The Effect of Sucrose on the Viability of Respiratory Syncytial Virus

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Background on RSV

- Significant cause of mortality and morbidity
  - Infants and elderly
    - Causes most complications
  - Immunocompromised
    - More severe in more compromised patients (Collins, 2008).
      - Transplant patients—no cure, easily spread
- No vaccine
- No effective treatment (McNamara, 2002).
  - Complicated by the immune system
  - Self limiting

Bronchiolitis

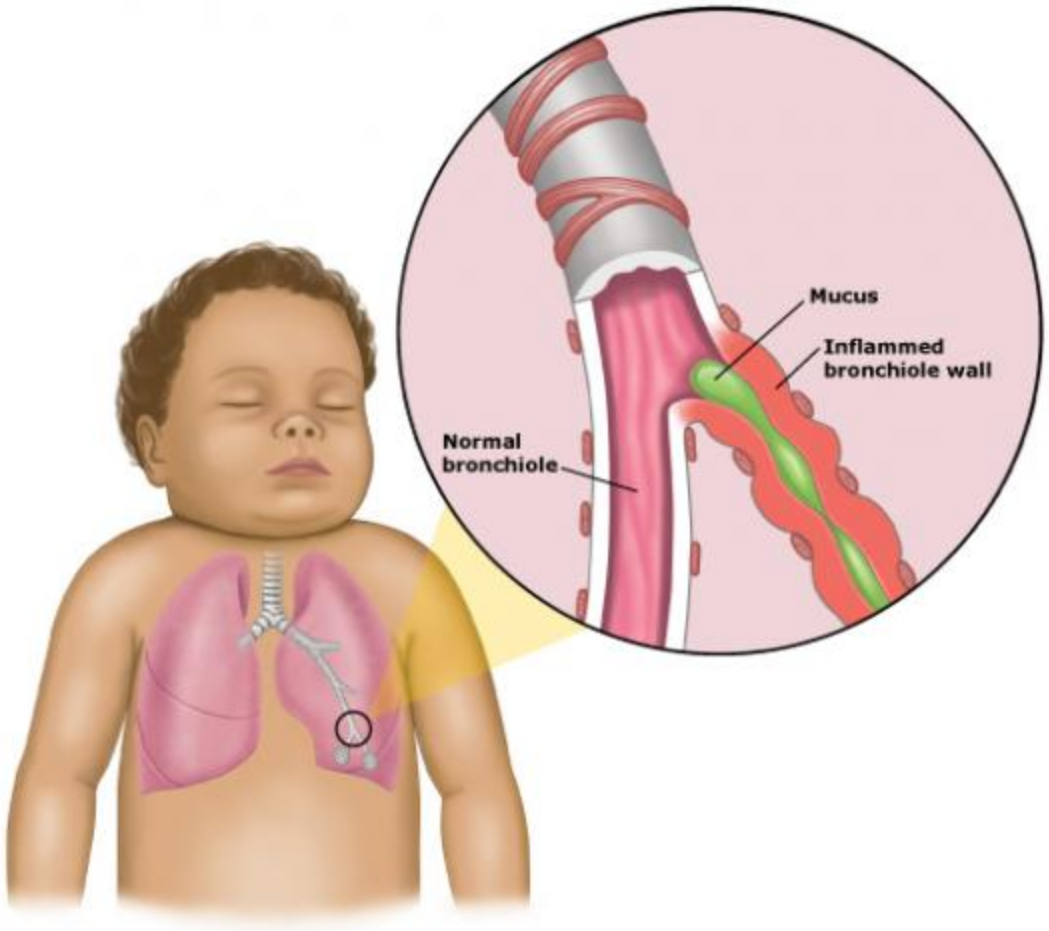


Image of infant with bronchiolitis

Background Cont.

- RSV causes significant time and monetary losses (Ausar, 2007).
  - 78,000 hospitalizations per year
  - Annual hospital cost ~$650 million
- Causes 199,000 deaths worldwide (Luongo, 2013)
  - Global annual infection—64 million
  - Younger child, more complications
    - Estimated 34 million episodes lower respiratory disease

Background Cont.

