

Reporter Enzyme Fluorescence to Detect TB

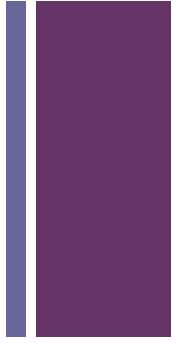
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Department of Microbial and Molecular Pathogenesis
College of Medicine



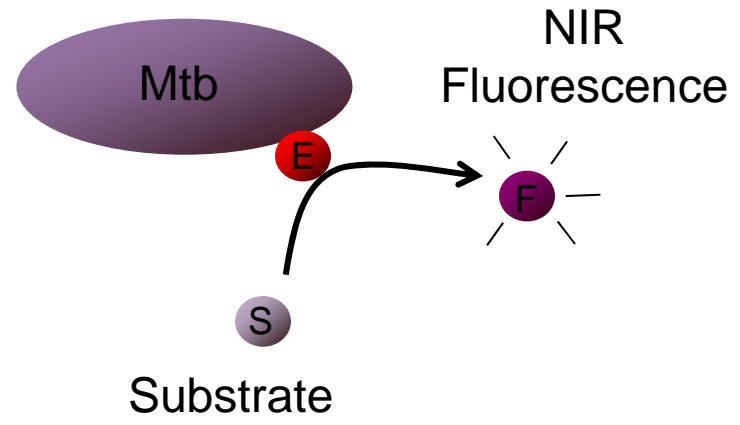
HEALTH SCIENCE CENTER
TEXAS A & M UNIVERSITY

+

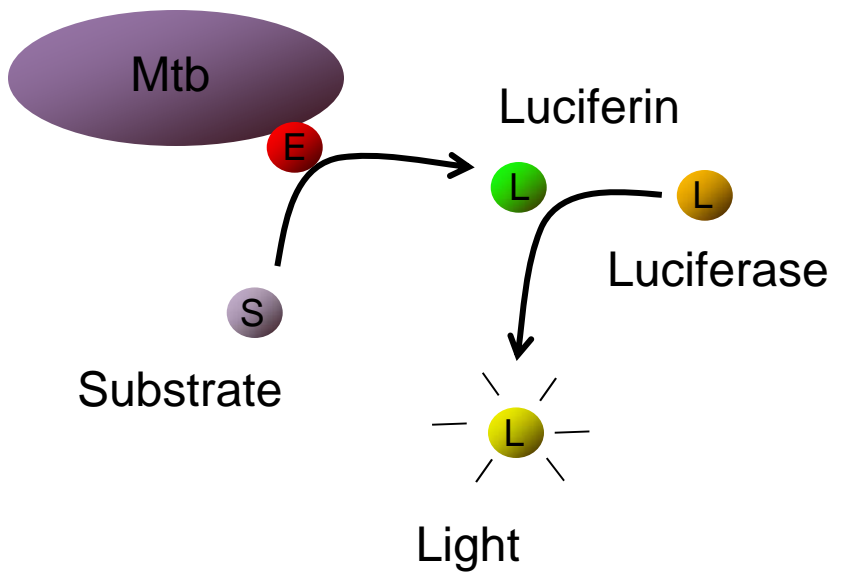
Reporter Enzyme Fluorescence (REF)



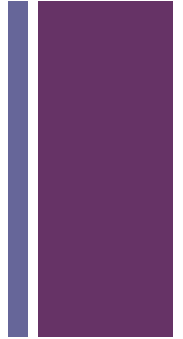
REF



SREL

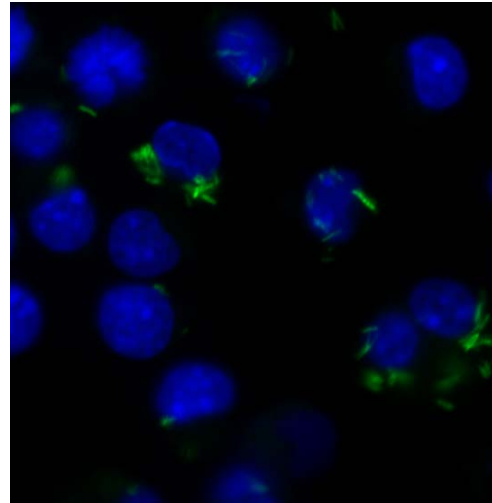
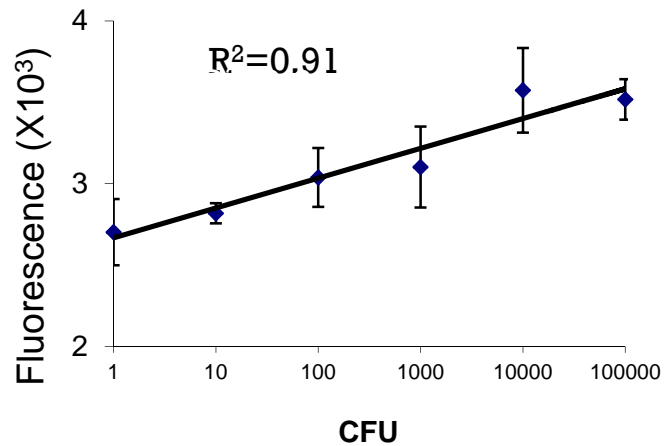


