



Determination of Effective Elution Buffers for Extracting Viral RNA from Personal Protective Equipment

Samuel Sprowls, Francoise Blachere M.B., John Noti PhD.
Allergy and Clinical Immunology Branch
CDC NIOSH, Morgantown, WV



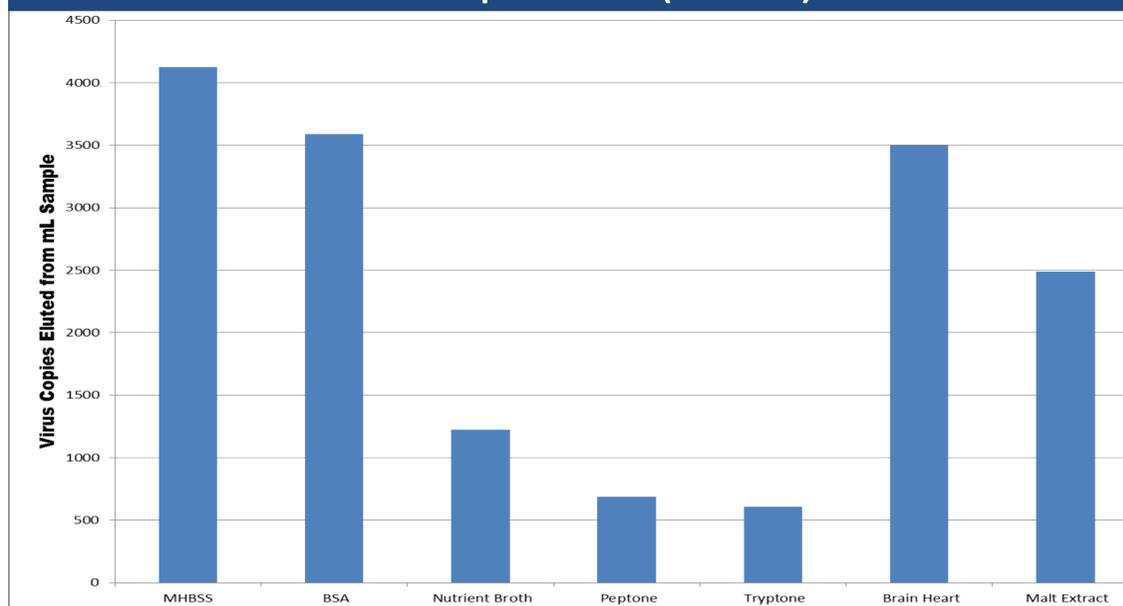
Background and Objective

The Shaeffer Study is a project conducted by the CDC NIOSH in order to determine the most effective means of protection using personal protective equipment (PPE). This study spreads over many hospitals across the country. After collecting the PPE from various hospitals during the peak influenza season, the samples will be shipped to CDC NIOSH to be analyzed in order to determine which hospitals are instructing their personnel best on protecting themselves from contracting the influenza virus. Not all hospitals use the same brand and/or style of PPE, therefore discrepancies in which defends healthcare workers the best have come into question. These masks will have influenza virus entrapped. In order to retrieve the virus from the PPE, a viral elution buffer (VEB) has to be used. This buffer works as anion exchange. The VEB competes with the charge of the PPE in such a way that the entrapped viral RNA of the influenza virus is release and cast into the remaining solution. This portion of a currently ongoing project is to determine if the current VEB, a Modified Hanks Balanced Salt Solution (MHBSS), is the best possible VEB to be used.

