thus, the aim of this study was to evaluate the inactivation efficacy of commercial lysis buffers with FMDV using a harmonized protocol in two laboratories.

Inactivation experiments were conducted in high-containment laboratories at FLI and TPI that meet or exceed the *Minimum Biorisk Management Standards for Laboratories Working with Foot-and-Mouth Disease Virus* of the European Commission for the Control of Foot-and-Mouth Disease (EuFMD, 2013). Procedures were designed to be as similar as possible between the two sites; however, there were minor differences due to local biosafety requirements and/or custom assays used in-house.

The three proprietary lysis buffers included in the inactivation experiments were AL, AVL (both from QIAGEN, Hilden, Germany) and MagMAX CORE (Thermo Fisher Scientific, Waltham, MA, USA) (Table 1). These lysis buffers all contain a guanidine-based denaturing agent, and were selected to encompass the reagents currently used most often for nucleic acid extraction at FLI (AL and AVL), and a new-to-market reagent (MagMAX CORE). The MagMAX kit currently used at TPI will be discontinued in 2020 and replaced in the manufacturer's catalogue with the MagMAX CORE kit.

A survey of the 27 National Reference Laboratories for FMD in the European Union (February 2018) indicated that 16 different lysis buffers were being used for virus inactivation and downstream molecular testing (Table 2). The most commonly used buffer was AVL, which was used by nine laboratories (including FLI), and AL was used by one other laboratory in addition to FLI. MagMAX CORE was not in use at the time of the survey.

Experiments were conducted using LFBK-$\alpha_v\beta_6$ cells, an adherent fetal porcine kidney cell line that stably expresses bovine $\alpha_v\beta_6$ integrin (provided by L. Rodriguez and M. LaRocco; LaRocco et al., 2013, 2015), and FMDV strain A/IRN/8/2015, isolated in primary bovine thyroid cells and passaged three times in LFBK-$\alpha_v\beta_6$ cells (7.4 $\log_{10}$ $TCID_{50}$/mL). This isolate was selected as a representative FMD virus that is diagnostically relevant; A/IRN/8/2015 belongs to the A/ASIA/G-VII lineage that has emerged from the Indian sub-continent in 2015 and caused outbreaks in Armenia, Iran, Israel, Saudi Arabia and Turkey (WRLFMD Genotyping Reports). The three sample matrices selected for testing were cell culture supernatant (virus isolate), bovine epithelial tissue suspension and bovine whole milk. For FMDV diagnosis, one of the preferred sample types is epithelium from an unruptured or recently ruptured vesicle, which is then homogenized in the laboratory to generate a tissue suspension (OIE, 2017). Milk samples are relatively easy to collect and may be used for FMD surveillance (Armson et al., 2018, 2019); this sample type was included because it represents a complex matrix with high protein, sugar and fat content. Given the limited amount of available FMDV-positive samples for each sample type, negative tissue suspension (generated at TPI) and milk (commercial UHT milk with 3.5 % fat) were spiked with A/IRN/8/2015 (1:1 virus to sample matrix).

In order to first determine the point at which each lysis buffer was no longer toxic to LFBK-$\alpha_v\beta_6$ cells, 2-fold dilution series (neat to 1:1024 in sterile PBS) were prepared starting at the working ratio recommended by the manufacturer, but adding sterile PBS instead of sample (Table 1). Each dilution was then added to confluent LFBK-$\alpha_v\beta_6$ cells (FLI: 0.1 mL per well of 12-well cell culture plate, 3.8 cm^2 cell surface area; and TPI: 0.2 mL per flat-sided, polystyrene cell tube, 5.5 cm^2 cell surface area) containing maintenance media (DMEM with 2 % fetal bovine serum and in-house antibiotics), incubated stationary at 37 °C, and examined daily for 4 days. The lowest dilution at which each lysis buffer was no longer toxic (i.e., cells forming a visibly healthy monolayer similar to control wells or tubes) was used for post-inactivation dilutions to avoid non-specific cytotoxicity in subsequent experiments. These dilutions were as follows: AL 1:32 (TPI) or 1:64 (FLI), AVL 1:64 (FLI and TPI) and MagMAX CORE 1:128 (FLI and TPI); the difference for AL between sites is likely because the 1:32 dilution is near the limit of cytotoxicity.