+ Characteristics of BlaC

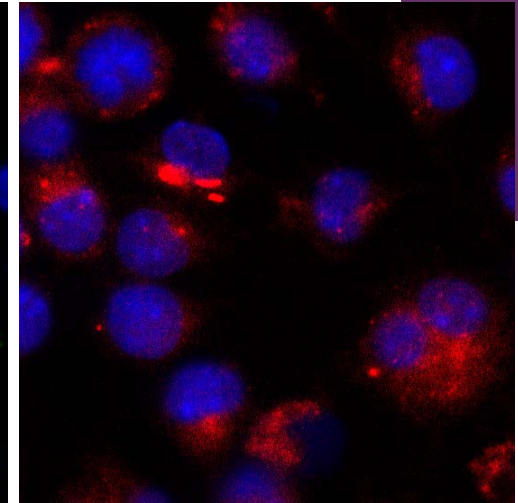


- Bla are robust enzymes with kinetics approaching rate of substrate diffusion.
- Half-life 3-4 hours allows viability assessment.
- TAT secreted (surface) viability (ATP) dependent.
- First reporter allowing detection gene expression in single cell.
- Threshold thought to be ~4 molecules of enzyme.
- Colorimetric, fluorescent, luminescent substrates developed.
- Numerous inhibitors and antibiotics targeting enzyme (chemistry).
- Lactam antibiotics not used for mycobacteria, no selective pressure for mutations.

