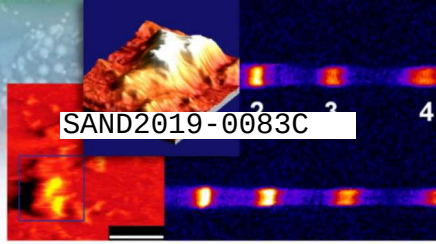
SANDIA BIOSCIENCE

This paper describes objective technical results and analysis. Any subjective views or opinions that might be expressed in the paper do not necessarily represent the views of the U.S. Department of Energy or the United States Government.



SAND2019-0083C

Development of a Multiplexed Microfluidic Detector for Biological Agents

Chung-Yan Koh

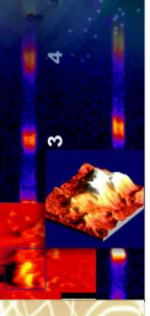
Biotechnology and Bioengineering

ckoh@sandia.gov

Research reported in this presentation was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number R01AI098853 and by the Department of Homeland Security, HSARPA-Chemical Biological Division under Agreement HSHQPM-13-X-00222. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Department of Homeland Security.

Outline

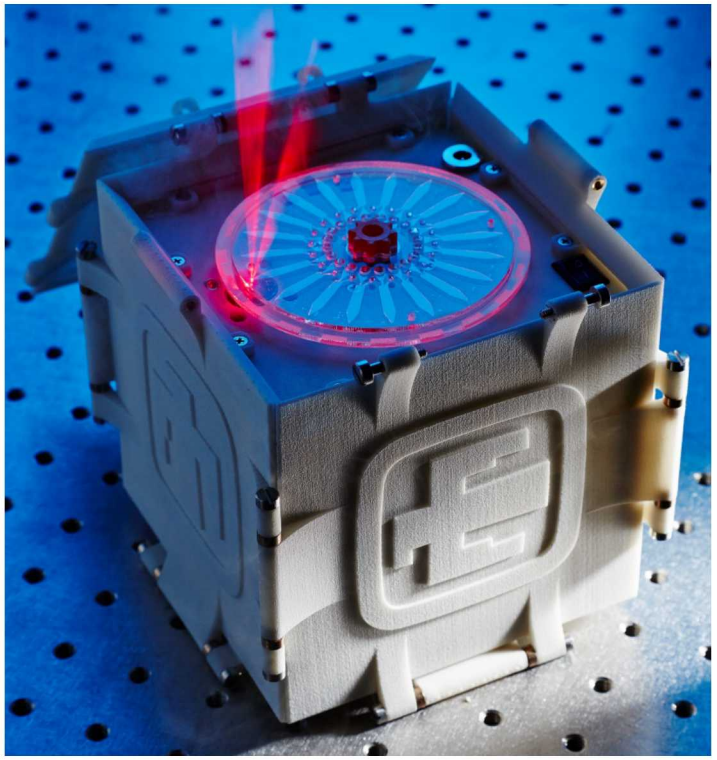
- Technology description
- Use cases
  - Protein detection
  - Small molecule detection
  - Multiplexed serology
  - Dry storage
- Conclusions



SANDIA BIOSCIENCE

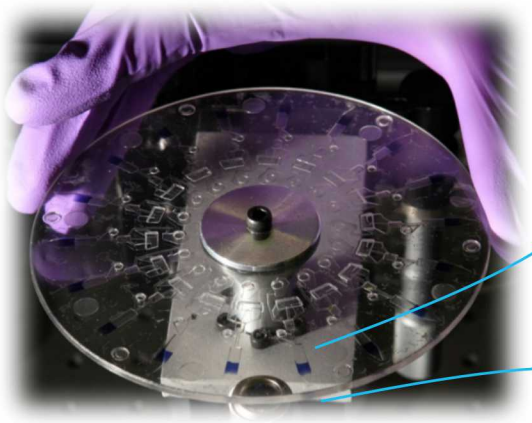
Key platform advantages

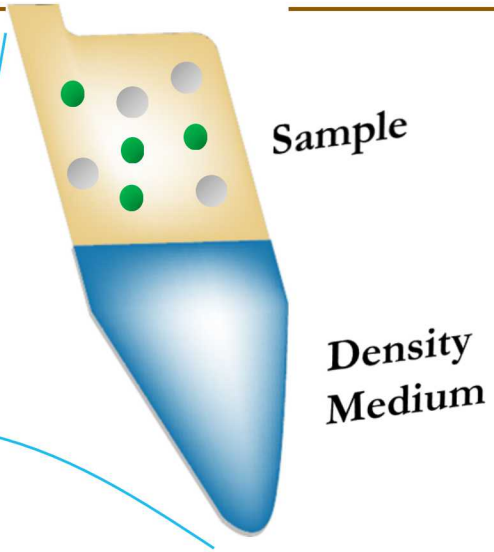
- **Fast:** Sample-to-answer in <15 min
- **Manufacturable:** Commidity plastics, standard metals, injection moldable
- **Multiplexed:** up to 20 parallel assays
- **Sample sparing:** 2 μL per assay
- **Sensitive:** 1-2 orders more sensitive
- **No sample prep**: Direct analysis of samples including clinical (blood, serum, urine, saliva, stool) and environmental (white powder, food)
- **Flexible:** Proteins, nucleic acids, cells
- **Designed for biodefense:** Radiation biodosimetry, biosurveillance, toxin and pathogen diagnostics



Portable: 5” (13cm) square, 2 pounds

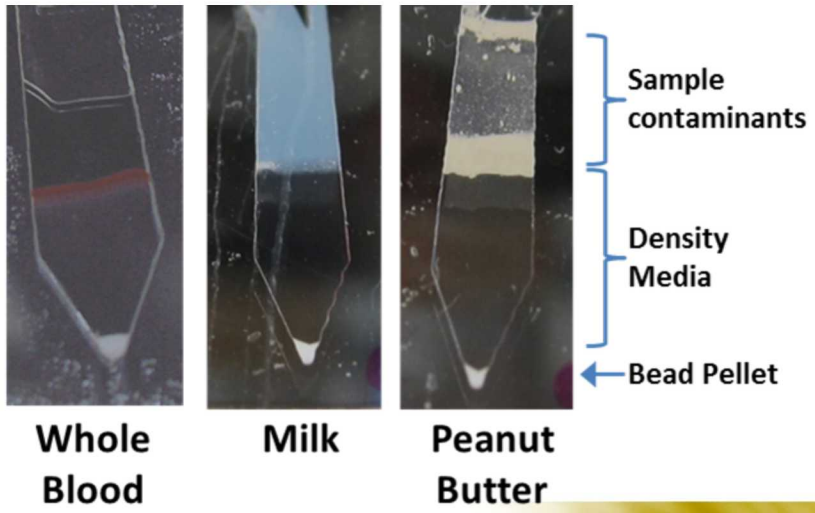
Centrifugal microfluidics: sedimentation-based assays



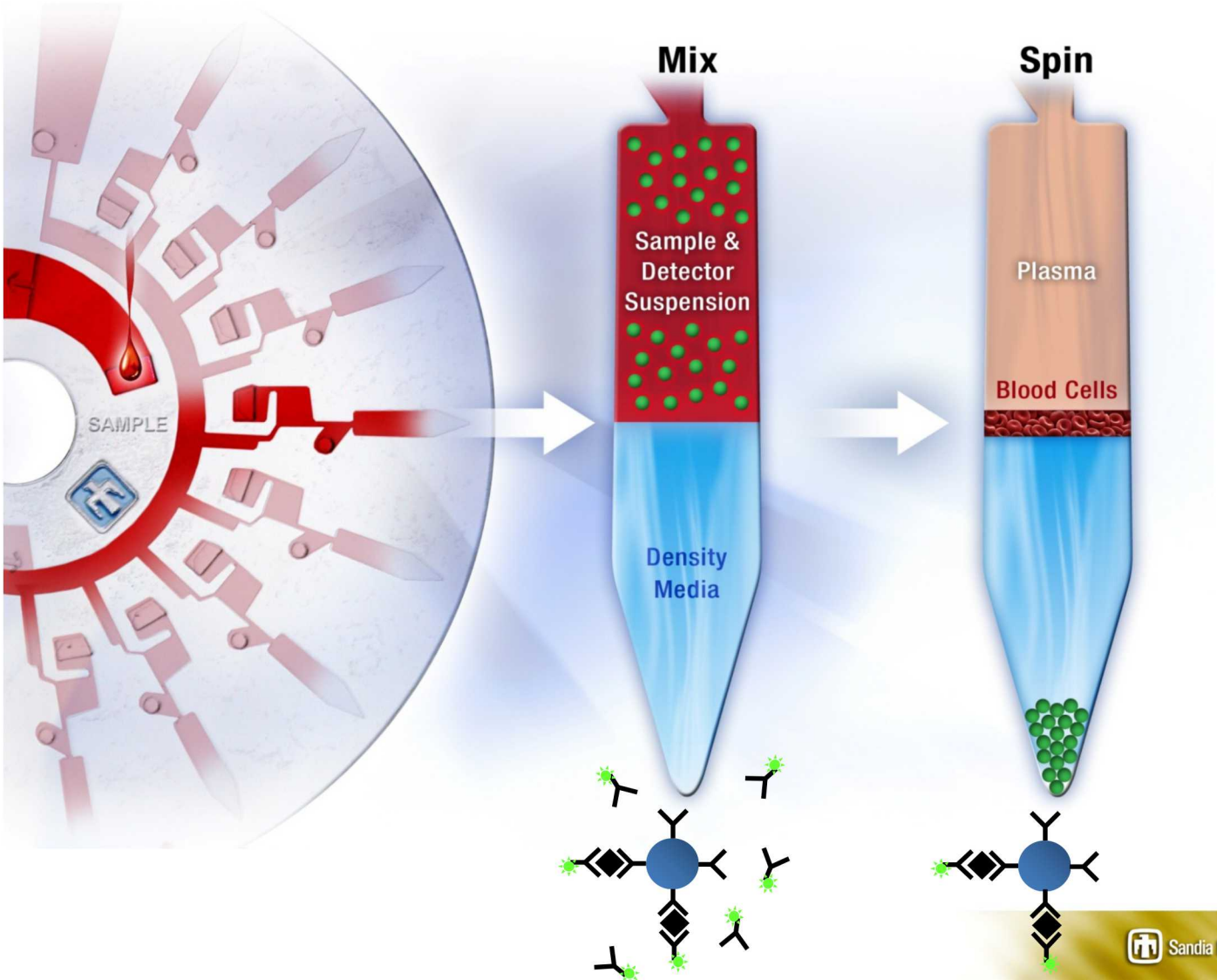


Particle transport governed by Stoke's Law:

