Hawaii – Quantification of infected *Fungia scutaria* larvae

**Materials/Reagents**

- 1x PBS
- Slides/coverslips
- Putty – molding clay
- Cell counter
- Light microscope

**10X PBS**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Make up in 800 ml</td>
<td>800 ml</td>
</tr>
<tr>
<td>0.02M NaH$_2$PO$_4$ (monobasic)</td>
<td>120 g/m</td>
</tr>
<tr>
<td>0.077M Na$_2$HPO$_4$ (dibasic)</td>
<td>142 g/m</td>
</tr>
<tr>
<td>1.4 M NaCl</td>
<td>58 g/m</td>
</tr>
<tr>
<td>add DH$_2$O to 800 ml</td>
<td>69.6</td>
</tr>
</tbody>
</table>

**1X PBS**

- 100 ml 10X PBS
- 900 ml DH$_2$O water

**Procedure**

A. Rinse fixed *Fungia scutaria* larvae
   1. Spin larvae 1min, ~2,000xg
   2. Remove sup (toxic – paraformaldehyde)
   3. Rinse w/ 1x PBS 3x
   4. Re-suspend in 50ml 1x PBS

B. Quantification of algae
   1. Place ~20ml larvae onto slide
   2. Scrap putty onto 4 corners of coverslip
   3. Gently depress coverslip, putty side down, onto larvae until no air is visible between slide and coverslip
   4. **ALTERNATIVE**: do not add putty to coverslip, gently drop onto larvae
      Use a Kimwipe to remove excess water until larvae are depressed enough to see all algae
   5. Count 100-150 larvae – record number of larvae with algae (% infection)
   6. Count number of algae in 100 infected larvae (density of algae per larvae)

**Comments:**