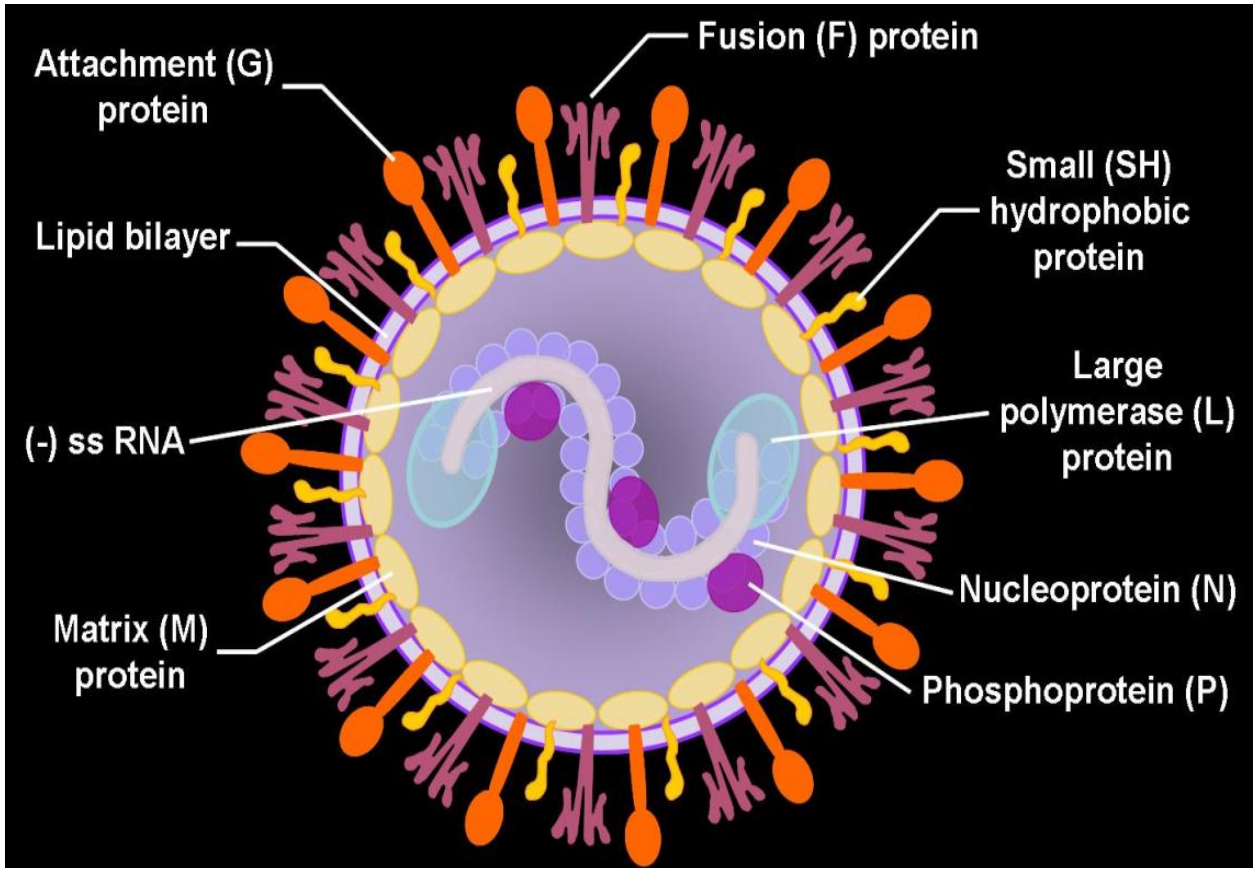


Figure of RSV structure.

Previous Research

- Sucrose could be stabilizing feature on RSV envelope (Ausar, 2007).

