HHS Public Access

Author manuscript

J Virol Methods. Author manuscript; available in PMC 2022 September 02.

Published in final edited form as:

J Virol Methods. 2021 November ; 297: 114262. doi:10.1016/j.jviromet.2021.114262.

Use of guanidine thiocyanate-based nucleic acid extraction buffers to inactivate poliovirus in potentially infectious materials

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Abstract

The efforts of the Global Poliovirus Eradication Initiative (GPEI) have brought about the near elimination of poliovirus worldwide. The World Health Organization has issued guidelines for the safe handling and containment of infectious materials (IM) and potentially infectious materials (PIM) following poliovirus eradication. Inactivation of poliovirus in IM and PIM is needed to prevent inadvertent re-introduction of polioviruses post-eradication. In this study, we investigated the use of guanidine thiocyanate-based nucleic acid extraction buffers from commercially available nucleic acid extraction kits to inactivate poliovirus in cell culture isolates and stool suspensions, two common types of poliovirus IM and PIM, respectively. Incubation with selected nucleic acid extraction buffers or extraction buffers supplemented with ethanol reduced the infectivity of high-titer wild poliovirus type 1 (WPV1), wild poliovirus type 3 (WPV3), Sabin 1 (SL1), and Sabin 3 (SL3) cell culture isolates below the limit of detection in $CCID_{50}$ assays. Stool suspensions containing WPV1, WPV3, SL1, SL2, or SL3 were also inactivated by the extraction buffers tested. Blind passage of WPV1-spiked stool suspensions confirmed complete inactivation of WPV1 after incubation with extraction buffers. Moreover, treatment with a buffer consisting of 4 M guanidine thiocyanate with 30 % ethanol inactivated a high-titer WPV1 culture isolate and a WPV1-spiked stool suspension. Taken together, these results show that guanidine thiocyanate-based nucleic acid extraction buffers are an effective means of inactivating poliovirus IM and PIM, and thus will be instrumental in ensuring containment compliance and preventing potential re-emergence of contained polioviruses.

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CRediT authorship contribution statement

Michelle J. Honeywood: Investigation, Conceptualization, Writing - original draft, Writing - review & editing. **Stacey Jeffries-Miles:** Investigation. **Kimberly Wong:** Investigation. **Chelsea Harrington:** Investigation. **Cara C. Burns:** Supervision, Resources, Writing - review & editing. **M. Steven Oberste:** Supervision, Resources, Writing - review & editing. **Michael D. Bowen:** Supervision, Resources, Writing - review & editing. **Everardo Vega:** Supervision, Conceptualization, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Keywords

Poliovirus; Virus inactivation; Nucleic acid extraction; Containment

1. Introduction

With declaration of eradication of wild type 2 poliovirus (WPV2) and wild type 3 poliovirus (WPV3) in 2015 and 2019, respectively, the Global Poliovirus Eradication Initiative (GPEI) is closer to the goal of the worldwide elimination of wild polioviruses and the cessation of oral poliovirus vaccine (OPV) use. Critical to the success of the GPEI is the WHO Global Action Plan III to Minimize Poliovirus Facility-Associated Risk (GAP III), which provides guidelines for containment safeguards and safe sample handling procedures to prevent re-emergence of eradicated viruses (World Health Organization, 2015). Reintroduction of polioviruses into the environment could potentially occur by way of accidental biological spills or laboratorians being exposed to potentially infectious materials containing poliovirus (Duizer et al., 2016; Oberste, 2018). Despite being vaccinated, asymptomatic workers may shed the virus and cause silent poliovirus transmission in communities. Samples collected at a time and place where WPV2, cVDPV2, WPV3, or cVDPV3 were circulating or where OPV2 was in use are considered PIM (World Health Organization, 2018). Currently, infectious materials (IM) such as cell culture isolates known to contain poliovirus, and potentially infectious materials (PIM) (e.g., respiratory samples, sewage, and stool samples) must be destroyed or transferred to designated poliovirus-essential facilities (PEFs) for containment (World Health Organization, 2015). WPV1 PIM will also be subject to GAP III containment guidelines upon certification of eradication. The containment guidelines pose a unique challenge for non-poliovirus facilities that work with other enteric or respiratory pathogens. These laboratories may have repositories of archived samples, potentially containing polioviruses, that need to be preserved for future research or other purposes.

As an alternative to destruction, nucleic acids can be extracted from PIM as a means of sample preservation. RNA isolated from poliovirus PIM is considered low risk for PV transmission, barring transfection into PV-permissive cells. Nucleic acid extraction is a common procedure used in many laboratories to purify, concentrate, and inactivate specimens prior to molecular analysis. A common component of buffers used for nucleic acid extraction is guanidine thiocyanate, a strong protein denaturant and potent inhibitor of nucleases. Previous work has demonstrated that guanidine thiocyanate based-nucleic acid extraction buffers can effectively inactivate high-consequence enveloped viruses, such as Ebola virus, Marburg virus, and Rift Valley Fever virus, as well as West Nile virus, influenza, Venezuelan equine encephalitis virus, and recently, SARS-CoV-2 (Abad, 2012; Blow et al., 2004; Haddock et al., 2016; Kumar et al., 2015; Ngo et al., 2017; Pan et al., 2020; Pastorino et al., 2020; Patterson et al., 2018; Rosenstierne et al., 2016; Smither et al., 2015). In this study, we investigated whether commonly used nucleic acid extraction buffers could also inactivate polioviruses in cell culture isolates and stool suspensions, two types of IM and PIM frequently handled in both poliovirus-essential facilities and non-poliovirus laboratories.

