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Progress in the Development of a Vi-CRM Conjugate Vaccine

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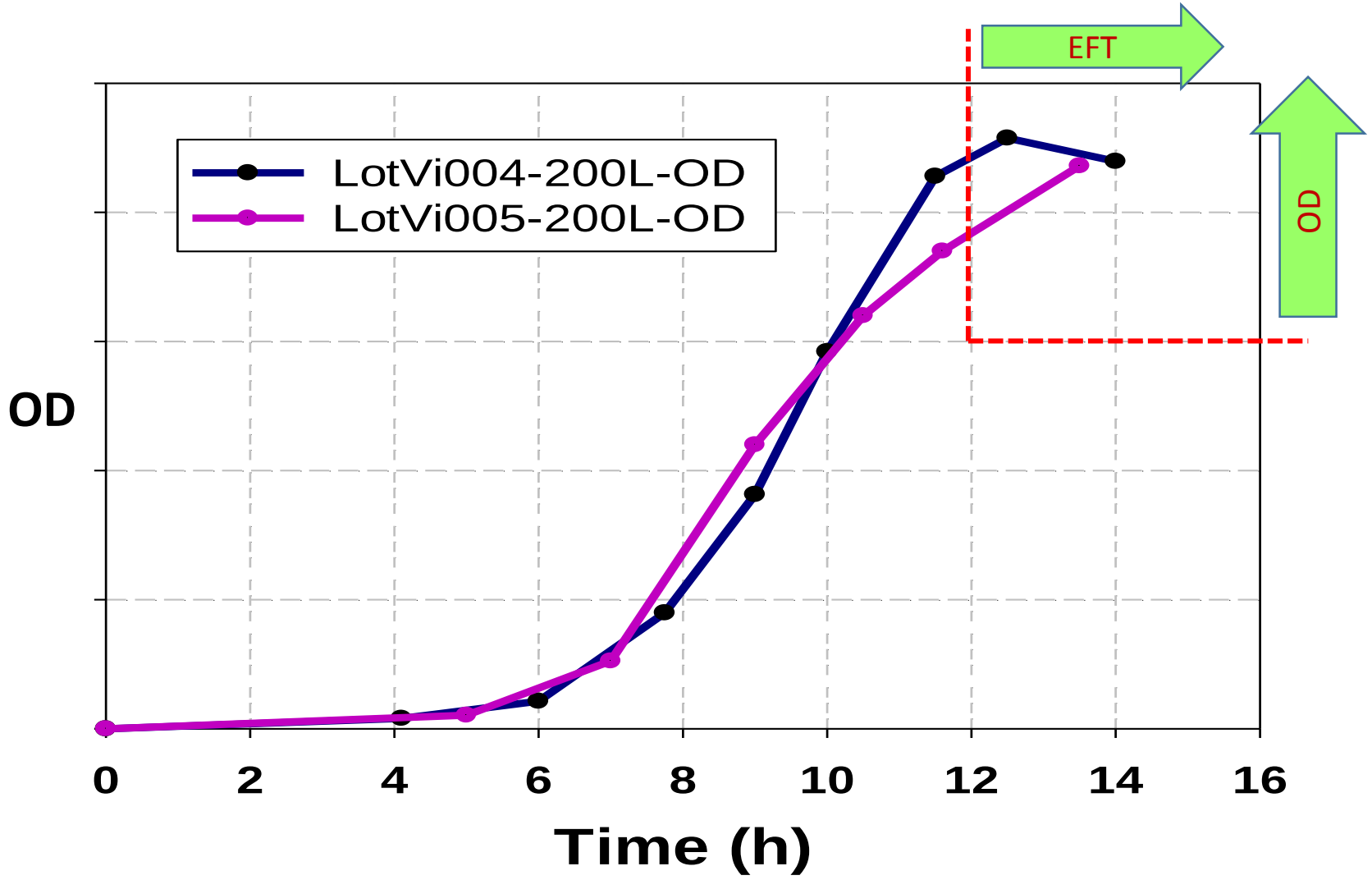
Vi Polysaccharide