- RSV is denatured easily
  - Thermolabile virus
  - Detergents
  - Reduces titer after 3 months

Hypothesis

- Sucrose may have stabilizing effects on RSV envelope and help shield against environment temperature fluctuations.
  - This could allow it to
    - Remain viable longer in environment
    - Assist person-to-person transmission
    - Development of effective thermostable live-attenuated vaccine
    - Longer storage= easier to study

Objectives

- Optimize RSV cell culture protocol
- Determine RSV titer using plaque assays
- Assay a variety of sucrose concentrations to determine stability
  - 0.1, 0.3, 0.5, 1, 2, 7% sucrose

Materials

- Sucrose Media
  - Stock sucrose concentration made is 14%.
- Cell culture model used are HeLa cells because of availability and convenience.
- The virus strain used is RSV A-2.
- Media used
  - Gibco® Dulbecco's Modified Eagle Medium (DMEM)

Plaque Assay Terms

- Plaque assay
  - Measures the virus concentration
- Virus titer
  - Concentration of virus in a sample
- Plaque
  - 1 plaque= 1 virus infection multiplied several times

Figure of plaques from RSV A-2 from McKimm-Breschkin, 2004

Virology Terms

- Multiplicity of Infection
  - Ratio of number of virions to the number of target cells present in defined space.
- Overlay
  - Restricts virus progeny to neighboring cells
- Viability
  - Ability of a virus to infect a cell.
- Cell-associated virus
  - Virus particles that remain attached to host cell after replication.

Other Terms Cont.

- HeLa cells
  - Immortal cell line from cervical cancer.
- Ten-fold dilution
  - Concentration is 1/10 of the original solution.

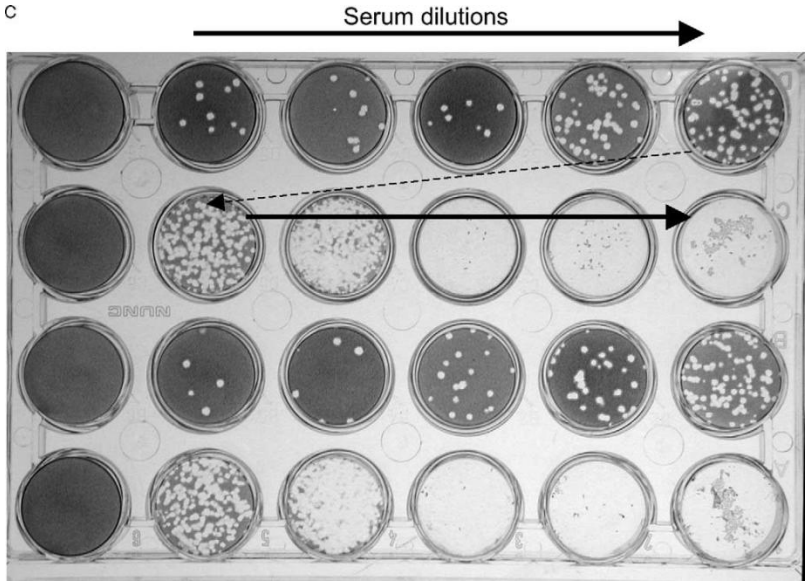


Figure of serum dilutions from RSV A-2 from McKimm-Breschkin, 2004.

Methods

- Typical RSV plaque assay takes about a week to complete.

- The titer assay was repeated twice
  - The first assay: two dilutions 10^{-6} and $5x10^{-7}$.
    - 1% and 2% seaplaque agarose®.
  - The second assay used one dilution (10^{-6}) for the whole plate using four replicates.
    - Both tested consistency between replicates.

Methods: Titer Assay

- Determine titer of virus without treatment
- Step 1
  - Add known concentration of HeLa cells to each well.
- Step 2
  - Distribute cells evenly by shaking
- Step 3
  - Incubate cells, allowing them to stick to wells
- Step 4
  - Inoculate with virus for 1 hour.

Methods: Titer Assay

- Step 5
  - Add overlay to wells to hold virus in place
- Step 6
  - Leave for 5 days
- Step 7
  - fix cells using 4% paraformaldehyde, stain with antibody, and count plaques

Titer Assay results

- 2% seaplaque agarose® only slightly lower than 1% agarose.
- Replicates were consistent with each other.
- Titer was higher than which didn't support hypothesis.
  - 4.32×10^8 pfu/mL.