2. Materials and methods

2.1. Virus stocks

Wild poliovirus type 1 (WPV1) and wild poliovirus type 3 (WPV3) cell culture isolates used in this study were WEAF-B (West African group B) polioviruses isolated from two stool specimens from Nigeria. Sabin 1 (SL1), Sabin 2 (SL2), and Sabin 3 (SL3) viruses were obtained front the National Institute for Biological Standards and Control (NIBSC, Hertfordshire, United Kingdom). To prepare WPV1, WPV3, SL1, SL2, and SL3 virus stocks, human rhabdomyosarcoma cells (RD) cells were seeded in 75 cm^2 culture flasks at a density of 3.0×10^5 cells/mL in growth media (minimum essential medium with Earle's salts [MEM; Gibco, Rockville, MD], supplemented with 1 M HEPES, 1.5 *g*/L sodium bicarbonate, 100 U/mL penicillin/streptomycin, and 10 % fetal bovine serum) and incubated at 37 °C in a CO_2 incubator. Cells were grown to about 90 % confluency, and culture media was then replaced with maintenance media (MEM with Earle's salts supplemented with 1 M HEPES, 1.5 *g*/L sodium bicarbonate, 100 U/mL penicillin/streptomycin, and 2 % fetal bovine serum). Cells were inoculated at multiplicity of infection of 0.2 with WPV1, WPV3, SL1, SL2, and SL3 isolates and incubated for 3 days at 37 °C in a CO_2 incubator. Flasks were frozen and thawed twice to lyse cells and release virus. Supernatants were collected and centrifuged at 10,000 x *g* for 15 min to pellet debris. Clarified virus stocks were titered, aliquoted and stored at −20 °C until further use.

2.2. Stool suspensions

To prepare 10 % stool suspensions, approximately 0.5 *g* of stool was added to 5 mL of serum-free MEM and 0.5 *g* of glass beads. Chloroform (250 μL) was added and the suspension was shaken for 30 min on a mechanical shaker (Heidolph North America, Wood Dale, IL). Following shaking, the suspension was centrifuged at 1800 x *g* for 30 min at 4 °C to pellet insoluble material. Clarified suspensions were titered, aliquoted, and stored at −20 °C until use. RNA was extracted front each stool suspension using the Quick Viral RNA kit (Zymo Research, Irvine, CA) and the poliovirus serotype and genotype were confirmed by rRT-PCR (Gerloff et al., 2017).

2.3. Reagents and extraction kits

Extraction buffers and kits used in this study include: Trizol LS (Cat No. 10296098; Thermo Fisher, Carlsbad, CA); QIAamp Viral RNA Mini Kit (Cat No. 52906; QIAGEN, Germantown, MD); *Quick*-RNA Viral Kit (Cat No. R1035; Zymo Research); High Pure Viral Nucleic Acid Kit (Cat. No. 11858874001; Roche, Wilmington, MA); MagMAX Lysis/Binding Buffer (Cat. No. 8500; part of the MagMAX Pathogen RNA/DNA Kit; Applied Biosystems, Foster City, CA); UNEX (Cat No. 726288-260; VWR, Radnor, PA). The QIAamp Viral RNA Mini Kit is the most commonly used kit for poliovirus RNA extraction from cell culture isolates in the Global Poliovirus Laboratory Network (GPLN). Trizol LS and the Qiagen Viral RNA Mini Kit are popular among many non-poliovirus laboratories for RNA extraction and have been used by several inactivation studies (Esona et al., 2013; Kochel et al., 2017; Ngo et al., 2017). Universal extraction buffer (UNEX) was developed at the Centers for Disease Control and Prevention for nucleic acid extraction from environmental samples and has previously been shown to inactivate

SL1 (Cromeans et al., 2019; Hill et al., 2015). Guanidine thiocyanate (Cat. No. G9277), 2-(N-morpholino)ethanesulfonic acid (MES) hydrate (Cat No. M8250), ethanol (Cat. No. E7023), and Amicon Ultra-15 100,000 NMWL centrifugal filters (Cat. No. UFC9100) were all purchased from Millipore Sigma (St. Louis, MO).

2.4. Inactivation of poliovirus in cell culture isolates, stool suspensions, and spiked-stool suspensions

One volume (200 μL) of sample was mixed and incubated with selected commercial nucleic acid extraction buffers according to the sample-to-buffer ratios outlined in the kit protocol (Table 1) or with four volumes of 4 M guanidine thiocyanate (in 0.1 M MES, pH 6.0) for 5–30 min at room temperature (24–26 °C). Ethanol was added after mixing the sample with Buffer AVL, UNEX Buffer, or 4 M guanidine thiocyanate buffer. To remove the extraction buffer and reduce cytotoxicity, samples were diluted in 10–12 mL PBS and centrifuged in an Amicon Ultra-15 100,000 NMWL filter at 1800 x *g* for 10 min. This process was repeated 2–4 additional times until the residual extraction buffer was removed. The resulting retentates, ranging front 120–200 μL, were used in $CCID_{50}$ assays or tested in blind passages to assess the extent of inactivation.