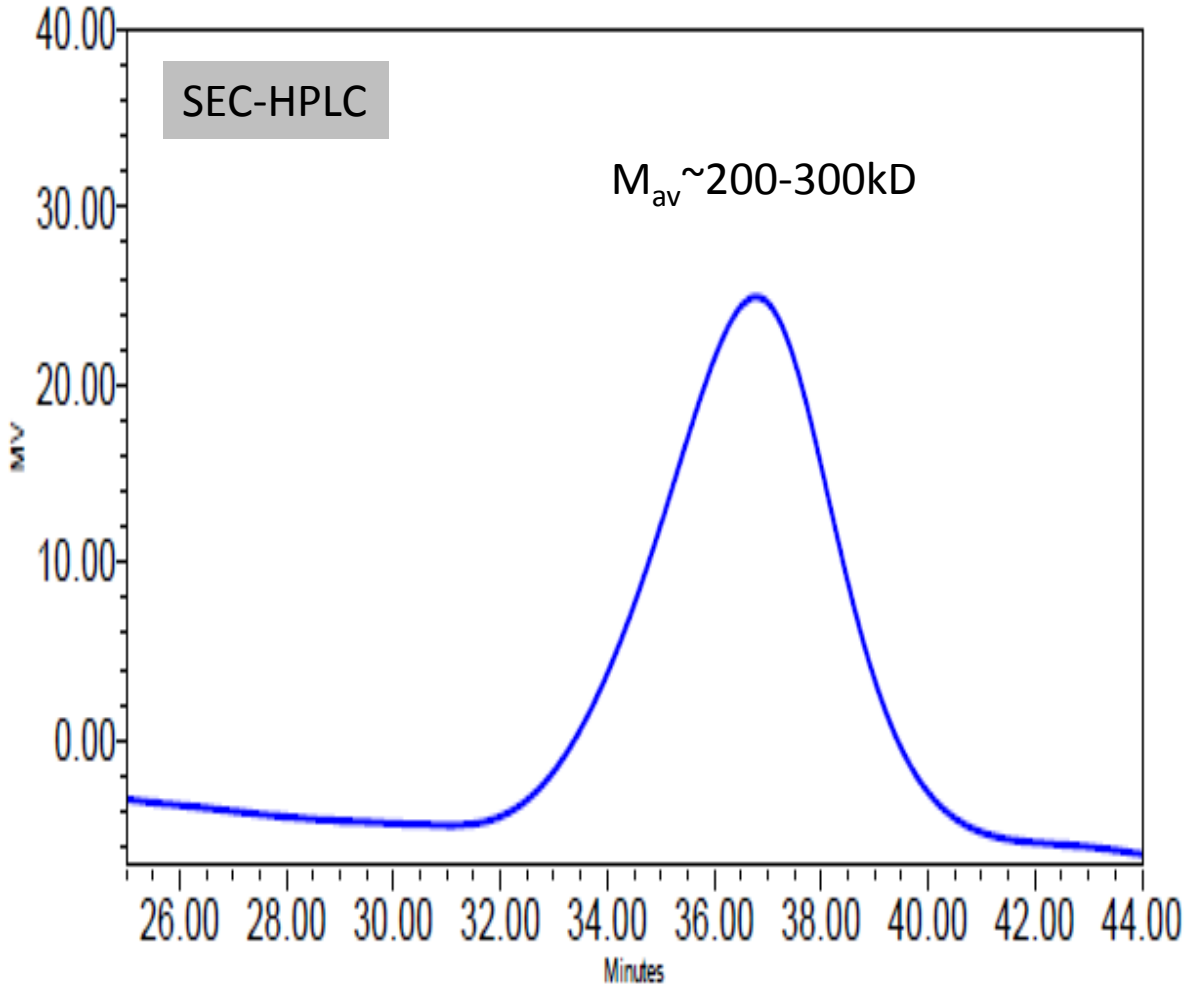
- Vi Polysaccharide derived from *Citrobacter freundii* sensu lato
- BSL-I, rapid growth, high yields
- Vi PS NMR identical to *Salmonella* Typhi



