

# From Antibody Engineering to Tools for First Responders: The DTRA Ruggedized Antibody Program

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## Abstract

The development of cold-chain independent reagents and assay systems for use in the field remain a priority for the military. The costs and logistical challenges of keeping a large inventory refrigerated from manufacture to point of use are incompatible with a smaller, more agile military. The Ruggedized Antibody Program (RAP) is an integrated portfolio of research and development efforts designed to enhance currently fielded antibody products through affinity, maturation and stabilization with the goal of producing detection products which can tolerate temperature extremes while maintaining function.

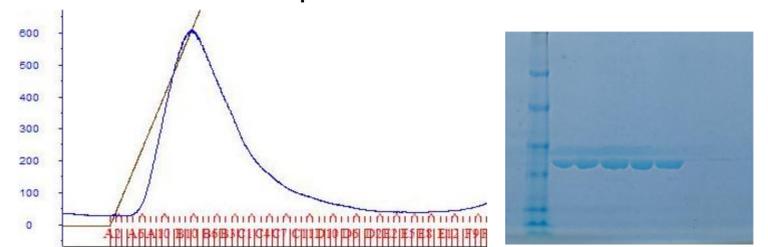
Under this program, antibody sequences from the Critical Reagents Program (CRP) are provided to contracted performers who apply their technologies to improve the antibody. The Edgewood Chemical Biological Center provides program support, standardized antigens, uniform testing of the starting material, and improved products from performers.

In addition to contracting antibody 'hardening' work, the RAP is researching means to use antibody fragments in detection products in lieu of full-length antibodies in order to capitalize on the greater speed and lower cost of synthetic antibody screening and production techniques. Finally, work is underway to graft antibody fragments or complementarity determining regions into known thermostable full-length scaffolds in cases where antibody fragments are ultimately unsuitable.

## Antigen Production

The first target for the RAP program is a set of anti-ricin antibodies. The ricin toxin is a select agent and an alternative to its use would be preferable for a variety of reasons. RiVax™ is a recombinant, mutant ricin A chain developed as a vaccine in which toxicity was eliminated through two point mutations.

## Expression and Purification



**Figure 1.** A typical chromatogram and SDS-PAGE gel from the RiVax™ purification procedure. B-strain *E.coli* expresses RiVax™ at high levels from pET21b when induced overnight in TB 'Overnight Express' media at 20°C. Following harvest and lysis, RiVax™ was purified to >90% purity (verified by SDS-PAGE, below) in one step of Ni-IMAC chromatography. Yields were >50 mg/L of culture.

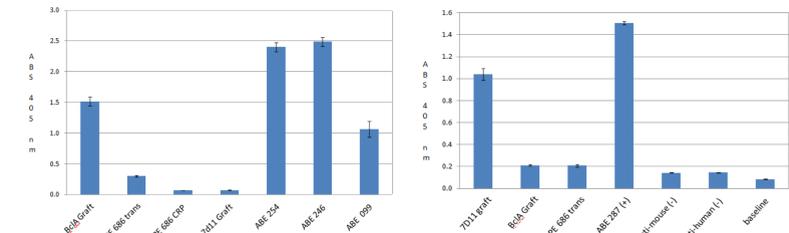
Despite the high soluble yields, purified RiVax™ precipitates rapidly in a TRIS-buffered saline buffer. Based on prior findings (Peek, Brey and Middaugh. 2007. A Rapid, Three-Step Process for the Preformulation of a Recombinant Ricin Toxin A-Chain Vaccine. *J of Pharm Sci*,96:1), our protocol was revised to include dialysis immediately post-purification into 25mM citrate phosphate, 1 M NaCl, and 20% glycerol. Dynamic light scattering data suggests a clean sample but shows a molecular weight of about 78 kDa, suggesting dimer formation in this buffer condition. RiVax™ prepared in this manner was compared to the ricin holotoxin and used to establish initial performance data on RAP anti-ricin antibodies.

## CDR Grafts to Thermostable Host

In addition to researching an 'adapter' system to make scFvs more readily useful in lateral-flow immunoassays, we are engaging in efforts to graft the complementarity- determining regions (CDRs) of antibodies with desirable antigen affinities and specificities into thermostable/robust IgG molecules. Should the adapter system strategy fail or underperform, this represents a risk-mitigation approach in which the CDRs from rapidly-discovered synthetic antibody fragments could be pressed into use in a format known to be generally compatible with lateral flow immunoassay (LFI) function.

Heavy Chain	Light Chain
<p>CDR 1</p> <p>..GSLRLSCAAS GFTFSSYAMS WVRQAPGKGL..</p> <p>..GSLRLSCAAS GFTFSSYAMS WVRQAPGKGL..</p> <p>..GSLRLSCAAS GFTFSSYAMS WVRQAPGKGL..</p> <p>..GSLRLSCAAS GFTFSSYAMS WVRQAPGKGL..</p> <p>CDR 2</p> <p>..KGLEWVC TISTGGGTYFPDSVK GRFTCS..</p> <p>..KGLEWVC SISDYGSIYSADSVK GRFTCS..</p> <p>..KGLEWVC YINPSTGYTEYNQKFKD GRFTCS..</p> <p>..KGLEWVC SISTGGDTHYQDSVK GRFTCS..</p> <p>CDR 3</p> <p>..EDTAVYYCAR QGDFGDWYFDV WGAGTIVTV..</p> <p>..EDTAVYYCAR GGQTFDY WGAGTIVTV..</p> <p>..EDTAVYYCAR TTVDGDFY WGAGTIVTV..</p> <p>..EDTAVYYCAT NRGYFDY WGAGTIVTV..</p>	<p>CDR 1</p> <p>..PASISC RSSQLVHNSGNTYLH WYQQR..</p> <p>..PASISC KSSQTLNSTRKKNYLA WYQQR..</p> <p>..PASISC RASQSISSYLH WYQQR..</p> <p>..PASISC SARSSVSYMY WYQQR..</p> <p>CDR 2</p> <p>..RPGQSPRLLIY KVSNRFS GVPDRFSGSGS..</p> <p>..RPGQSPRLLIY WASTRES GVPDRFSGSGS..</p> <p>..RPGQSPRLLIY AASSLQS GVPDRFSGSGS..</p> <p>..RPGQSPRLLIY RTSNHLAS GVPDRFSGSGS..</p> <p>CDR 3</p> <p>..EAEDVGVYFC QSSTHVPWT FGGGKLEIK..</p> <p>..EAEDVGVYFC KQSYNLMT FGGGKLEIK..</p> <p>..EAEDVGVYFC QQSYSTPMT FGGGKLEIK..</p> <p>..EAEDVGVYFC QQYHSYPPT FGGGKLEIK..</p>

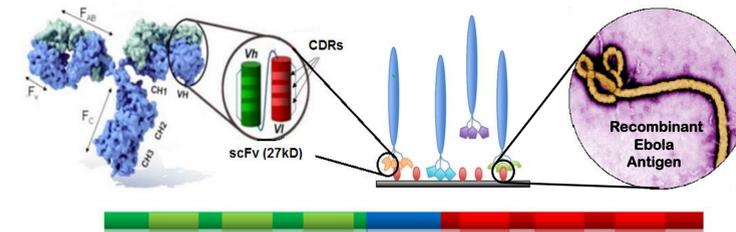
**Table 1:** CDR regions grafted into the anti-MS2 antibody. Predicted CDRs of target-binding antibodies were converted into coding DNA and incorporated into a single-stranded megaprimers (~500bp). New CDRs were polymerase chain reaction (PCR) amplified into heavy and light chain plasmids coding for the thermostable Anaptys IgG backbone, and were subsequently sequence verified by the Synthesis & Sequencing Facility at the Johns Hopkins University School of Medicine.



**Figures 2 and 3.** ELISA results for a BclA grafted IgG (top left) and 7D11 grafted IgG (top right). Assays clearly show binding of grafted IgGs to their respective antigen in relation to positive control antibodies. IgGs grafted for different antigens as well as transfected non-grafted anti-MS2 IgG were used as negative controls.

Antigen	Light Chain Sequences			Heavy Chain Sequences		
	CDR 1	CDR 2	CDR 3	CDR 1	CDR 2	CDR 3
Bot A	RSSTGAVTSSNYDN	GTDNRPP	ALWYSHHVV	GYAFSSWMN	RYPGDDGTNYGKFKD	EGVVDYG
Bot A	RSSTGAVTSSNYAN	GTDNRPP	ALWYSHHVV	GFTFTYDIN	WIYPNGRTEYSEKFK	GSHYDF
Bot	KSSQLNSTRKKNYLA	WASTRES	KQSYNLRT	GYTFSSWIE	EILPGSGSTNCNEKFKG	PYGGDLYYAMDY
Ebola	KASQDVSTAVA	SASRYT	QQHYSTPWT	GYTLTXGMX	WIWYTGATFADDFKG	GYGNVAGTWFPY
Ebola	KSSQXSDSDGRTYLN	LVSKLDS	WQGTHLPL	GYTFDDNLD	DIYPSNGNTIYNQKFKG	AGFDY
Ebola	RSSQLVHNSGNTYLH	KVSRFS	SQSTHVPT	GYTFSSWIMN	QIYLGDDGTNYGKFKD	GNRYRDEGFDK
Lassa Fever	TSSQLVHNSGNTYLH	LVSTRFS	SQSTHVPT	GFSLTYGVH	VIWRGGRTDYNAAFMS	NGNPNAMDY
Lassa Fever	RASDSVDTYGNSFIH	RASNLES	RASNLES	QSNEDPYT	YITYSGSTNYNPSLKS	KYRGDYPMDY
Lassa Fever	KSSQVLSDDQKNYLA	WASTRES	HQYLSWT	GFTFTNYMWT	RIDPYDSETHYNQDFKD	RLGLYSAMDY
Lassa Fever	RASESDYSGNSFMH	RASNLES	QSNADPYT	YISYNGKTSYNGKFKG	FHYDYRGLLTGAKGL	-
Rift Valley Fever Virus	KASQDVSNVA	SASRYT	QQHYSTPWT	GYPFSSWIE	EILPGSGSTNYNENFK	RANWVFAY
Rift Valley Fever Virus	KASQVDYDGDYSYMN	AASNLES	QQSDEDPWT	GFTFSYVMS	TISGGTYTYPDSVKG	-
SEB	KASQVNNFLS	RVSRVLD	LQYDDFPWT	GFSSDYMY	TISDGGGTYTYPDSVKG	DDAMDY
VEEV	KASQVNNFLS	RVSRVLD	LQYDDFPWT	GFNIKTYH	EILPGSGSTNCNEKFKG	SEGYGNFPAY
Vaccinia	RASQDISNYLN	YTSRLHS	QQGNTLPYT	GYAFSSWIMN	QIYPGDDGTNYGKFKG	GGLLRGFTY
Vaccinia	RSSTGAVTSSNYAN	GTDNRPP	ALWYSHHVV	GYAFSSWIMN	QIYPGDDGTNYGKFKG	GGLLRGFTY
West Nile Virus	KASQDVSTAVA	WTSTRHT	QQHYTMPLT	GYAFSSWIMN	RHPGDDGNTYNGEFRA	EGNDYDGFVD
West Nile Virus	KASQDVSTAVA	WASTRHT	QHYYATPRT	GFTFSYVMS	SIYHGNTYYPDSVKG	ERYDGFDFD
Anaptys MS2	RSSQLVHNSGNTYLH	KVSNRFS	QQHYSTPWT	GFTFSSYAMS	TISTGGGTYTYPDSVK	QGDFGDWYFDV
Vaccinia	RASQSISSYLH	AASSLQS	QQSYSTPMT	GYTFTRYWMH	YINPSTGYTEYNQKFKD	TTVDGDFAY