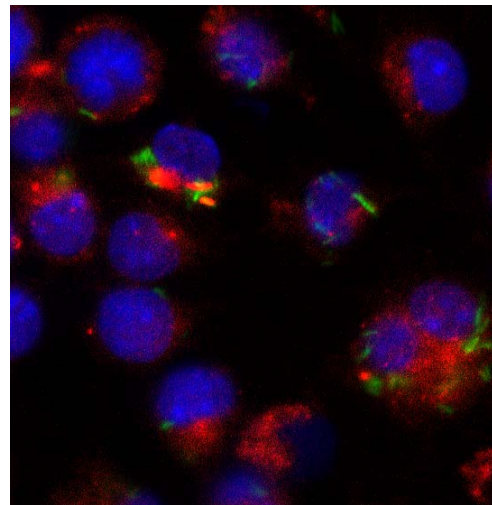
+ Detection of Infected Cells



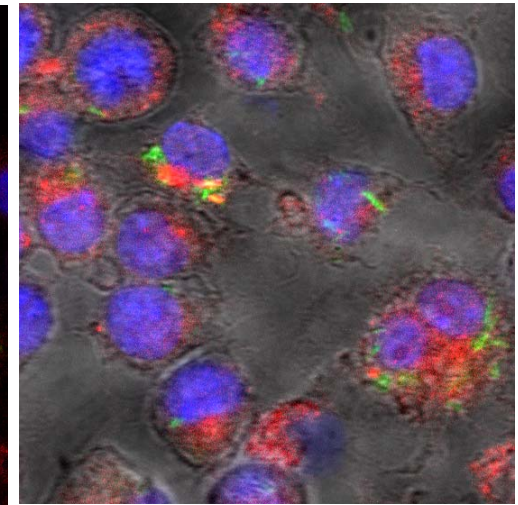
DAPI + GFP



DAPI + REF

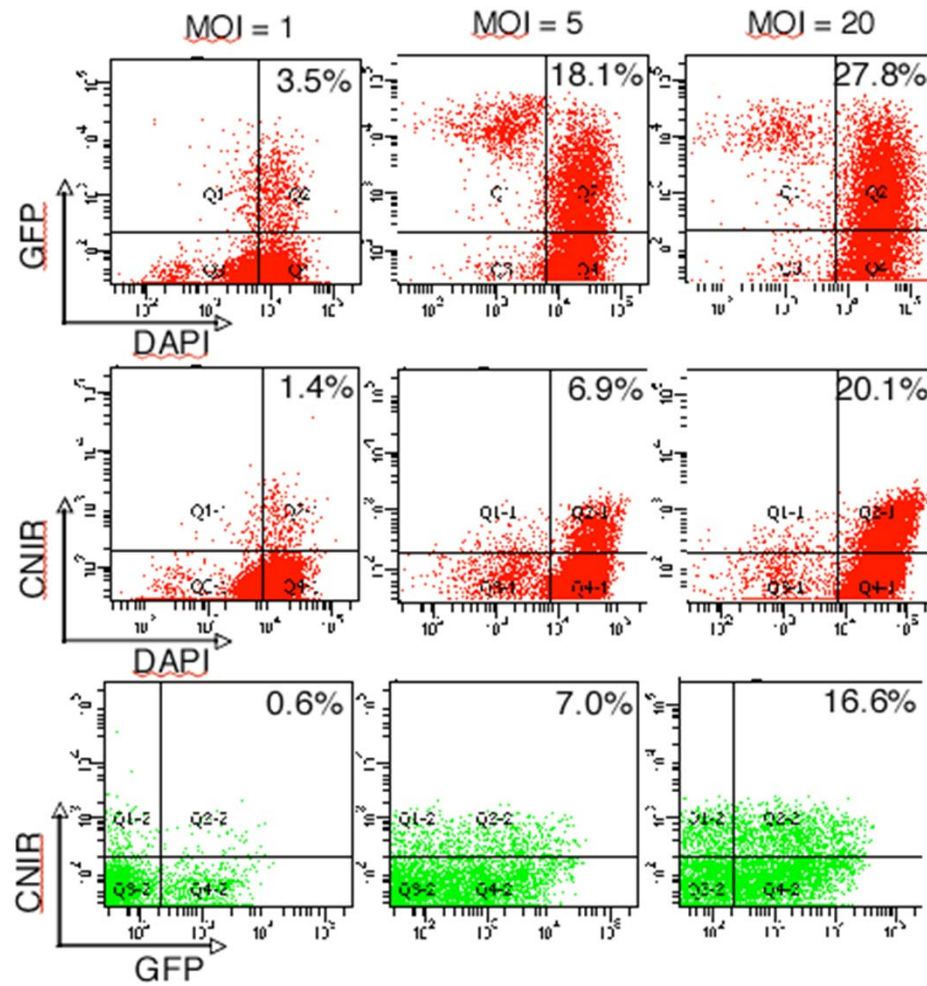