1% seaplaque	2% seaplaque
4.16×10^8 pfu/mL	3.93×10^8 pfu/mL

Comparison of titer between 1% and 2% sea plaque agarose®

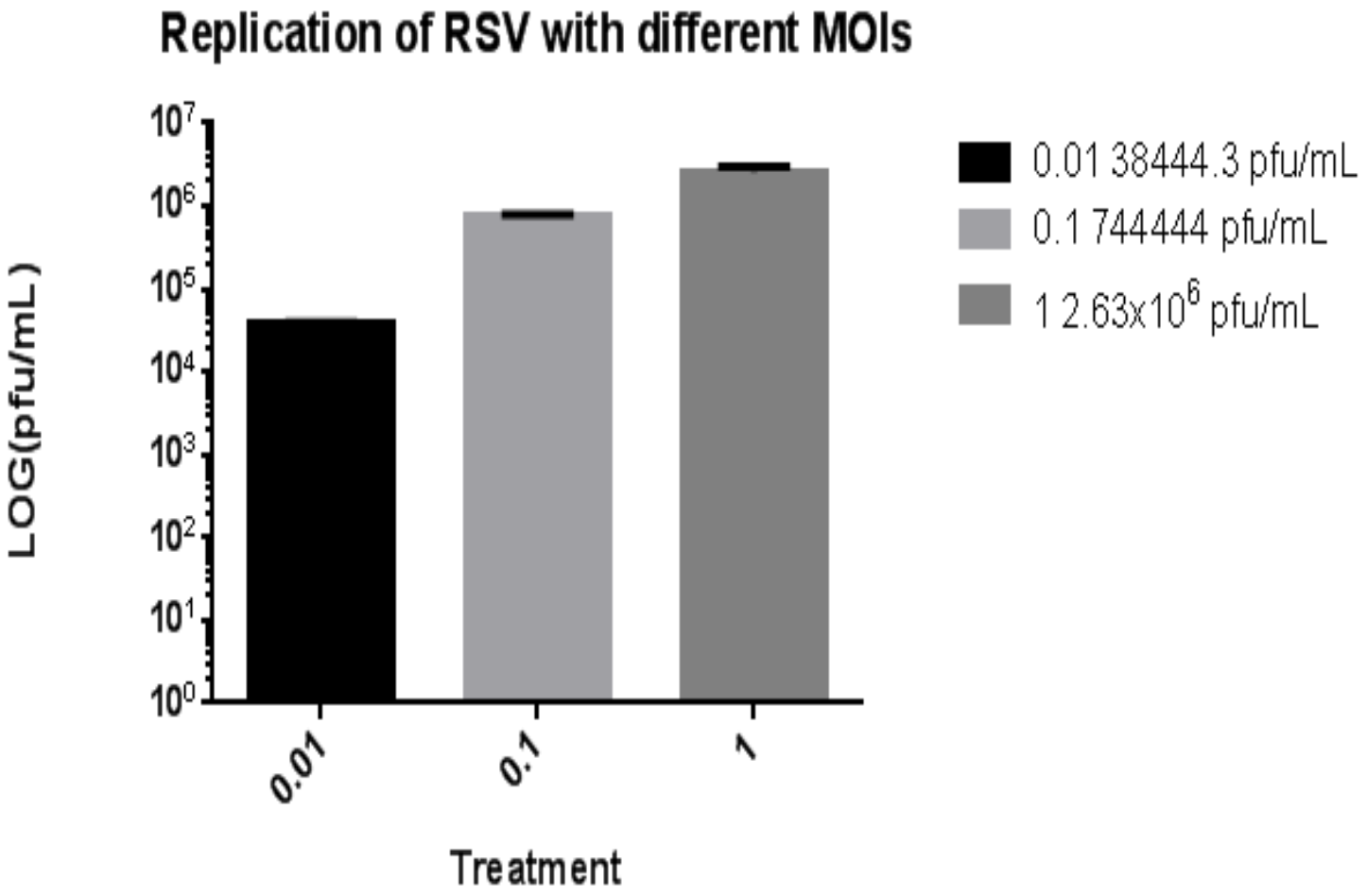
Methods: MOI Assay

- Determine titer using MOI of 0.01, 0.1, and 1
- Should be a log increase in virus concentration
- Step 1-4 of titer assay is the same
- Step 5
  - Add media and incubate
  - Prepare twenty-four well plates
- Step 6
  - Scrap cells with pipette tip and make dilutions