Table 1
Details of the commercially available lysis buffers used in this study, including lysis buffer and sample volumes, and dilutions used to eliminate lysis buffer cytotoxicity.

Lysis buffer	Manufacturer information			Used in this study	
	Active ingredient (concentration[c])	Nucleic acid extraction kit (catalog #)	Lysis buffer to sample ratio	Lysis buffer + sample volumes (µL)	Dilution used to eliminate cytotoxicity
Buffer AL[a]	Guanidinium hydrochloride (30–50 %)	QIAamp and DNeasy kits (e.g, 69581 and 69582)	1:1	150 + 150	1/32 (TPI) 1/64 (FLI)
Buffer AVL[a]	Guanidinium thiocyanate (50–70 %)	QIAamp Viral RNA Mini kit (52904)	4:1	560 + 140	1/64
MagMAX CORE lysis solution[b]	Guanidine thiocyanate (55–100 %)	MagMAX CORE Nucleic Acid Purification kit (A32700)	7:4	350 + 200	1/128

[a] QIAGEN (Hilden, Germany).
[b] Thermo Fisher Scientific (Waltham, MA, USA).
[c] Weight percentages as per the safety data sheet (SDS) provided by the manufacturer.

Table 2
Lysis buffers used in the National FMD Reference Laboratories of European Union Member States (February 2018).

Active ingredient(s)	Lysis buffer (kit, if applicable)	Guanidinium concentration[a]	# of labs[b]
Guanidinium hydrochloride	Binding buffer (High Pure Viral RNA kit[c])	30–50 %	2
	Buffer AL (BioSprint 96 DNA Blood kit[d])	30–50 %	2
	N1 (LSI MagVet Universal Isolation kit[e])	60–100 %	1
	VXL (QIAmp *cador* Pathogen Mini kit[f])	30–50 %	3
Guanidinium thiocyanate	Buffer AVL (QIAmp Viral RNA Mini kit[d])	50–70 %	9
	Buffer MFL (MagAttract Viral RNA M48 kit[d])	30–50 %	1
	Buffer RLT (RNeasy Mini kit[d])	30–50 %	3
	Lysis/binding buffer (MagNA Pure Compact Nucleic Acid Isolation kit[c])	30–50 %	2
	Lysis/binding buffer (MagNA Pure LC Total Nucleic Acid Isolation kit[c])	30–50 %	2
	Lysis/binding solution concentrate (MagMAX-96 Viral RNA Isolation kit[e])	30–60 %	1
	Lysis buffer (PureLink RNA Mini kit[e])	30–60 %	1
	Lysis buffer RAV1 (Nucleospin RNA Virus kit[g])	30–60 %	2
	Lysis solution (Bullet Stool kit[h])	40–53 %	1
	Viral lysis buffer (iPrep PureLink Virus kit, Invitrogen[e])	5–10 %	1
Guanidinium thiocyanate & phenol	TriPure Isolation Reagent[i]	20–25 %	1
Guanidinium thiocyanate, ammonium thiocyanate & phenol	TRIzol Reagent[e]	15–40 %	2

[a] Weight percentages as per SDS; percentages do not account for lysis buffer preparation/dilutions prior to use (e.g., NM1 lysis buffer, LSI MagVet).
[b] Some laboratories use more than one lysis buffer and one laboratory does not currently perform molecular testing.
[c] Roche (Basel, Switzerland).
[d] QIAGEN (Hilden, Germany).
[e] Thermo Fisher Scientific (Waltham, MA, USA).
[f] Indical Bioscience GmbH (Leipzig, Germany); formerly QIAGEN.
[g] Macherey-Nagel GmbH (Dueren, Germany).
[h] DiaSorin (Saluggia, Italy); kit has been discontinued.
[i] Sigma Aldrich Corporation (St. Louis, MO, USA).

Table 3
Summary of the cytopathic effect (CPE; yes/no) for the lysis buffer inactivation experiments conducted at FLI and TPI. All three lysis buffers were able to inactivate FMDV, at least below the limit of detection, as indicated by the lack of CPE even after a blind passage. The presence of FMDV type A antigen was confirmed by antigen ELISA for virus controls. Not done, -.

