

Background

This project leverages previous antibody ruggedization efforts at ECBC to determine whether the ruggedization of detection antibodies translates into improved performance in the field. Capitalizing on ECBC's previous work with DARPA's Antibody Technology Program (ATP) and DTRA's Ruggedized Antibody Program (RAP), this effort looks to develop an LFI against the ricin toxin with increased thermostability that enlists the use of a reader device capable of communicating results with a smartphone. Incorporating ruggedized ricin antibodies into fieldable, Lateral flow immunoassay (LFI) formats offers significant benefits for both reducing the cold chain logistic tail currently required for biological reagents and increasing the reliability of assay results under harsh and/or extremely variable environments.

Ricin toxin, a glycoprotein produced by the castor bean plant *Ricinus communis*, is highly toxic and can cause death when given in sufficient quantities by either systemic or inhalational routes of exposure

Ricin is derived from the castor bean plant, a common ornamental plant whose easy cultivation is the major reason why ricin continues to remain a public health threat today.



The toxin ricin is classified by the Centers for Disease Control (CDC) as a level B biothreat because of its wide availability and extraordinary toxicity.

The "Umbrella Murder"

In 1978, Georgi Markov, a communist defector working for the BBC World Service was murdered in London by an operative connected to the KGB and the Bulgarian secret police. Markov was assassinated by a pellet of Ricin injected from an umbrella.

Relevance

Immuno-assays are the primary detection component of many of the Department of Defense's (DoD) fielded biological Warfare agent (BWA) detection systems. The lack of ruggedized reagents for use in these assays remain a significant issue for operational reliability and decisional certainty under widely varying environmental conditionals. Based on previous efforts for ruggedizing, or increasing the thermal stability of antibody reagents, we ask this question:

Does the ruggedization of antibody reagents used in LFIs translate into an improved performance in field operations?

Lateral flow immuno-assays (LFI):

- Pros**
- Simple, hand-held, easily transported
 - Generally no additional reagents required
 - Relatively inexpensive
 - Easy to manufacture
 - Amenable to multiplexing



- Cons**
- Restricted shelf life
 - Decrease in sensitivity in austere environments
 - Need for cold-chain logistics

Technical Challenge

Develop a ruggedized Hand-Held Lateral Flow Immunoassay (LFI) for the detection of ricin toxin that incorporates the use of a smartCAR reader device capable of communicating assay results to a Net Warrior (NW) - ready smartphone.

Method

- ❑ Ruggedized, engineered antibodies from DTRA Ruggedized Antibody Program (RAP) were compared to a pair of native anti-Ricin antibodies sourced from the JPEO-CBD Critical Reagent Program (CRP).
- ❑ Selected antibodies were provided to Maxim Biomedical, Inc. who pair-wise tested different combinations of antibodies in the LFI format and delivered two lots of LFIs using the optimal antibody pair for detecting ricin. One lot incorporated native, CRP-supplied antibodies as the control assay, and the second lot employed the engineered antibodies.
- ❑ Established LFIs limit of detection (LOD) by antigen (ricin A chain) titration.
- ❑ LFIs with both native and ruggedized antibodies were baked at 70°C and 75°C for up to 24 hours prior to measuring activity.
- ❑ LFI performance measured using a CAMAG4 LFI Reader.
- ❑ ECBC's Advanced Design & Manufacturing Division (ADM) was tasked with developing of new LFI reader software and adapting the new LFI reader cartridges to the smartphone reader.

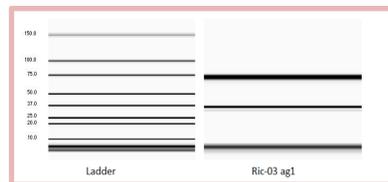


Figure 1. Ric-03 AG1 grown from hybridoma cell line at ECBC purified over protein A, 97% pure by experion analysis. anti-Ricin MAB2 was provided by the CRP.

- Striping**
- #A Anti- Ricin MAB2*
 - #B APE 36633.03
 - #C D12f101013
 - #D 1GX0207
 - #E 1GX2691
 - #F RIC-03-A-G1*
*non-thermostable

- Gold conjugation**
- #1 Anti Ricin MAB2
 - #2 APE36633.03
 - #3 D12 f102413
 - #4 1GX0207
 - #5 1GX2691
 - #6 RIC-03-A-G1

Results

Maxim Biomedical pair-wise comparisons: Two nitrocellulose membranes were striped from the 6 Ricin antibodies provided then conjugated to colloidal gold. Each combination of striped membrane and conjugated gold were tested to determine which antibody worked best as the capture (conjugated to gold) or detector (striped on the membrane) antibodies in the LFI.

Membrane	Gold
Native Anti-Ricin Mab2	Native RIC-03-A-G1
Thermostable APE36633.03	Thermostable 1AX2691

Table 1. Results of pair testing to determine which antibodies in the assay would work best as detector and capture antibody



Figure 2. Photo of developed LFI test tickets. Several range finding experiments were performed to determine the LOD of the assay. The final titration from 25 ng/ul to .1ng/ul revealed that the LOD for Ricin A chain is .75ng/ml

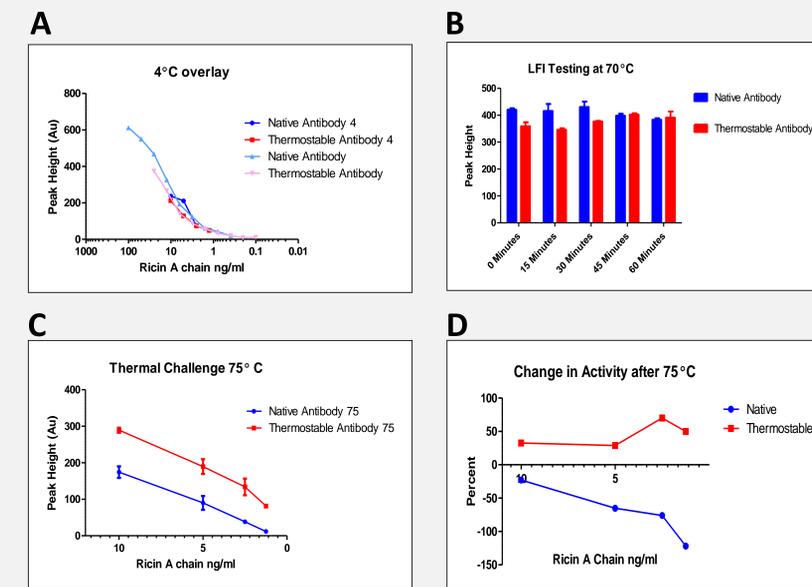


Figure 3. Limit of Detection and Thermal Challenge curves. A) Curves show limit of detection of 0.75ng/ml for both native and ruggedized antibody pairs when LFIs are stored at 4°C. B) Initial Thermal challenge showed no change in activity for both sets of LFIs at 70°C. C) After 24 hours at 75°C the thermostable antibody pairs maintain full activity down to 1.25ng/ml. D) Illustration of the percent change in activity between 4°C and 75°C degrees. The native antibody pair LFI loses activity at 75°C while the "ruggedized" LFI appears to gain activity.

Conclusions

Present results support the hypothesis that engineering antibodies for thermal stability can protect LFIs from loss of activity due to extended exposure to high heat.

Future Directions

- Both sets of LFIs should be further-assessed in a long term stability study.
- Further testing should also be done to assess other environmental variables (e.g., humidity).
- Continued partnership with ADM to optimize the smartCAR software.

Our Partners:

