Potency testing for NTD vaccines: determining relative potency for the *Na*-GST-1 Human Hookworm Vaccine

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Abstract

Over the next decade, a new generation of vaccines will target the neglected tropical diseases (NTDs). The goal of most NTD vaccines will be to reduce the morbidity and decrease the chronic debilitating nature of these often-forgotten infections - outcomes that are hard to measure in the traditional potency-testing paradigm. The absence of measurable correlates of protection, a lack of permissive animal models for lethal infection, and a lack of clinical indications that do not include the induction of sterilizing immunity required us to reconsider the traditional bioassay methods for determining vaccine potency. Owing to these limitations, potency assay design for NTD vaccines will increasingly rely on a paradigm where potency testing is one among many tools to ensure that a manufacturing process yields a product of consistent quality. This potency test is a bioassay using BALB/c mice, which evaluates the immunogenicity of the vaccine at set time interval post manufacture. Herein, we discuss the results of 12 month potency testing of Necator americanus-glutathione-S- transferase-1 (*Na*-GST-1) vaccine. The Effective Dose 50 (ED50), with its 95% fiducial limits (FL) for each time point was determined along with the Relative Potency with its 95% FL for 3, 6, 9 and 12 months post manufacture. Potency testing has shown that storage at 4° C decreases the ED50 and increases the relative potency of *Na*-GST-1 vaccine. We proposed that the change in ED50 and relative potency coincide with higher affinity binding of the *Na*-GST-1 to the Alhydrogel® that occurred during storage at 4° C. These preclinical results lay the foundation for moving forward with Phase 1 clinical trial in Brazil.

Na-GST-1 Hookworm Vaccine

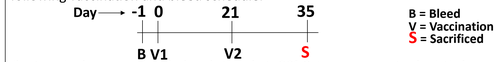
Necator americanus glutathione-S-transferase-1 (*Na*-GST-1) is a 24-kDa protein from N. americanus that has peroxidase activity and catalyzes the conjugation of reduced glutathione to a variety of electrophiles. *Na*-GST-1 exhibits a high affinity for heme in vitro. Because both heme and hematin contain oxidative iron, they can generate toxic reactive oxygen species that damage parasite structures. Hookworm GSTs may bind and detoxify heme and hematin byproducts generated during the blood degradation process. The recombinant polypeptide *Na*-GST-1 was expressed in Pichia pastoris. *Na*-GST-1 Hookworm Vaccine Drug Product was formulated at 0.1 mg/ml of *Na*-GST-1 with 0.8 mg/ml of Alhydrogel®. The cGMP manufactured vaccine was stored at 4° C.

Methods

Potency testing was performed using Female Balb/c mice. This assay utilizes a quantal response or the achievement of a threshold of murine IgG against *Na*-GST-1 above which we could assign an individual serum as being 'positive'. The response threshold was obtained using the standard reference serum . We developed a standard reference serum (SRS) that we assayed on each ELISA plate as a standard reference curve.

Standard Reference Serum

Standard Reference Serum was generated by vaccinating sixty Balb/c mice with 0.05µg *Na*-GST-1 + 80µg Alhydrogel® + 5µg CpG10104 intramuscularly using the following vaccination and bleed schedule.



The terminal sera was pooled and stored at -80° C. Four-parameter logistic log (4-PL) fit of the standard reference curve data generated by serial dilution of standard reference serum on ELISA plate were plotted on a logistic-log scale. The 4-parameter standard reference curves were generated at multiple time points.

Methods

Cumulative curves were obtained from individual time points. Test (ANOVA) of parallelism was performed by linearizing these cumulative curves [1]. A global standard reference curve with the 95% CI was generated using these cumulative curves[1]. A response threshold was estimated using the above global curve [1]. The *Na*-GST-1 drug product was fractionated by groups to generate the following doses.

Table 1. Study Design

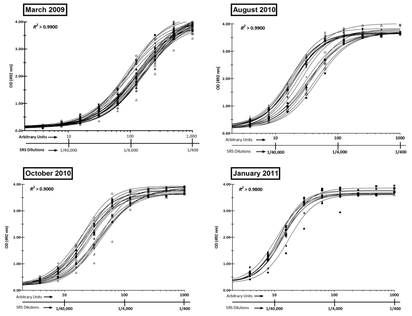
Na-GST-1 (µg)	Alhydrogel® (µg)	Volume (ml)	Animals (n)
N/A	240	0.3	10
30	N/A	0.015	10
30	240	0.3	10
17	136	0.17	10
10	80	0.1	10
6	48	0.06	10
3	24	0.03	10
2	16	0.02	10
1	8	0.01	10

Ninety mice were divided into 9 groups. Mice were vaccinated Intra-peritoneally using the above doses and the following bleed and vaccination schedule.

The above potency animal study was performed at 0, 3, 6, 9 and 12 months post manufacturing. The probit transformation of the percentage of responders on day 28 in each dose group were plotted against the log10-transformed dose of *Na*-GST-1. ED50 (Effective Dose 50) was obtained using the graphical interpolation as well as using the methods described in European pharmacopeia[2]. Similarly, relative potency was calculated using the methods described in European pharmacopeia[2]. Here, the relative potency compare the potency (immunogenicity) of *Na*-GST-1 vaccine at time 3, 6, 9 and 12 months post manufacture to that of its potency at time 0 month (time of release).

