The Charles T. Campbell Ophthalmic Microbiology Laboratory

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The Charles T. Campbell Eye Microbiology Lab

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Welcome to the Charles T. Campbell Eye Microbiology Lab website.

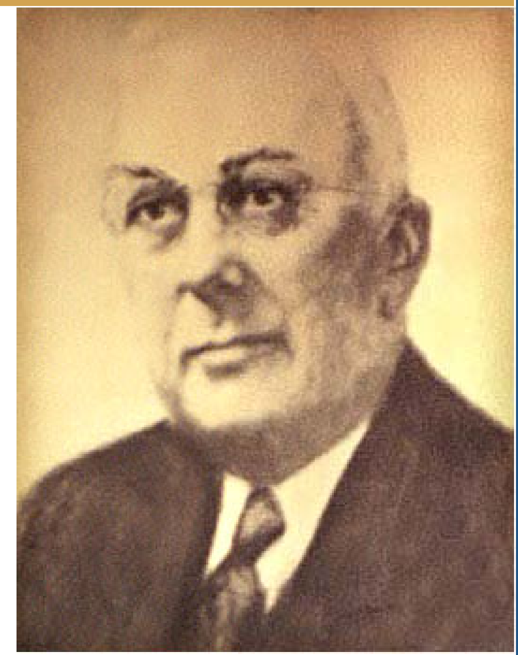
The Charles T. Campbell Ophthalmic Microbiology Laboratory at UPMC, Pittsburgh, PA is a clinical microbiology laboratory dedicated solely to the diagnosis of infectious diseases of the eye. Our dedicated laboratory, which opened in 1973, is fully certified by the Commonwealth of Pennsylvania, the College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

Ocular Microbiology and Immunology Group (OMIG) Information Read more-->



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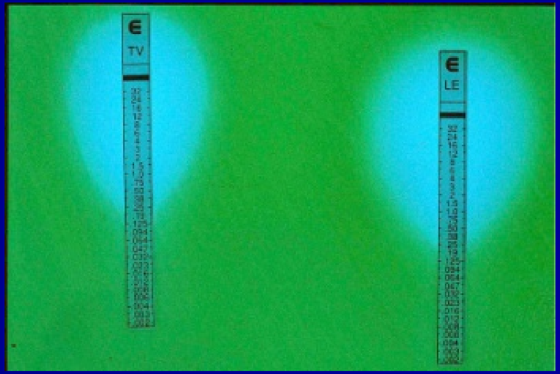
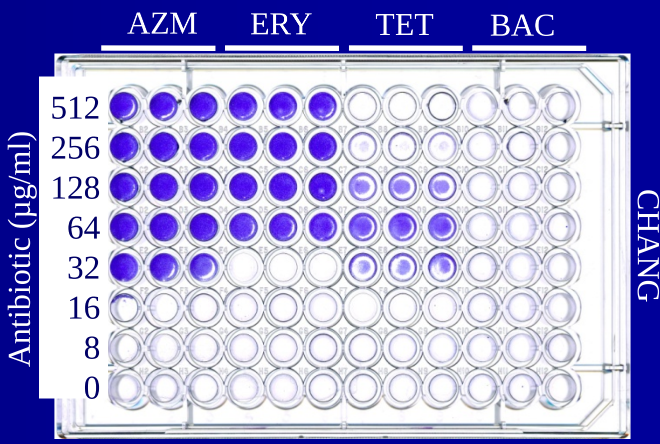
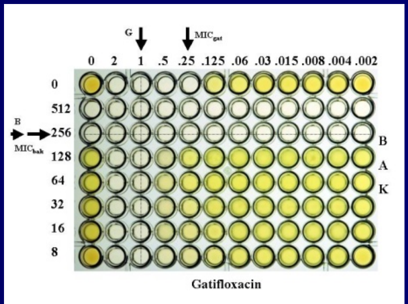
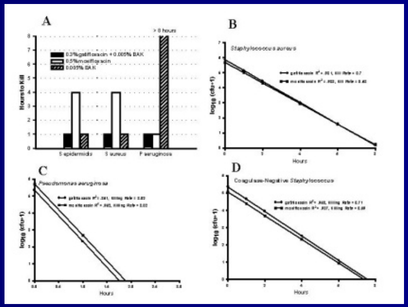
1. *In Vitro* Antibiotic/Antifungal Assessment

- Independent Evaluation of MICs with our Isolates
  - Etests, Broth Dilution
- Time-Kill Studies
- Synergy Testing with BAK
- CAPA Assay