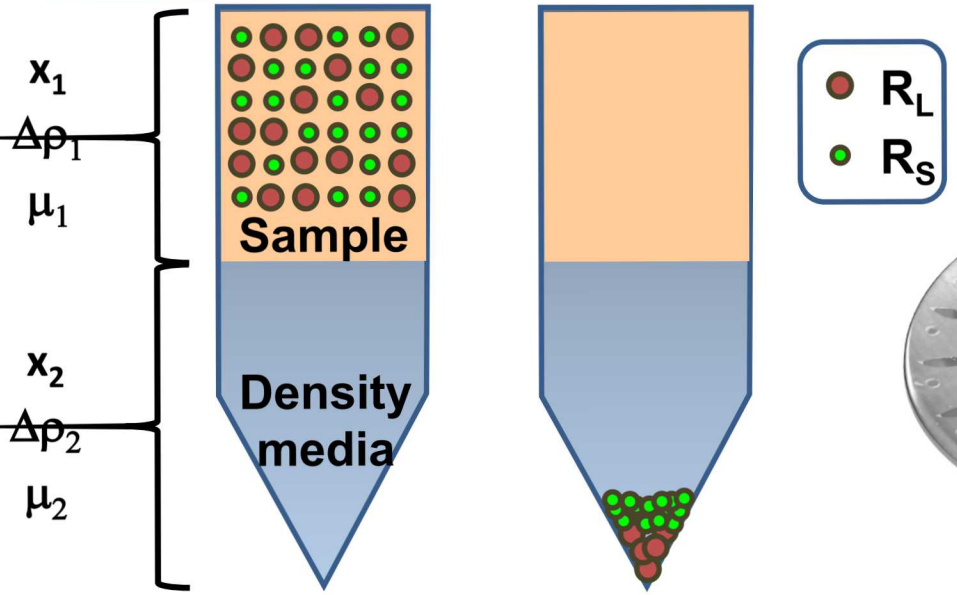
$$U_s = \frac{2}{9}\frac{(\rho_p - \rho_f)}{\mu} gR^2$$

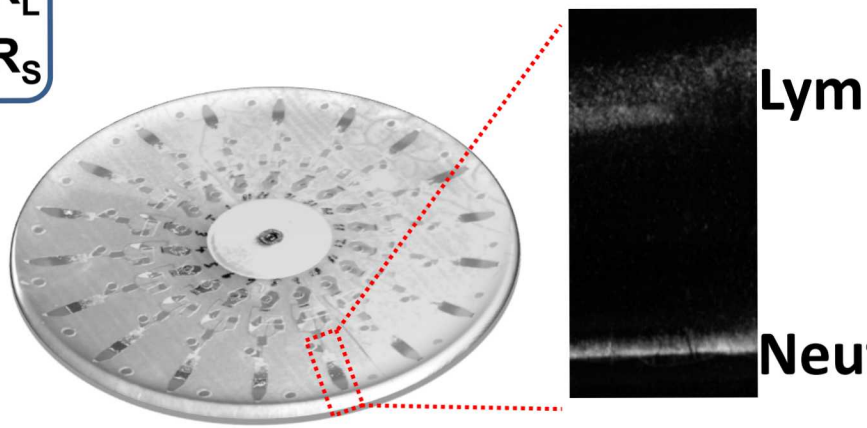


Immunoassay architecture



Further utilization of density-based separations

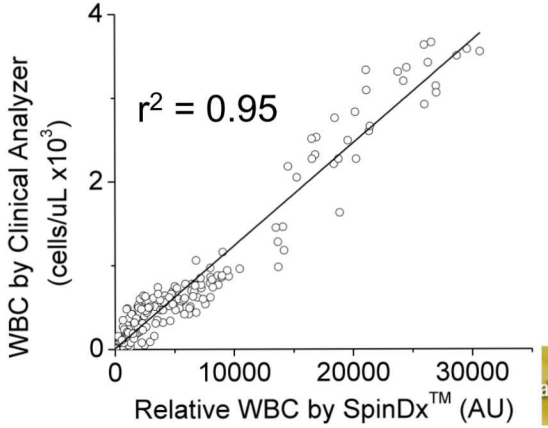




Cell sorting and analysis

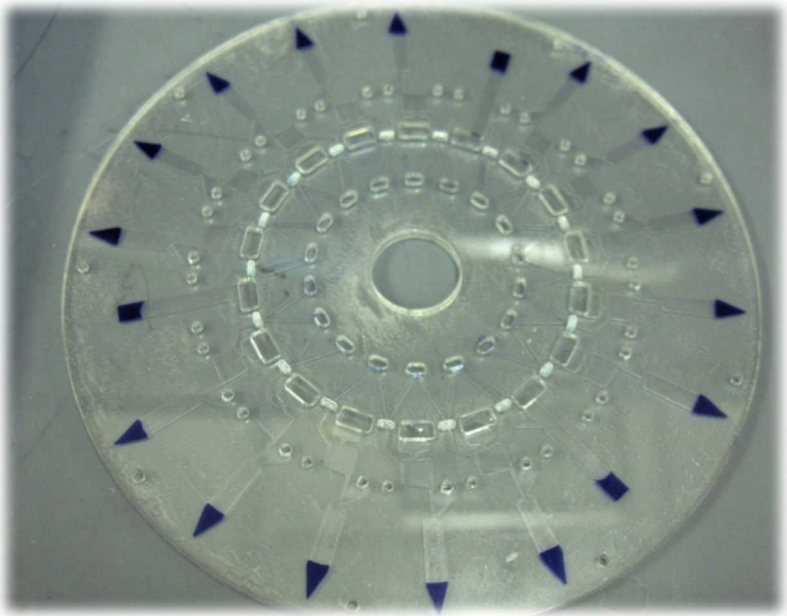
In-channel multiplexing

$$\frac{R_L}{R_S} \geq \sqrt{1 + \frac{x_1 \mu_1 \Delta\rho_2}{x_2 \mu_2 \Delta\rho_1}}$$



SANDIA BIOSCIENCE

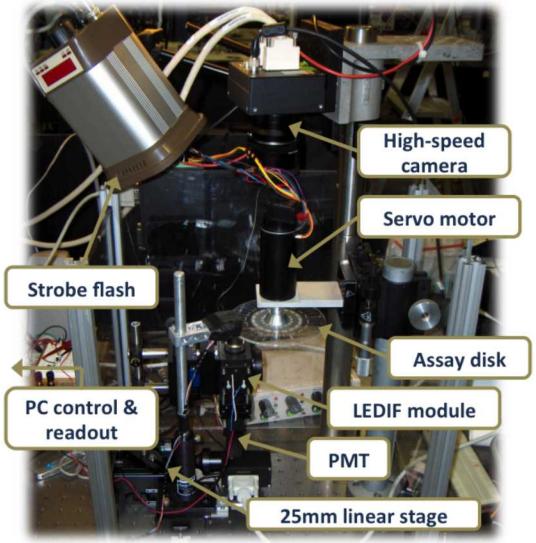
Multiplexing and sample routing



- Single inlet port, sample routed via capillary action
- Repeatable volume dispensing using channel geometry

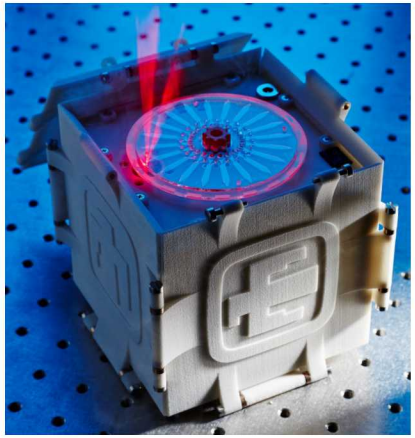
Platform evolution

2011

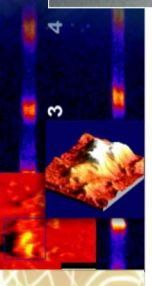


2012

2013



2014



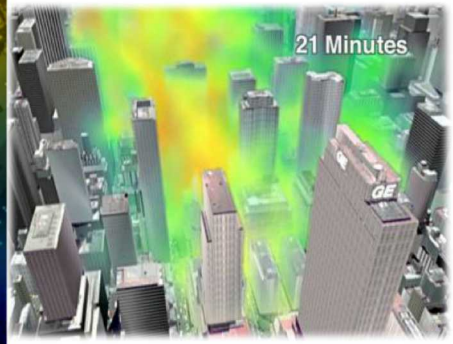
SANDIA BIOSCIENCE

Broadly applicable to many use cases

Biodefense

Medical Diagnostics

Mass Population Exposure



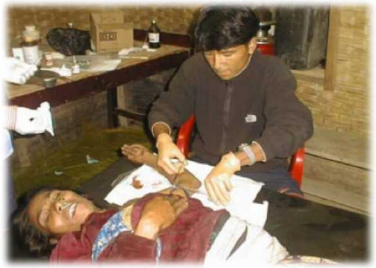
Field

High Risk
Traffic Screening

BSL Compatible
Research

Portable, Rapid Triage

Routine
Clinic Visit



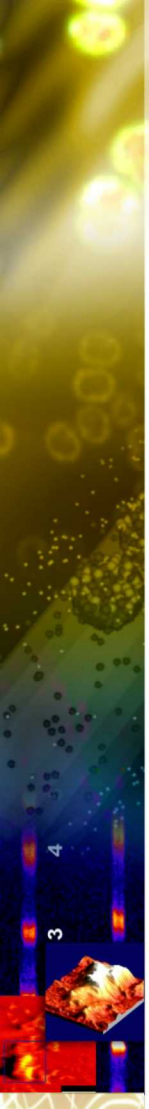
Global Healthcare

SANDIA BIOSCIENCE

Sandia National Laboratories

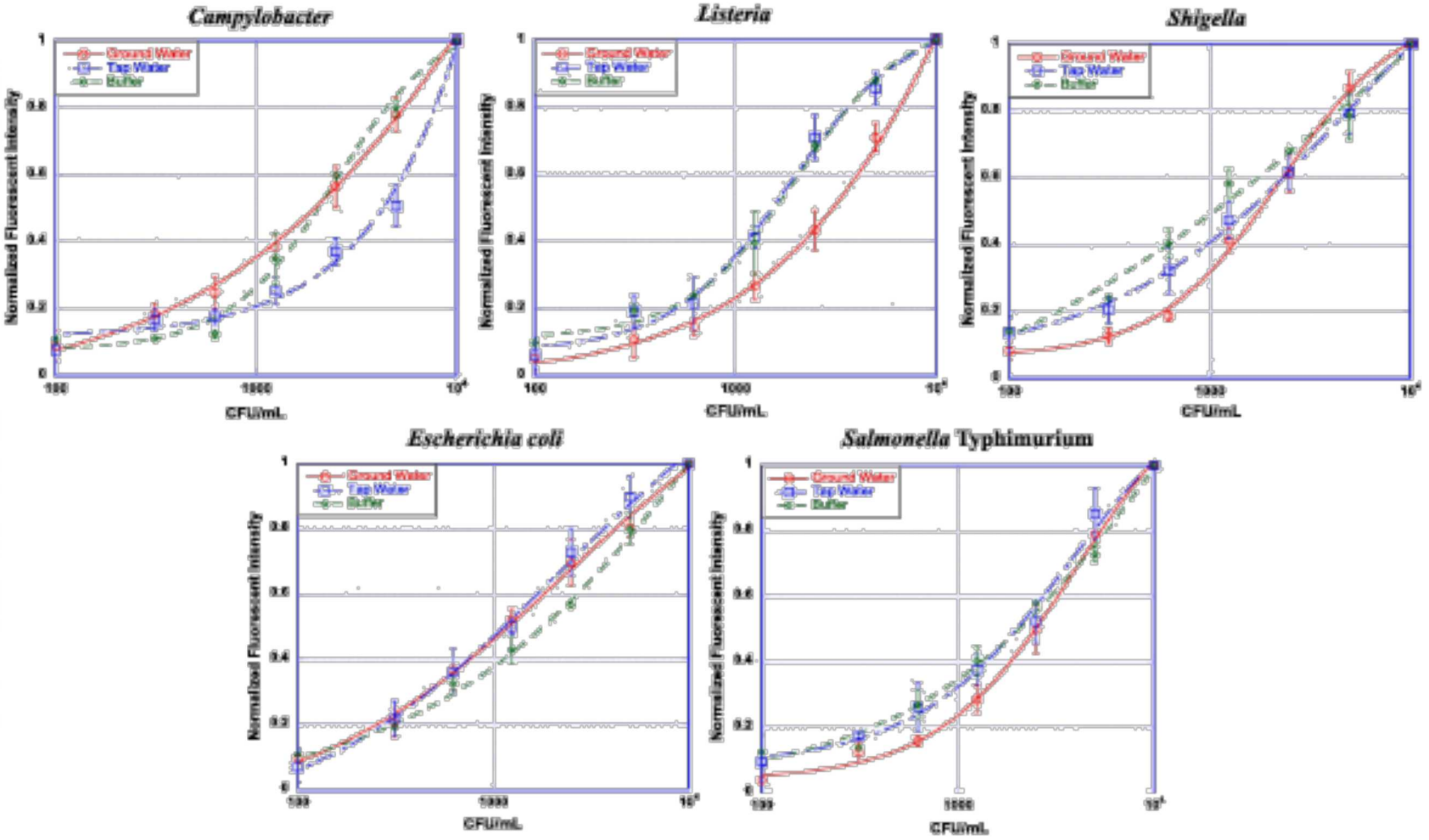
Platform technology: easily adapted to new targets

- Biodefense agents (e.g., toxins, pathogens)
- Public health targets (e.g., Salmonella, influenza, HIV, Zika, Dengue, Chikungunya)
- Biosurveillance (e.g., epidemiology, serology)
- Agriculture (e.g., animal health, disease prevalence)
- High value biomarkers (e.g., cancer, cardiac)



SANDIA BIOSCIENCE

Whole-cell assays for water quality

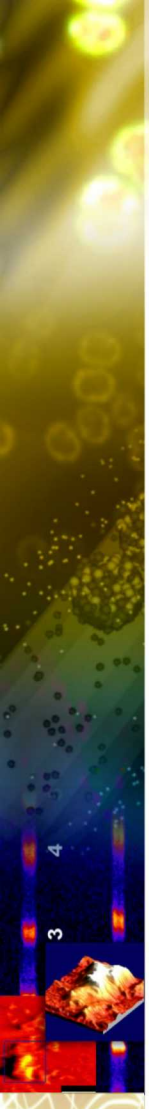


SANDIA BIOSCIENCE

Sandia National Laboratories

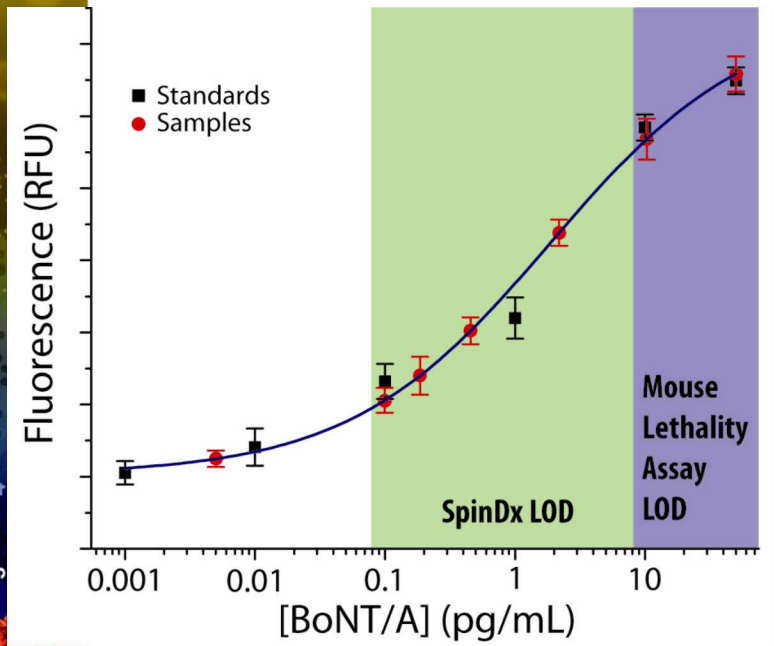
Toxin and pathogen diagnosis for biodefense applications

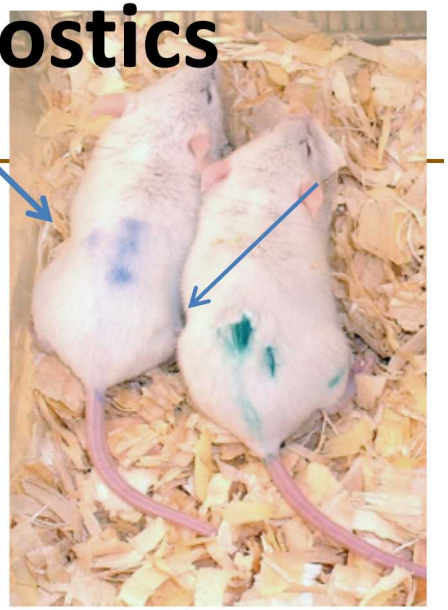
- Making the most of limited medical resources
- Separating ‘worried well’ from real cases
- Shortening cycle time from sample-to-answer-to-intervention
- Focused on category A select agents
- Animal study in collaboration with USDA-Albany



SANDIA BIOSCIENCE

Botulinum neurotoxin diagnostics





BoNT/A (pg/mL)	Estimated Mouse LD_{50}	Deaths (X/10)	Wasp Waist (X/10)
100	3.4	10/10	10/10
50	1.7	8/10	9/10
10	0.6	0/10	8/10
2	0.1	0/10	0/10

