Development of Rapid Diagnostics using the Waveguide-based Optical Biosensor

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LANL Biosensor Team

Courtesy: K-RITH, Durban, S. Africa

Overview

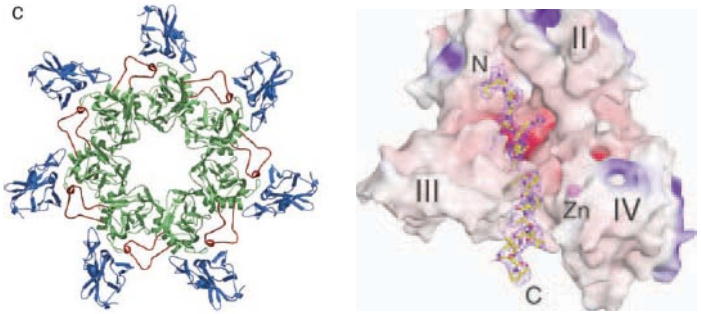
- **The Waveguide-based Platform**
  - Introduction to biomarkers
  - Components of a diagnostic platform
  - Optical waveguide-based biosensor
  - Sensing films
  - Assay format

- **Applications to Disease Detection**
  - Anthrax lethal toxins
  - Influenza
  - Tuberculosis

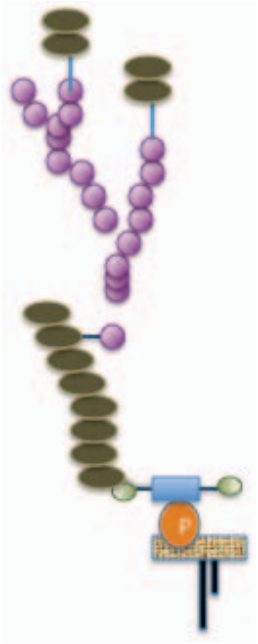
Wikipedia, NY City Campaign, Public Domain

Biomarkers

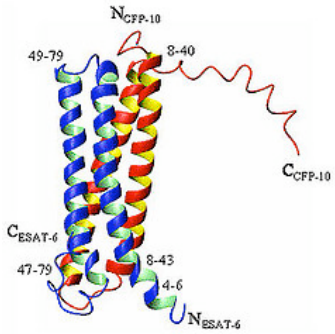
Biological Molecules that are secreted or differentially expressed during the course of disease.



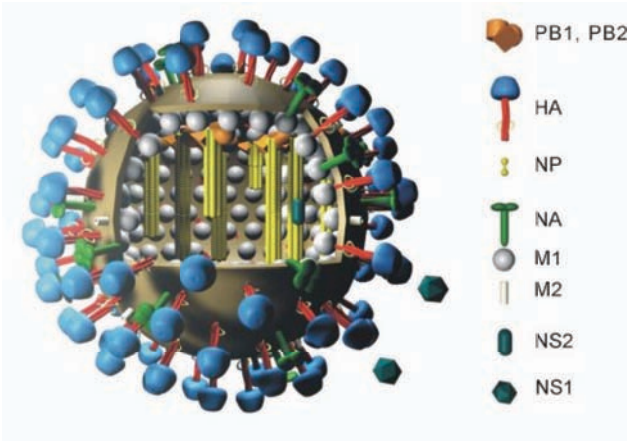
Anthrax lethal toxins



Lipoarabinomannan for TB

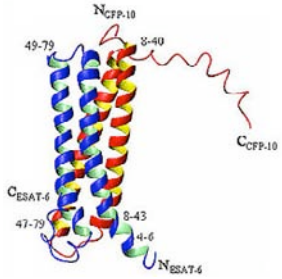


ESAT-6 & CFP 10 for TB

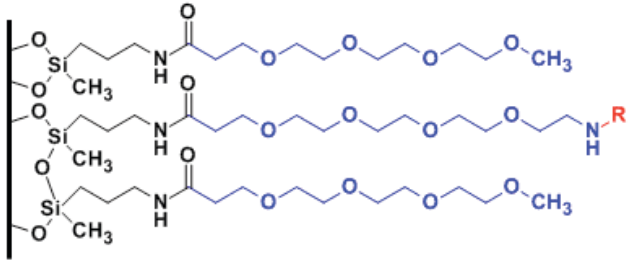


Influenza Virus

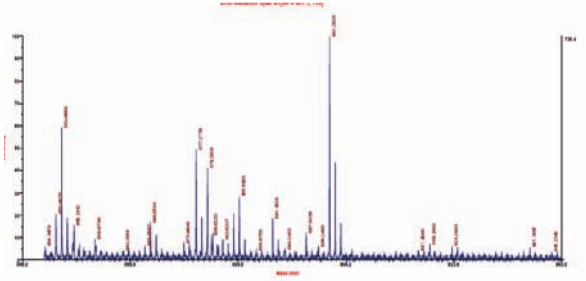
Courtesy: Dr. M. Carr, U. Leicester, Dr. R.J. Collier, Harvard Medical School; California Dept. Health Services