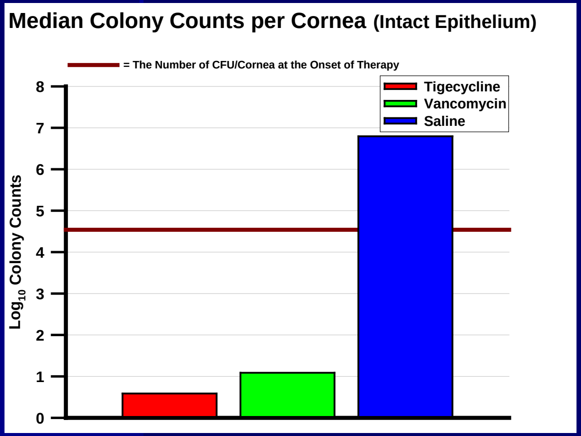


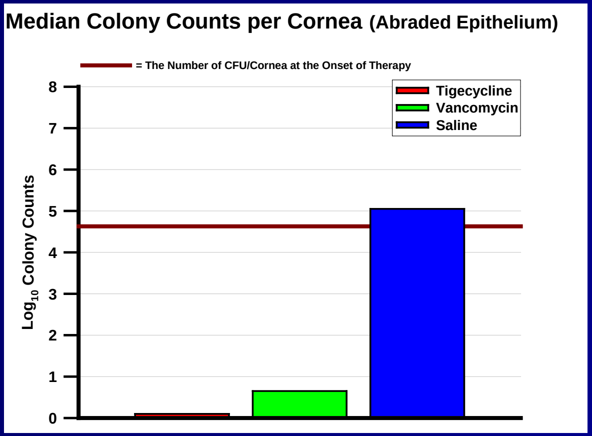
1. *In Vitro* Antibiotic/Antifungal Assessment - References

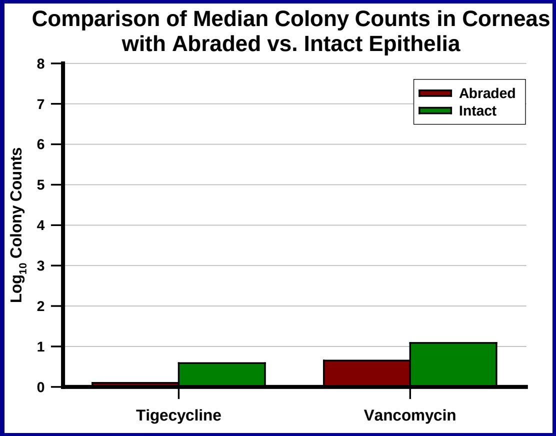
- Mather R, Karenchak LM, Romanowski EG, Kowalski RP. 4th Generation fluoroquinolones: New weapons in the arsenal of ophthalmic antibiotics. *Am J Ophthalmol.* 2002;133:463-466.
- Kowalski RP, Dhaliwal DK, Karenchak LM, Romanowski EG, Mah FS, Ritterband DC, Gordon YJ. Gatifloxacin and moxifloxacin: An *in vitro* susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. *Am J Ophthalmol.* 2003;136:500-505.
- Kowalski RP, Yates KA, Romanowski EG, Karenchak LM, Mah FS, Gordon YJ. An ophthalmologist's guide to understanding antibiotic susceptibility and minimum inhibitory concentration data. *Ophthalmology.* 2005;112:1987-1991.
- 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.
- Wingard JB, Romanowski EG, Kowalski RP, Mah FS, Ling Y, Bilonick RA, Gordon YJ, Shanks RMQ. A novel Cell-Associated Protection Assay (CAPA) demonstrates the ability of certain antibiotics to protect ocular surface cell lines from subsequent clinical *Staphylococcus* aureus challenge*. Antimicrob Agents Chemother.* 2011;55:3788-3794.
- Kowalski RP, Kowalski TA, Shanks RMQ, Romanowski EG, Karenchak LM, Mah FS. *In vitro* comparison of combination and monotherapy for the empiric and optimal coverage of bacterial keratitis based on incidence of infection. *Cornea.* 2013;32:830-834.