Purified Vi Polysaccharide

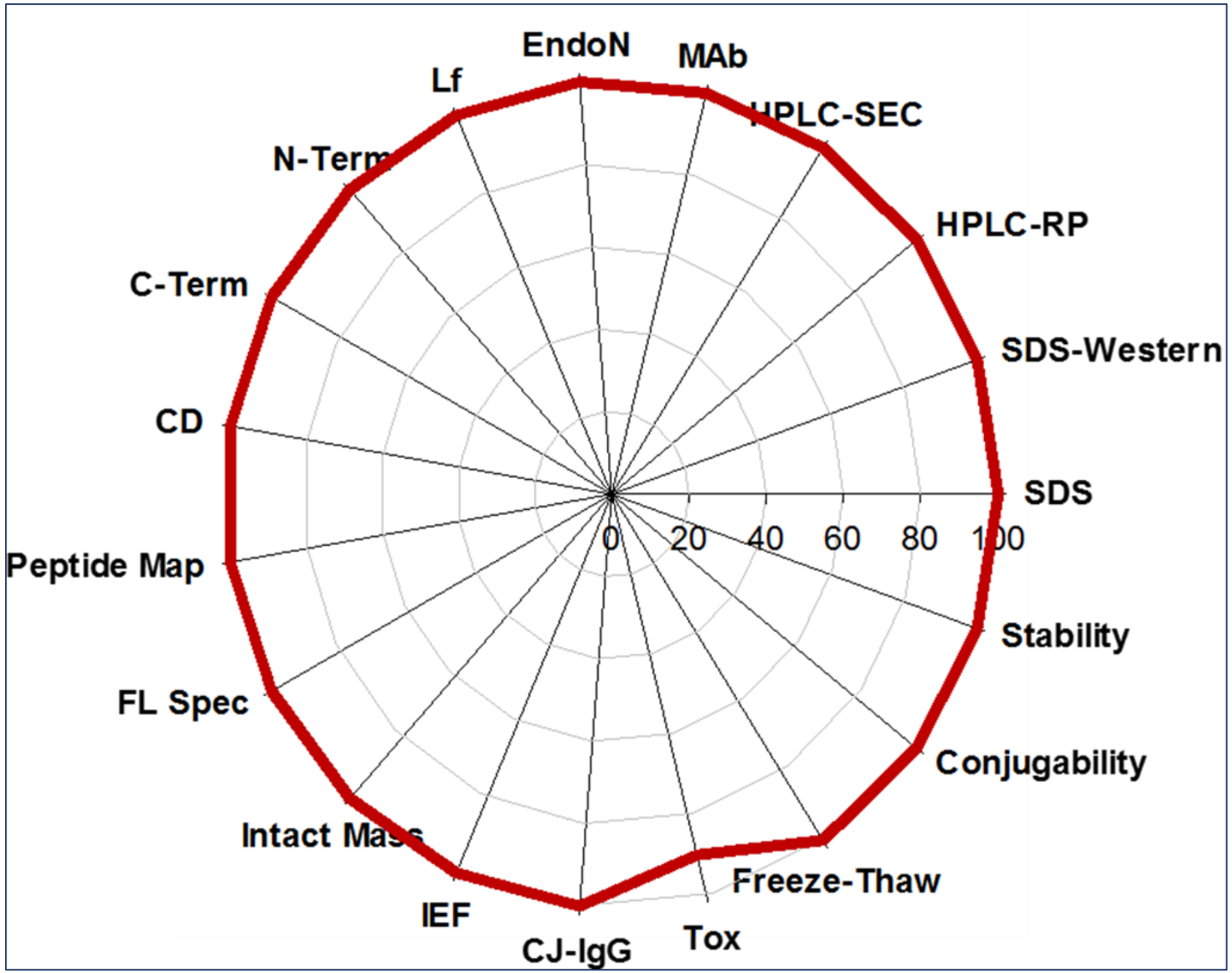
Purified Vi-PS meets WHO TRS requirements