DAPI + GFP + REF

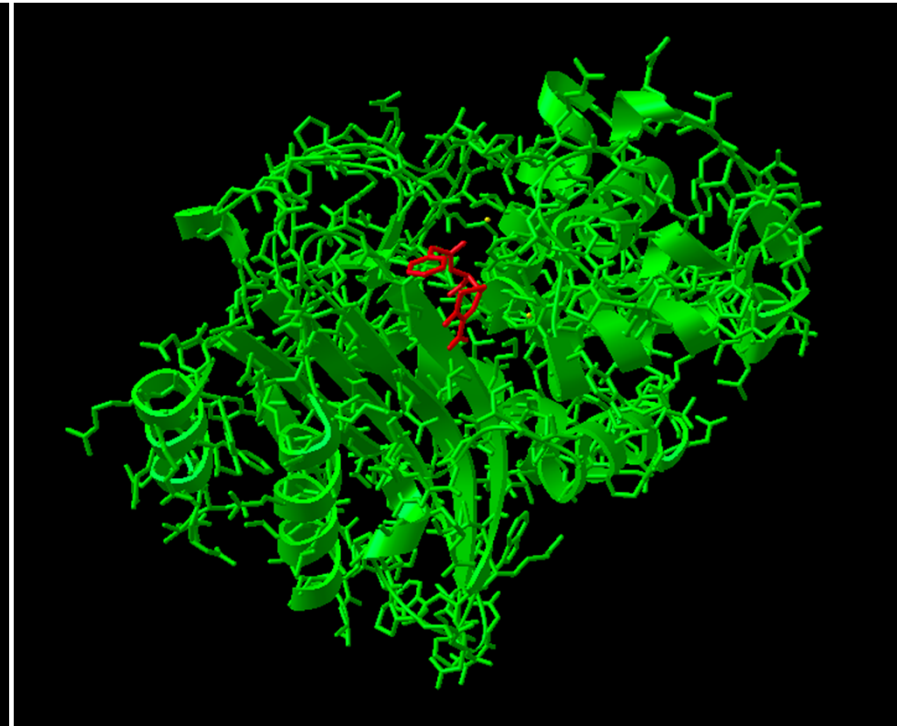
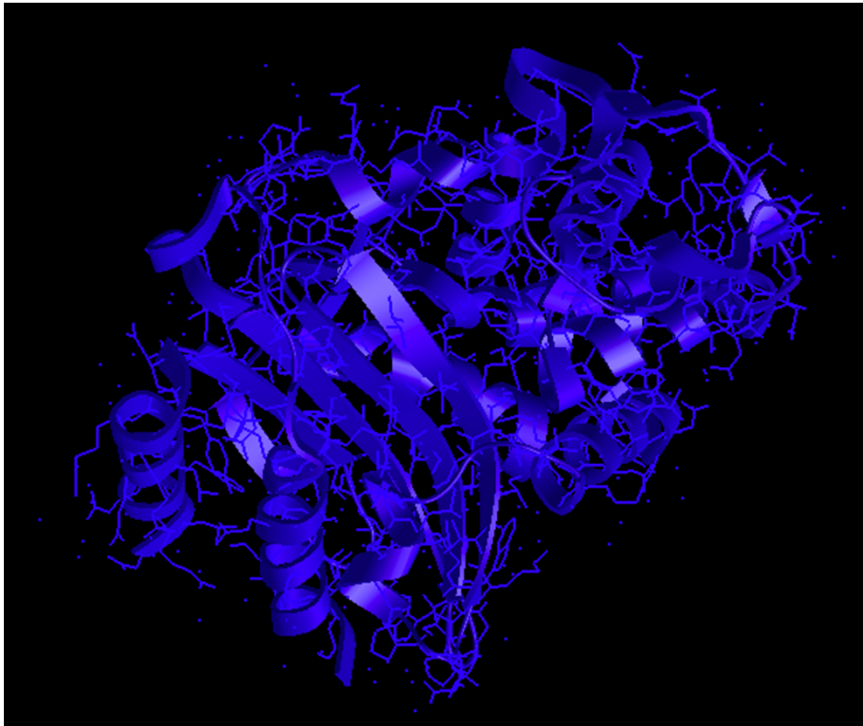
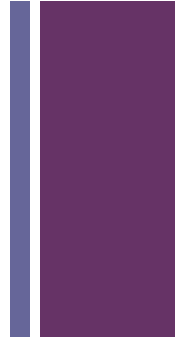