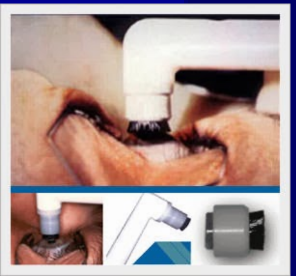
2. In Vivo Antibiotic Testing

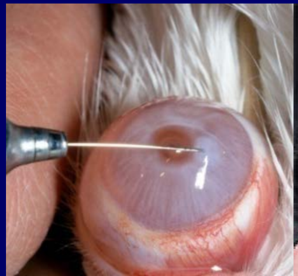
- Rabbit Keratitis Models







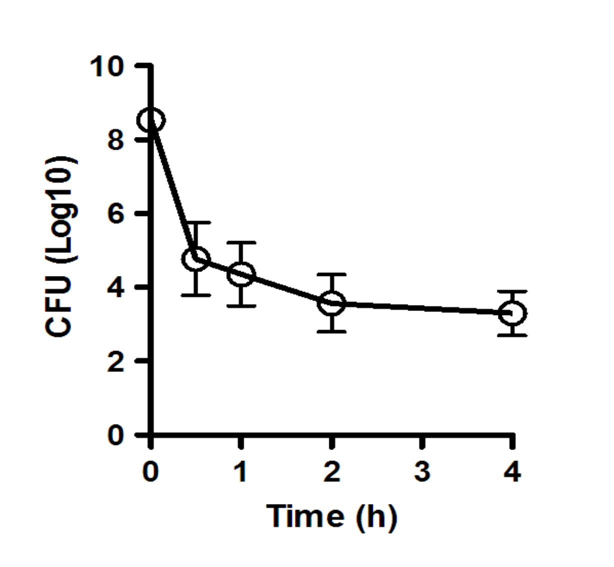




2. *In Vivo* Antibiotic Testing - References

- Kowalski RP, Romanowski EG, Yates KA, Gordon YJ. Lomefloxacin is an effective treatment for experimental bacterial keratitis. *Cornea* 2001;20:306-308.
- Rhee MK, Kowalski RP, Romanowski EG, Mah FS, Ritterband DC, Gordon YJ. A laboratory evaluation of antibiotic therapy for ciprofloxacin-resistant Pseudomonas aeruginosa. *Am J Ophthalmol* 2004;138:226-230.
- Romanowski, EG, Mah FS, Yates KA, Kowalski RP, Gordon YJ. The successful treatment of gatifloxacin-resistant Staphylococcus aureus keratitis with Zymar (gatifloxacin 0.3%) in a NZW rabbit model. *Am J Ophthalmol* 2005;139:867-877.
- Romanowski EG, Mah FS, Kowalski RP, Yates KA, Gordon YJ. Benzalkonium chloride enhances the antibacterial efficacy of gatifloxacin in an experimental rabbit keratitis model. *J Ocular Pharm Therapeutics* 2008;24:380-384.
- Kowalski RP, Romanowski EG, Mah FS, Shanks RMQ, Gordon YJ. Topical levofloxacin 1.5% overcomes *in vitro* resistance in rabbit keratitis models. *Acta Ophthalmologica.* 2010;88:e120-125.
- Kowalski RP, Romanowski EG, Yates KA, Mah FS. An independent evaluation of a novel peptide mimetic, Brilacidin (PMX30063), for ocular anti-infective therapy. *J Oc Pharm Ther.* 2015 (In Press)
- 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.

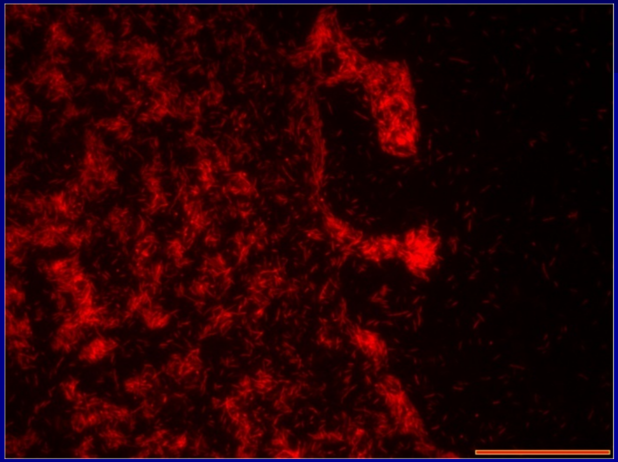
4. *In Vivo* Endophthalmitis Prevention Assays

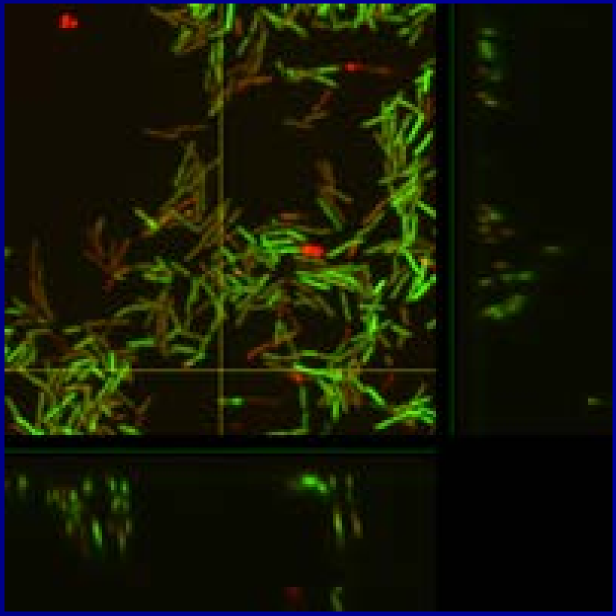
- Topical Prophylaxis – AC Challenge
  - Before and After Bacterial Challenge
- Topical Prophylaxis – Contaminated Needle Challenge
- Intravitreal Prophylaxis – Vitreal Challenge