- NMR
- O-Acetyl Content
- Size
- % Pr
- %NA
- Endotoxin
- Residual reagents

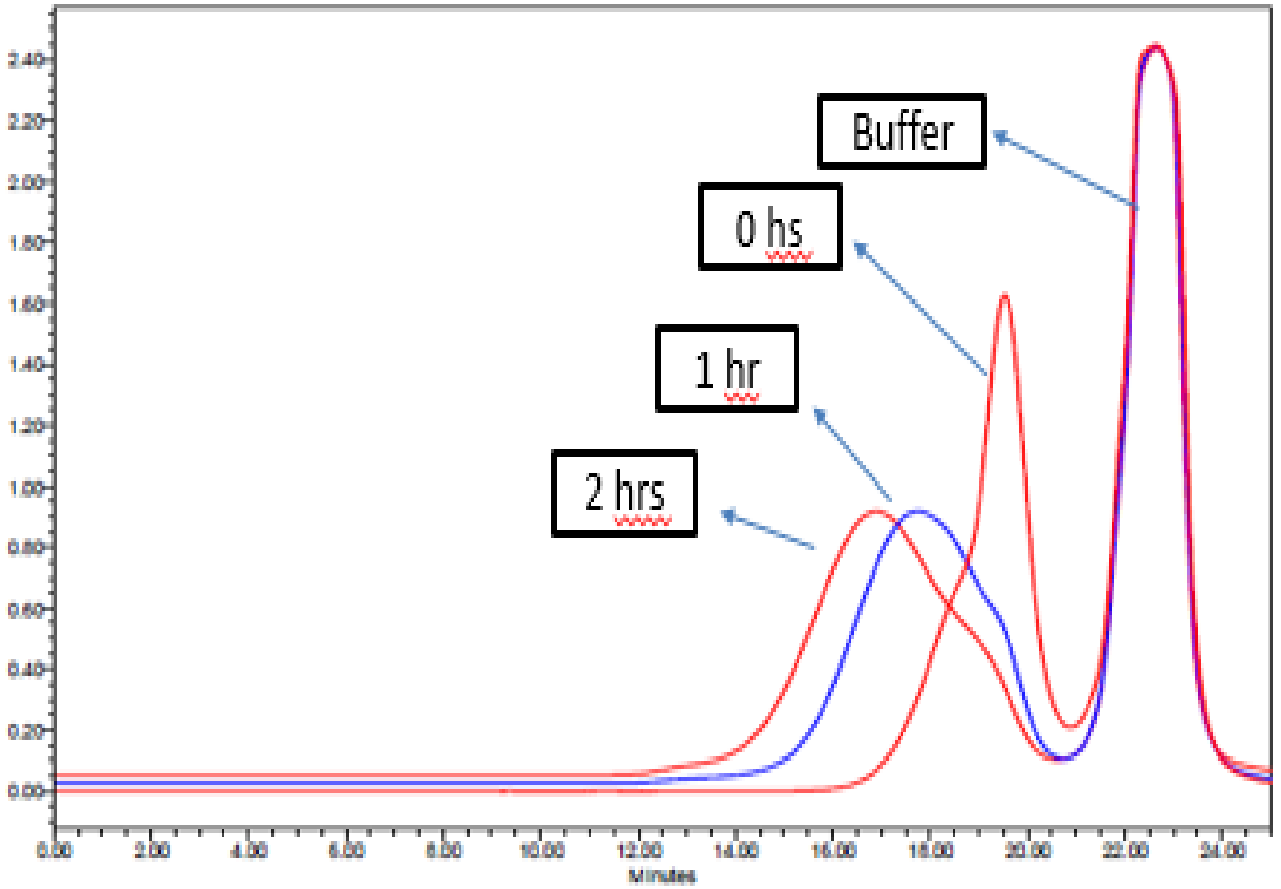


$rCRM_{197}$

- Developed at BioE using *E. coli* host
- Process demonstrated to be robust, meets yield criteria
- $rCRM_{197}$ meets all quality criteria



Vi-CRM_{197} Conjugation Kinetics



S. No.	Process step
1	CRM_{197} Derivatization
2	CRM_{197}-ADH Purification
3	Vi activation
4	Conjugation
5	Depth filtration of Conjugation Mixture
6	Vi-CRM_{197} Conjugate Purification
7	Buffer Exchange of Vi-CRM_{197} Conjugate
8	0.22 µm Filtration

Vi-CRM Conjugates : Critical to Quality

Bulk Conjugate

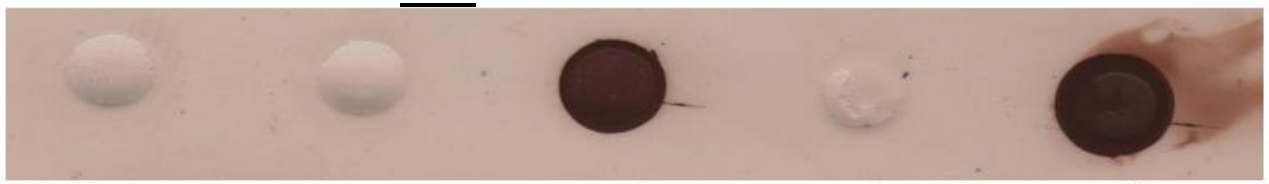
- Identity
- Vi Concentration
- Vi:CRM ratio
- Size
- % free PS
- O-Acetylation level
- Residual reagents
- Endotoxin
- Stability

Formulated Bulk

- Identity
- Vi Concentration (25 μg/0.5 mL)
- Vi:CRM ratio
- Size
- % Free PS
- Sterility
- Osmolarity, pH
- Stability