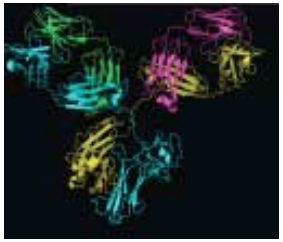
Purified Antigens



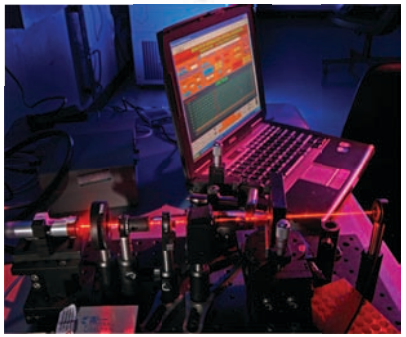
Functional Surfaces



Marker Discovery

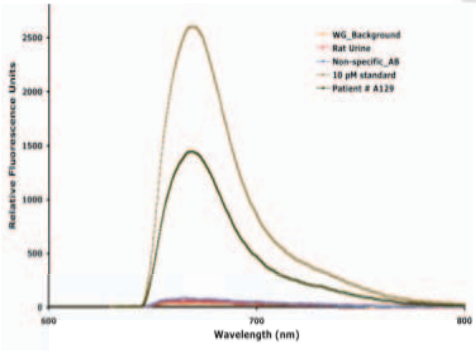


Recognition Ligands

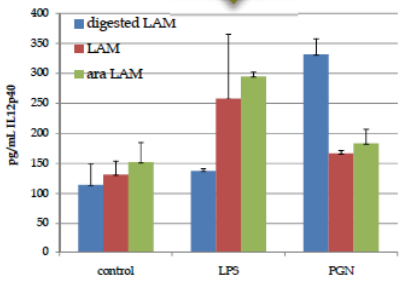


Biosensors

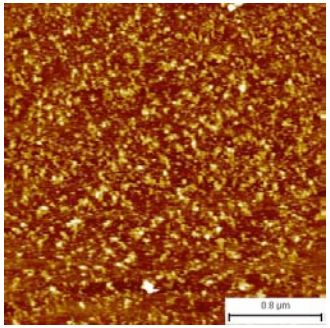
Fluorescence Reporters



Sensitive Detection

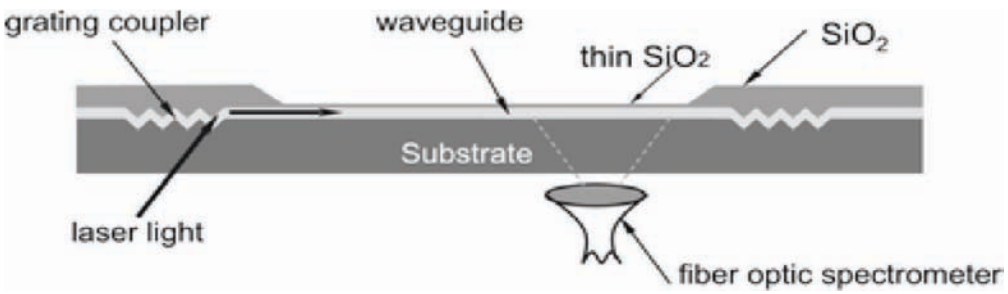


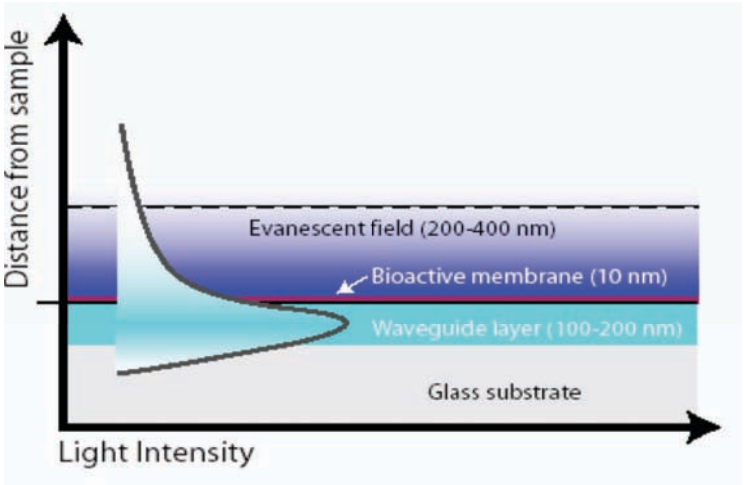
Basic Biology

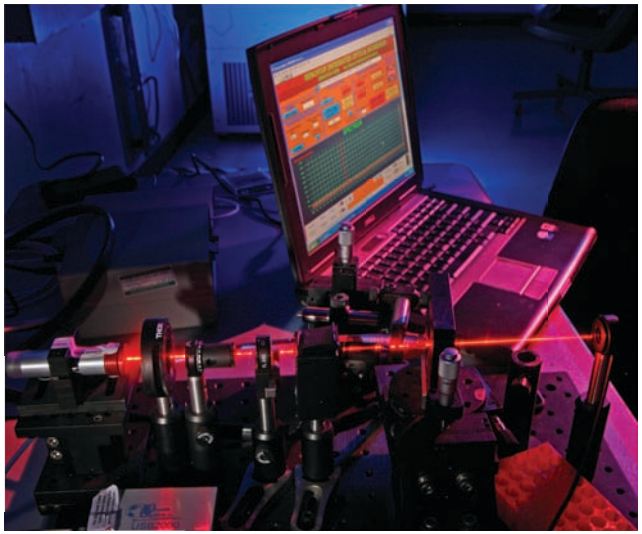


Physical Characterization

Waveguide-based Optical Biosensor





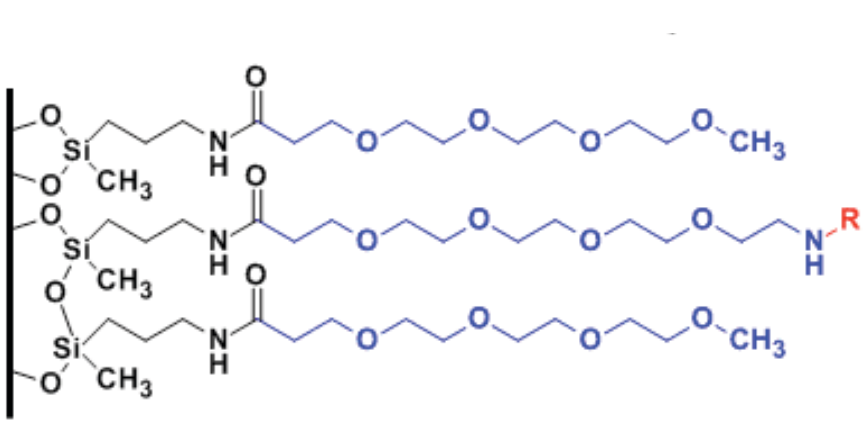


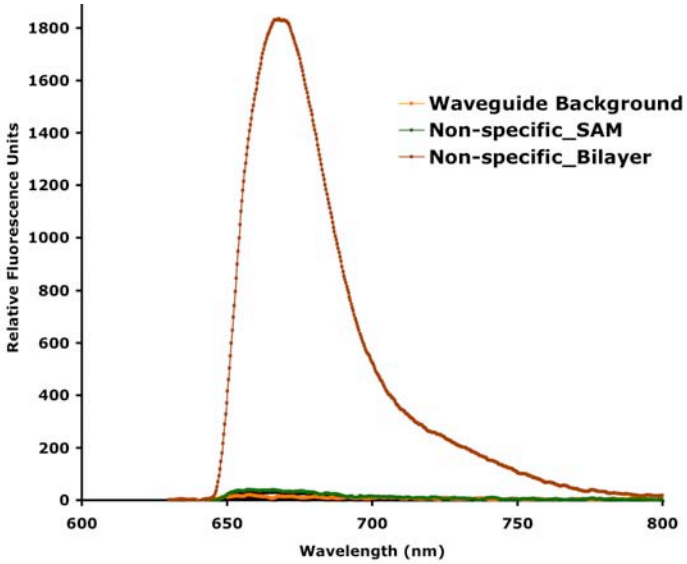
Waveguide sensor test-bed

Hand-held sensor system

Martinez J.S. et al, 2005; Kelly et al, 1998, Mukundan H et al, 2009

Sensing Films for use with the Optical Biosensor Platform





- Silane-based self assembled monolayers

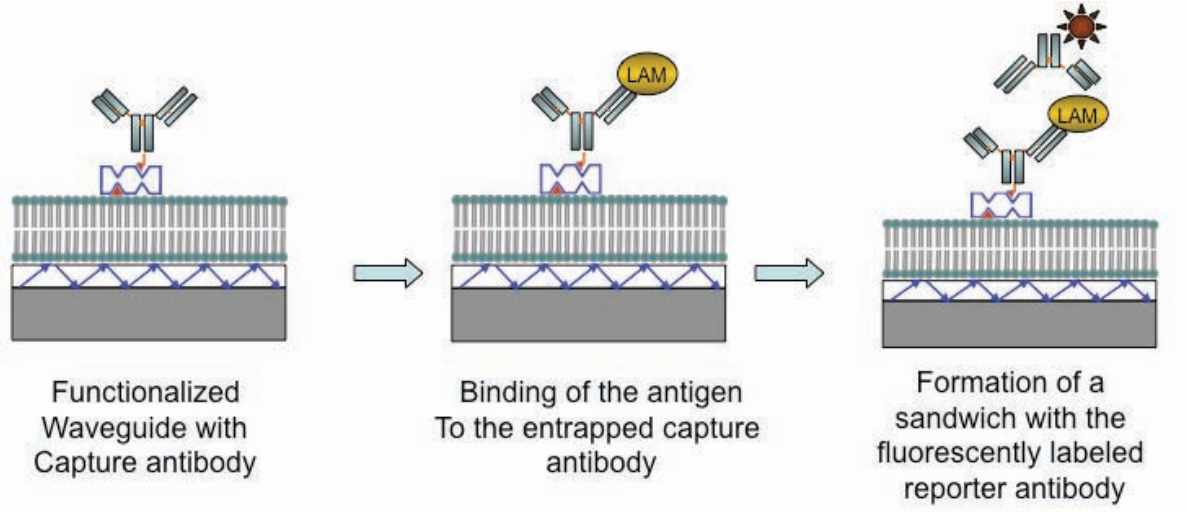
- Robust, cheap, easy to store and use, resistant to detergents and complex biological solutions, potentially reusable.