DAPI + GFP + REF + Phasor

+ Identification of Infected Cells

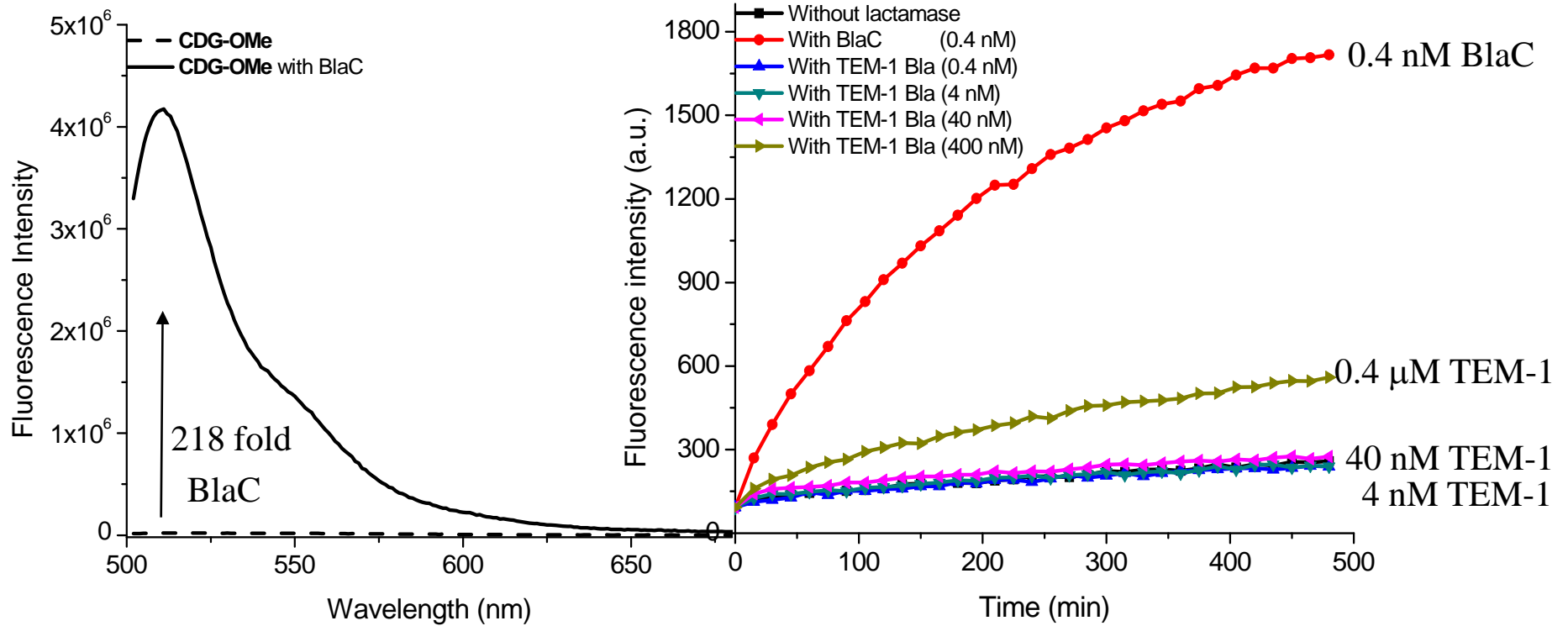
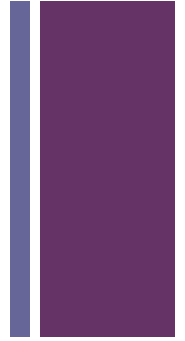