Methods: MOI Assay

- Step 7
  - Inoculate with virus for 1 hour
- Step 8
  - Add overlay and incubate for 5 days
- Step 9
  - Add 4% paraformaldehyde, stain and count plaques.

Results



Graph of the replication of RSV using different MOI treatments. The averages are graphed in LOG(pfu/mL) to show differences between the 0.01, 0.1, and 1. The averages of the MOIs are given in the legend.

Sucrose Assays

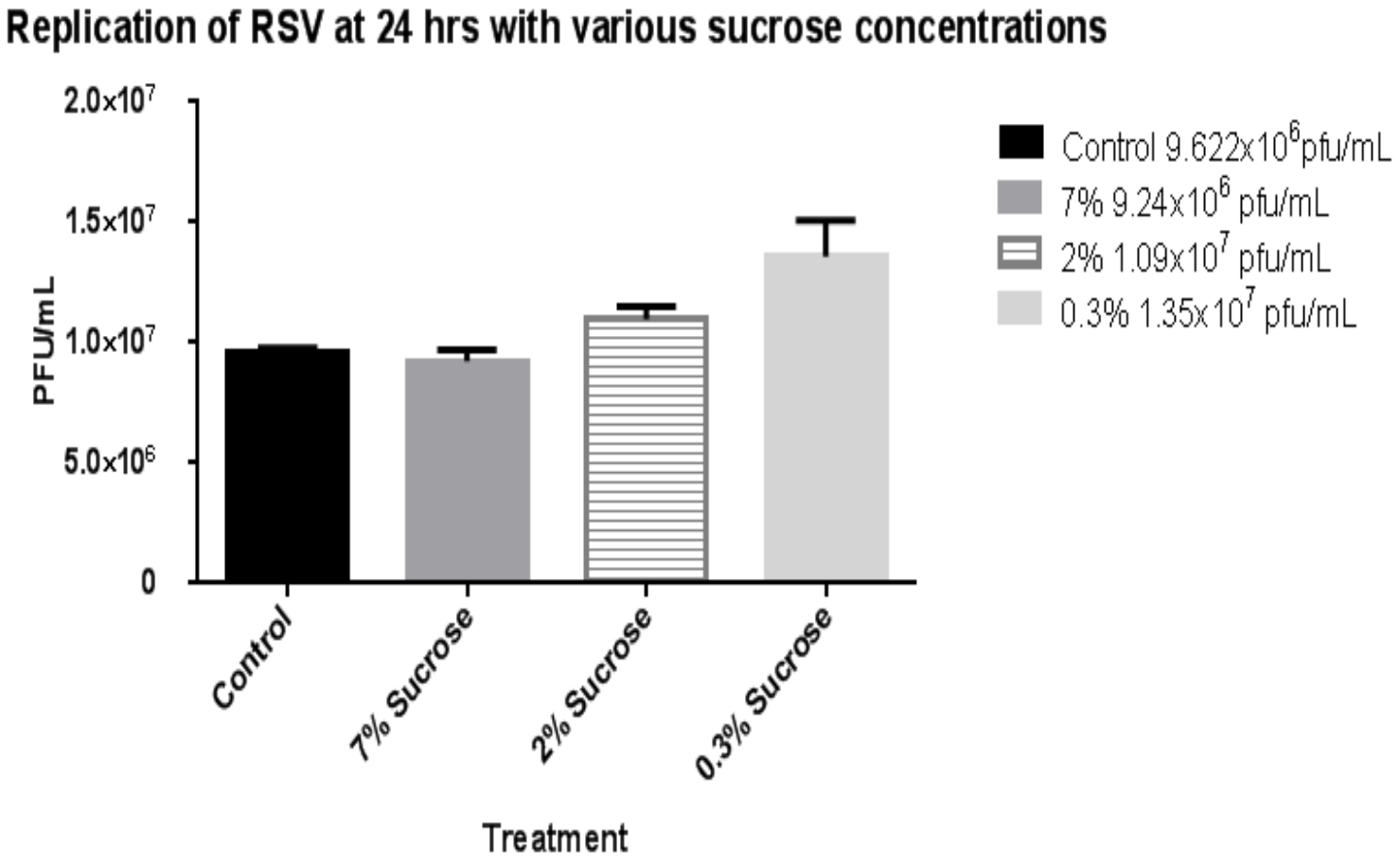
- The plaque assays testing various sucrose concentrations followed the MOI model.
  - Use MOI of 2
- The first sucrose assay tested concentrations 0.3, 2, and 7% sucrose. The second tested 0.1, 0.5, and 1% sucrose.
- Sucrose incubate for an hour with virus and media.