- Excellent at resisting non-specific binding!

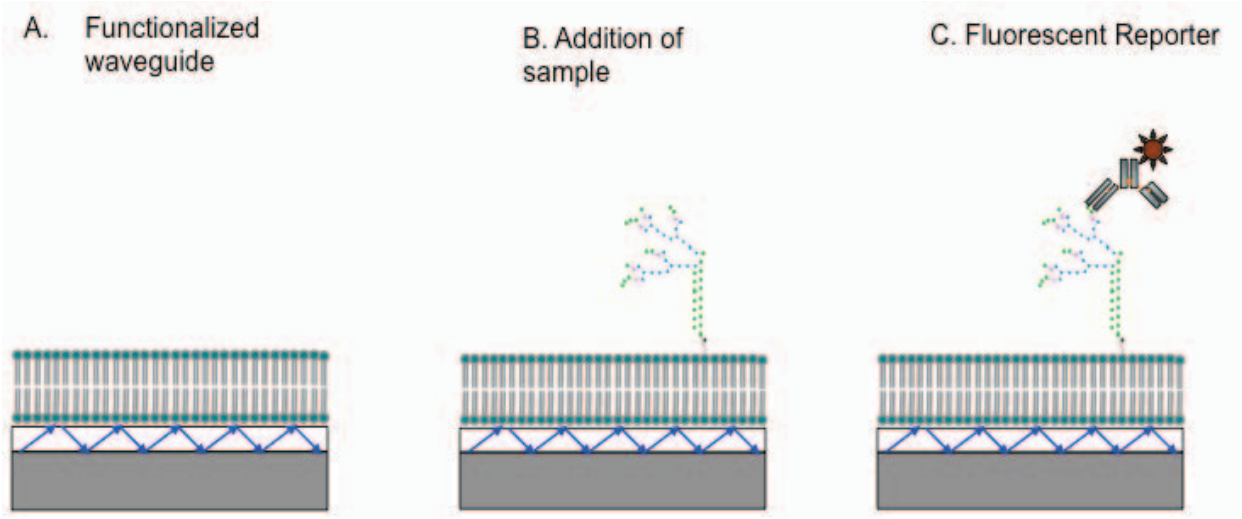
Anderson A.S. et al, Langmuir 2008; Anderson A.S. et al, SPIE 2009

Assay Format

Traditional Sandwich Immunoassay

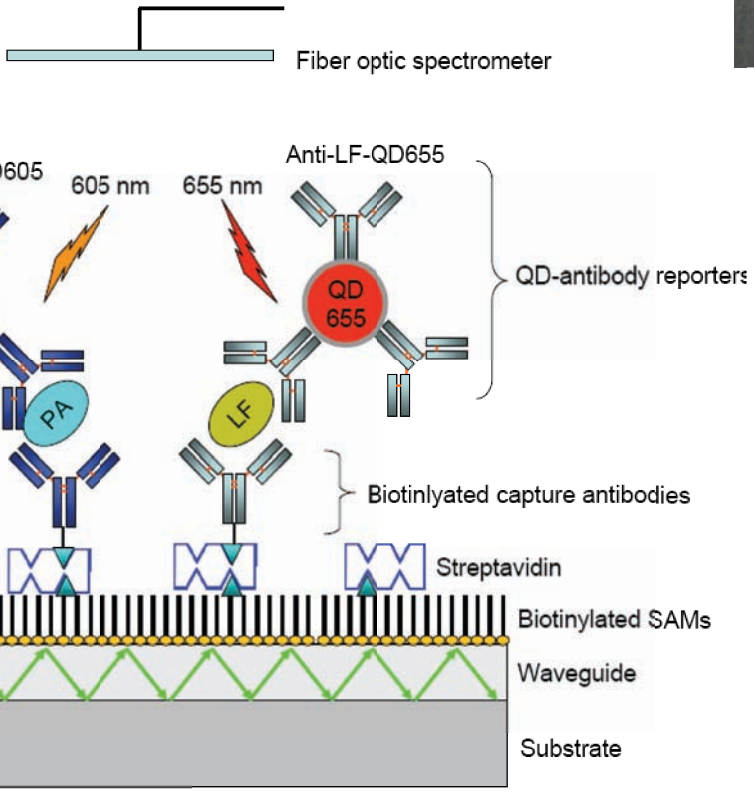


Membrane Mimetic Approach

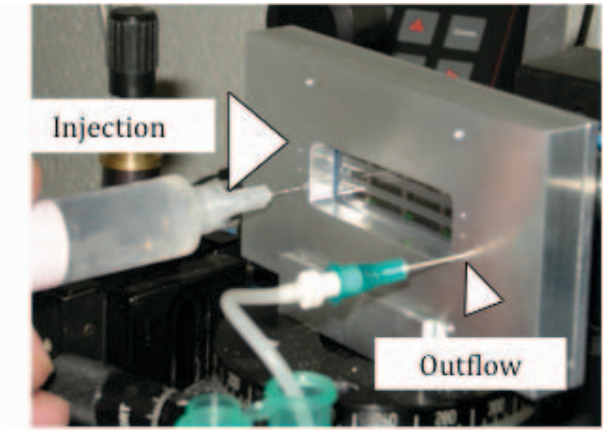


Multiplex Detection and Multichannel Waveguides

Simultaneous rapid detection of multiplex biomarkers using quantum dots as the fluorescence reporter.