2.5. $CCID_{50}$ assays and blind passages

For $CCID_{50}$ assays, RD cells were diluted in maintenance medium and seeded at 1.5×10^4 cells per well in 96-well plates. Treated samples were serially diluted 1:10 in serum-free MEM and added (100 μL per well) to cells freshly plated in maintenance medium. For each experiment, a positive control in which virus was incubated with PBS alone in addition to a negative control consisting of extraction buffer alone was included to assess cytotoxicity; controls were treated identically to the virus samples, including buffer exchange by centrifugal filtration. Plates were incubated at 37 °C and monitored for cytopathic effect (CPE) after 5 days. Viral titer was calculated according to the Spearman-Kärber method (World Health Organization, 2004). For experiments with treated stool suspensions, RD cells were seeded at 1.25×10^5 cells per well in 24-well plates and incubated at 37 °C. When cells reached at least 90 % confluence, the medium was replaced with maintenance medium. Half of each treated stool suspension or control was added undiluted to duplicate wells. Wells were visually scored for the presence or absence of CPE using an inverted microscope after plates were incubated for 5 days at 37 °C. For blind passage experiments, RD cells were seeded in 25 cm^2 flasks at 1.25×10^6 cells per flask and grown until 90 % confluence. The medium was replaced by 5 mL of maintenance medium prior to addition of treated samples. Inoculated flasks were checked for CPE using an inverted microscope after incubation at 37 °C for 5 days. Approximately 1 mL of supernatant from a negative flask was transferred to a fresh confluent flask containing 4 mL of maintenance medium. These flasks were monitored for CPE using an inverted microscope after another incubation for 5 days at 37 °C.

3. Results

3.1. Guanidine thiocyanate-based nucleic acid extraction buffers inactivate poliovirus cell culture isolates

Several commercial nucleic acid extraction buffers were selected (Table 1) to initially test whether they could inactivate a high titer ($10^{8.6}$ $CCID_{50}$/0.1 mL) WPV1 isolate. All buffers used in this study contain at least 4 M guanidine thiocyanate, with the exception of Trizol LS, which contains 1.3–3.4 M guanidine thiocyanate, and High Pure Binding Buffer, which has none (Table 1). Each extraction buffer was mixed with 200 μL of WPV1 isolate according to the sample-to-buffer ratios recommended by the manufacturers (Table 1) and incubated for 30 min to ensure maximal inactivation. Samples were then diluted with PBS, the lysis buffer was removed using an Amicon filter, and virus was titered in a $CCID_{50}$ assay. Treatment with Viral RNA Buffer, Trizol LS, MagMAX Lysis Buffer alone inactivated WPV1 below the limit of detection of the assay ($10^{0.5}$ $CCID_{50}$/0.1 mL; Table 2). Incubation with Buffer AVL or UNEX alone reduced the WPV1 titer by 5.4 and 7.2 $\log_{10}$ respectively, but the addition of ethanol (to a final concentration of 44 % (v/v) or 50 % (v/v), respectively) was necessary to reduce WPV1 titer below the limit of detection. Moreover, WPV3, SL1, and SL3 isolates were also inactivated by Buffer AVL with the addition of ethanol to 44 % v/v (Fig. 1). Contrary to the other buffers tested, High Pure Binding Buffer had no effect on WPV1 titer. Proteinase K treatment after incubation in High Pure Binding Buffer, however, was sufficient to fully inactivate the virus.

3.2. Guanidine thiocyanate plus ethanol inactivates WPV1

Because the chemical composition of commercial nucleic extraction buffers varies widely, we next determined whether guanidine thiocyanate alone could inactivate WPV1 isolate. Guanidine thiocyanate concentrations typically used for nucleic acid extractions and found in commercial buffer formulations (see Table 1) range between 4 M (~50 %) and 6 M (~70 %). For this experiment, we chose to test the concentration of 4 M guanidine thiocyanate, which is the lowest concentration in nucleic acid extraction buffers. Four volumes (800 μL) of 4 M guanidine thiocyanate (in 0.1 MES, pH 6.0) were added to 1 vol of WPV1 isolate (200 μL), similar to the QIAamp Viral RNA Mini Kit protocol (Fig. 2). An increasing amount of ethanol (10–50 % v/v final concentration) was also added to assess potential enhancement of inactivation. Ethanol is often added as a part of RNA extraction procedures to facilitate nucleic acid binding to silica columns. It is also a partial protein denaturant and has been shown to inactivate poliovirus at high concentrations (Roberts and Lloyd, 2007). After a 30-minute incubation at room temperature (24–26 °C), residual buffer in the samples was removed by ultrafiltration and the viral titers determined by $CCID_{50}$ assay. Incubation with 4 M guanidine thiocyanate alone reduced the viral titer by more than 4 $\log_{10}$ (Fig. 2). Adding ethanol to a final concentration of 10 % further reduced the viral titer by approximately 1 $\log_{10}$. Viral titer was below the limit of detection after the addition of at least a 20 % final concentration of ethanol.