ID of Vi and CRM_{197} in Conjugate by Dot Blot

Identification of CRM_{197} in conjugate

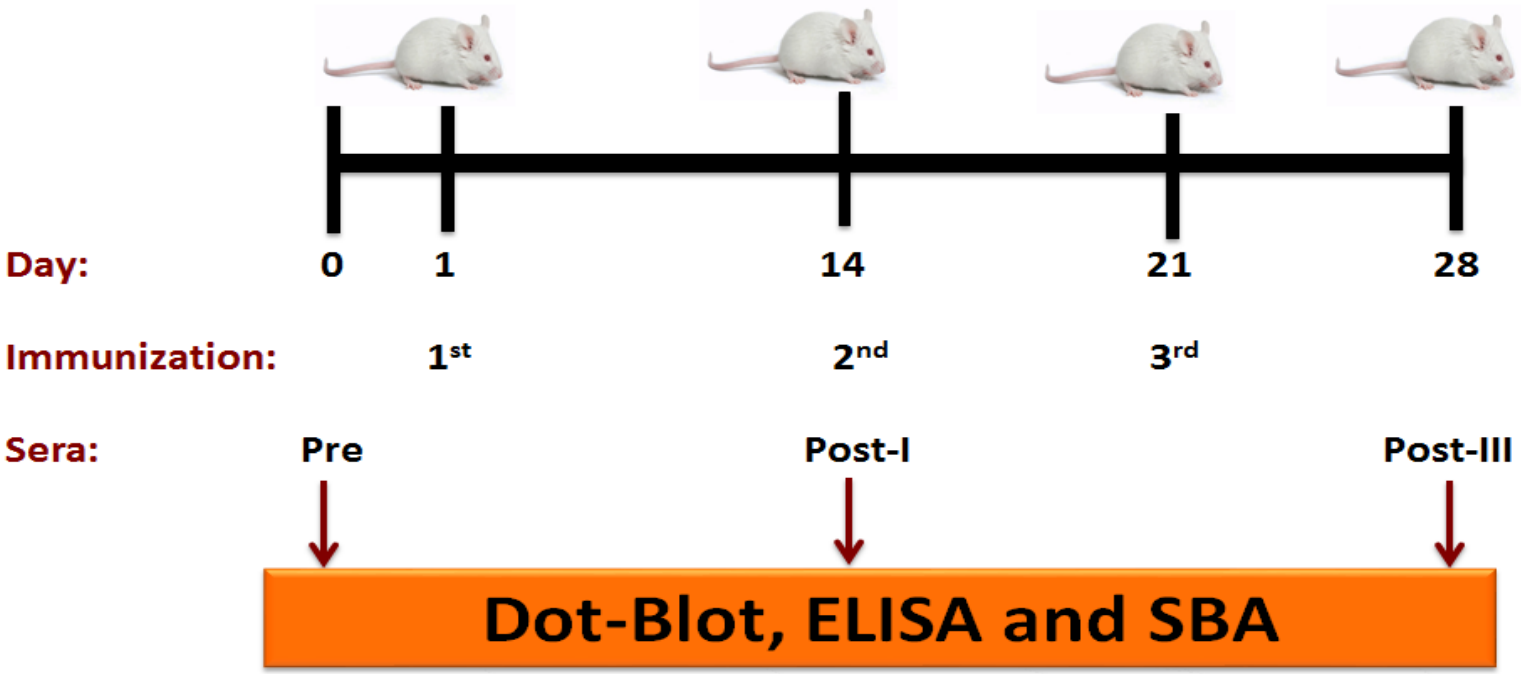


Expected	Negative	Negative	**Positive**	**Positive**
Obtained	Negative	Negative	**Positive**	**Positive**

Identification of Vi in conjugate

Expected	Negative	**Positive**	Negative	**Positive**
Obtained	Negative	**Positive**	Negative	**Positive**

Balb/c Mice Immunization Plan for TCV



Study Plan:

1. Mice:
   - Inbred Balb/C Female SPF Mice
   - < 6weeks old
   - 20 mice/per group
2. Route: Subcutaneous
3. Dose: 2.5µg vaccine/100µl , 3 dose
4. Sera collected by terminal bleeding

Responses Evaluated:

- Anti-Vi IgG (Fold increase over Placebo and over PS only)
- Booster Effect

Samples Evaluated:

1. BE Vi-rCRM
2. BE Vi-rCRM
3. Vi PS negative control (BE Vi)
4. Vi-conjugate positive control
5. Vi PS negative control (native)
6. PBS Placebo

World Health Organization

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Guidelines on the quality, safety and efficacy of typhoid conjugate vaccines:

B.3 Nonclinical immunogenicity studies

Immunogenicity studies in animal models should be conducted because they provide valuable proof-of-concept information that can be used to support a clinical development plan. In addition, immunogenicity data derived from appropriate animal models are useful in establishing the immunological characteristics of the Vi polysaccharide conjugate product, and may guide the selection of doses, schedules and routes of administration that will be evaluated in clinical trials. To ensure immunogenicity in nonclinical testing weaning mice (younger than 6 weeks) should receive intramuscularly 2 injections 2 weeks apart of the conjugate vaccine and Vi should be used for a control group. Anti-Vi IgG should then be measured. The conjugate should induce a response that is at least four times higher than the response induced by Vi, and a booster response should occur after the second dose (*100*). Immunogenicity studies of Vi polysaccharide conjugates have been conducted in mice (*71, 93, 113–115*); in humans, correlation has been made between the level of anti-Vi IgG and protection against clinical disease (*53, 116*). Therefore, the primary endpoint for nonclinical studies of the immunogenicity of Vi conjugate vaccines should be the level of anti-Vi elicited.