+ Structural Analyses

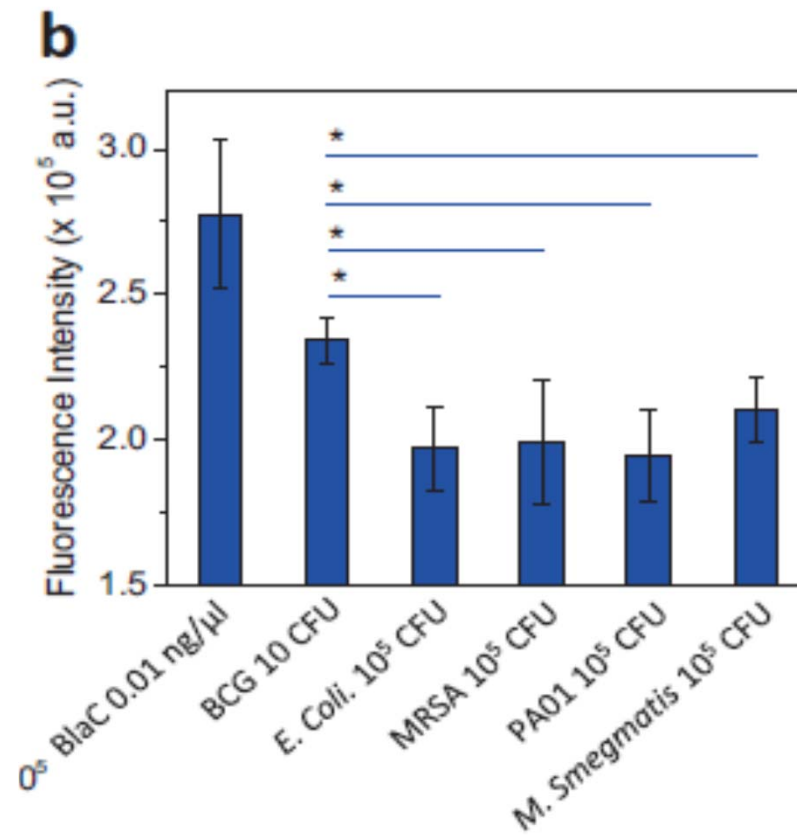




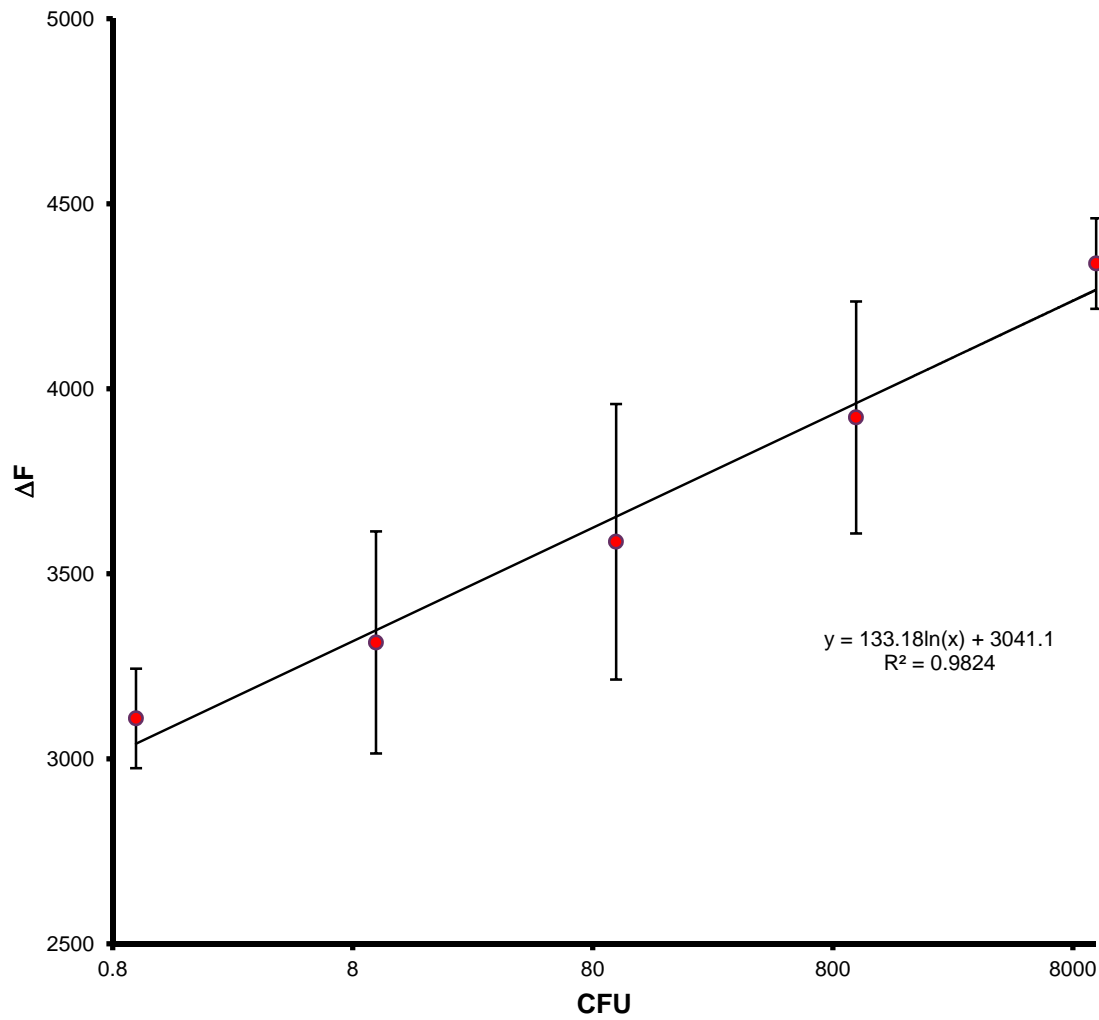
CDG-OMe with Enzyme



+ Specificity with Bacteria



+ REF Detection in Sputum



+ Sensitivity in Non-Decontaminated Sputum

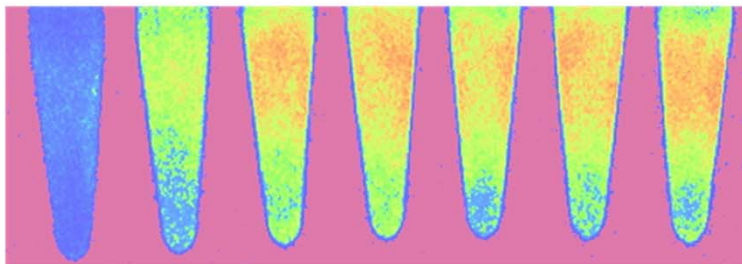
Limit of Detection \approx 50 CFU/ml

- Non-decontaminated sputum
- Time points = post addition of CDG-OMe
- Sample size 200 μ l in PCR Tube
- Imaged with iPhone 4S in simple light box (see next slide)
- Analyzed with iPhone app: iPhotolux

BCG dilutions in sputum, no CDG-OMe

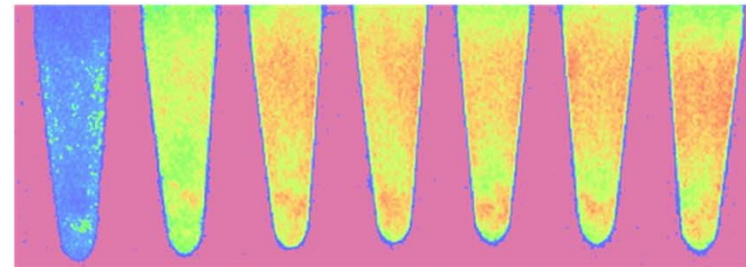
0 <10 10 10² 10³ 10⁴ 10⁵

2 minutes



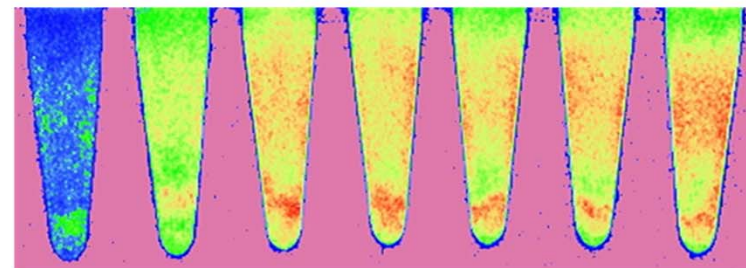
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10 minutes