3.3. Guanidine thiocyanate-based nucleic acid extraction buffers inactivate poliovirus in stool suspensions

Next, nucleic acid extraction buffers were evaluated for their effectiveness in inactivating poliovirus in stool suspensions. Although stool suspensions typically have viral titers lower than isolates, increased organic load due to the presence of bile salts and other compounds may impair viral inactivation. The *Quick*-RNA Viral Kit and QIAamp Viral RNA Mini Kit were selected for further analysis, as both are widely used by the GPLN and non-poliovirus laboratories for viral RNA extractions from stool suspensions and isolates. Viral RNA Buffer and Buffer AVL with ethanol were evaluated for their ability to inactivate poliovirus in 10 % stool suspensions consisting of either WPV1, SL1, SL3, SL2 or SL2 isolate. The 4 M guanidine thiocyanate buffer with 20 % ethanol was also tested for comparison. The viral titers of the suspensions ranged from to 1.4–4.5 log $CCID_{50}$ /0.1 mL. One volume (200 μL) of each stool suspension was combined with three volumes of Viral RNA Buffer, four volumes of Buffer AVL and ethanol (44 % final concentration), four volumes of 4 M guanidine thiocyanate in 0.1 M MES, pH 6.0 and ethanol (20 % final concentration), or four volumes of PBS. Samples were incubated for 5 min (Viral RNA Buffer and Buffer AVL with 44 % ethanol) or 30 min (4 M guanidine thiocyanate with 20 % ethanol). To increase sensitivity, treated stool suspensions were concentrated by ultrafiltration and added undiluted to RD cells. There was no CPE in any of the cultures following treatment of the samples with any of the buffers at Day 5, in contrast to those treated with PBS (Table 3).

3.4. Blind passage confirms poliovirus inactivation by guanidine thiocyanate-based nucleic acid extraction buffers

Finally, as further confirmation of the effectiveness of these methods to inactivate poliovirus in stool suspensions and to increase the possibility of detecting any remaining viable virus, blind passage experiments were performed. A high-titer WPV1 virus stock was spiked into enterovirus-negative stool suspension. Extraction buffers were added to the resulting spiked suspension (10^7 $CCID_{50}$ /0.1 mL) and incubated for the minimum amount of time to achieve inactivation previously determined in $CCID_{50}$ assays (Table 5). Treated suspensions were processed by ultrafiltration, added to RD cells, and CPE was recorded after 5 days. Supernatants from negative flasks were then passaged onto a fresh cell monolayer and the cells were monitored for CPE for an additional 5 days. There was no CPE detected in the first or second passages of WPV1-spiked stool suspension treated with any of the commercial extraction buffers with the incubation times tested (Table 4). However, viable virus was present in the first passage after treatment with a buffer containing 4 M guanidine thiocyanate with 20 % ethanol. Increasing the final ethanol concentration to 30 % with 4 M guanidine thiocyanate resulted in no detectable CPE in the first or second passages.

4. Discussion

In this report, we show that guanidine thiocyanate-based nucleic acid extraction buffers can readily inactivate high-titer polioviruses in isolates and stool suspensions, two types of IM and PIM likely to be handled in both PEFs and diagnostic laboratories that are not PEFs (non-essential facilities). Our results confirm and extend previous findings that guanidine thiocyanate-based nucleic acid extraction buffers are effective inactivators of viruses. We

initially tested whether nucleic acid extraction buffers could inactivate poliovirus using a high-titer WPV1 isolate. Importantly, we demonstrated that the other poliovirus type isolates and stool suspensions tested in this study (WPV3, SL1, SL2, and SL3) were also inactivated by extraction buffers using the same procedures used to inactivate WPV1 isolate. One limitation of our study is that we were unable to test whether these extraction buffers could inactivate wild poliovirus type 2 (WPV2) isolates or WPV2-positive stool suspensions due to current GAP III containment restrictions. To our knowledge, however, there have been no studies that show whether one poliovirus serotype or genotype is more resistant to chemical inactivation in comparison with other serotypes or genotypes. Therefore, the inactivation methods outlined in this report using guanidine thiocyanate-based nucleic acid extraction buffers should be effective in inactivating isolates and stool suspensions of all poliovirus serotypes and genotypes. Buffer AVL and UNEX Buffer completely inactivated poliovirus only when ethanol (44 % v/v final concentration and 50 % v/v final concentration, respectively) was added, as recommended by the manufacturer. Trizol LS, Viral RNA Buffer, and MagMAX lysis buffer successfully inactivated poliovirus without ethanol.