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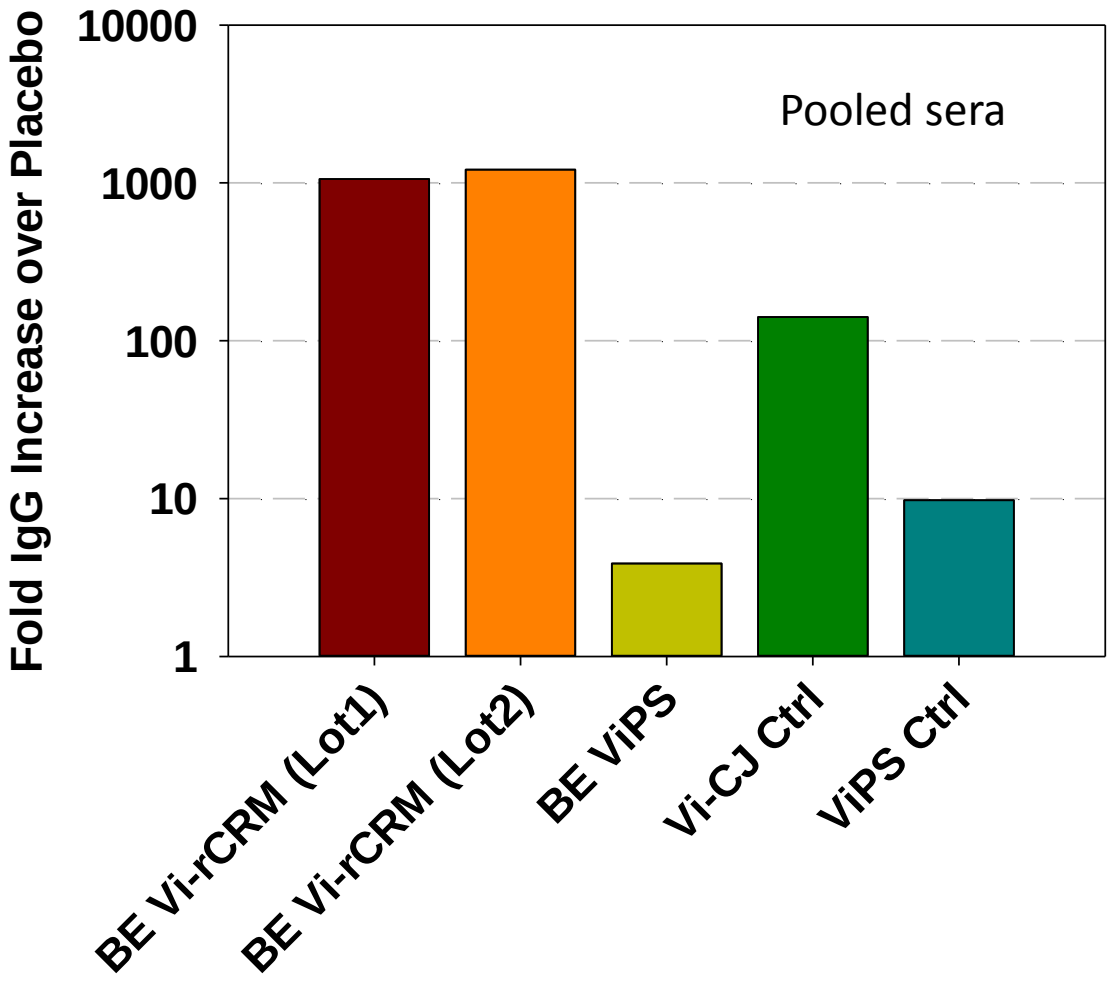
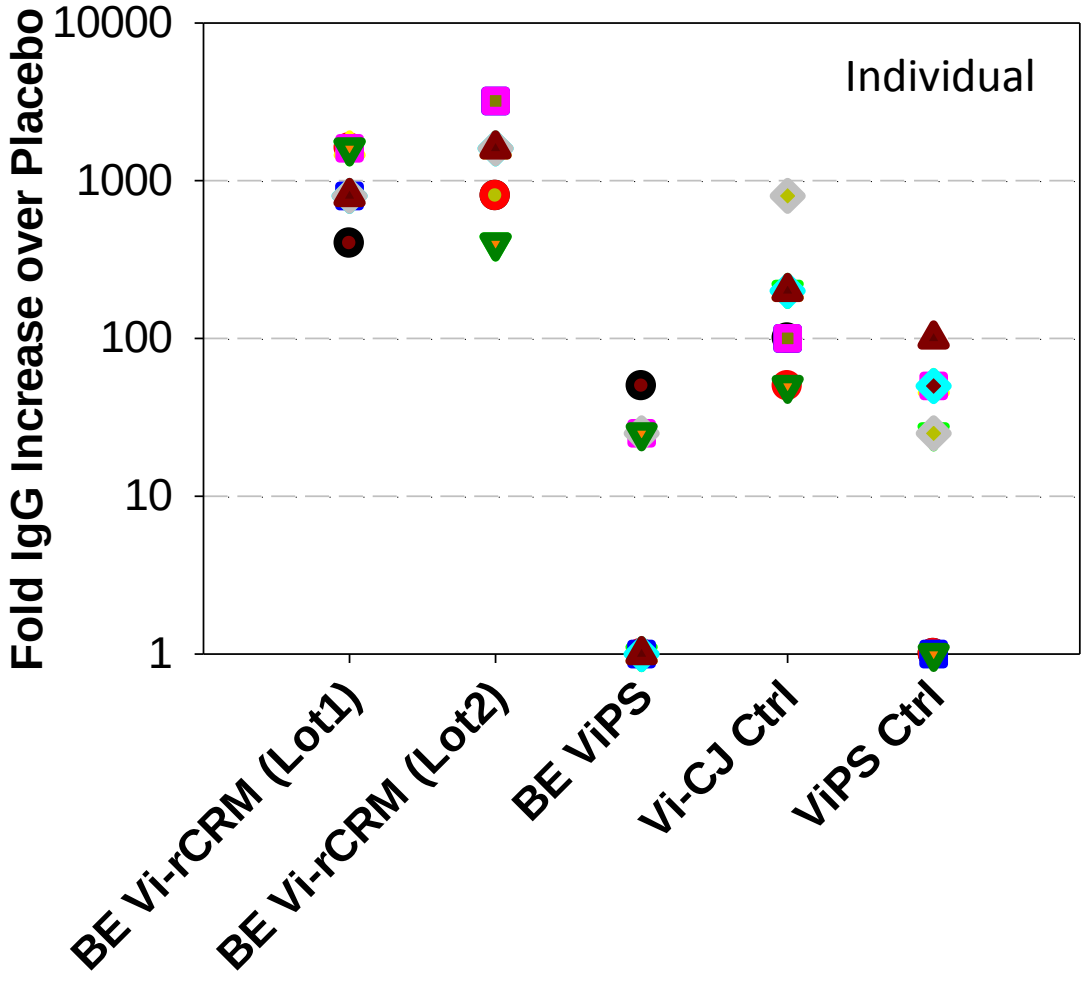
The Conjugate should induce a response that is at least four times higher than the response induced by Vi ……..