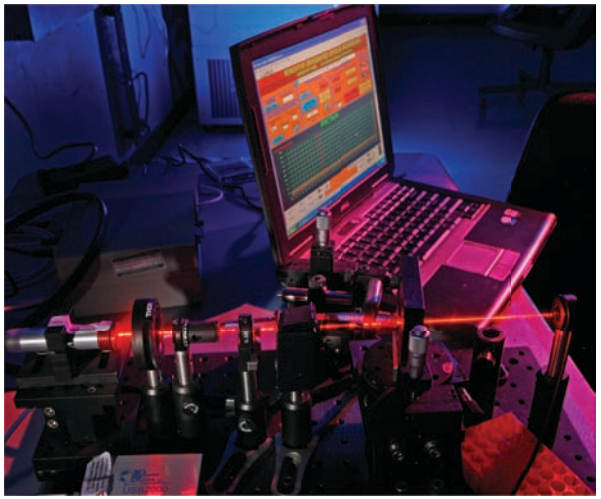
Multichannel Waveguides

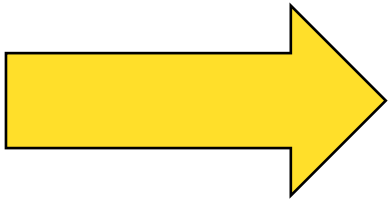


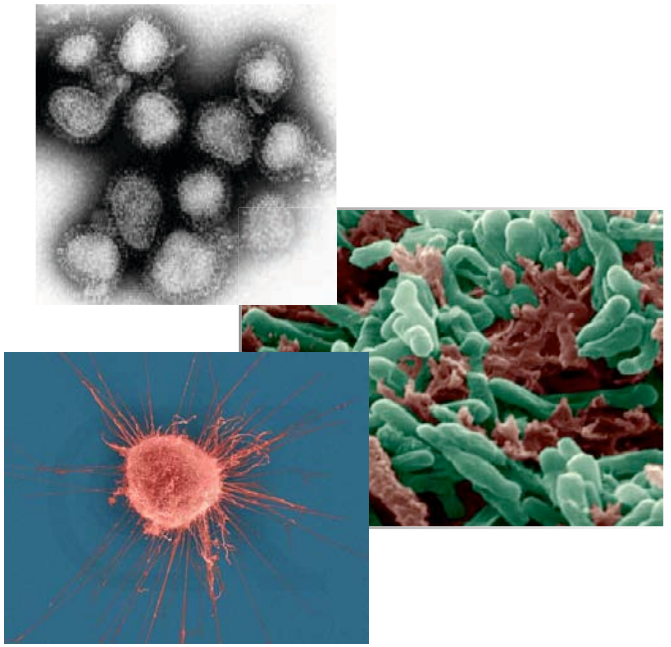
Multichannel Waveguide mounted in flow cell

Mukundan H et al, Analytical Chem, 2010

Applications of the Waveguide-based Optical Biosensor

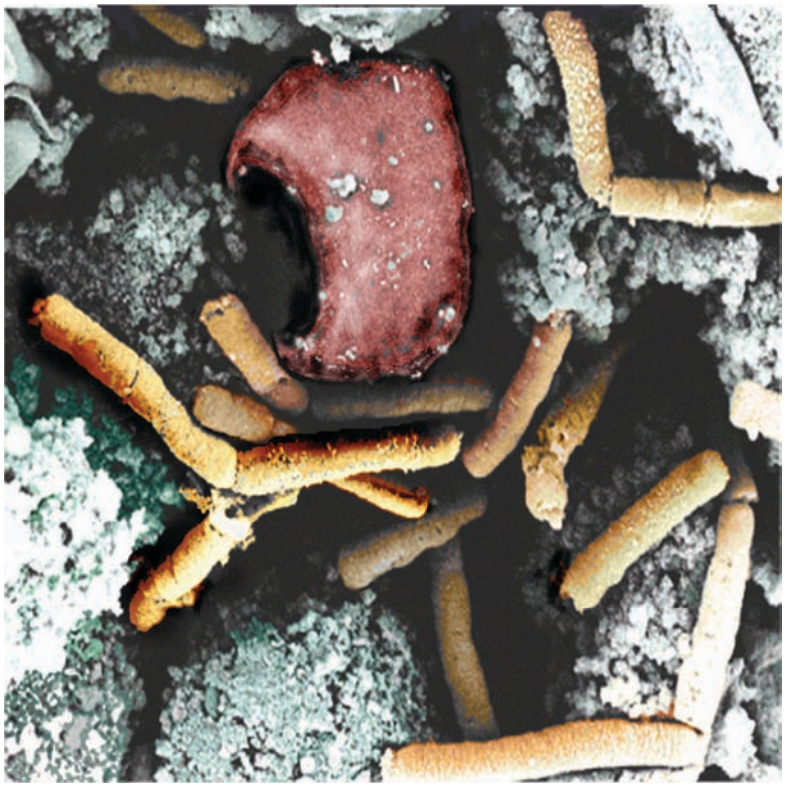


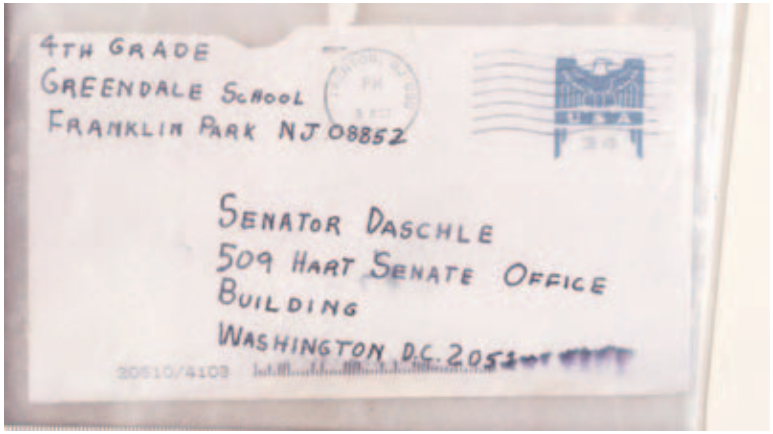




Anthrax

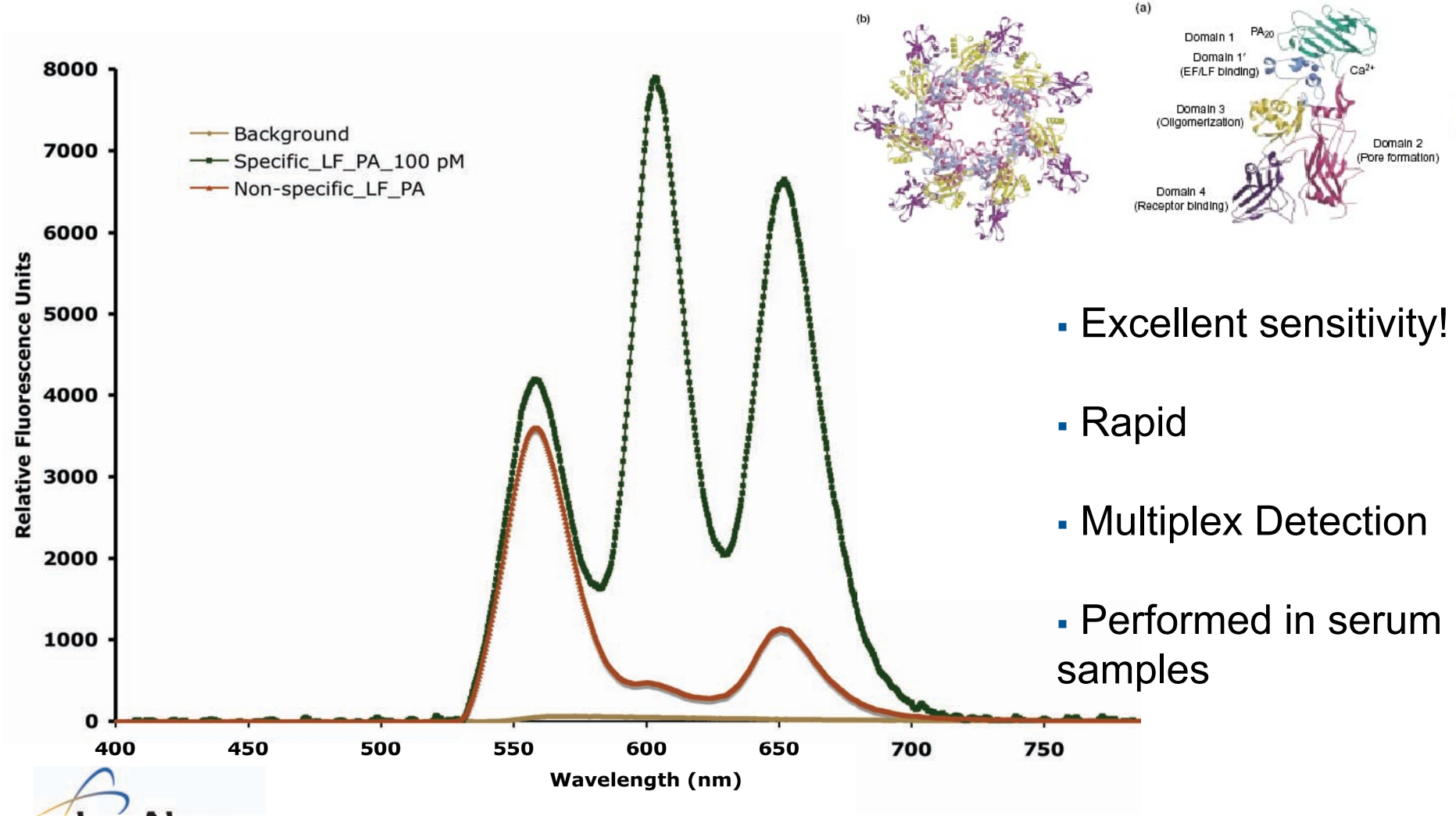
- **Bio-threat agent**
- **Three associated toxins**
  - Lethal factor
  - Edema factor
  - Protective antigen
- **75% mortality with inhalational anthrax, even when treated!**