Poliovirus is rapidly inactivated by 6 M guanidine thiocyanate (Roberts and Lloyd, 2007). We report here that treatment with 4 M guanidine thiocyanate alone reduced the virus titer by more than 4 $\log_{10}$ in $CCID_{50}$ assays. We also observed that incubation with 50 % ethanol alone had no effect on the virus (data not shown). However, adding ethanol to 4 M guanidine thiocyanate had a synergistic effect on inactivation. Four molar guanidine thiocyanate with 20 % ethanol inactivated a high-titer WPV1 isolate in $CCID_{50}$ assays and a panel of 17 PV-positive stool suspensions but was not adequate for complete inactivation when tested in blind passage. Thus, it is likely that some viable virus remained below the limit of detection in the $CCID_{50}$ assays ($<10^{0.5}$ $CCID_{50}$/0.1 mL) and that virus was amplified to a detectable level in the blind passage experiments. Increasing the final ethanol concentration to 30 % inactivated a high-titer WPV1-spiked stool suspension in blind passage. The typical guanidine thiocyanate concentration of commercial nucleic acid extraction buffers ranges from 4 M to 6 M (1 M – 2M final concentration after sample and ethanol addition) and the final ethanol concentration added to the sample usually exceeds 30 % for column-based nucleic extraction kits. Many commercial guanidine thiocyanate-based extraction buffers, therefore, especially those that include ethanol, should be able to inactivate poliovirus. Moreover, these buffers may contain additional denaturing agents, such as sodium iodide (e.g., Viral RNA Buffer), phenol (e.g., Trizol LS), and detergents that aid in solubilizing samples and likely enhance inactivation. The minimum inactivation time for all tested commercially available nucleic acid extraction buffers did not exceed fifteen minutes, suggesting they were more effective than the combination of 4 M guanidine thiocyanate and 30 % ethanol, which required 30 min for complete inactivation. The only buffer tested that failed to have any effect on poliovirus was High Pure Binding Buffer, which contains 6 M guanidine hydrochloride as the chaotropic salt (see Table 1). Poliovirus is resistant to guanidine hydrochloride, which is a weaker chaotrope than guanidine thiocyanate (Roberts and Lloyd, 2007). Extraction buffers with guanidine hydrochloride as the primary chaotropic agent are therefore not appropriate for poliovirus inactivation, unless used in conjunction with proteinase K or other enzymatic treatment. Furthermore, caution should be exercised when using extraction buffers that use detergents as the primary inactivating agent, since

non-enveloped viruses such as poliovirus are resistant to treatment with detergents, although detergents can inactivate enveloped viruses. Regardless, nucleic acid extraction buffers that were not included in this study will need to be validated for their effectiveness in inactivating poliovirus in PIM.

Immediate nucleic acid extraction from poliovirus PIM is presently recommended for non-PV labs as a means of inactivating PV, but storage in nucleic acid extraction buffers may allow for long-term preservation of inactivated samples for later extraction and analysis by compatible downstream methods such as rRT-PCR and next-generation sequencing. Guanidine thiocyanate is frequently a component of specimen transport media and fecal storage buffers used when there is no need to retain viable virus (Daum et al., 2011; Hosomi et al., 2017). Several studies have reported using commercial nucleic acid extraction buffers to store samples for extended periods at ambient and frozen temperatures. Dengue virus, Venezuelan Equine Encephalitis Virus, and Rift Valley Fever Virus RNA were detectable after at least 35 days when samples were stored in Buffer AVL at 20 °C or 4 °C (Blow et al., 2008). Norovirus RNA in stool suspensions was successfully genotyped using rRT-PCR assays after being stored for two weeks at room temperature on disks soaked with UNEX buffer. Although ethanol was not used, the ability to recover RNA was shown (Cromeans et al., 2019). A guanidine thiocyanate-based extraction buffer preserved rotavirus RNA in gorilla feces for up to 180 days at room temperature (Whittier et al., 2004). Thus, storing inactivated samples in nucleic acid extraction buffers or extraction buffers with ethanol added (e.g., Buffer AVL, UNEX) could be potentially used by non-PEF labs to retain PIM and obviate sample destruction. Collectively, these results suggest that inactivation of PV PIM using nucleic acid extraction buffers will be an important part of biorisk management procedures to mitigate facility-associated poliovirus release yet still ensure sample integrity for molecular detection.

Funding

This research was supported in part by the Center for Preparedness and Response at the CDC through the U.S. National Authority for Containment of Poliovirus.

