Is Influenza A Rendered Non-Viable Following Treatment With RNA Isolation Kit Lysis/Binding Buffers?

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Human or animal viral swab specimens which potentially contain highly-pathogenic avian influenza A virus (HPAI) may require characterization in a BSL-3 laboratory. Procedures conducted in a BSL-3 laboratory can be time-consuming and extremely costly. With the rapidly increasing need to monitor the spread and transmission of HPAI, the large number of specimens requiring processing has potential to overwhelm the capacity of our BSL-3 facilities to process samples in an effective time frame. Many protocols for typing influenza A samples rely on real-time reverse transcription PCR amplification of viral RNA isolated from samples. The ability to remove viral samples from the BSL-3 laboratory at earlier stages in the RNA isolation protocol would encourage the use of high throughput technology to speed the processing and typing of samples. To remove a samples from a BSL-3 laboratory it must be demonstrated the complete inactivation of live virus particles. In this work we demonstrate that the lysis/binding buffers from two popular viral RNA isolation kits are effective at inactivating influenza A virus. This inactivation permits the removal of treated field specimens from the BSL-3 laboratory soon after addition of the lysis/binding buffers and incubation.

Introduction

As government agencies and health care providers around the world continue to escalate surveillance for highly pathogenic avian influenza (HPAI) viruses in response to their spread, the number of isolates warranting characterization could rapidly overwhelm the capacity of BSL-3 facilities. Many agencies, having opted for the ease and speed of real-time PCR screening [1-3], will need to isolate viral RNA from a large number of potential HPAI samples. Unfortunately, high throughput sample processing protocols are more compatible with lower level containment facilities. To remove samples from a BSL-3 laboratory a team must confirm that the virus is either not a highly pathogenic strain or show, without questionable doubt, that there are no active virus particles present. The ability to demonstrate the inactivation of virus at earlier steps in the RNA isolation, enabling their removal from the BSL-3 laboratory, will greatly improve the throughput capacity of typing laboratories. While many viral RNA isolation kits allude to the ability of their lysis/binding buffers to inactivate virus, to our knowledge no published studies exist demonstrating that influenza A virus is indeed inactivated by these reagents. In this study we tested the lysis/binding reagents from two popular viral isolation kits, the QIAamp® Viral RNA Mini Kit (QIAgen, Valencia, CA) and the MagMax™-96 Viral RNA Isolation Kit (Ambion, Austin, TX), for their ability to inactivate prototypical examples of 12 HA subtypes of avian influenza A, three common vaccine strains of human influenza A, and three prototypical swine influenza A samples.

Materials and Methods

Eighteen Influenza A virus stock solution (Table 1) were added to QIAgen QIAamp® viral RNA isolation kit AVL buffer and Ambion MagMax™- 96 viral RNA isolation kit lysis/binding solution per the respective manufacturer's instructions. Treated virus samples were added to a 7ml 20kDa MWCO iCon™ concentrator (Pierce, Rockford, IL) pre-rinsed with 1 x PBS (Gibco Carlsbad, CA) and containing 4.5 ml 1 x PBS in the upper chamber. Concentrators were centrifuged at 2800 x g at 4°C until the volume of upper chamber was reduced to <0.5 ml. The filtrate was decanted from lower chamber of the concentrator and ~ 4.5ml of 1 x PBS was added to the upper chamber. The concentration and dilution steps were repeated 2 additional times with a final concentration step resulting in overall ~1000 fold dilution of the original buffer composition. 200 ml of the final concentrate was used to infect a 200 mm^2 surface area well of a 24 well tissue culture cluster plate containing a confluent monolayer of MDCK London cells and 1 ml Olsen's viral growth medium (passage 1). Plates were centrifuged at 615 x g for 30 min to facilitate efficient infection and incubated at 36°C under 5.0% CO_2 for 48-72 hours. 200 ml of cell culture media from passage 1 was used to infect fresh cultures of MDCK London monolayers in 24 well tissue culture plates and incubated as before for 48-72 hours (passage 2). Cells from passage 1 and 2 were washed two times with sterile 1 x PBS, scraped from wells into 0.5 ml sterile 1 x PBS and 25 ml was spotted onto a microscope slide. Following air drying in a BSC the slides were fixed in cold 100% acetone for 10 minutes. Fixed cells were stained with FITC labeled anti-Influenza A mouse monoclonal antibody (Chemicon/Millipore Temecula, CA) and examined under UV microscopy.

Results

The presence of cytocidic compounds in the lysis buffers of both kits prohibit the ability to culture samples treated with these compounds on cell culture monolayers. This was overcome though a process of repeated dilution and concentration by centrifugal ultrafiltration of lysis buffer treated samples resulting in exchange of buffers with PBS equating to ~500-fold dilution of buffer components. To determine that this level of dilution of the lysis buffers was sufficient to prevent cytocidic effects to the cell monolayers, samples containing aliquots of each lysis/binding buffer were buffer-exchanged in the manner described above and the resulting diluate used to infect cell monolayers in parallel with the experimental samples. Cell monolayers to which the exchanged lysis/binding buffers has been added looked identical to untreated monolayers thus demonstrating that the cytocidic components of these reagents had been sufficiently removed. In addition, to determine that the buffer exchange process itself was not responsible for inactivation of virus, a sample of H1N1 Influenza A/New Caldonia/20/99 was subjected to the buffer exchange process described above.