Head-to-head comparison with gold standard live mouse bioassay at USDA

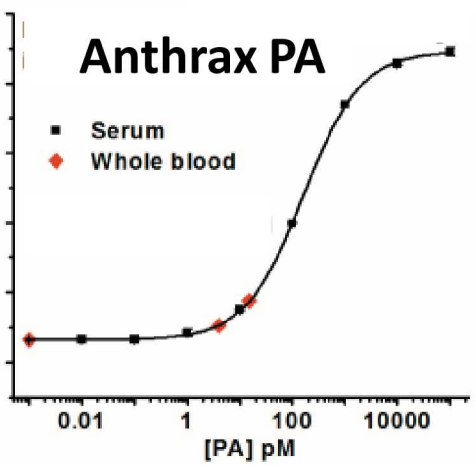
~100-fold improvement in LOD, 500-fold decrease in sample volume

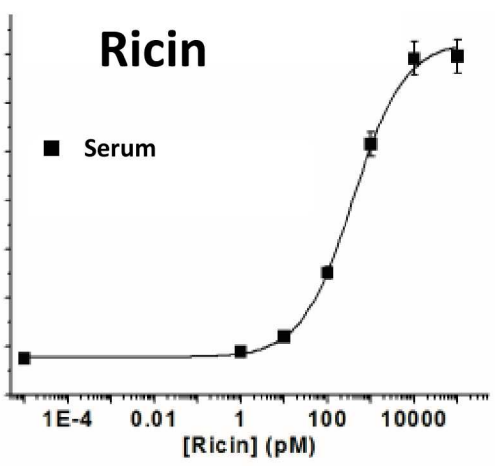
Results available in minutes instead of four days

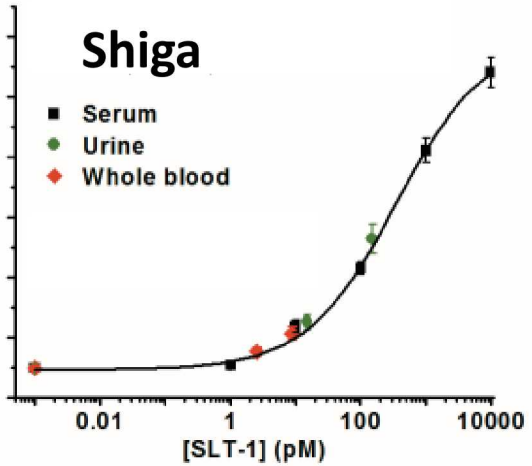
No mice need to die

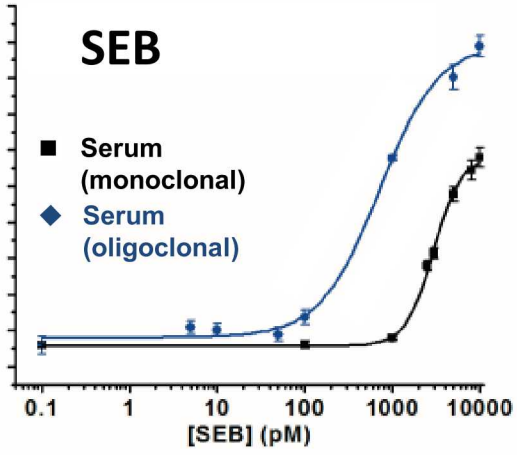
SANDIA BIOSCIENCE

Other category A toxins



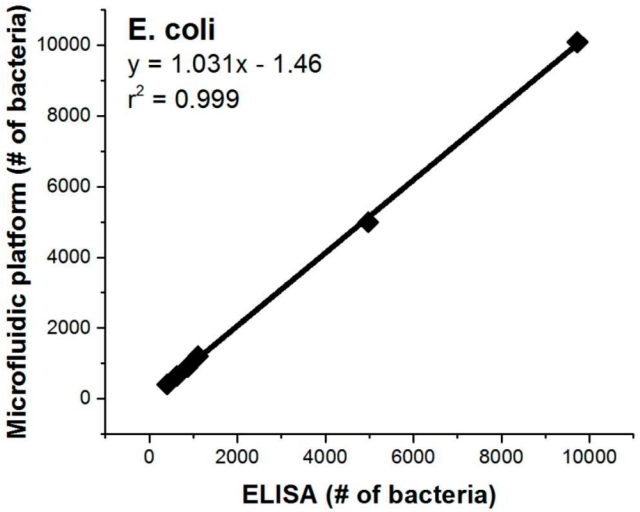




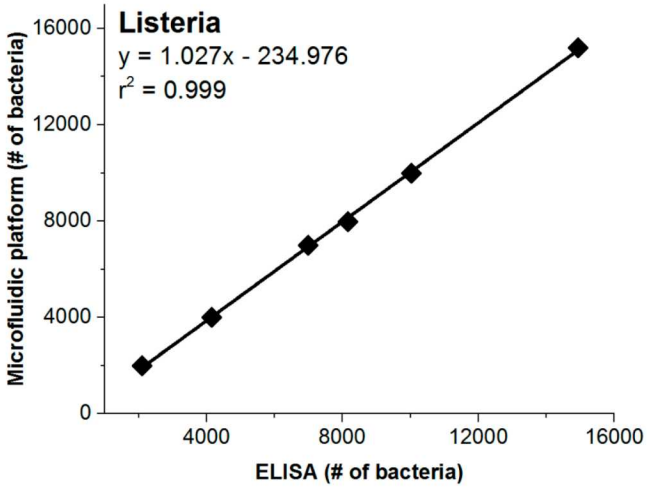


Biotoxin	Serum LOD	Serum LOQ
BoNT/A	0.5 fM	20 fM
Anthrax PA	2 pM	5 pM
Ricin	1 pM	7 pM
Shiga-like Toxin 1	0.8 pM	5 pM
SEB	84 pM	317 pM

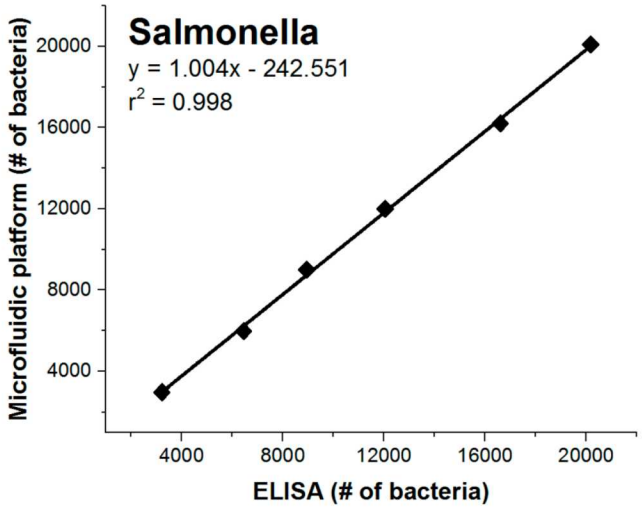
Method comparison with ELISA



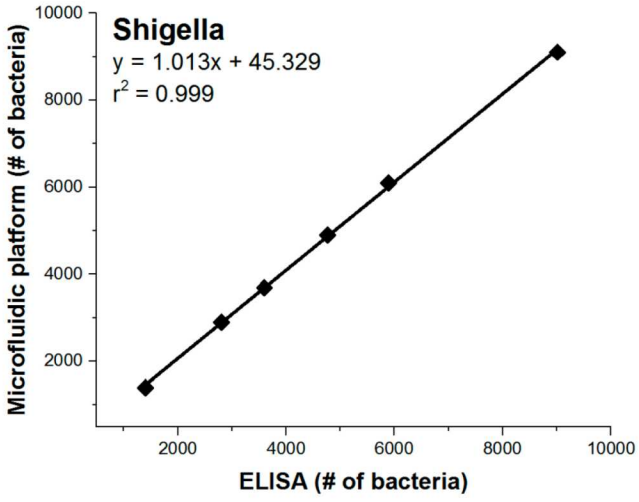
(a)



(b)



(c)

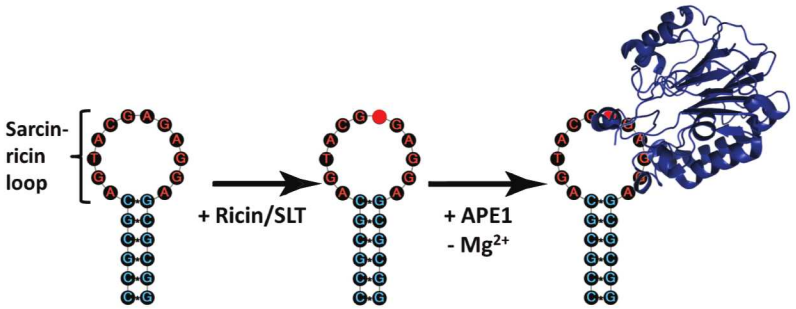


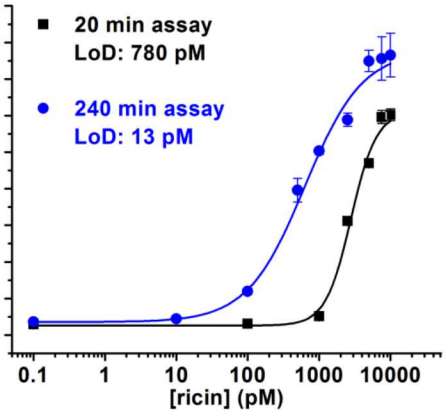
(d)