4. *In Vivo* Endophthalmitis Prevention Assays - References

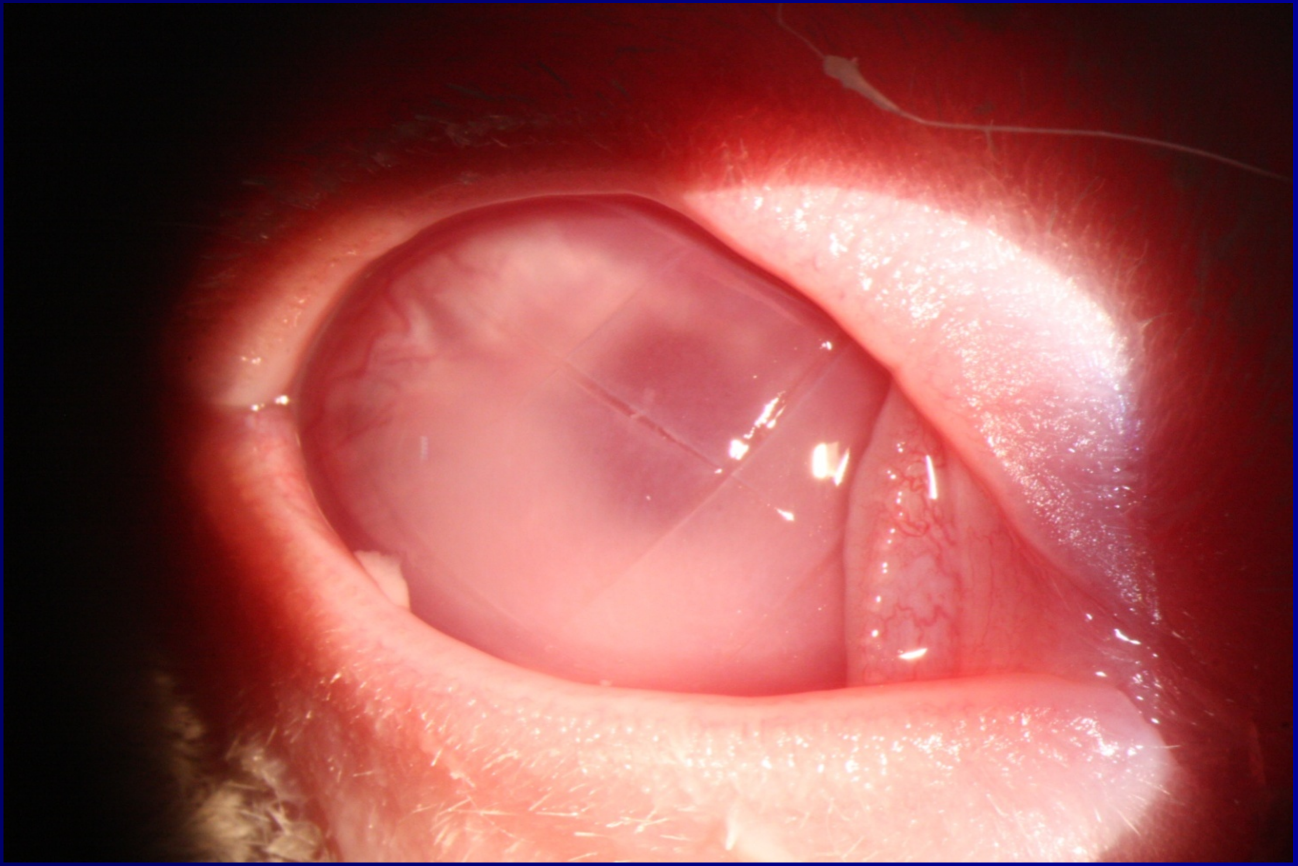
- Kowalski RP, Romanowski EG, Mah FS, Yates KA, Gordon YJ. Topical prophylaxis with moxifloxacin prevents endophthalmitis in a rabbit model. *Am J Ophthalmol* 2004;138: 33-37.
- Kowalski RP, Romanowski EG, Mah FS, Yates KA, Gordon YJ. Intracameral Vigamox (0.5% moxifloxacin) is non-toxic and effective in preventing endophthalmitis in a rabbit model*. Am J Ophthalmol* 2005;140:497-504.
- Kowalski RP, Romanowski EG, Mah FS, Yates KA, Gordon YJ. A new rabbit model demonstrates that topical 0.5% moxifloxacin prevents endophthalmitis after intravitreal injection. *J Ocular Pharm Therapeutics* 2008;24:1-7.
- Kowalski RP, Romanowski EG, Mah FS, Sasaki H, Fukuda M, Gordon YJ. A comparison of moxifloxacin and levofloxacin topical prophylaxis in a fluoroquinolone-resistant *Staphylococcus aureus* rabbit model*. Jpn J Ophthalmol.* 2008;52:211-216.
- Kowalski RP, Romanowski EG, Shanks RMQ, Gordon YJ, Mah FS. The Comparison of Fluoroquinolones to Non-Fluoroquinolone Antibacterial Agents for the Prevention of Endophthalmitis in a Rabbit Model. *J Oc Pharm Ther.* 2012;28:604-608.

5. *In Vitro* Biofilm Assays





6. *In Vivo* Contact Lens Models



7. *In Vitro* Acanthamoeba Susceptibility Assay

- Kowalski RP, Abdel Aziz S, Romanowski EG, Shanks RMQ, Nau AC, Raju LV. Development of a practical complete-kill assay to evaluate anti-acanthamoeba drugs. *JAMA Ophthalmol.* 2013;131(11):1459-1462.

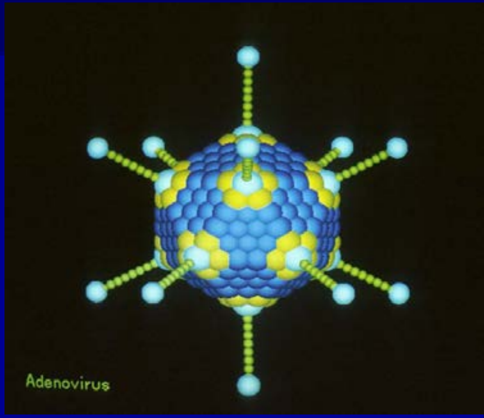
Table. Descriptive Statistics of 15 *Acanthamoeba* Isolates After Exposure to Common Anti-*Acanthamoeba* Drugs

Drug	Median Growth Grade[a]	Kill Incidence Rate,[b] No. (%) of Isolates
Saline (control)	3.0	0 (0)
Polyhexamethylene biguanide, 0.02%	0.0	12 (80)
Chlorhexidine digluconate, 0.02%	1.0	6 (50)
Hexamidine diisethioonate, 0.1%	0.0	14 (93)
Voriconazole, 1.0%	2.0	2 (13)

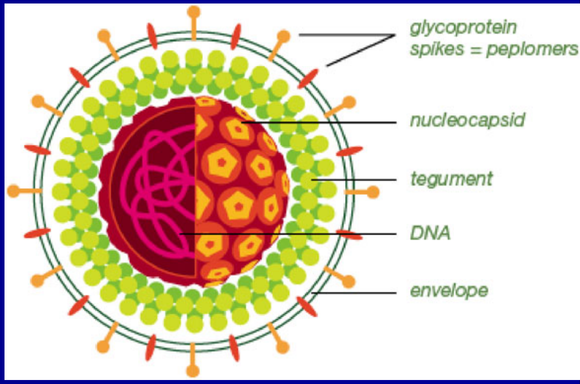
[a]The median value of the growth grade of 15 *Acanthamoeba* isolates based on the cumulative score of 3 time points.