Sucrose Assays Cont.

- Negative control
  - No treatment.

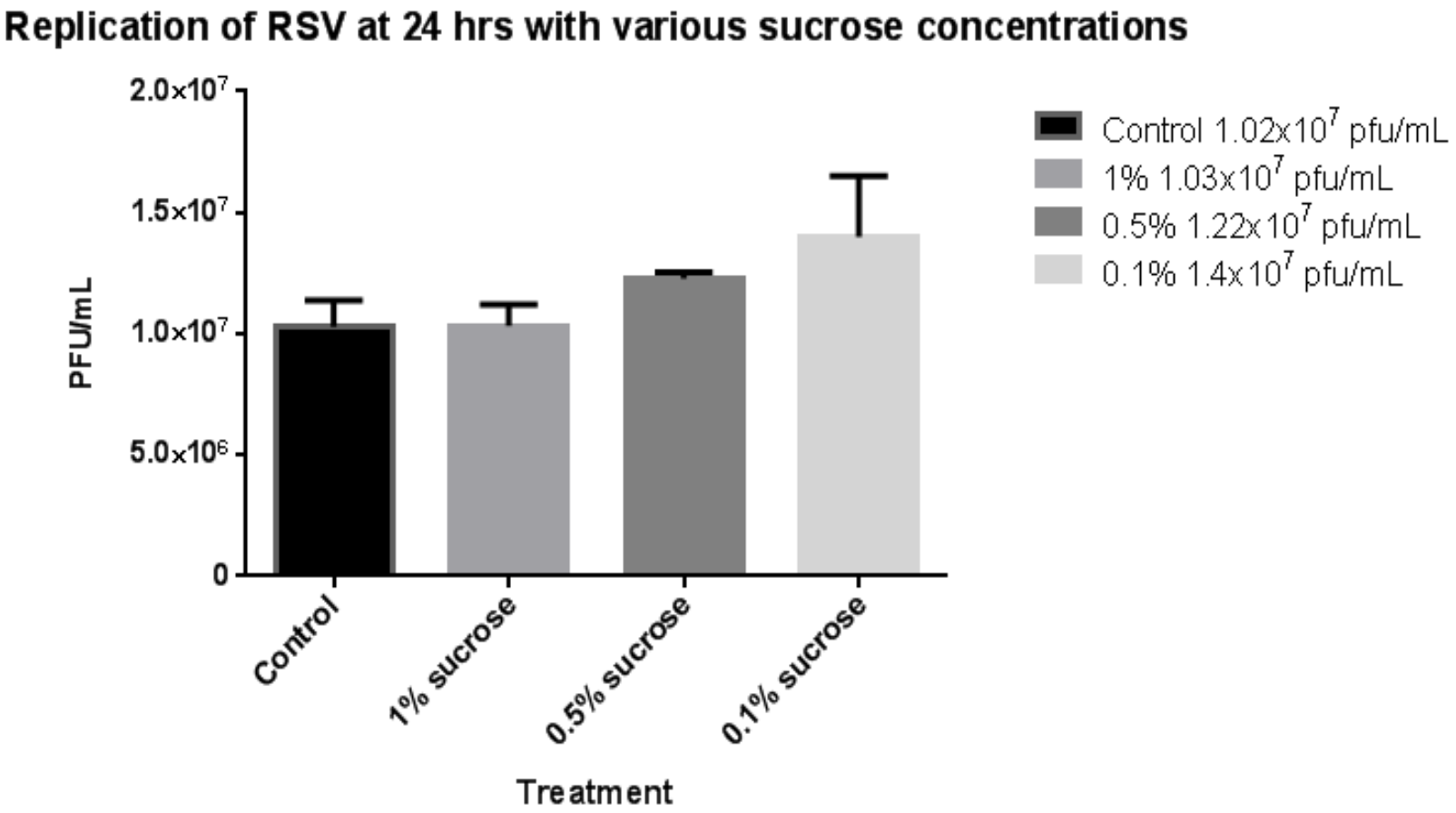
- Except for 7% sucrose and control, the dilutions plated in triplicate were $5x10^{-5}$, and 10^{-5}.
  - The dilutions plated in triplicate for 7% sucrose and control were 10^{-4}, and $5x10^{-5}$.