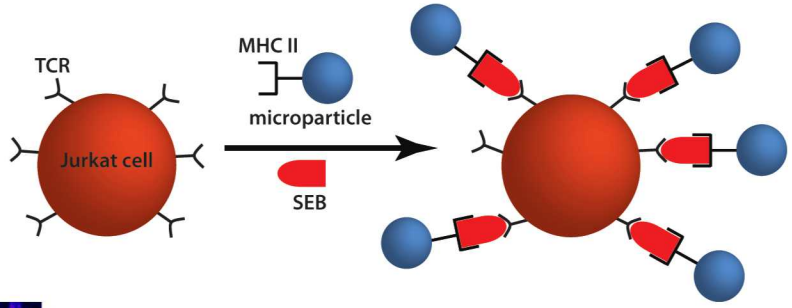
SANDIA BIOSCIENCE

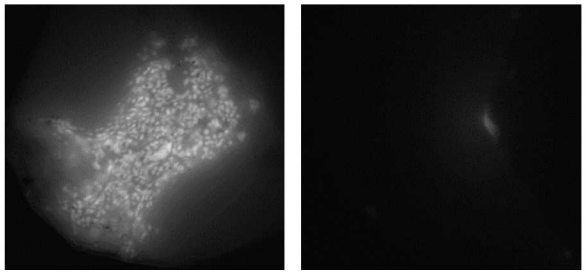
Sandia National Laboratories

Toxin activity assays

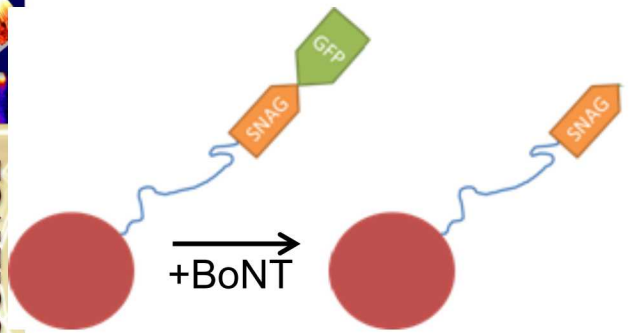


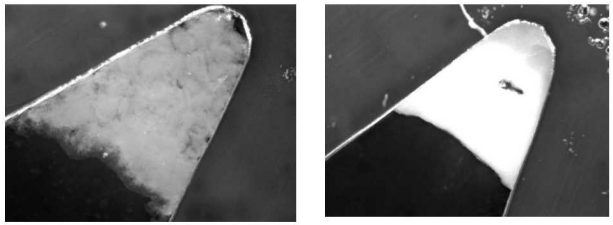






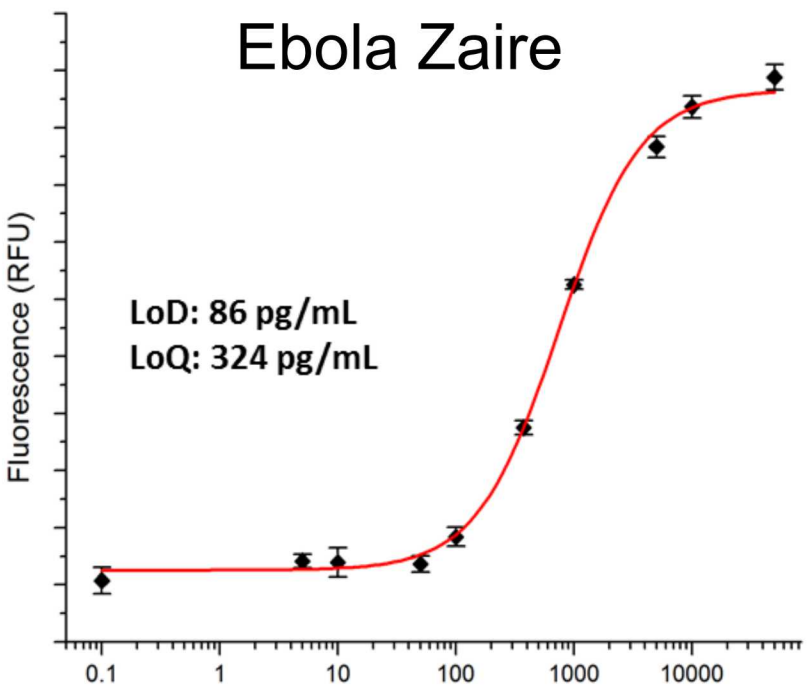
+ SEB - SEB

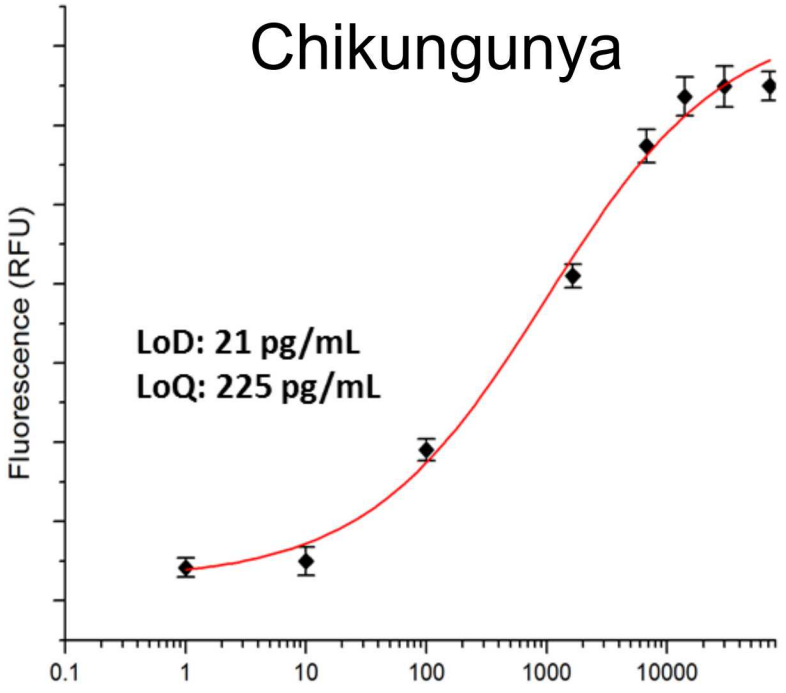




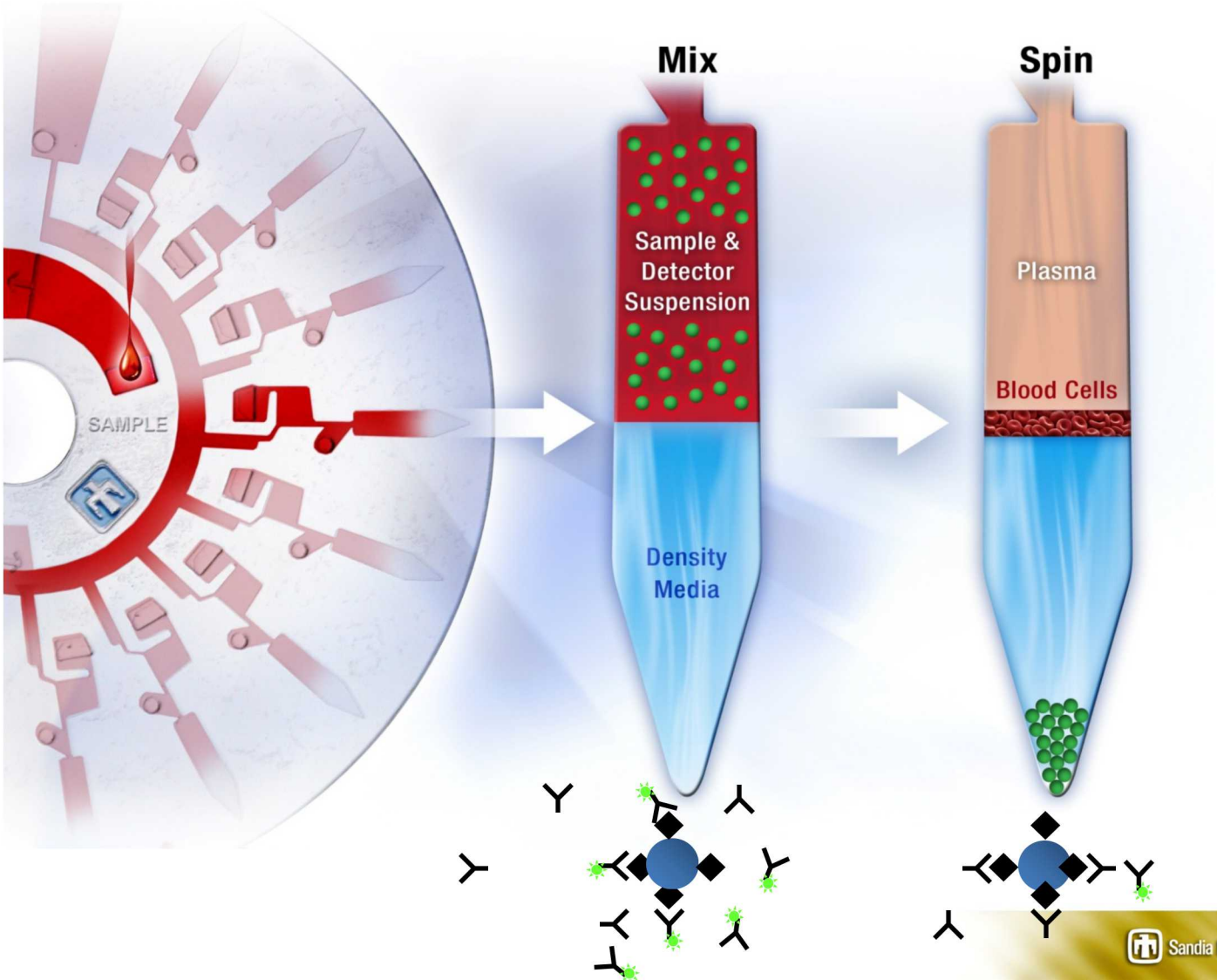
+BoNT -BoNT

Pathogen antigen detection



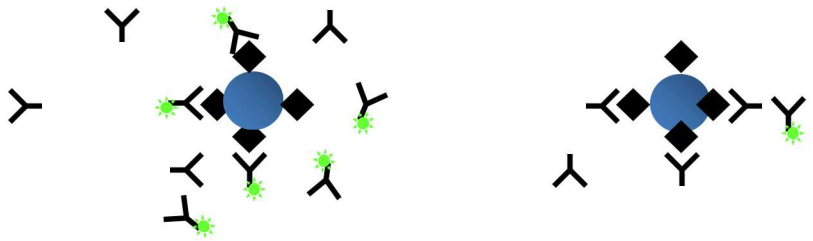


Detection of small molecules – competitive inhibition



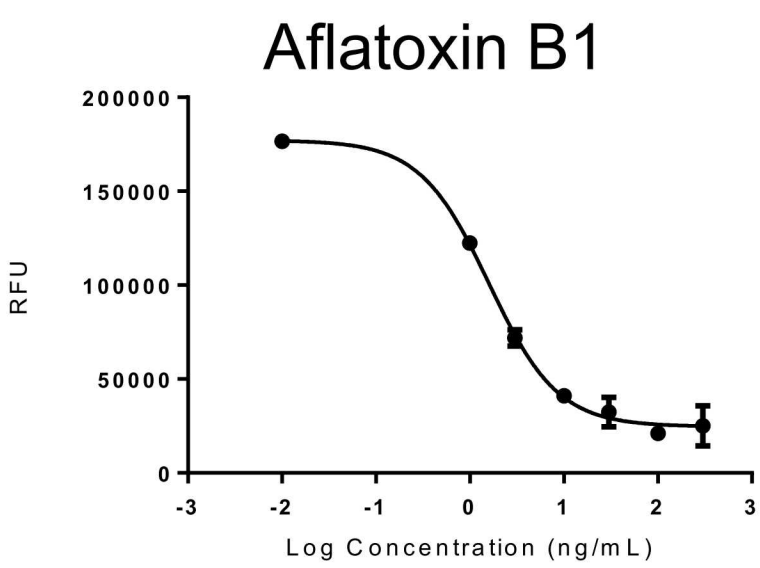
Competition assay for small molecules

- Dual antibody recognition in sandwich format