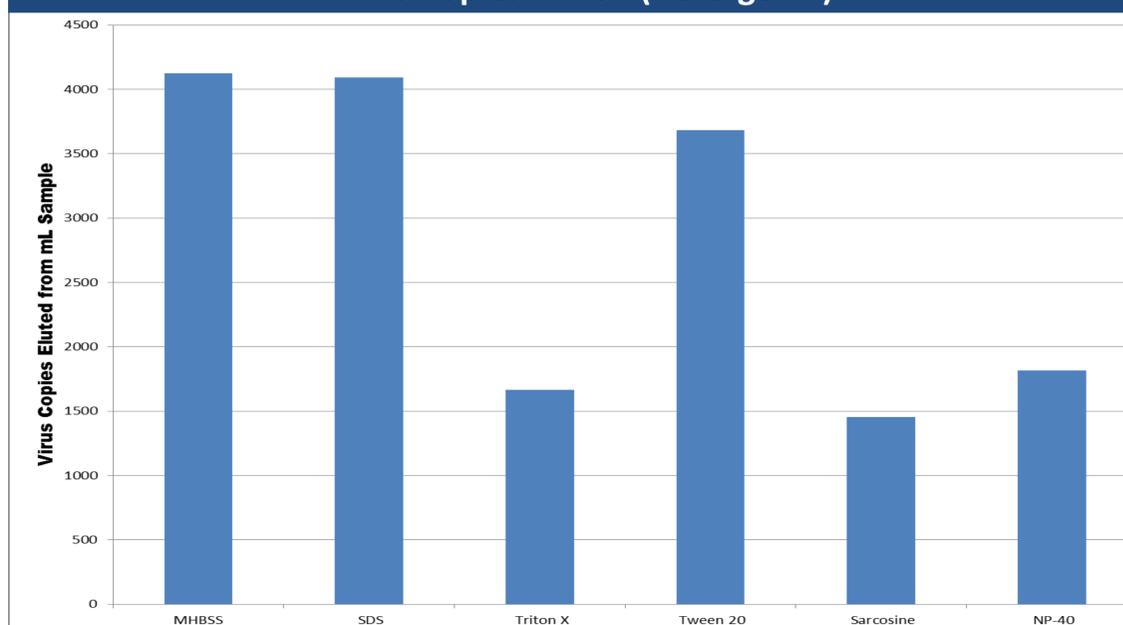
Methods

The following proteins were made in 1% stock solutions: Nutrient Broth, Bovine Serum Albumin, Peptone, Tryptone, Brain Heart Extract, and Malt Extract. The following detergents were made in 0.1% stock solutions: SDS, Triton X, Tween 20, Sarcosine and NP-40. A viral solution was made containing roughly 250,000 influenza virus copies. This viral solution was then used to inoculate a 2.5cm coupon cut from a Kimberly Clark 47080 Procedure Mask. 10 coupons were suspended separately in 2mL of each of the stock solutions. 10 Coupons were also suspended separately in the predetermined MHBSS. Therefore there were 120 coupons tested. The coupons were allowed to elute overnight. Following elution, two 500µL reps were used to isolate the Viral RNA from the rest of the solution. Viral RNA isolation was performed using Ambion's MagMax™ -96 Viral RNA Isolation Kit following the protocol previously set by the CDC NIOSH. After RNA was isolated, cDNA transcription was carried out using 30 µL of isolated RNA and 10 µL of prepared 5X RNA to cDNA Master Mix. After this process, samples were put through thermal cycling using an Eppendorf Thermal Cycler set at the following conditions: 5 minutes at 25°C, 30 minutes at 42°C, 5 minutes at 85°C and then maintained at 4°C until sample was retrieved. From here, results were quantified using qPCR methods. Following qPCR, obtained Ct values were inserted into a previously derived formula to calculate the number of Influenza virus particle eluted from coupon.

Virus Copies Eluted (Proteins)



Virus Copies Eluted (Detergents)



Discussion

As seen in the graphed results, the MHBSS is the most qualified viral elution buffer. The experiments performed were limited in some aspect because only the stock solutions of either just the specified protein or detergent were used. Further testing can now be done to assess whether a combination of certain protein and detergent viral elution buffers can be used to get a higher extraction efficiency of the virus particles from the PPE gathered. The amount of VEB to use per size of PPE was not tested in this study. Therefore, there are no direct correlations between amount of VEB used and size of the procedure mask coupons used in this project compared to the size of the future gathered PPE and the amount of VEB needed to extract the virus particles from them. Also, since the virus particles used herein were inactivated, further experimentation could be performed to examine the difference between eluting activated influenza virus particles and inactivated particles. With the confirmation of a thoroughly tested VEB, progress can be further made in the Schaeffer study. More recent studies using this determined VEB include a series of cough chamber experiments and aerosol virus particle testing, both of which are continued by the overseers of this particular study.

Conclusion

In regards to this study, the predetermined Modified Hanks Balanced Salt Solution, prepared by previous research by the CDC NIOSH, is the most qualified viral elution buffer to be used when extracting influenza virus particles from personal protective equipment.

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