Matrix	Sample	AL		AVL		MagMAX CORE	
		Pass 1	Pass 2	Pass 1	Pass 2	Pass 1	Pass 2
Isolate	Cell control	No	No	No	No	No	No
	Virus control	Yes	–	Yes	–	Yes	–
	Lysis buffer cytotoxicity control	No	No	No	No	No	No
	Virus + lysis buffer	No	No	No	No	No	No
Spiked epithelium suspension	Cell control	No	No	No	No	No	No
	Virus control	Yes	–	Yes	–	Yes	–
	Lysis buffer cytotoxicity control	No	No	No	No	No	No
	Virus + lysis buffer	No	No	No	No	No	No
Spiked milk	Cell control	No	No	No	No	No	No
	Virus control	Yes	–	Yes	–	Yes	–
	Lysis buffer cytotoxicity control	No	No	No	No	No	No
	Virus + lysis buffer	No	No	No	No	No	No

Lysis buffer inactivation experiments were conducted in both laboratories for all three lysis buffers with each sample matrix. In each experiment, three independent replicates of virus (isolate or spiked matrix) and lysis buffer were prepared at the recommended lysis buffer-to-sample ratio (Table 1). After incubating at room temperature for 10 min, the samples were diluted as required to eliminate lysis buffer cytotoxicity. Each replicate was then added to LFBK-$\alpha_v\beta_6$ cells containing maintenance media (FLI: 0.1 mL per well, n = 1 well; and TPI: 0.2 mL per tube, n = 3 tubes). Each experiment also included the following controls (FLI, one well per control; TPI, three tubes per control): negative cell culture (maintenance media only), lysis buffer cytotoxicity (lysis buffer only, at the previously determined dilution where the lysis buffers were not expected to be cytotoxic) and virus (virus only, at the same dilution used to eliminate cytotoxicity). All samples and controls were incubated stationary at 37 °C and examined daily for CPE for 3 days. All samples, except for the negative cell culture controls at TPI, were read blind by those checking for CPE.

If CPE was observed, the sample was tested by ELISA to determine whether detectable FMDV antigen was present (Roeder and Le Blanc Smith, 1987; Ferris and Dawson, 1988). If no CPE was observed, the plates/tubes were frozen at -80 °C before passing onto new LFBK-$\alpha_v\beta_6$ cells (FLI: 0.5 mL per well + 0.5 mL maintenance media, n = 1 well; and TPI: 1 mL per tube + 1 mL maintenance media, n = 3 tubes). Samples and controls were incubated stationary at 37 °C and examined daily for CPE for 3 days. If no CPE was observed for all replicates in the initial and blind passage of a virus + lysis buffer sample, then the lysis buffer was considered effective for virus inactivation.

In each experiment, the negative cell culture controls and lysis buffer cytotoxicity controls were CPE-negative, and the virus controls were CPE-positive on or before day 3 of incubation (Table 3). The

Table 4
Estimated minimum reduction (log_{10}) of FMDV infectivity after accounting for the sample in lysis buffer and lysis buffer cytotoxicity dilutions. The different values obtained by the two laboratories are due to different limits of detection that were calculated based on inoculation volumes and number of replicates tested. Neither lab detected any residual infectivity for any combination of lysis buffer and sample matrix.

Matrix	AL		AVL		MagMAX CORE	
	1:32 TPI	1:64 FLI	1:64 TPI	1:64 FLI	1:128 TPI	1:128 FLI
Isolate	> 5.1	> 3.8	> 4.4	> 3.4	> 4.3	> 3.4
Spiked epithelium suspension	> 4.8	> 3.5	> 4.1	> 3.1	> 4.0	> 3.1
Spiked milk	> 4.8	> 3.5	> 4.1	> 3.1	> 4.0	> 3.1

specificity of the observed CPE was confirmed by FMDV-specific antigen ELISA. All three lysis buffers were effective at reducing FMDV infectivity in each sample matrix to undetectable levels, as indicated by the lack of CPE for all virus + lysis buffer samples tested (Table 3). After accounting for the sample in lysis buffer and lysis buffer cytotoxicity dilutions, the minimum reductions in infectivity were estimated to be 3.1 to > 5.1 log_{10} (Table 4).