  - Higher specificity
  - Potentially higher sensitivity
- Not always possible with small molecules
- Loss of signal assay

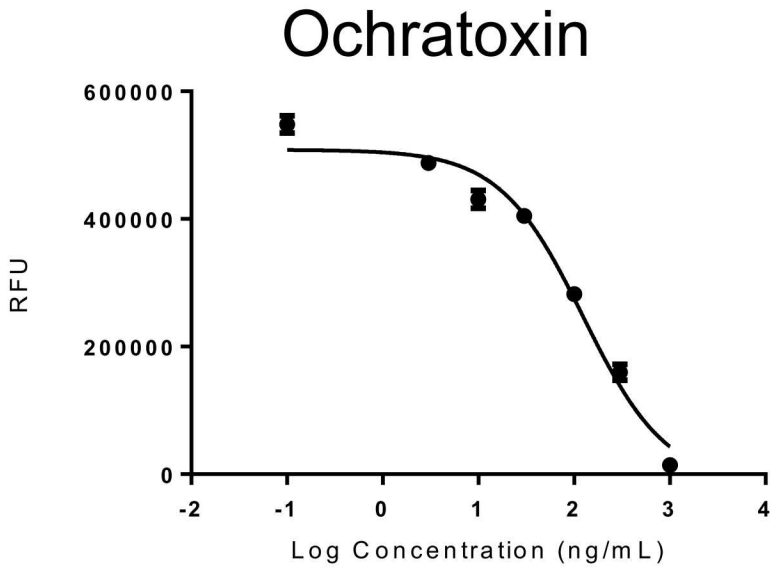


SANDIA BIOSCIENCE

Detection of small molecule toxins

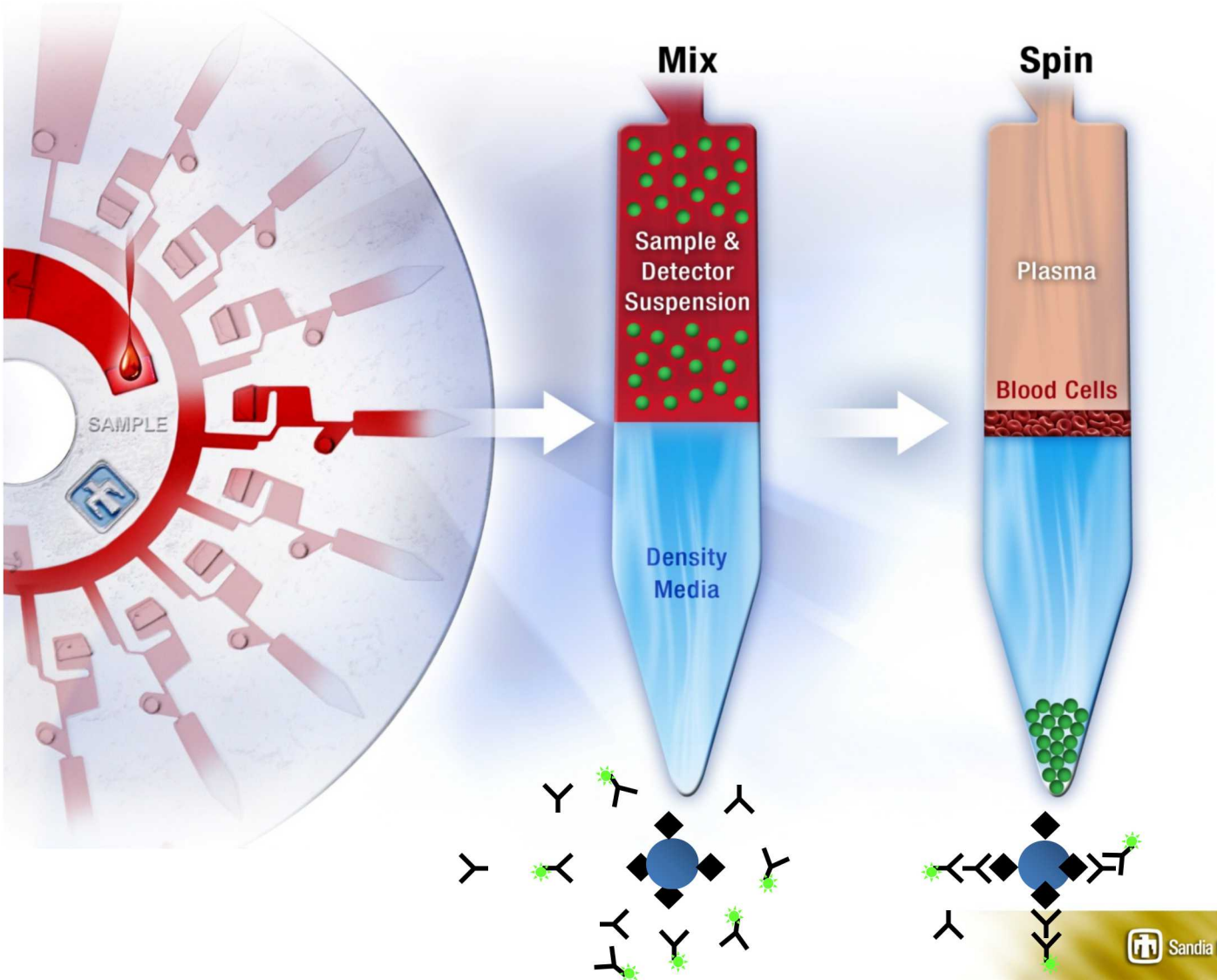


LoD: 0.200 ng/mL



LoD: 0.405 ng/mL

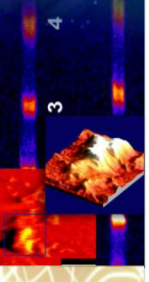
Multiplexed serology



SANDIA BIOSCIENCE

Advantages

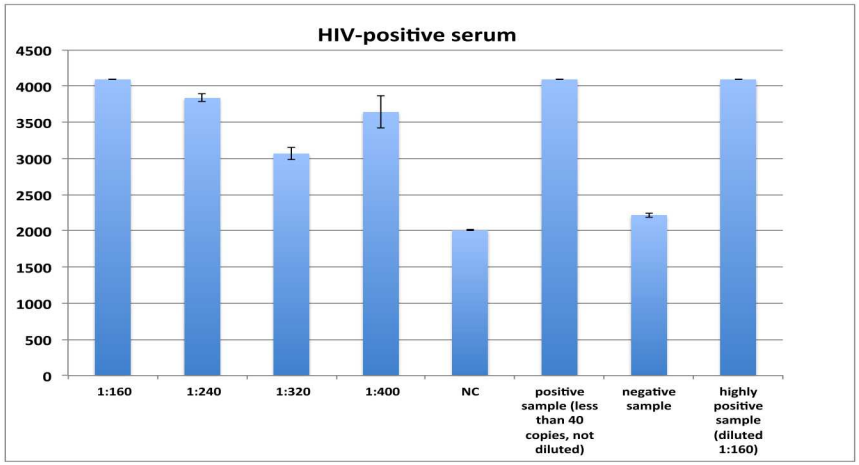
- Built-in dilution scheme
- No sample preparation required
- Integrated pre-treatment (e.g., acid/base, surfactants, salts) if necessary
- Precise, repeatable sample metering