Courtesy: A. Frielander, NIAID, NIH, US Public Domain; R. Taylor, from FBI.org

Detection of Anthrax Lethal Toxins

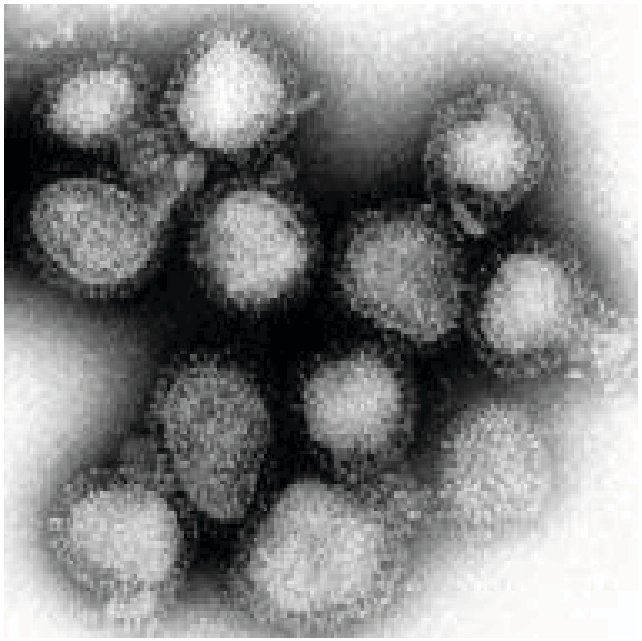


- Excellent sensitivity!
- Rapid
- Multiplex Detection
- Performed in serum samples

Dr. R.J. Collier, Harvard Medical School, Mukundan H et al, Anal Chem 2010

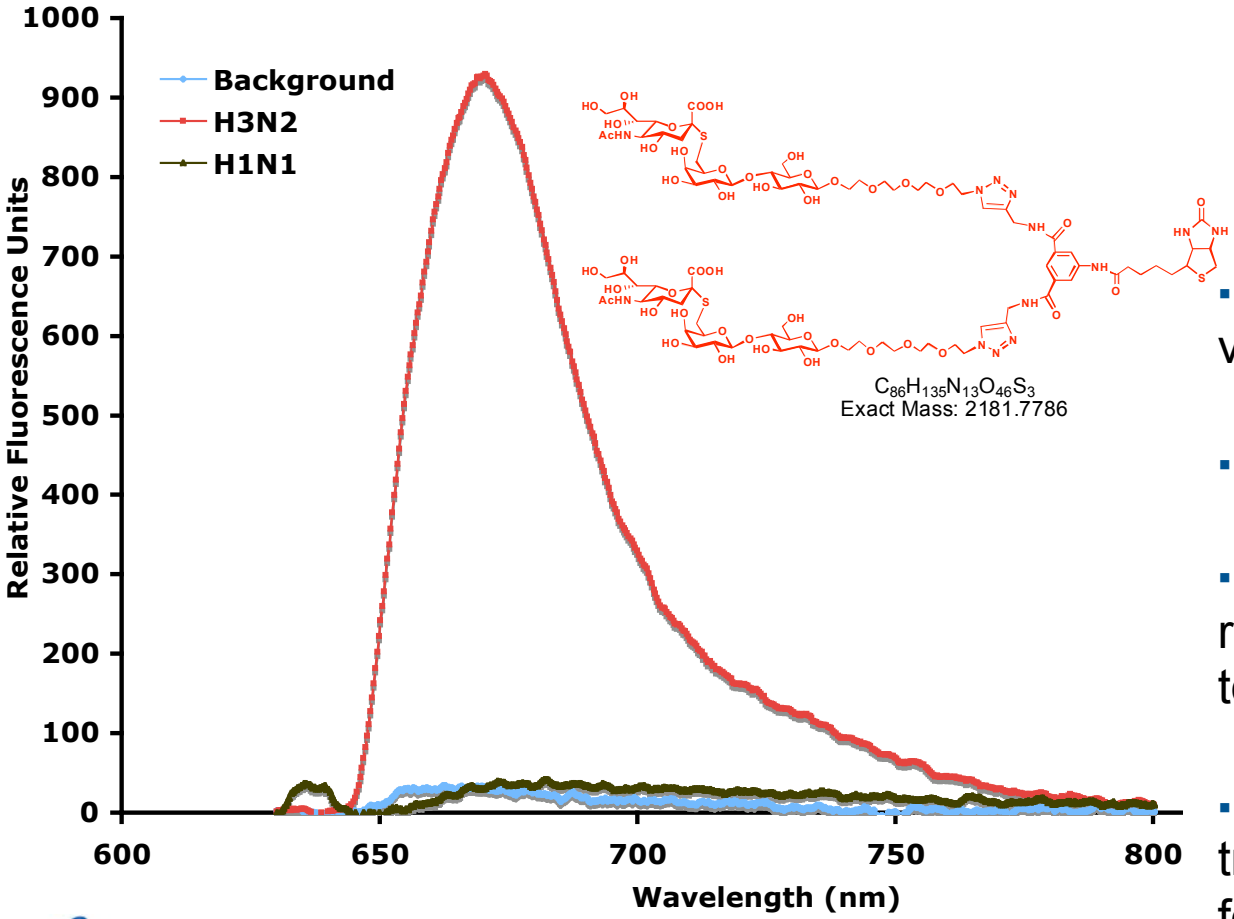
Influenza

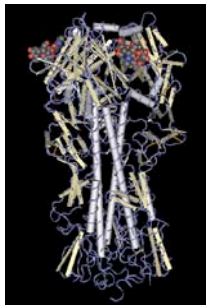
- **Major global concern of pandemic potential**
  - Current H1N1 pandemic is a classic example
  - Risk of new pandemics, avian flu
  - Persistent annual infection cycle