[b]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

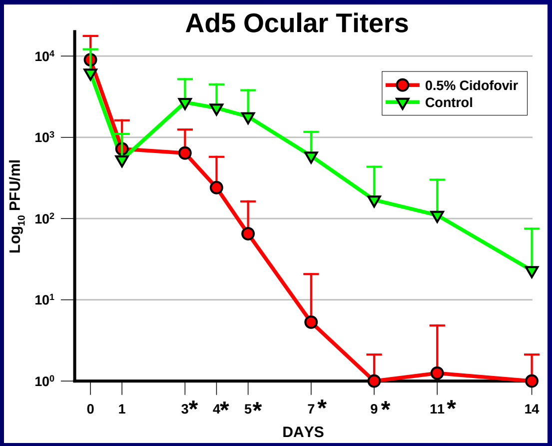


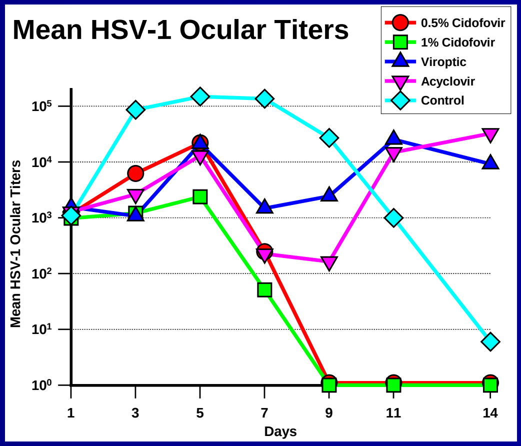
8. In Vitro Antiviral Testing - References

- Gordon YJ, Capone A, Sheppard J, Gordon A, Romanowski EG, Araullo-Cruz T. 2'nor cGMP, A new cyclic phosphate derivative of 2'NDG, inhibits HSV-1 replication in vitro and in the mouse keratitis model. *Cur Eye Res.* 1987;6:247-253.
- Gordon YJ, Romanowski EG, Araullo-Cruz T, Seaberg L, Erzurum S, Tolman R, De Clercq E. Inhibitory effect of (S)-HPMPC, (S)-HPMPA, and 2'-nor-Cyclic GMP on different ocular adenoviral serotypes in vitro. *Antiviral Res.* 1991;16:11-16.
- Romanowski EG, Yates KA, Teuchner B, Nagl M, Irschick EU, Gordon YJ. N–Chlorotaurine is an effective antiviral agent against adenovirus *in vitro and in the Ad5/NZW rabbit ocular model. Invest Ophthalmol Vis Sci.* 2006;47:2021-2026.
- Romanowski EG, Yates KA, Gordon YJ. The *in vitro* and *in vivo* evaluation of ddC as a topical antiviral for ocular adenovirus infections. *Invest Ophthalmol Vis Sci.* 2009;50:5295-5299.

9. *In Vivo* Antiviral Testing

- Ad5/NZW Rabbit Ocular Model
  - – Cidofovir, Aganocides, NCT, ddC, IVIG
- HSV-1/NZW Rabbit Keratitis Model
  - – Cidofovir, Acyclovir, Trifluridine