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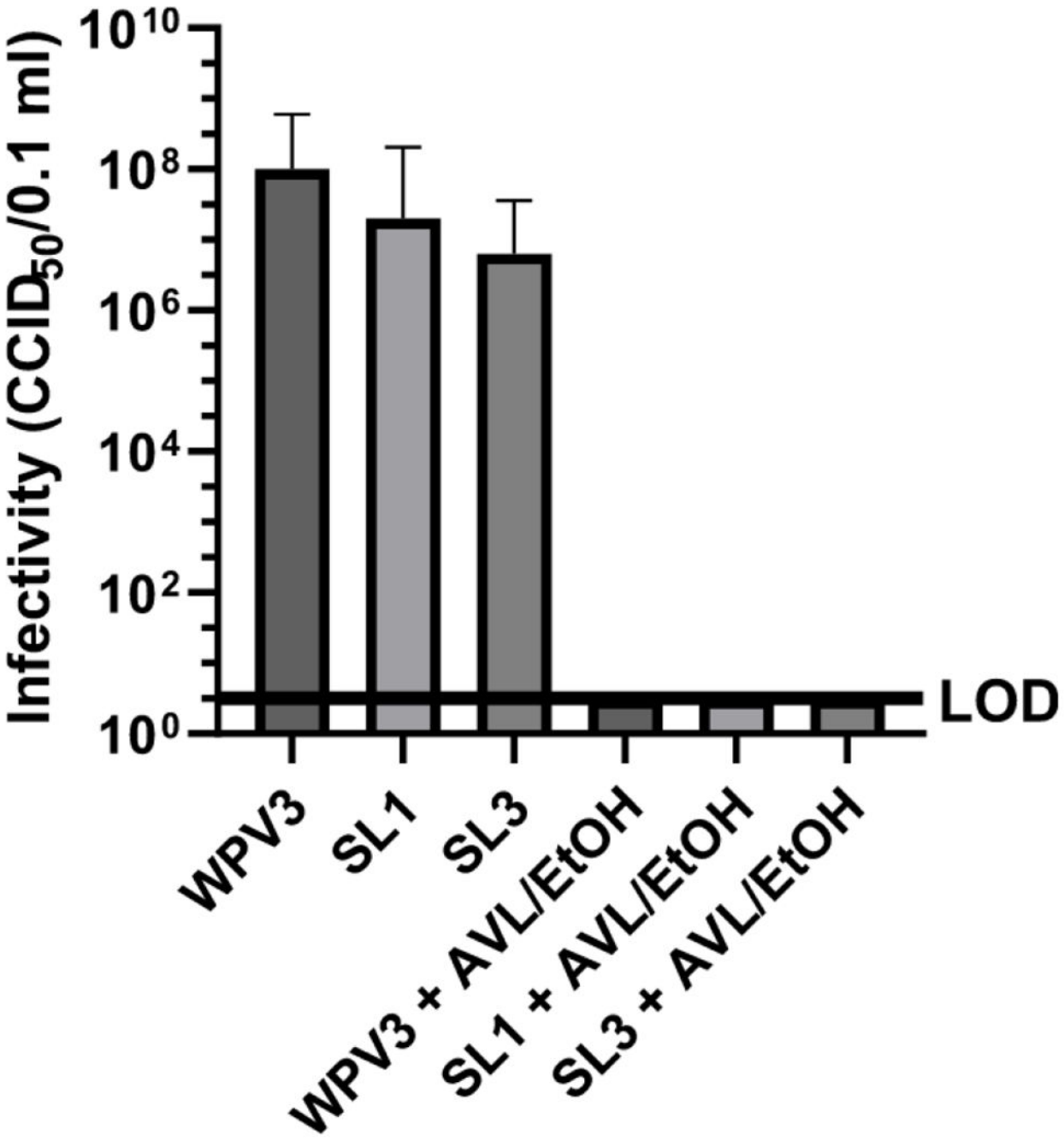

Fig. 1.

WPV3, SL1, and SL3 inactivation after incubation with Buffer AVL and ethanol. WPV3, SL1, and SL3 cell culture isolates were incubated with Buffer AVL and ethanol for 30 min and concentrated. Viral titers were determined by $CCID_{50}$ assay. of detection ($10^{0.5}$ $CCID_{50}$/0.1 mL). Error bars represent standard deviation of three experiments.