In this study, the virus selected had as high a titre as could be generated when grown in LFBK-$\alpha_v\beta_6$ cells (7.4 log_{10} $TCID_{50}$/mL) in order to challenge the lysis buffers with the highest dose possible after accounting for the sample in lysis buffer and lysis buffer cytotoxicity dilutions. Although the post-inactivation dilution reduces the sensitivity of subsequent infectivity assays, all of the virus controls developed CPE at this dilution. This indicates that the dilutions used did not compromise the sensitivity of the tests, and that more complex methods for removing cytotoxic reagents were not required, such as neutralization (Krug et al., 2011), dialysis (Haddock et al., 2016) or centrifugation (Smither et al., 2015; Vinner and Fomsgaard, 2007). Preliminary experiments conducted with size exclusion filters (Amicon Ultra-15, Millipore) were effective at removing the lysis buffers, but virus recovery was variable between replicates and the filters were not compatible with complex matrices (i.e., milk clogged the filter).

The results from this study show that all three lysis buffers tested were able to inactivate FMDV strain A/IRN/8/2015 in the sample matrices evaluated, at least below detection with cell culture. The methods and results presented here could be useful for other laboratories when selecting nucleic acid extraction protocols and/or conducting similar in-house validations. Although many lysis buffers contain the same active ingredients (e.g., Table 2), additional validation experiments may be required in the future in the event that a new lysis buffer is introduced that has a different concentration of the denaturing agent (and excipients) or recommended ratio of sample to lysis buffer.

CRediT authorship contribution statement

Britta A. Wood: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Valérie Mioulet:** Conceptualization, Methodology, Investigation, Writing - review & editing, Visualization. **Elisabeth Henry:** Investigation, Writing - review & editing. **Ashley Gray:** Investigation, Writing - review & editing. **Mehreen Azhar:** Investigation, Writing - review & editing. **Barsha Thapa:** Investigation, Writing - review & editing. **Sandra Diederich:** Conceptualization, Writing - review & editing. **Bernd Hoffmann:** Writing - review & editing. **Martin Beer:** Conceptualization, Writing - review & editing, Funding acquisition. **Donald P. King:** Conceptualization, Writing - review & editing, Funding acquisition. **Michael Eschbaumer:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

This work is dedicated to the memory of Bernd Haas. Holger Freese of the FLI is acknowledged for his invaluable contribution to the inactivation experiments. The authors would also like to thank colleagues in the EU reference laboratory network for participating in the lysis buffer survey; Damien Magnée (Thermo Fisher Scientific) for providing MagMAX CORE reagents; Julie Charrot, Andrea Biserni and Jörn Voss (Thermo Fisher Scientific) for technical advice; and L. Rodriguez and M. LaRocco (Plum Island, USDA/ARS) for providing the LFBK-$\alpha_V\beta_6$ cell line. This research was supported by funding from the United Kingdom Department for Environment, Food and Rural Affairs (DEFRA; projects SE1128 and SE1129).