**Table 2.** CDRs that were identified via Rosetta or manual alignment. Sequences of the mRNAs expressed in various hybridoma lines supplied by the C-Reactive Protein (CRP) were obtained by PCR-amplifying the antibody variable regions using primers designed by Essono et al. 2003, and then sequencing the PCR products.



**Figure 4:** Phage display strategy to optimize CDR recognition. Ebola ligand development is currently underway to utilize scFvs displayed on phage which are derived from a newly designed thermostable IgG scaffold. This allows CDR regions from the binding scFvs to be directly incorporated (grafted) back into the full-length thermostable IgG with little to no distortion of CDR conformation.

## Performer Antibody Testing

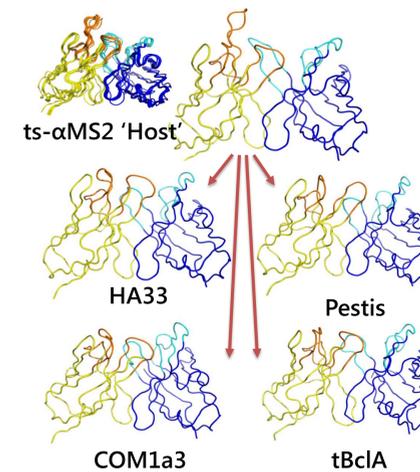


ECBC's Biotechnology Quality Capability: ISO: 17025

ECBC achieved a major landmark with the achievement of International Standard ISO/IEC 17025:2005 accreditation. Quality testing is performed in accordance with rigid quality control procedures and quality management oversight to assure that a product meets pre-defined physical and/or functional properties. For a laboratory to be ISO 17025 accredited, they must be consistent as well as proficient in testing the quality of their products. ECBC's scope of accreditation includes; Experion, Dynamic Light Scattering (DLS), NanoDrop, and Differential Scanning Calorimetry (DSC).

## Structure-Guided Grafting

While simple grafting based on bioinformatic CDR predictions can be successful, we are investigating the use of structure-based computational methods to increase efficiency and the likelihood of obtaining chimeric molecules with favorable properties. The Rosetta Antibody Server automates antibody modeling algorithms which we intend to utilize and possibly extend in function in collaboration with the Gray Research Group at Johns Hopkins University.



**Figure 5.** We are investigating the use of modeling and energetic evaluation to guide the grafting process. We aim to use simple evaluations as a predictor for success and to build a capacity for semi-automated prediction of compensatory/adaptive mutations.

## Future Directions

Efforts to ruggedize antibodies for Department of Defense use will continue, with external performers leveraging their technologies to improve existing CRP antibodies and ECBC supporting these efforts by supplying standardized antigens and validating the 'hardened' end-product antibodies. Additional aims include continuing efforts to apply fast and efficient antibody-fragment-based discovery, maturation, and stabilization techniques to creating antibody forms compatible with commodity or LFI assays. ECBC is increasing its ability to support these efforts through more advanced antigen production including the use of in-house gene synthesis, cell-free expression screening, and the use of mammalian and insect expression systems. ECBC is also pursuing the transition of some performer technologies in-house to be applied to select agent targets or other antigens of interest.

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