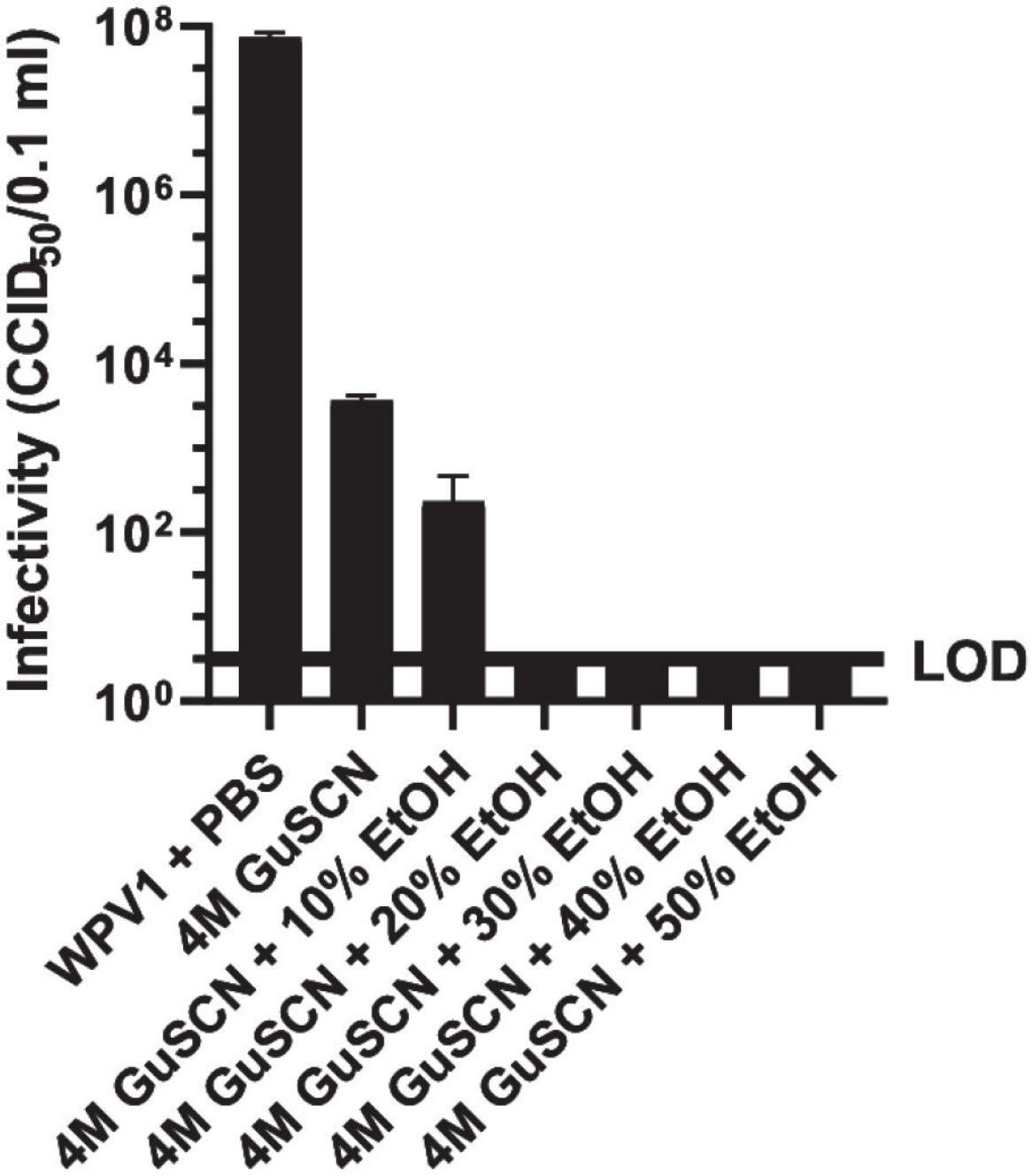

Fig. 2.

WPV1 inactivation by guanidine thiocyanate with increasing concentrations of ethanol. WPV1 was incubated with 4 M guanidine thiocyanate (GuSCN) in 0.1 M MES, pH 6.0 with increasing concentrations of ethanol (0-50 %) for 30 min at room temperature. Samples were concentrated by ultrafiltration and viral titers determined by $CCID_{50}$ assay. Error bars represent standard deviation for three experiments.

Table 1

Summary of commercial nucleic acid extraction buffers used in this study.

Extraction Buffer (EB)	Kit	Vendor	Guanidine Thiocyanate content	Sample:EB Ratio	Additional Components Added	Sample/Buffer Incubation Time (Kit)
Viral RNA Buffer	*Quick-RNA* Viral Kit	Zymo Research	≤6 M (70 %)	1:3	0.5% β-ME added	Not Specified
Binding Buffer	High Pure Viral Nucleic Acid Kit	Roche Diagnostics	None (6 M Guanidine HCl)	1:1	Proteinase K	Proteinase K +10 min
MagMAX Lysis/ Binding Concentrate	MagMAX Pathogen RNA/DNA Kit MagMAX-96 Viral Isolation RNA Kit	Applied Biosystems	4.65–6.77M (55–80 %)	1:2	None	Not Specified
Buffer AVL	QIAamp Viral RNA Mini kit	Qiagen	4.2–6 M (50–70 %)	1:4	Ethanol added (44 % final)	10 min
UNEX	N/A	VWR/Microbiologics	4.1 M (48 %)	1:1	Ethanol added (50 % final)	10 min
Trizol LS	N/A	Invitrogen	1.3–3.4 M (15–40 %)	1:1	None	Not Specified

Table 2

WPV1 inactivation by commercial nucleic acid extraction buffers.

Extraction Buffer	Sample: Buffer Ratio	Additional Components Added	Incubation Time	Viral Titer Post-Treatment (log $CCID_{50}$/ 0.1 mL)
PBS (control)	1:4	None	30 min	8.6 ± 0.1
Trizol LS	1:3	None	30 min	Below LOD
Viral RNA Buffer	1:3	0.5 % β-mercaptoethanol	30 min	Below LOD
Buffer AVL	1:4	None	30 min	3.6 ± 0.4
Buffer AVL + Ethanol	1:4	Ethanol (44 % final)	30 min	Below LOD
UNEX	1:1	None	30 min	1.4 ± 0.1
UNEX + Ethanol	1:1	Ethanol (50 % final)	30 min	Below LOD
MagMAX Lysis/Binding Concentrate	1:2	None	30 min	Below LOD
High Pure Binding Buffer	1:2	None	30 min	7.8 ± 0.3
High Pure Binding Buffer with Proteinase K	1:2	Proteinase K	30 min	Below LOD

WPV1 isolate was incubated with extraction buffers Buffer AVL, Trizol and UNEX, Viral RNA Buffer, MagMAX Lysis/Binding Concentrate, or High Pure Binding Buffer with or without ethanol and reducing agents, for 30 min at room temperature. Samples were concentrated and resulting viral titers determined by $CCID_{50}$ assay. LOD, Limit of detection (0.5 $\log_{10}$ $CCID_{50}$/0.1 mL).