The resulting CPE and DFA staining seen in monolayers infected with the buffer-exchanged virus was similar to a positive control of untreated virus from the same stock demonstrating that the buffer exchange process itself did not affect viral viability. Inactivation of all viruses tested (Table 1), following treatment with lysis/binding buffers, was demonstrated by the inability to culture virus after double blind passage on MDCK London cell culture monolayers. A positive viral culture was noted by typical influenza induced CPE, compared to positively infected controls, as well as observance of FITC tagged flu specific monoclonal antibody stained cells under fluorescence microscopy. Passage of the lysis buffer treated viruses on MDCK cell culture yielded no CPE as compared to the positive control. The absence of influenza A was confirmed by an inability to detect virus by direct fluorescence antibody staining, using a FITC tagged influenza-specific monoclonal antibody of cells from the cell cultures inoculated with viral samples treated with the lysis/binding buffers. These data suggest that influenza A specimens treated with the QIAgen QIAamp® viral RNA isolation kit AVL buffer and Ambion MagMax™ -96 viral RNA isolation kit lysis/binding buffers are inactivated and would be safe to remove from the BSL-3 laboratory.

Discussion

The ability to remove influenza A specimens from the BSL-3 laboratory soon after the addition of lysis buffers has the potential to greatly speed up the capacity to process and characterize these specimens. Without this ability surveillance laboratories would need to complete the RNA isolation from the specimens before they could be removed from the BSL-3 laboratory for molecular characterization. The ability to remove specimens earlier in the process becomes even more valuable as the number of specimens increases, as would be the case in the event of a widespread influenza epidemic. For example, by our estimates, for every one hundred specimens requiring molecular characterization the ability to complete RNA isolation from specimens in our BSL-2 laboratory using automated RNA purification equipment would save at least four man-hours over having to complete these isolations by hand in the BSL-3 facility. Furthermore, the ability to inactivate specimens rapidly by the addition of the lysis/binding buffers opens up the possibility of inactivating specimens for characterization at the point of collection thus eliminating the need to ship potentially infectious materials. We are currently testing the stability of viral RNA in these buffers to determine the feasibility of using these reagents as a shipping media for viral RNA from field surveillance sites. While the study we report here tested only low pathogenic influenza strains, additional extensive testing with HPAI strains would need to be conducted to assure that the lysis/binding buffers from these RNA isolation kits could, without a doubt, inactivate HPAI strains as well before they could be removed from the BSL-3 facility. These studies are currently underway. In summary, this study suggests that samples treated with the lysis/binding buffers from either the QIAgen QIAamp® viral RNA isolation kit or the Ambion MagMax™ -96 viral RNA isolation kit are free of active virus and are safe to remove from the BSL-3 facility upon surface decontamination of sealed tubes or 96 well plates. In addition, by inactivating virus with the lysis/binding buffers it may be possible to transport samples from the field to the laboratory without having to ship infections materials or the need to receive the samples at a BSL-3 facility saving surveillance laboratories limited resources required to process specimens.

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Table 1. Prototypical influenza A strains tested for viability following treatment with RNA isolation kit lysis buffers.

Strain	Culture source	$TCID_{50}$ /ml
Avian H1N1 Influenza A/Duck/Alberta/35	Allantoic fluid	5.62E+04
Avian H2N2 Influenza A/mallard/NY/6750/78	Allantoic fluid	5.62E+04
Avian H3N8 Influenza A/Duck/Ukraine/1/63	Allantoic fluid	1.26E+04
Avian H4N6 Influenza A/Duck/CZ/1/56	Allantoic fluid	6.82E+04
Avian H5N2 Influenza A/chucker/MN/14591-7/98	Allantoic fluid	N/D
Avian H6N2 Influenza A/turkey/MA/65	Allantoic fluid	2.16E+04
Avian H7N2 Influenza A/TKY/VA/4592/2002	Allantoic fluid	4.64E+06
Avian H8N4 Influenza A/Ty/Ontario/68	Allantoic fluid	1.78E+05
Avian H9N2 Influenza A/chicken/NJ/12220/99	Allantoic fluid	5.62E+04
Avian H10N7 Influenza A/chicken/Germany/49	Allantoic fluid	1.78E+06
Avian H11N9 Influenza A/duck/Memphis/546/76	Allantoic fluid	2.15E+06
Avian H12N5 Influenza A/duck/Alberta/60/76	Allantoic fluid	5.62E+04
Swine H1N1 Influenza A/sw/WI/238/97	MDCK cell supernatant	2.51E+05
Swine H1N2 Influenza A/sw/WI/R33F/01	MDCK cell supernatant	5.62E+05
Swine H3N2 Influenza A/sw/MN/593/99	MDCK cell supernatant	3.76E+06
Human H3N2 Influenza A/Nanchang/933/95	Allantoic Fluid	1.21E+06
Human H3N2 Influenza A/Panama/2007/99	MDCK cell supernatant	3.57E+05
Human H1N1 Influenza A/New Caldonia/20/99	Allantoic Fluid	1.17E+05