Development of Focus Assays to Analyze Pathogens in Exhaled Breath
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Introduction
The CATCH (Characterizing And Tracking College Health) research study examines Acute Respiratory Infection (ARI) transmission in college students. This tracking of disease can show how the disease spreads within a population and how to prevent it in the future.

As part of sample collection, breath samples are taken to examine infectious agents within the breathe/lungs using a specialized machine called the G2. Previous data has demonstrated that infectious flu virus can be isolated from these breath samples. Focus assays were used to determine if the flu virus isolated from these breath samples was infectious.

Methods
Focus Assay
- Plate MDCK cells
- Infect with virus 48 hrs (flu)
  - Other virus incubation times may vary
- Fix cells with Methanol:Acetone
- Stain with antibodies:
  - E-cadherin (cell structures)
  - Influenza Nucleoprotein (Influenza)
  - DAPI (nuclear staining)

Previous focus assays do not include staining for cellular proteins to help define cell boundaries. This makes determining cellular localization difficult, and can contribute to mis-identifying foci. Additionally, data from the current study suggests that several cases were co-infected with multiple viruses. Determining infectivity of these co-infections is important to understanding ARIs.

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The overall goals of this project are:
- Expand the utility of the focus assay by incorporating a cellular protein stain, e-cadherin, to visualize cell boundaries.
- Eventually adapt the method to screen for other non-flu viruses, and/or
- Examine co-infected samples

Previous Results

<table>
<thead>
<tr>
<th>Type of Coinfection</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoV_HKU1, CoV_OC43</td>
<td>7</td>
</tr>
<tr>
<td>Adenovirus pool 1, Adenovirus pool 3</td>
<td>2</td>
</tr>
<tr>
<td>CoV_HKU1, CoV_OC43, Adenovirus pool 4</td>
<td>1</td>
</tr>
<tr>
<td>Adeno 4, HPV_4</td>
<td>1</td>
</tr>
<tr>
<td>Adeno 4, RSV_B</td>
<td>1</td>
</tr>
<tr>
<td>CoV_229E, CoV_NL63</td>
<td>1</td>
</tr>
<tr>
<td>Flu A (H3), CoV_229E</td>
<td>1</td>
</tr>
<tr>
<td>Flu A (H3), RSV_A, RSV_B</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>

Discussion
This study is currently in early stages of development. Preliminary results demonstrate that DAPI staining works well to visualize nuclei in MDCK cells. Further testing with e-cadherin is on-going.

Future Directions include:
1. Repeat focus assays with PR8 Influenza along with e-cadherin and DAPI to examine how the antibodies interact
2. Repeat focus assays using previous study samples along with e-cadherin and DAPI to confirm findings
3. Use focus assay to examine if other, non-flu viruses isolated from G2 samples are infections
4. Examine if focus assays can be used to determine infectivity of multiple pathogens in same sample (co-infection).

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