Table 3

PV Inactivation in stool suspensions after incubation with guanidine thiocyanate-based buffers.

Sample No.	PV Type	Viral Titer (log $CCID_{50}$/0.1 mL RD cells)	Number of CPE – Positive Wells on Day 5			
			PBS	Viral RNA Buffer	Buffer AVL + ethanol	4 M GuSCN + 20 % ethanol
1	WPV1	3.6	2/2	0/2	0/2	0/2
2	WPV1	5	2/2	0/2	0/2	0/2
3	WPV1	3.6	2/2	0/2	0/2	0/2
4	WPV1	4	2/2	0/2	0/2	0/2
5	WPV1	3	2/2	0/2	0/2	0/2
6	WPV1	3.8	2/2	0/2	0/2	0/2
7	WPV1	4.5	2/2	0/2	0/2	0/2
8	WPV1	3.6	2/2	0/2	0/2	0/2
9	WPV3	2.5	2/2	0/2	0/2	0/2
10	WPV3	2.8	2/2	0/2	0/2	0/2
11	SL1	2.7	2/2	0/2	0/2	0/2
12	SL1	1.6	2/2	0/2	0/2	0/2
13	SL3	1.4	2/2	0/2	0/2	0/2
14	SL3	4.3	2/2	0/2	0/2	0/2
15	SL1/SL3	2.3	2/2	0/2	0/2	0/2
16	SL2 (CCS)	6.8	2/2	NT	0/2	0/2
17	SL2	3.6	2/2	NT	0/2	0/2
18	SL2	4.3	2/2	NT	0/2	0/2

PV-positive stool suspensions were incubated with either PBS, Viral RNA Buffer (5 min), Buffer AVL and ethanol (5 min), or 4 M guanidine thiocyanate (GuSCN) and 20 % ethanol (30 min) at room temperature. Samples were concentrated by ultrafiltration and incubated with RD cells. CPE was scored after 5 days. NT, Not Tested. CCS, Cell culture supernatant.

Table 4

Inactivation of WPV1-spiked stool suspensions in blind passage.

Extraction Buffer	Spiked Suspension:Buffer Ratio	Additional Components Added	Incubation time	Number of CPE Positive Flasks	
				Passage 1	Passage 2
PBS (control)	1:4	NA	30 min	6/6	6/6
Trizol LS	1:3	None	5 min	0/3	0/3
Viral RNA Buffer	1:3	0.5% β-mercaptoethanol	5 min	0/3	0/3
Buffer AVL	1:4	Ethanol (44 % final)	5 min	0/3	0/3
UNEX	1:1	Ethanol (50 % final)	10 min	0/3	0/3
Binding Buffer	1:1	Proteinase K	10 min (with Pro K)	0/3	0/3
MagMAX Lysis/Binding Concentrate	1:2	None	15 min	0/3	0/3
4 M Guanidine Thiocyanate+ 20 % Ethanol	1:4	Ethanol (20 % final)	30 min	6/6	NT
4 M Guanidine Thiocyanate+ 30 % Ethanol	1:4	Ethanol (30 % final)	30 min	0/3	0/3
4 M Guanidine Thiocyanate+ 50 % Ethanol	1:4	Ethanol (50 % final)	30 min	0/3	0/3

WPV1-positive stool suspensions were incubated at room temperature with extraction buffers for the amount of time indicated. Samples were concentrated by ultrafiltration and incubated with RD cells. CPE was scored after 5 days for both 1st and 2nd passages. NT, Not Tested.

Table 5

Minimum incubation time for WPV1 inactivation.

Extraction Buffer	Sample:Buffer Ratio	Additional Components	Incubation Time			
			5 min	10 min	15 min	20 min
Trizol LS	1:3	None	Below LOD	NT	NT	NT
Viral RNA Buffer	1:3	0.5% β-mercaptoethanol	Below LOD	NT	NT	NT
Buffer AVL	1:4	Ethanol (44 % final)	Below LOD	NT	NT	NT
UNEX	1:1	Ethanol (50 % final)	0.7 ± 0.3	Below LOD	NT	NT
MagMAX Lysis/Binding Concentrate	1:2	None	0.6 ± 0.06	0.5 ± 0.06	NT	NT
4 M Guanidine Thiocyanate+ 20 % Ethanol	1:4	Ethanol (20 % final)	1 ± 0.3	0.9 ± 0.3	1.1 ± 0.1	0.8 ± 0.1

WPV1 isolate was incubated with extraction buffers Buffer AVL with ethanol, Trizol LS, UNEX, Viral RNA Buffer, MagMAX Lysis/Binding Concentrate, or 4 M guanidine thiocyanate with 20 % ethanol for the times indicated below. Samples were concentrated and resulting viral titers determined by $CCID_{50}$ assay. LOD, Limit of detection (0.5 $\log_{10}$ $CCID_{50}$/0.1 mL). NT, Not Tested.