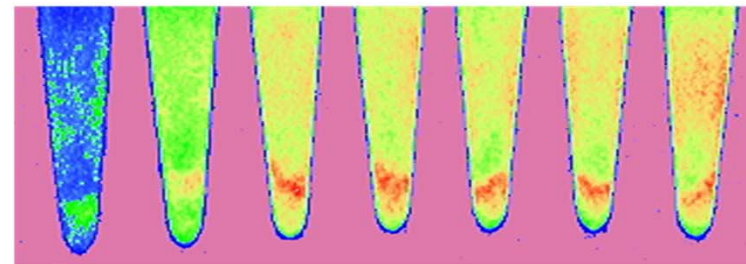
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20 minutes



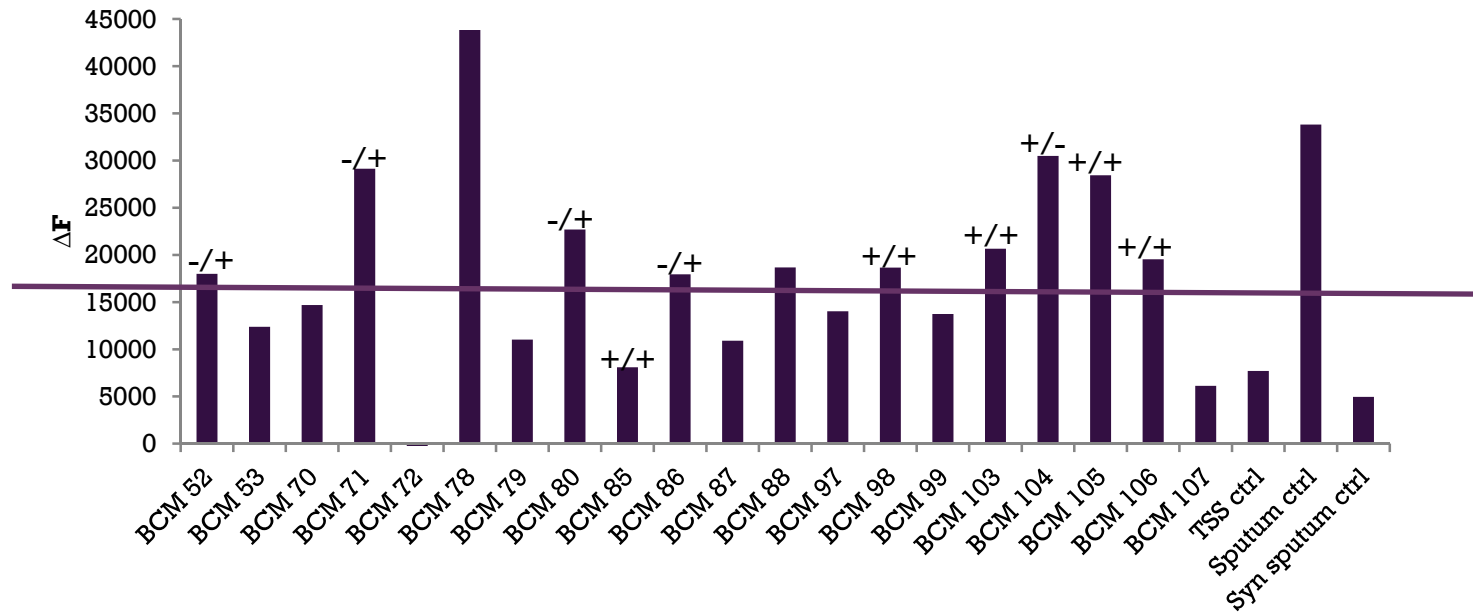
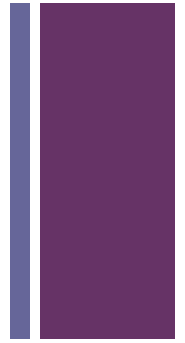
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30 minutes