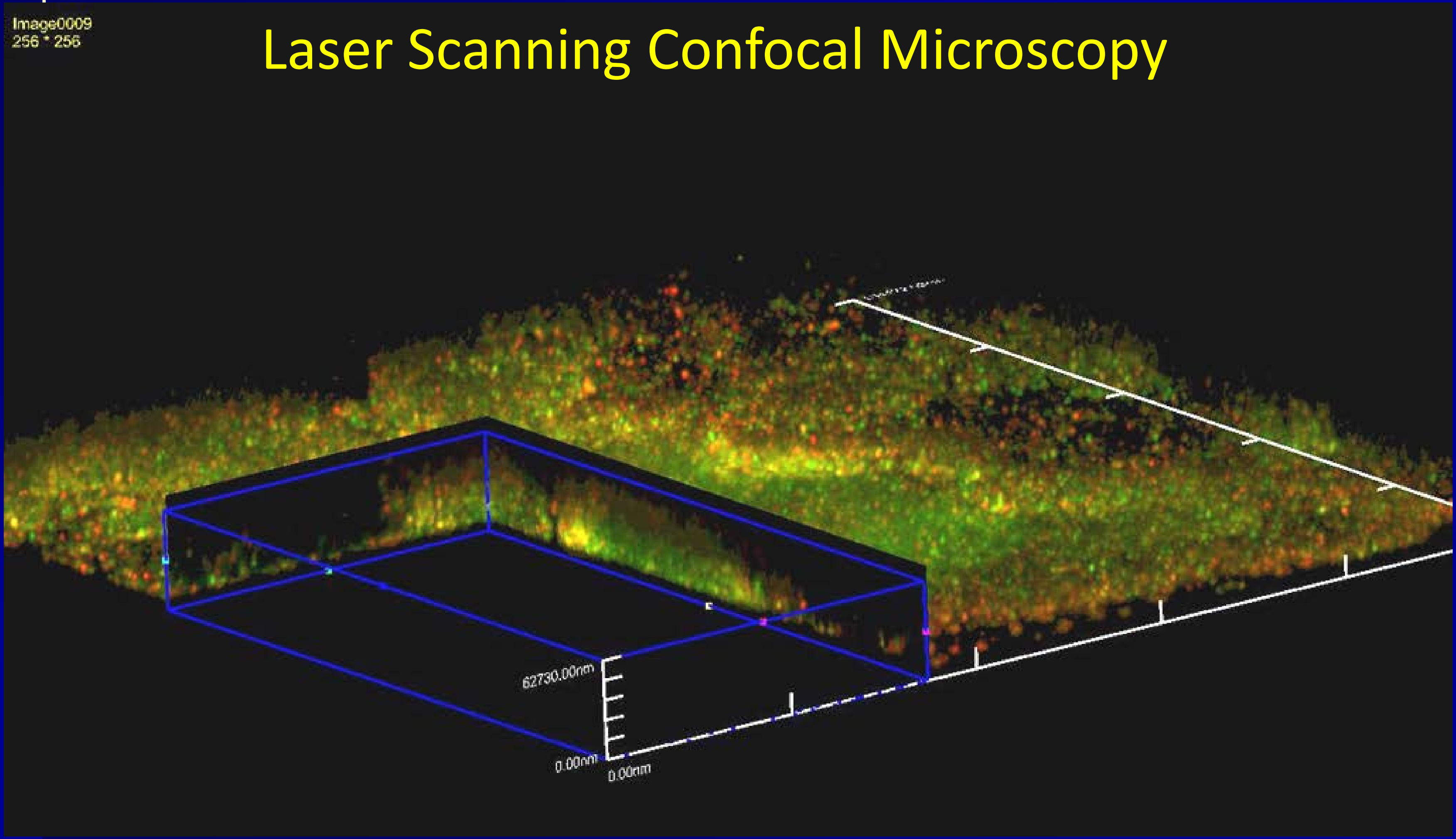




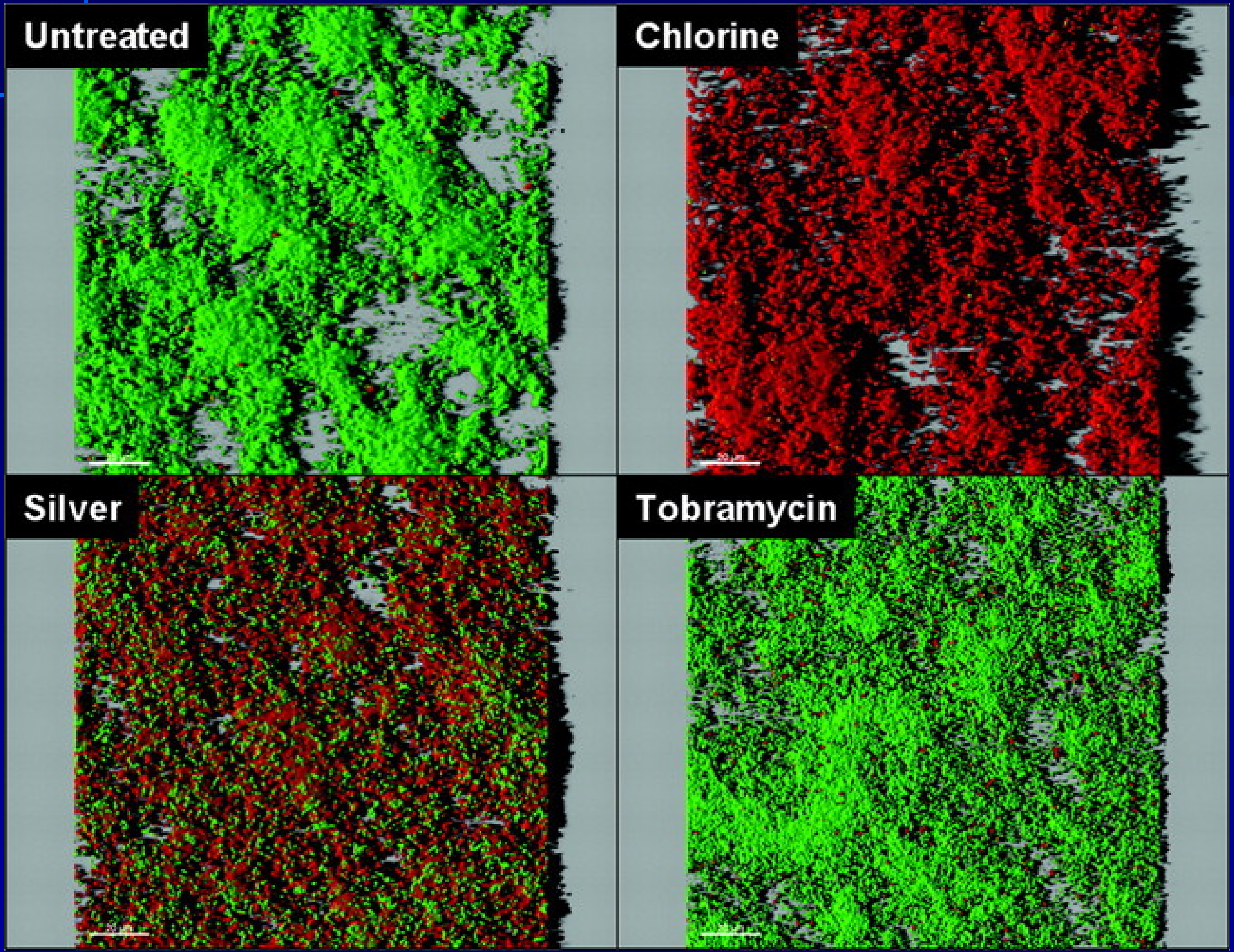
9. *In Vivo* Antiviral Testing - References