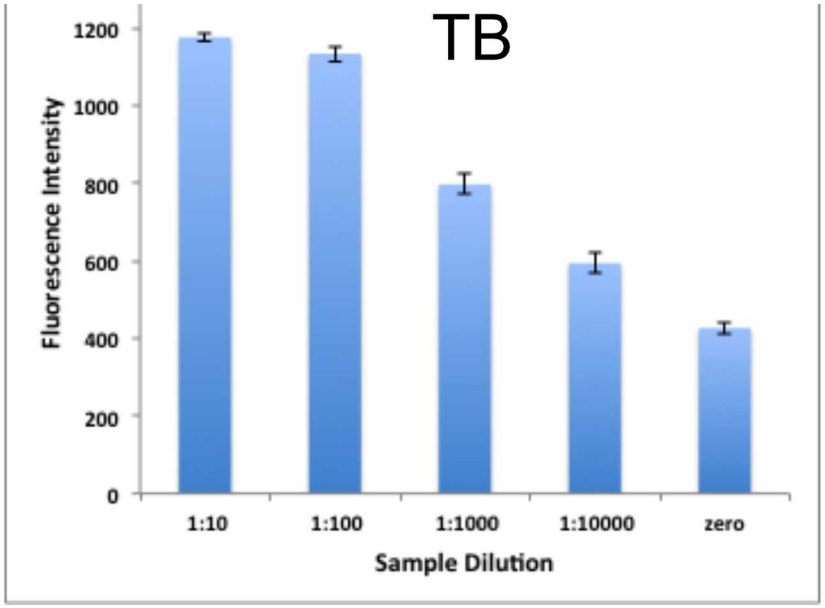
SANDIA BIOSCIENCE

Infectious diseases

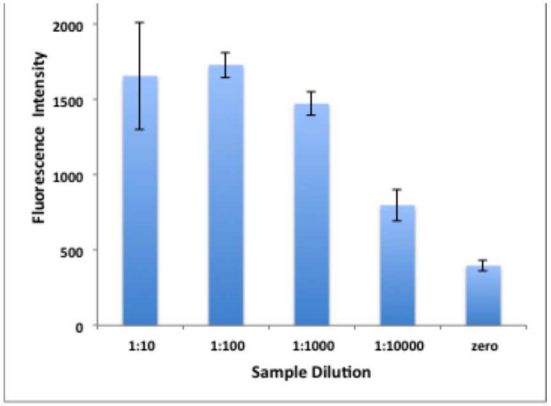
HIV



TB



Zika

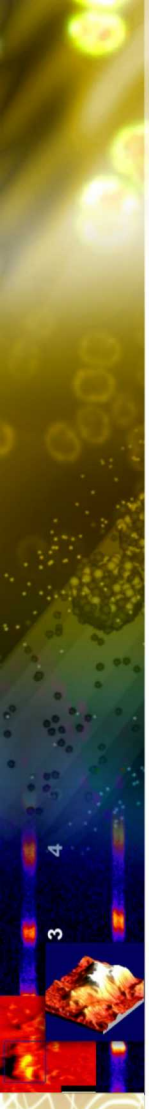


SANDIA BIOSCIENCE

Sandia National Laboratories

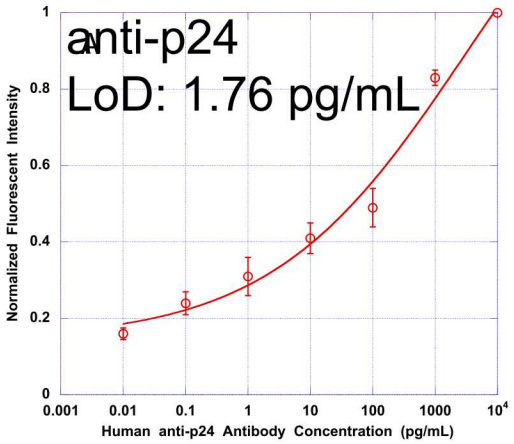
Clinical study

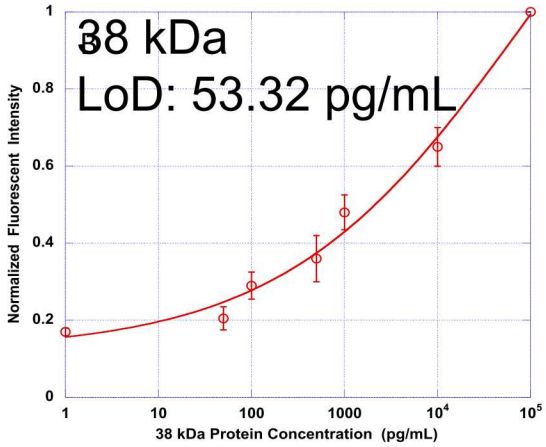
- Collaboration with University of Texas-Medical Branch (Galveston, TX) hospital lab
- Demonstration of antigen detection and serology
- HIV - p24; TB – ag85B and 38 kDa
- 24-month study, 180 patient samples tested
- Only found two TB-positive samples