References

Alexandersen, S., Mowat, N., 2005. Foot-and-mouth disease: host range and pathogenesis. Curr. Top. Microbiol. Immunol. 288, 9–42.
Armson, B., Mioulet, V., Doel, C., Madi, M., Parida, S., Lemire, K.A., Holder, D.J., Das, A., McIntosh, M.T., King, D.P., 2018. Detection of foot-and-mouth disease virus in milk samples by real-time reverse transcription polymerase chain reaction: optimisation and evaluation of a high-throughput screening method with potential for disease surveillance. Vet. Microbiol. 223, 189–194.
Armson, B., Wadsworth, J., Kibona, T., Mshanga, D., Fowler, V.L., Knowles, N.J., Mioulet, V., Reeve, R., King, D.P., Bachanek-Bankowska, K., Lembo, T., 2019. Opportunities for enhanced surveillance of foot-and-mouth disease in endemic settings using milk samples. Transbound. Emerg. Dis. 66, 1405–1410.
Blow, J.A., Dohm, D.J., Negley, D.L., Mores, C.N., 2004. Virus inactivation by nucleic acid extraction reagents. J. Virol. Methods 119, 195–198.
Burton, J.E., Easterbrook, L., Pitman, J., Anderson, D., Roddy, S., Bailey, D., Vipond, R., Bruce, C.B., Roberts, A.D., 2017. The effect of a non-denaturing detergent and a guanidinium-based inactivation agent on the viability of Ebola virus in mock clinical serum samples. J. Virol. Methods 250, 34–40.
Colavita, F., Quartu, S., Lalle, E., Bordi, L., Lapa, D., Meschi, S., Vulcano, A., Toffoletti, A., Bordi, E., Paglia, M.G., Di Caro, A., Ippolito, G., Capobianchi, M.R., Castilletti, C., 2017. Evaluation of the inactivation effect of Triton X-100 on Ebola virus infectivity. J. Clin. Virol. 86, 27–30.
EuFMD, 2013. Minimum Biorisk Management Standards for Laboratories Working With Foot-and-Mouth Disease Virus (Rome, Italy, 40th General Session of the European Commission for the Control of Foot-and-Mouth Disease).
Ferris, N.P., Dawson, M., 1988. Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular diseases. Vet. Microbiol. 16, 201–209.
Grubman, M.J., Baxt, B., 2004. Foot-and-mouth disease. Clin. Microbiol. Rev. 17, 465–493.
Haddock, E., Feldmann, F., Feldmann, H., 2016. Effective chemical inactivation of Ebola virus. Emerg. Infect. Dis. 22, 1292–1294.
Knight-Jones, T.J., Rushton, J., 2013. The economic impacts of foot and mouth disease – what are they, how big are they and where do they occur? Prev. Vet. Med. 112, 161–173.
Knowles, N.J., Samuel, A.R., 2003. Molecular epidemiology of foot-and-mouth disease virus. Virus Res. 91, 65–80.
Krug, P.W., Lee, L.J., Eslami, A.C., Larson, Luis Rodriguez, L., 2011. Chemical disinfection of high-consequence transboundary animal disease viruses on nonporous surfaces. Biologicals 39, 231–235.
LaRocco, M., Krug, P.W., Kramer, E., Ahmed, Z., Pacheco, J.M., Duque, H., Baxt, B., Rodriguez, L.L., 2013. A continuous bovine kidney cell line constitutively expressing bovine $\alpha_V\beta_6$

integrin has increased susceptibility to foot-and-mouth disease virus. J. Clin. Microbiol. 51, 1714–1720.
LaRocco, M., Krug, P.W., Kramer, E., Ahmed, Z., Pacheco, J.M., Duque, H., Baxt, B., Rodriguez, L.L., 2015. Correction for: a continuous bovine kidney cell line constitutively expressing bovine $\alpha_V\beta_6$ integrin has increased susceptibility to foot-and-mouth disease virus. J. Clin. Microbiol. 53, 755.
Ngo, K.A., Jones, S.A., Church, T.M., Fuschino, M.E., St. George, K., Lamson, D.M., Maffei, J., Kramer, L.D., Ciota, A.T., 2017. Unreliable inactivation of viruses by commonly used lysis buffers. Appl. Biosaf. 22, 56–59.
OIE, 2017. Foot and mouth disease (infection with foot and mouth disease virus). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019. pp. 433–464.
Roeder, P.L., Le Blanc Smith, P.M., 1987. Detection and typing of foot-and-mouth disease virus by enzyme-linked immunosorbent assay: a sensitive, rapid and reliable technique for primary diagnosis. Res. Vet. Sci. 43, 225–232.
Rosenstierne, M.W., Karlberg, H., Bragstad, K., Lindegren, G., Stoltz, M.L., Salata, C., Kran, A.M., Dudman, S.G., Mirazimi, A., Fomsgaard, A., 2016. Rapid bedside inactivation of Ebola virus for safe nucleic acid tests. J. Clin. Microbiol. 54, 2521–2529.
Smither, S.J., Weller, S.A., Phelps, A., Eastaugh, L., Ngugi, S., O'Brien, L.M., Steward, J., Lonsdale, S.G., Lever, M.S., 2015. Buffer AVL alone does not inactivate Ebola virus in a representative clinical sample type. J. Clin. Microbiol. 53, 3148–3154.
Vinner, L., Fomsgaard, A., 2007. Inactivation of orthopoxvirus for diagnostic PCR analysis. J. Virol. Methods 146, 401–404.
WRLFMD Genotyping Reports. http://www.wrlfmd.org/country-reports.