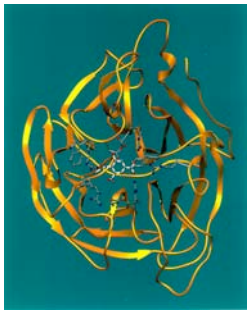


Courtesy: A. Murphy, UC Davis, through NIH; featurepics.com;

Strain-Specific Detection of Intact Influenza Virus using Carbohydrates







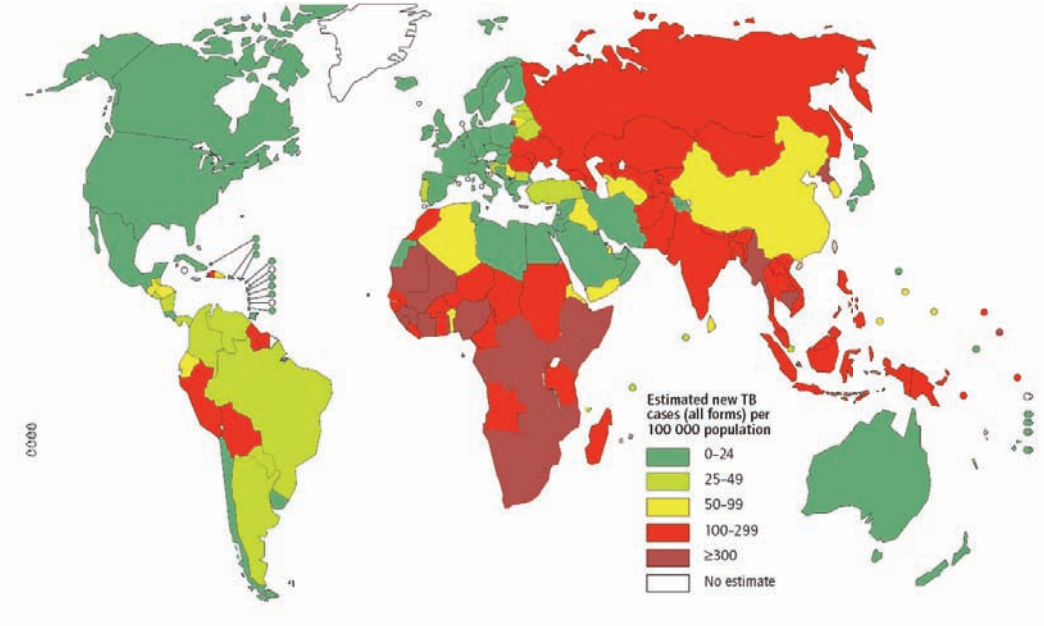
- Detection of intact influenza virus
- Serotype-specificity
- Ligands are extremely robust, even at room temperature
- Rapid, amenable to transition to point-of-care format

Courtesy: Kale RR et al, JACS 2008; National center for biotechnology information

Tuberculosis

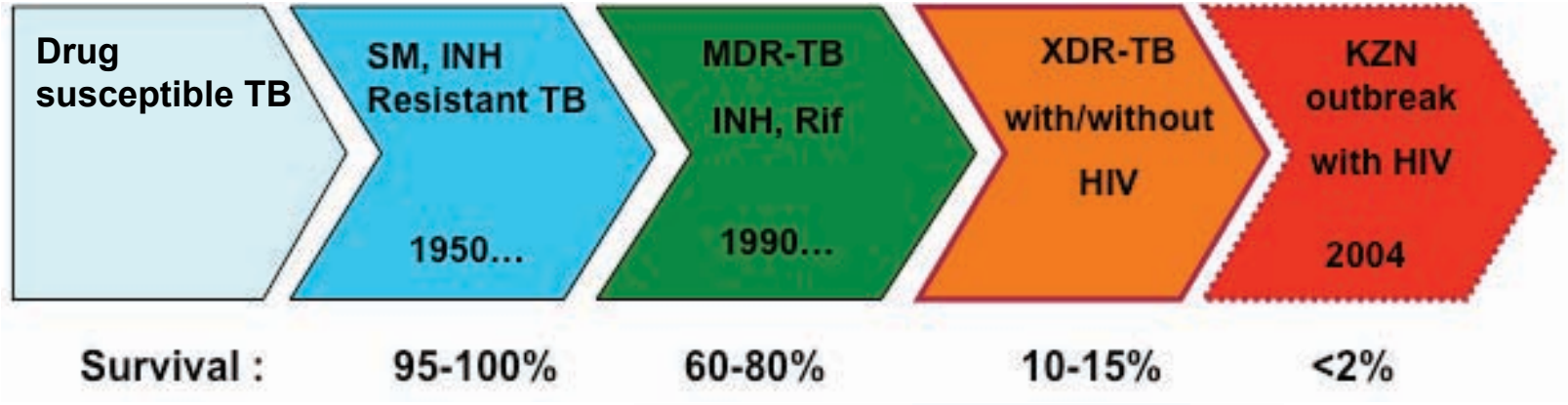
Courtesy: Dr. G. Kubica, CDC, public domain, Wikipedia