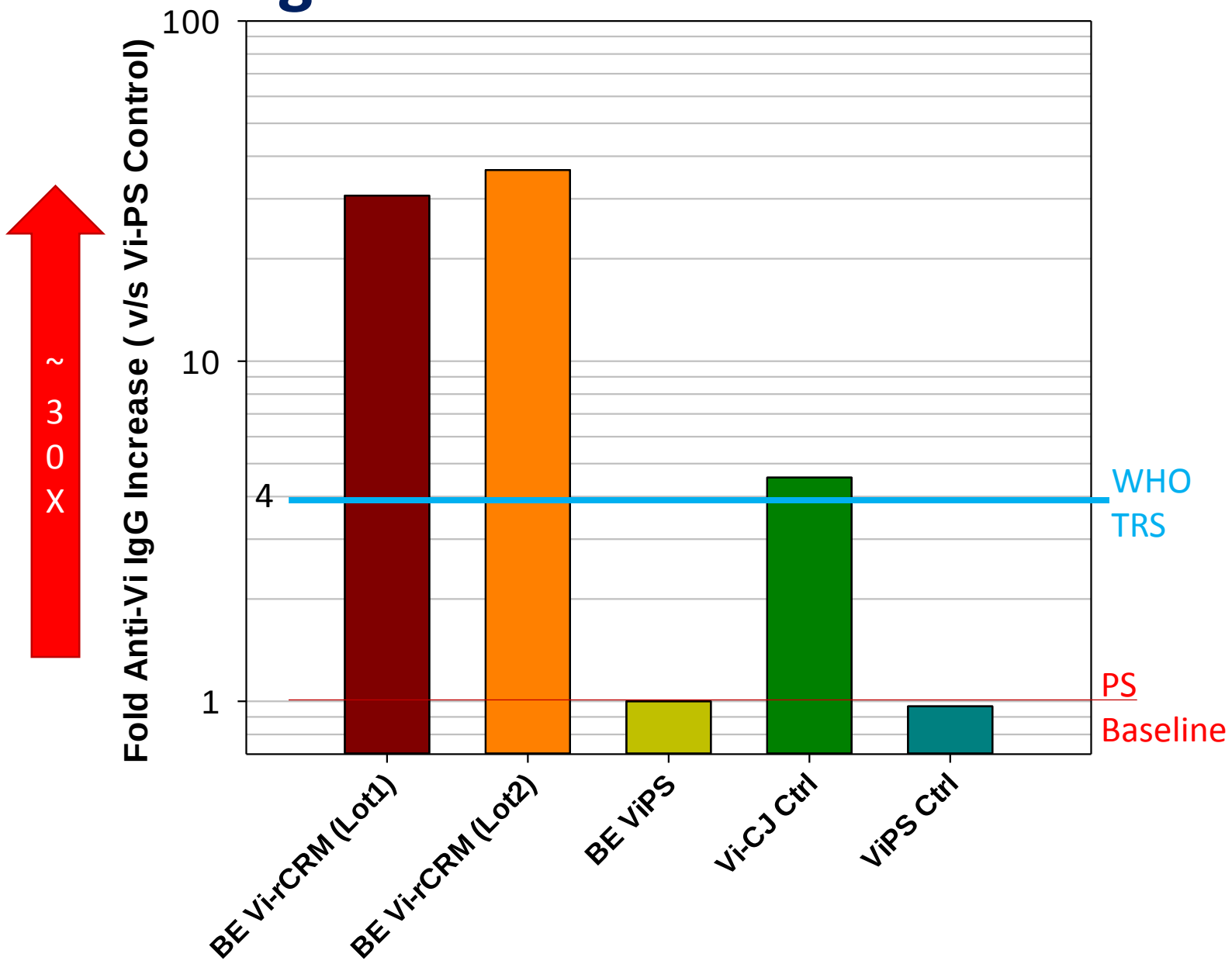
A booster response should occur after the 2nd dose ……..

