The Effect of Sucrose on the Viability of Respiratory Syncytial Virus

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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
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
Figure of RSV structure.
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 $5 \times 10^{-7}$.
    - 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.
  - 4.32x10^8 pfu/mL.

<table>
<thead>
<tr>
<th>1% seaplaque</th>
<th>2% seaplaque</th>
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<tr>
<td>4.16x10^8 pfu/mL</td>
<td>3.93x10^8 pfu/mL</td>
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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 MOIs 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.
Negative control

- No treatment.

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

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

Sucrose Assays Cont.
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.
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.
    - $2.8 \times 10^8$ pfu/mL versus $4.32 \times 10^8$ pfu/mL.

- MOI assay had the expected log increase
  - There was a smaller increase between 0.1 and 1.
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