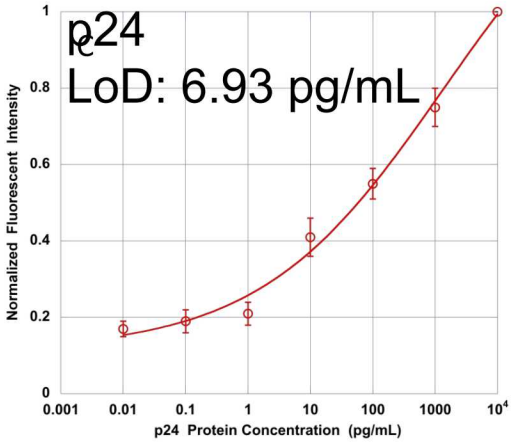


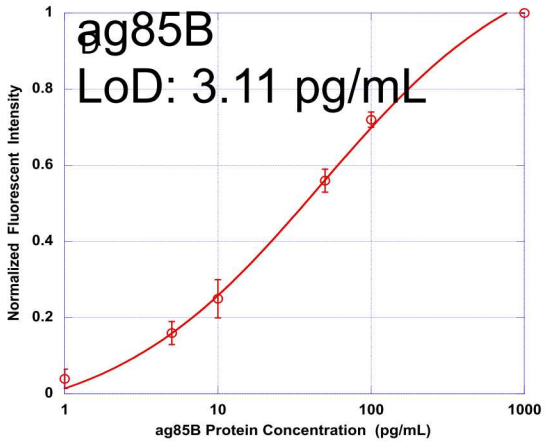
Preliminary Data

Clinical study









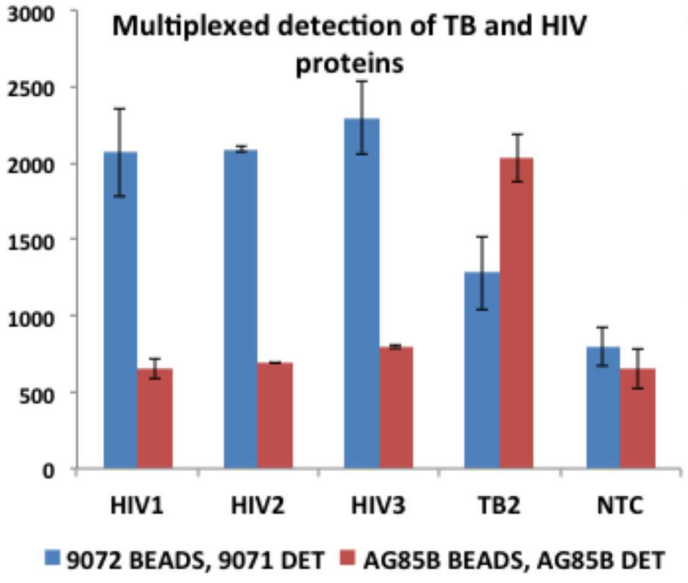
Preliminary Data

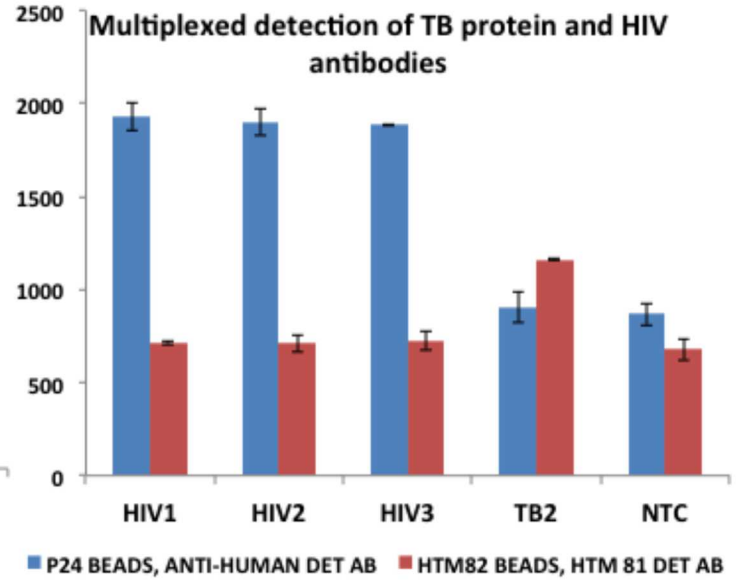
SANDIA BIOSCIENCE

Sandia National Laboratories

Preliminary Data

Clinical study





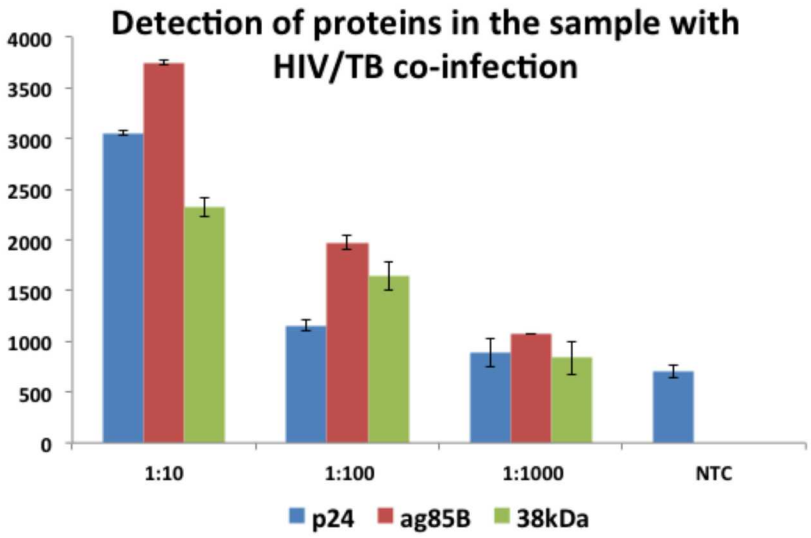
Preliminary Data

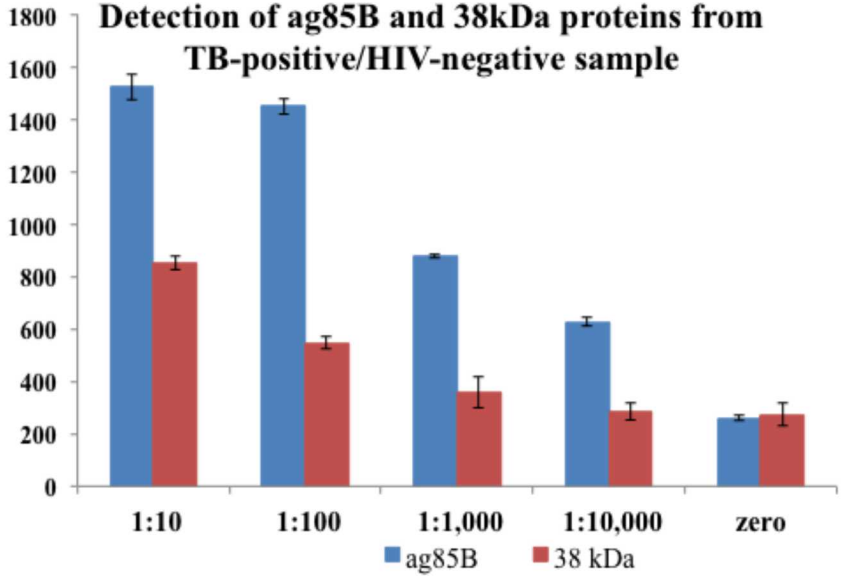
SANDIA BIOSCIENCE

Sandia National Laboratories

Preliminary Data

Clinical study





Preliminary Data

SANDIA BIOSCIENCE

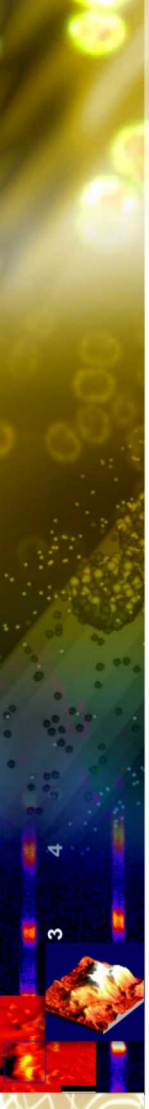
Sandia National Laboratories

Clinical study

- Antigen detection: 92% sensitivity, 89% specificity
- Serology: 100% sensitivity and specificity
- Demonstration of antigen detection and serology

Dried reagent storage

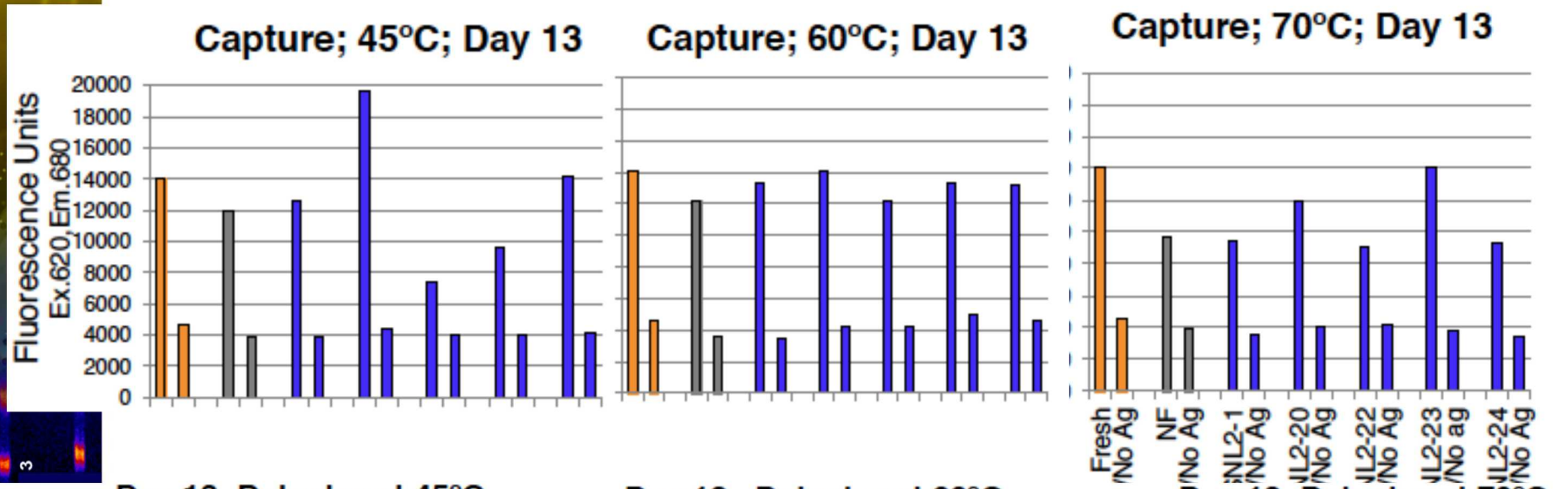
- Shelf-stable reagents ideal
  - Ease of storage
  - Elimination of cold chain
- To date, all experiments done with reagents stored at 4/-20°C
- Tested control antigen detection sandwich immunoassay (IL-6 detection)

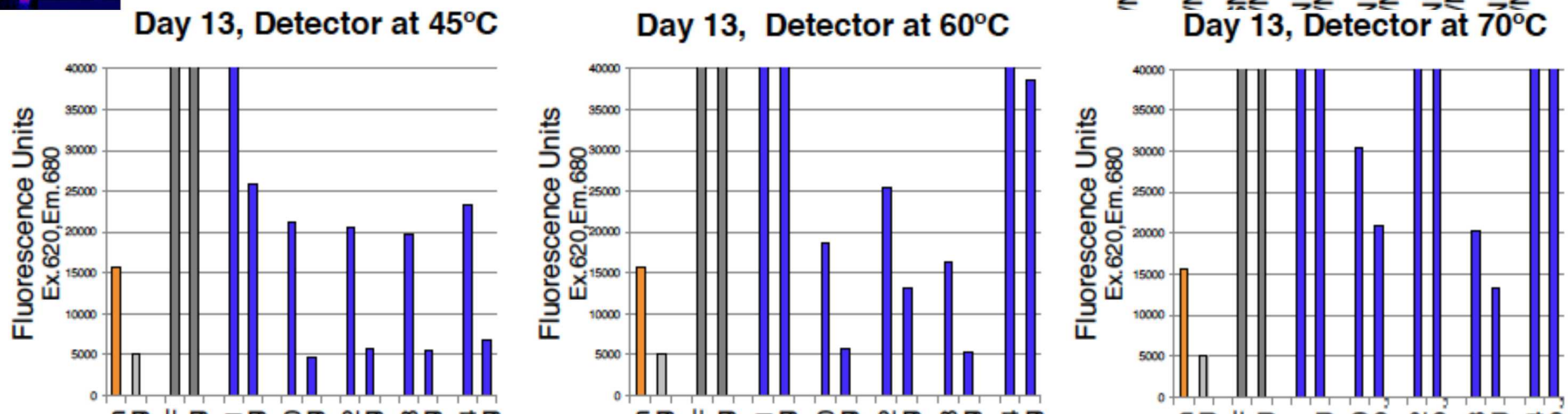


SANDIA BIOSCIENCE

Preliminary Data

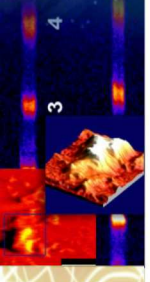
Shelf life study





Conclusions

- Rapid, sensitive, easy-to-use portable biological molecule detector
- Immunoassay-based detection chemistry
- Flexible platform technology
- Sample matrix insensitive
- Onboard sample preparation simplifies operation



SANDIA BIOSCIENCE

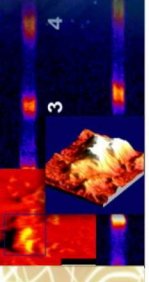
Acknowledgements

- SNL
  - Betty Mangadu
  - Huu Tran
  - Christopher Phaneuf
  - Jonathan Helm
  - Anup Singh

- UTMB
  - Julia Litvinov
  - Michael Loeffelholz
  - Scott Moen
  - Greg Berry

Funding provided by NIH-NIAID (R01AI098853) and DHS-HSARPA-CBD (HSHQPM-13-X-00222).

Research reported in this presentation was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number R01AI098853 and by the Department of Homeland Security, HSARPA-Chemical Biological Division under Agreement HSHQPM-13-X-00222. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Department of Homeland Security.



SANDIA BIOSCIENCE

Sandia National Laboratories