- Gordon YJ, Romanowski EG, Araullo-Cruz T. Topical HPMPC inhibits adenovirus type 5 in the New Zealand rabbit ocular replication model. *Invest Ophthalmol Vis Sci.* 1994;35:4135-4143.
- Gordon YJ, Romanowski EG, Araullo-Cruz T. HPMPC, A broad-spectrum topical antiviral, inhibits HSV-1 replication and promotes healing of dendritic keratitis in the NZ rabbit ocular model. *Cornea.* 1994;13:516-520.
- Romanowski EG, Araullo-Cruz T, Gordon YJ. Multiple adenoviral serotypes demonstrate host range extension in the New Zealand rabbit ocular model. *Invest Ophthalmol Vis Sci.* 1998;39:532-536.
- Romanowski EG, Bartels SP, Gordon YJ. Comparative antiviral efficacies of cidofovir, trifluridine, and acyclovir in the HSV-1 rabbit keratitis model. *Invest Ophthalmol Vis Sci.* 1999;40:378-384.
- Romanowski EG, Yates KA, Teuchner B, Nagl M, Irschick EU, Gordon YJ. N-Chlorotaurine is an effective antiviral agent against adenovirus *in vitro* and in the Ad5/NZW rabbit ocular model. *Invest Ophthalmol Vis Sci.* 2006;47:2021-2026.
- Nwanegbo EC, Romanowski EG, Gordon YJ, Gambotto A. Efficacy of topical immunoglobulins (IG) against experimental adenoviral ocular infection. *Invest Ophthalmol Vis Sci.* 2007;48:4171-4176.
- Romanowski EG, Yates KA, Gordon YJ. The *in vitro* and *in vivo* evaluation of ddC as a topical antiviral for ocular adenovirus infections. *Invest Ophthalmol Vis Sci. 2009;50:5295-5299.*
- Romanowski EG, Yates KA, Gordon YJ. Cyclopentenylcytosine (CPE-C): *In Vitro* and *In Vivo* Evaluation as an Antiviral against Adenoviral Ocular Infections. 2016 (In Preparation).

10. Outstanding Imaging



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



Green = Live

Red = Dead

Antimicrobial Agents and Chemotherapy
Journals.ASM.org

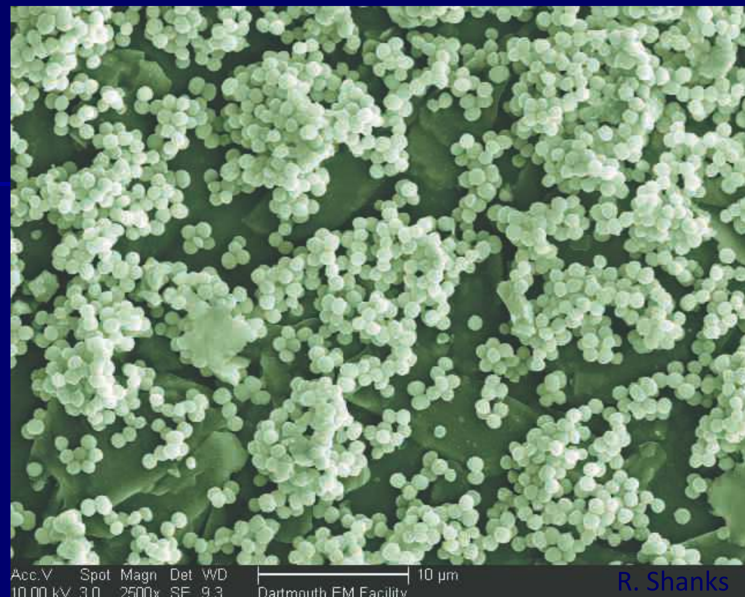
CLSM=confocal laser scanning microscopy.

Kim J et al. *Antimicrob Agents Chemother*. 2008;52(4):1446-1453.