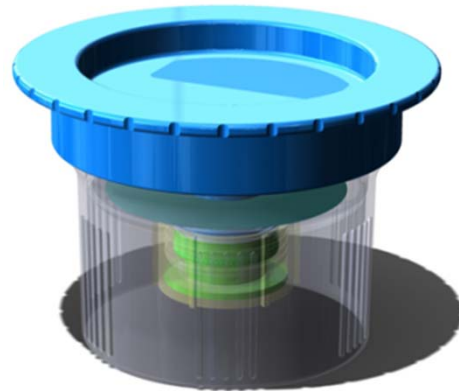


0 <10 10 10² 10³ 10⁴ 10⁵

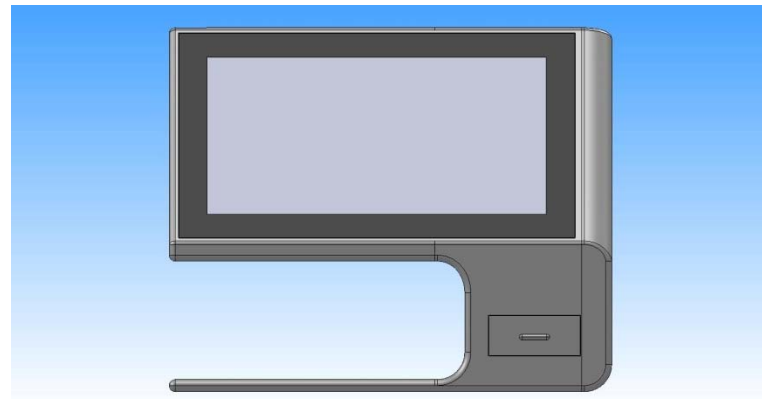
+ Clinical Trials



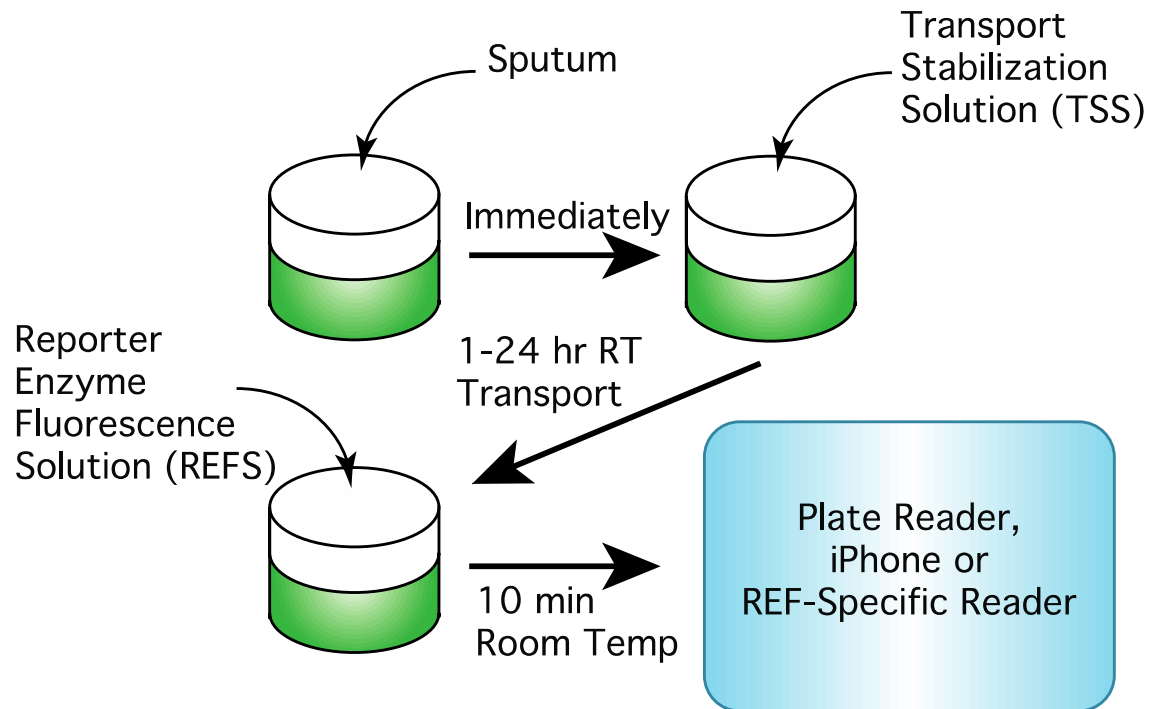
TB REaD™ Reader Platform



Global BioDiagnostics Corp.
(Concept development).



+ Clinical Trial Results



	Sensitivity		Specificity
<u>Total -</u>	<u>Sm+/Cult +</u>	<u>Sm-/Cult+</u>	<u>Non-TB</u>
126 samples	93%(26/28)	83%(24/29)	77%(53/69)

+ Center for Airborne Pathogens Research and Imaging (CAPRI)

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DISCLOSURE

Dr. Cirillo has patents filed on these technologies. He is also a founder of and consultant for Global BioDiagnostics Corp. and owns significant stock in the company that has licensed many of the technologies presented.



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