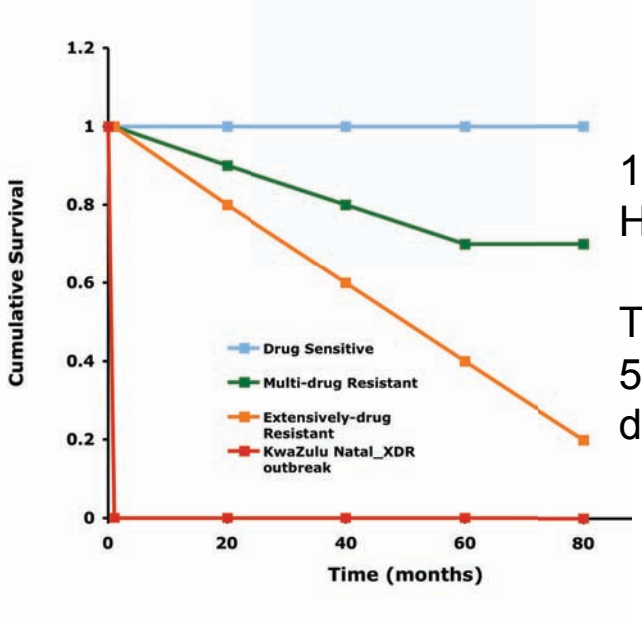
Tuberculosis: A re-emerging Scourge



Courtesy: K-RITH and Edendale Hospital, South Africa

Emergence of Drug Resistant Strains and Increased co -Infection with HIV





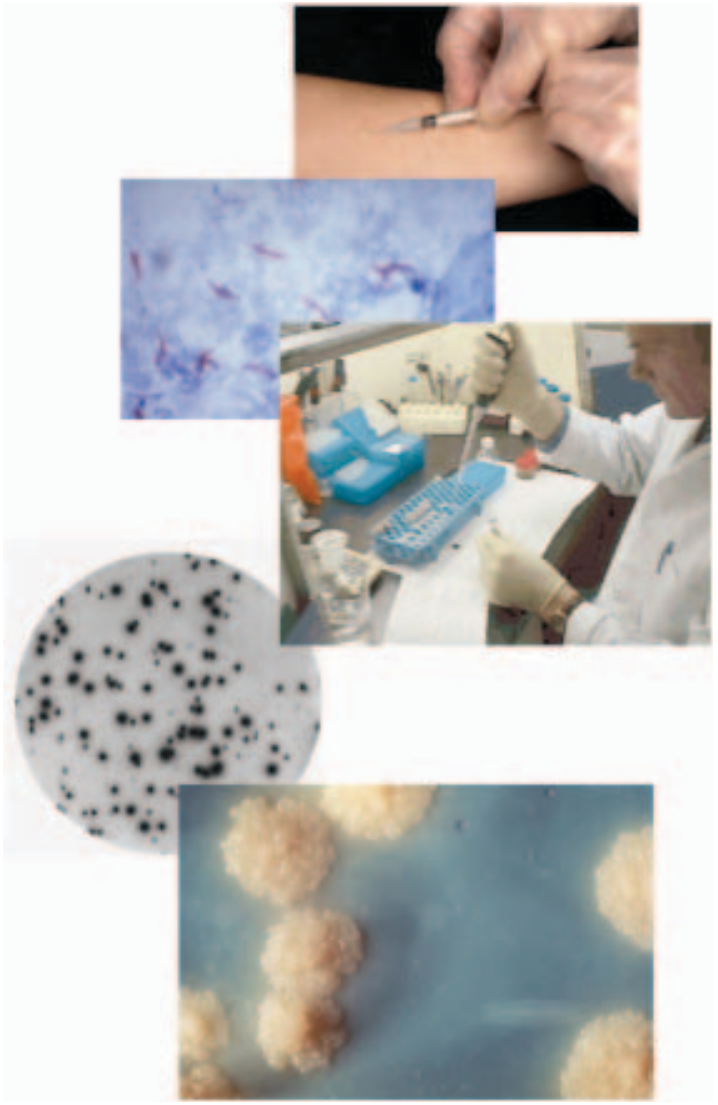
100% co-infection with HIV.

Tugella Ferry Outbreak: 53/54 dead within 16 days of positive sputum

Current Diagnostics for Tuberculosis

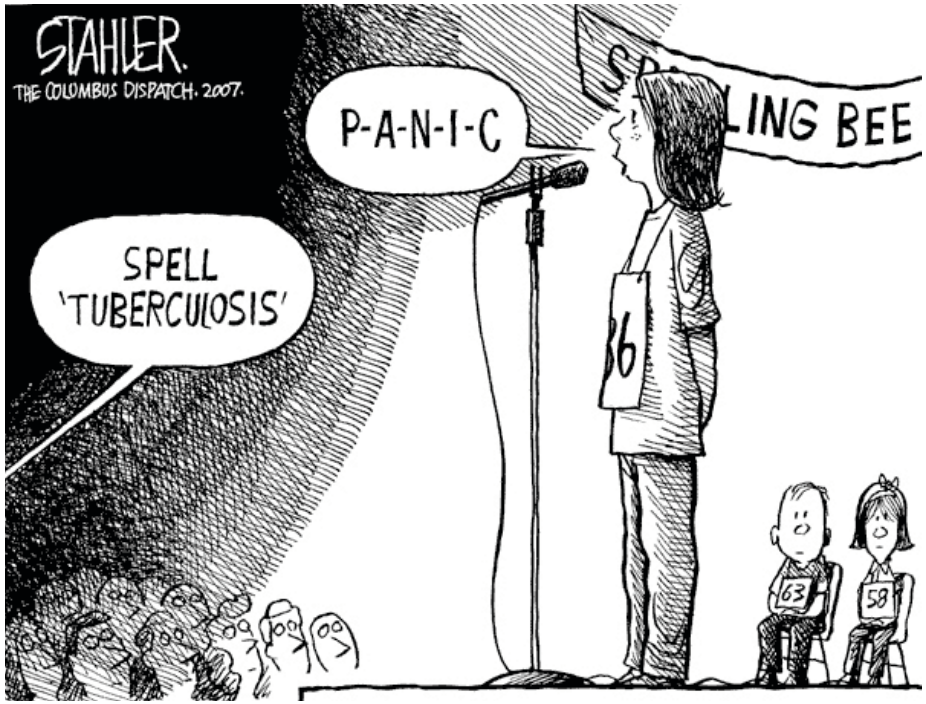
- Skin test
- "Smear microscopy"
- PCR-assay
- Interferon-gamma based assays
- Culture
  - Confirmation for all diagnostics
  - 4-6 weeks, additional 4-6 weeks for confirming drug resistance

Current methods of diagnosis are NOT SUFFICIENT to resolve the crisis in resource-poor settings



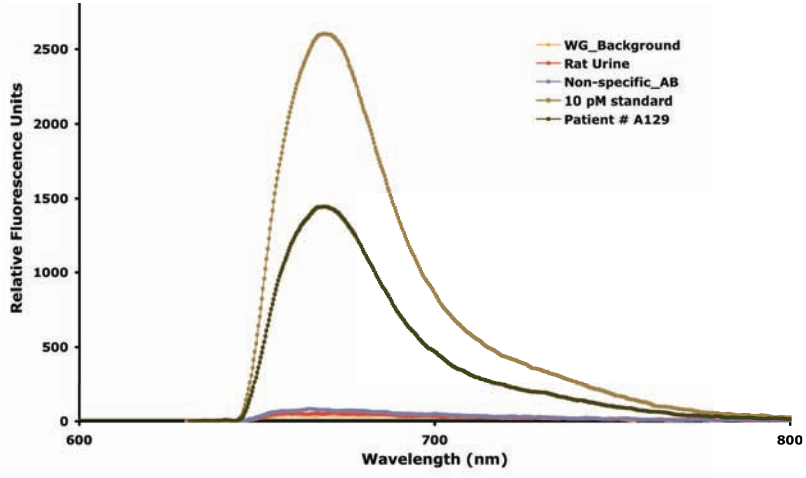
The Need

- Rapid
- Sensitive and specific
- Effective in HIV-positive individuals
- Inexpensive
- Not require extensive laboratory equipment or personnel training
- Effective for extra-pulmonary TB
- Early detection of resistant strains