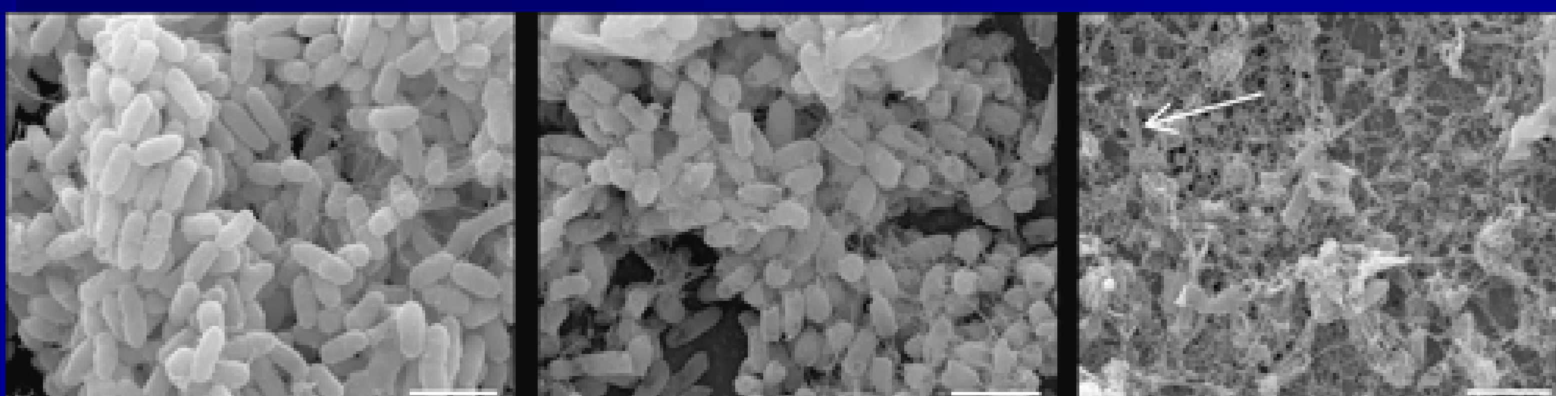
Scanning Electron Microscopy

Easy to see what is going on!

Possible outcome



Biofilm + treatment



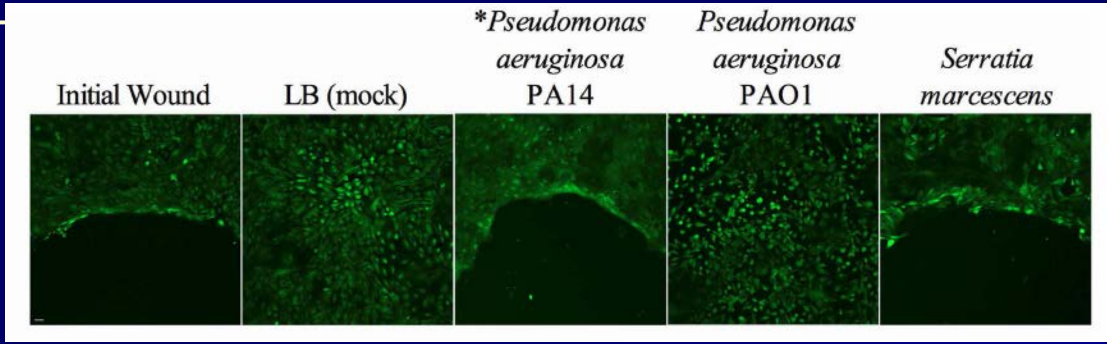
Kadouri and O'Toole 2008

11. *In Vivo* Ocular Tolerability Models

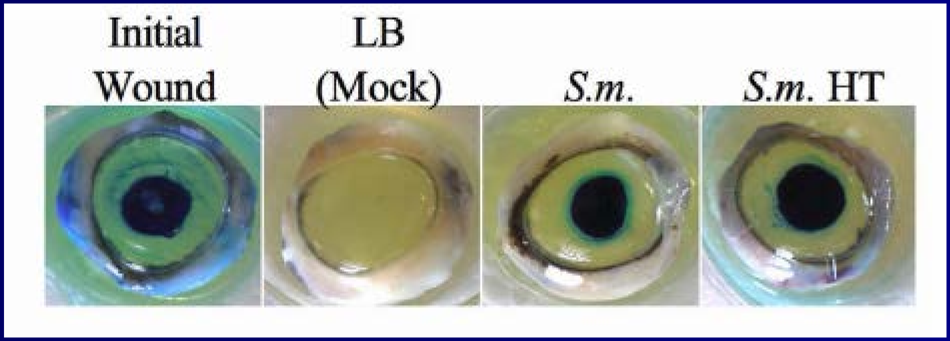
12. In Vivo Ocular Inflammation Assays

- Cytokine Analysis
- Ocular Inflammatory Signs
  - W/ and W/O LPS
  - W/ and W/O Bacterial Challenge
  - W/ and W/O Viral Challenge

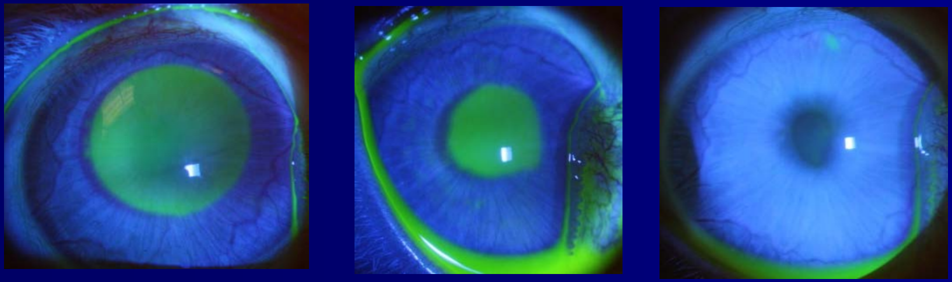
13. Corneal Wound Healing Assays



In Vitro (HCLE)



Ex Vivo (Pig Eyes)



Day 1 Day 2 Day 3

In Vivo (Rabbits)

14. Assay Development

- Design Assays to Achieve your Research Goals