Mouse Data (Post 3rd Dose) Fold-increase over Placebo





Anti- Vi IgG ELISA: Fold Increase Over PS Baseline



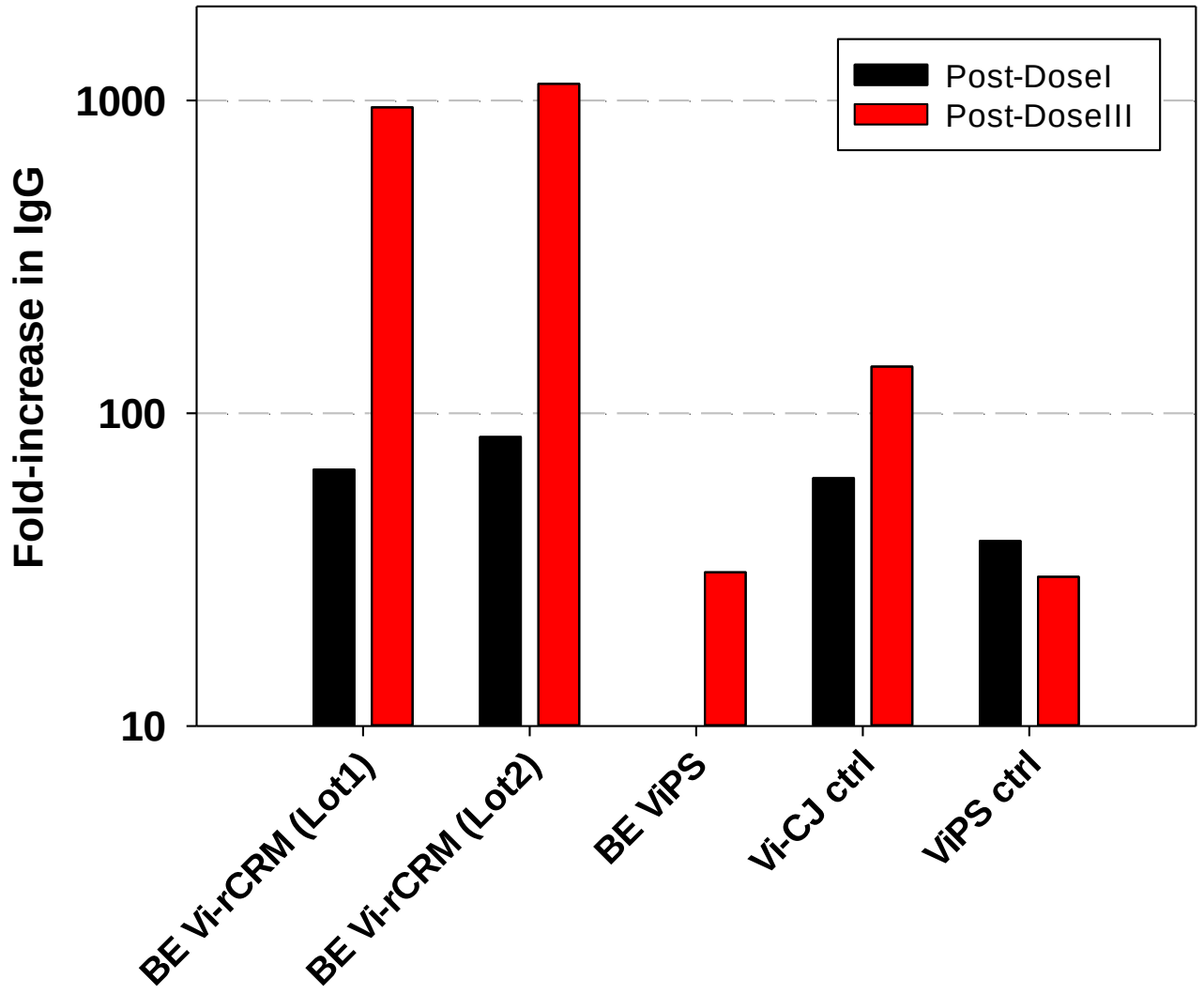
Samples

1. BE Vi-rCRM Lot 1
2. BE Vi-rCRM Lot 2
3. BE Vi PS control
4. Vi-CJ positive control
5. Vi PS negative control

Conclusions:

1. **Significant (30X) increase in anti-Vi IgG levels observed for both Vi-rCRM samples when compared to PS baseline.**
2. **BE product meets the 4X threshold mentioned in WHO TRS.**

Evidence of Booster Response – Dose I and III



Fold-increase over placebo baseline

Initial Immunogenicity Evaluation
Vi-rCRM$_{197}$: Conclusions

- BE Vi-rCRM is highly immunogenic in mice. BE Vi-rCRM preclinical immunogenicity results meet WHO TRS requirements.
- BE Vi-rCRM elicits a booster response in mice.
- BE Vi-CRM conjugate have similar immunogenicity to other reported conjugates
  - Vi-CRM by NVGH
  - Vi-rEPA by Szu et al
  - Vi-rCRM by Eubiologics
  - Vi-DT by IVI

Next Steps

- BioE Vi-CRM targeted to be in clinical trials in 1Q16
- Additional lots under preparation for preclinical immunogenicity evaluation in mice and rabbit models
- BioE also working on bivalent TCV candidate vaccine (Vi-CRM and O:2-CRM). Preliminary preclinical immunogenicity evaluation ongoing.