The Charles T. Campbell Ophthalmic Microbiology Laboratory

http://eyemicrobiology.upmc.com

Search engine: eye microbiology
Welcome to the Charles T. Campbell Eye Microbiology Lab website.

The Charles T. Campbell Ophthalmic Microbiology Laboratory at UPMC, Pittsburgh, PA is a clinical microbiology laboratory dedicated solely to the diagnosis of infectious diseases of the eye. Our dedicated laboratory, which opened in 1973, is fully certified by the Commonwealth of Pennsylvania, the College of American Pathologists, and the federal government (CLIA).

At UPMC, we serve the clinical practices, inpatient care areas, emergency departments, and surgical units. In addition, we provide microbiology services to the tri-state (Pennsylvania, West Virginia, and Ohio) ophthalmic community practices and to our ophthalmic alumni. The clinical laboratory does not depend on research sources for operation and all testing is billable for insurance reimbursement.

Our goal is to provide pertinent information to assist ophthalmologists and physicians of all specialties in the treatment of eye infections. Our endeavors include providing information on the best testing methods for detecting ocular pathogens, current antibiotic susceptibility data, and standard antibiotic therapies of ocular infections.

Ocular Microbiology and Immunology Group

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Click here to view PHOTOS
Click here to view VIDEOS
1. *In Vitro* Antibiotic/Antifungal Assessment

- Independent Evaluation of MICs with our Isolates
  - Etests, Broth Dilution
- Time-Kill Studies
- Synergy Testing with BAK
- CAPA Assay
1. *In Vitro* Antibiotic/Antifungal Assessment - References


- Kowalski RP, Kowalski BR, Romanowski EG, Mah FS, Thompson PP, Gordon YJ. The *in vitro* impact of moxifloxacin and gatifloxacin concentration (0.5% versus 0.3%) and the addition of benzalkonium chloride on antibacterial efficacy. *Am J Ophthalmol.* 2006;142:730-735.


2. In Vivo Antibiotic Testing

- Rabbit Keratitis Models

<table>
<thead>
<tr>
<th>Median Colony Counts per Cornea (Intact Epithelium)</th>
<th>Median Colony Counts per Cornea (Abraded Epithelium)</th>
<th>Comparison of Median Colony Counts in Corneas with Abraded vs. Intact Epithelia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tigecycline</td>
<td>Tigecycline</td>
<td>Tigecycline</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Vancomycin</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>Saline</td>
<td>Saline</td>
<td>Saline</td>
</tr>
</tbody>
</table>

- The Number of CFU/Cornea at the Onset of Therapy

Comparison of Median Colony Counts in Corneas with Abraded vs. Intact Epithelia
2. *In Vivo* Antibiotic Testing - References

- Romanowski EG, Kowalski TA, O’Connor KE, Yates KA, Mah FS, Shanks RMQ, Kowalski RP. The *in vitro* evaluation of tigecycline and the *in vivo* evaluation of RPX-978 (0.5% tigecycline) as an ocular antibiotic. *J Oc Pharm Ther*. 2015 (In Press).
3. *In Vivo* Ocular Bacterial Occupancy Model

Ocular Occupancy Model preliminary data. *S. aureus* was added to the ocular surface of 12 rabbit eyes. Ocular surface bacteria were enumerated (dacron-tipped swabs of the conjunctival fornices) at the indicated times. Error bars indicate one standard deviation. These data indicate that we can measure ocular surface bacteria and that sufficient bacteria survive to measure antimicrobial efficacy of a topical, surface acting anti-infective.
4. *In Vivo* Endophthalmitis Prevention Assays

- Topical Prophylaxis – AC Challenge
  - Before and After Bacterial Challenge
- Topical Prophylaxis – Contaminated Needle Challenge
- Intravitreal Prophylaxis – Vitreal Challenge
4. *In Vivo* Endophthalmitis Prevention Assays - References


- Kowalski RP, Romanowski EG, Mah FS, Yates KA, Gordon YJ. Intracameral Vigamox (0.5% moxifloxacin) is non-toxic and effective in preventing endophthalmitis in a rabbit model. *Am J Ophthalmol* 2005;140:497-504.


5. *In Vitro* Biofilm Assays
6. *In Vivo* Contact Lens Models
7. *In Vitro* Acanthamoeba Susceptibility Assay


### Table. Descriptive Statistics of 15 Acanthamoeba Isolates After Exposure to Common Anti-Acanthamoeba Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Median Growth Grade&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Kill Incidence Rate&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (control)</td>
<td>3.0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Polyhexamethylene biguanide, 0.02%</td>
<td>0.0</td>
<td>12 (80)</td>
</tr>
<tr>
<td>Chlorhexidine digluconate, 0.02%</td>
<td>1.0</td>
<td>6 (50)</td>
</tr>
<tr>
<td>Hexamidine diisethionolate, 0.1%</td>
<td>0.0</td>
<td>14 (93)</td>
</tr>
<tr>
<td>Voriconazole, 1.0%</td>
<td>2.0</td>
<td>2 (13)</td>
</tr>
</tbody>
</table>

<sup>a</sup>The median value of the growth grade of 15 *Acanthamoeba* isolates based on the cumulative score of 3 time points.

<sup>b</sup>Determined over 3 time points; any incidence of positive growth over the 3 time points was considered survival and not a kill. Negative growth at all 3 time points was denoted as a kill.
8. In Vitro Antiviral Testing

- **Viral Inactivation Assay**
  - Direct Killing/Neutralization MOA

- **Plaque Reduction Assay**
  - Intracellular MOA
    - Viral Protein
    - Host Protein Involved with Viral Replication

- **Progeny Yield Assay**
  - Intracellular MOA
  - Toxicity an Issue
8. In Vitro Antiviral Testing - References


9. *In Vivo* Antiviral Testing

- **Ad5/NZW Rabbit Ocular Model**
  - Cidofovir, Aganocides, NCT, ddC, IVIG

- **HSV-1/NZW Rabbit Keratitis Model**
  - Cidofovir, Acyclovir, Trifluridine
9. *In Vivo* Antiviral Testing - References

10. Outstanding Imaging

Laser Scanning Confocal Microscopy
CLSM Images of Untreated and Chlorine-, Silver-, and Tobramycin-treated PAO1 Biofilms Stained with BacLight Live/ Dead Stain

CLSM=confocal laser scanning microscopy.

Scanning Electron Microscopy

Possible outcome

Biofilm + treatment

Easy to see what is going on!

Kadouri and O'Toole 2008
11. *In Vivo* Ocular Tolerability Models
12. In Vivo Ocular Inflammation Assays

- Cytokine Analysis
- Ocular Inflammatory Signs
  - W/ and W/O LPS
  - W/ and W/O Bacterial Challenge
  - W/ and W/O Viral Challenge
13. Corneal Wound Healing Assays

*Pseudomonas aeruginosa* PA14

*Serratia marcescens*

<table>
<thead>
<tr>
<th>Initial Wound</th>
<th>LB (mock)</th>
<th><em>Pseudomonas aeruginosa</em> PA14</th>
<th>Pseudomonas aeruginosa PAO1</th>
<th>Serratia marcescens</th>
</tr>
</thead>
</table>

*In Vitro* (HCLE)

*Ex Vivo* (Pig Eyes)

*In Vivo* (Rabbits)

Day 1  Day 2  Day 3
14. Assay Development

- Design Assays to Achieve your Research Goals