Sucrose Concentrations 0.3, 2, and 7%



Graph of the replication of RSV at 24 hours with sucrose concentrations 0.3%, 2%, and 7% sucrose. The averages are given in the legend. P-value <0.05.

Sucrose Concentrations 0.1, 0.5, 1%



Graph of the replication of RSV at 24 hours with sucrose concentrations 0.1%, 0.5%, and 1%. The averages are given in the legend. P-value >0.05.

Discussion

- Justification of titer assays
  - The titer assays were consistent with each other but the titer was a lot higher than hypothesized.
    - $2.8x10^8$ pfu/mL versus $4.32x10^8$ pfu/mL.
- MOI assay had the expected log increase
  - There was a smaller increase between 0.1 and 1.

Discussion Cont.

- Sucrose helps to stabilize virus.
  - Only 0.3% sucrose was only slightly significant, with a p-value <0.05.
  - 0.1%- 0.3% have most plaques compared to other sucrose concentrations.
  - One hour of incubation
    - 0.1-0.3 range of stability.
- Future experiments
  - Reproducibility and validity.
  - 0.3% sucrose should be tested at 4 hours, 24 hours, 48 hours, and 3 months, to test stability.

Conclusions

- Hypothesis: sucrose may have stabilizing effects on RSV.
- Only 0.3% sucrose was statistically significant. But possible range.
- Implications
  - This range could show a max of sucrose effect.
  - Better storage, can study better

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References

- Ausar, SF.; Espina, M.; Brock, J.; Thyagarayapuran, N.; Repetto, R.; Khandke, L.; Middaugh, CR. “High-throughput screening of stabilizers for respiratory Syncytial virus: identification of stabilizers and their effects on the conformational thermostability of viral particles.” Hum Vaccin. (2007): 94-103.
- Collins, P.L.; Graham, B.S.; “Viral and Host Factors in Human Respiratory Syncytial Virus Pathogenesis.” Virology. (2008): 2040-2055.
- McKimm-Breschkin, J.L. “A simplified plaque assay for respiratory syncytial virus—direct visualization of plaques without immunostaining.” J. Virol. Methods (2004) 113-117.
- McNamara, PS.; Smyth, RL. “The pathogenesis of respiratory Syncytial virus disease in childhood.” British Medical Bulletin(2002); 61: 13-28.
- Luongo,C.; Winter, C.C.; Collins, P.L.; Buchholz, U.J. “Respiratory Syncytial Virus Modified by Deletions of the NS2 Gene and Amino Acid S1313 of the L Polymerase Protein Is a Temperature- Sensitive, Live-Attenuated Vaccine Candidate That Is Phenotypically Stable at Physiological Temperature.” J.Virol (2013); 87: 1985-1996