Courtesy: Stahler, Columbus Dispatch 2007

Sandwich Immunoassays for Tuberculosis Biomarkers

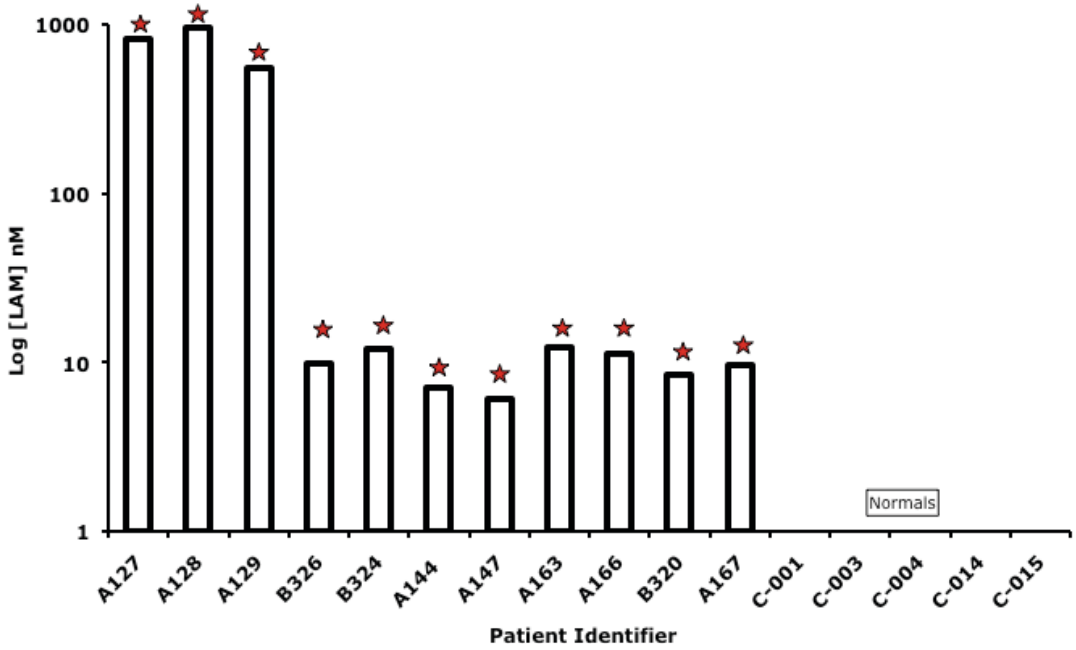


Sandwich immunoassay for Lipoarabinomannan

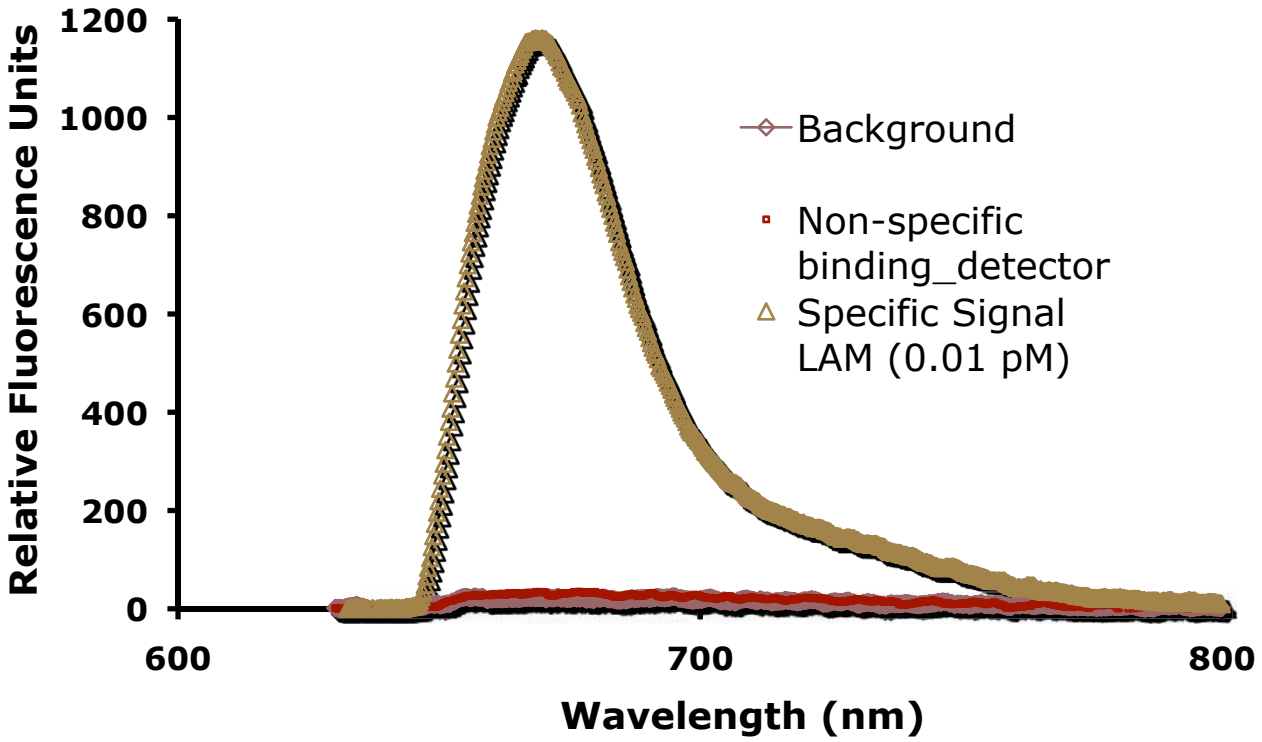
Limit of detection 1 pM in urine/serum

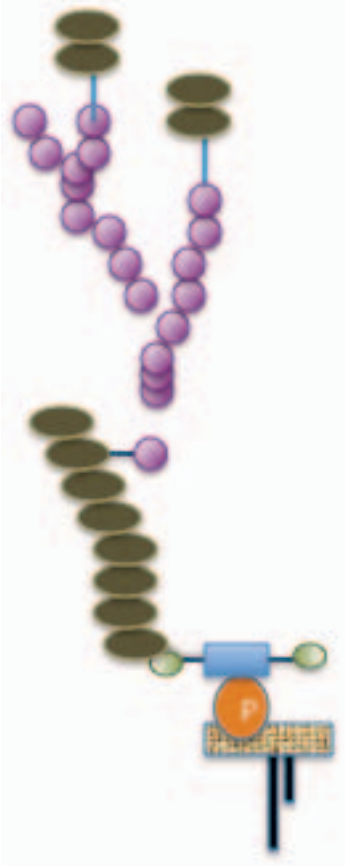
Evaluation in a cohort of patient urine samples

Blind study: 100% corroboration with disease!!



Detection of Lipoarabinomannan using the Membrane -Insertion Approach





Exquisite sensitivity of detection.

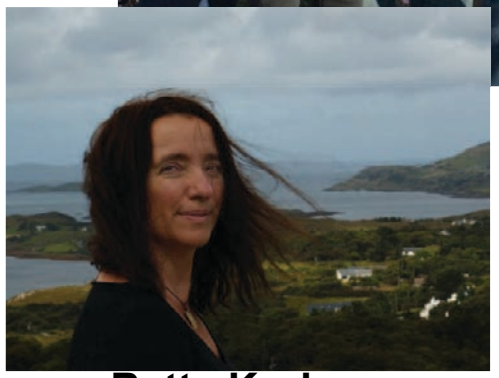
Limit of detection is femtomolar in serum!

Conclusions

- **Diagnostics of infectious disease remains an unsolved problem.**
- **Emergence of drug-resistance and co-infection (with HIV) exacerbate the problem.**
- **Diagnostics approaches based on pathogen biomarkers show tremendous promise!!**

Our optical biosensor approach offers promise for the rapid and sensitive detection of pathogen biomarkers in disease.

- Early pre-symptomatic diagnosis
- Detection of drug resistant strains
- Guide therapeutic intervention



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Los Alamos National Laboratory LDRD DR, NMSBA, NIH, CRADA