Results

Figure 1. Standard Reference Curves



Results

Figure 2. Cumulative Standard Reference Curves

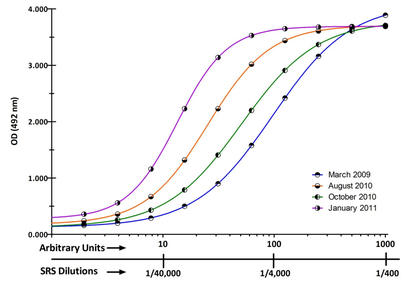


Figure 3. Parallelism of linearized standard reference curves

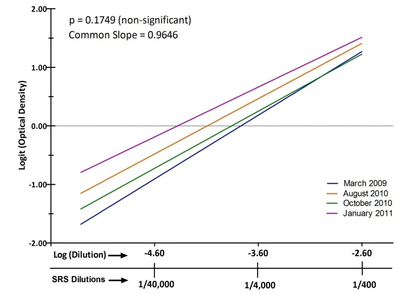
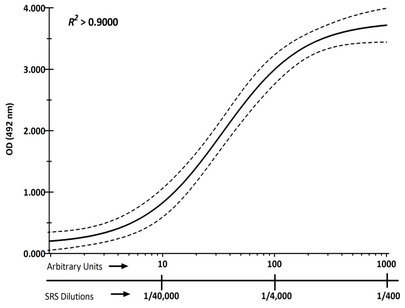


Figure 4. Global Standard Reference Curve



Results

Figure 5. Response Threshold (Global Standard Reference Curve)

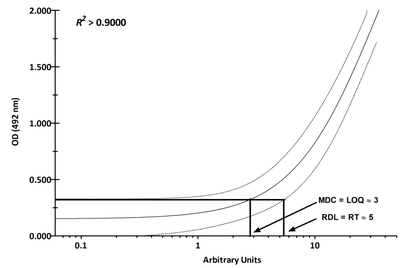


Figure 6. ED50 using probit transformed percent responders

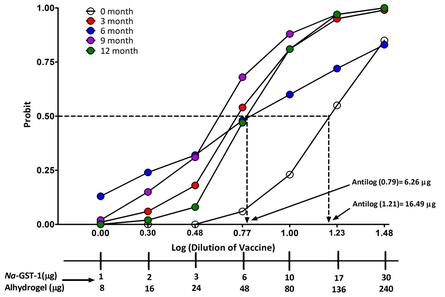
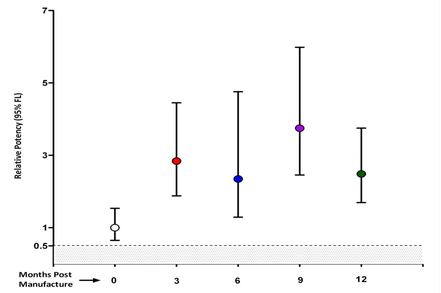


Figure 7. Relative Potency[2]



Results

Table 2. ED50[2] and Relative Potency[2]

Months post manufacture	0	3	6	9	12
ED50 (µg)	16.49	5.57	6.67	4.22	6.26
95% Fiducial Limits (µg)	(11.72--23.20)	(4.09--7.57)	(3.63—12.23)	(3.06--5.82)	(4.74--8.28)
Relative Potency	1.00	2.84	2.35	3.75	2.49
95% Fiducial Limits	(0.65--1.54)	(1.88--4.45)	(1.29—4.76)	(2.45—5.98)	(1.70--3.75)

- A cumulative response threshold of 5AU was estimated using the Global Standard Reference Curve (Figure 5).
- ED50 of the *Na*-GST-1 vaccine was found to be 16.49 µg, 5.57 µg, 6.67 µg, 4.22 µg and 6.26 µg at 0, 3, 6, 9 and 12 months post manufacture respectively (Figure 6, Table 2).
- Relative potency of *Na*-GST-1 vaccine was found to be 2.84, 2.35, 3.75 and 2.49 at 3, 6, 9 and 12 months post manufacture respectively (Figure 7, Table 2).
- The specification submitted in our IND states, "The criterion for acceptance is that the 95% upper fiducial limit of the estimated relative potency is not less than 0.5".
- The upper 95% fiducial limits of the relative potency at 3,6, 9 and 12 months post manufacture was found to be 4.45, 4.76, 5.98 and 3.75 respectively.

Conclusions

- The *Na*-GST-1 vaccine gain potency within the first three months of its storage at 4° C and remain immunogenic 12 months post manufacture.
- We proposed that the gain in potency within the first three months coincides with the higher affinity binding of the *Na*-GST-1 to the Alhydrogel® that occurred during storage at 4° C.
- The *Na*-GST-1 vaccine shows increased potency at each of the tested time points compared to its potency at the time of manufacture.

References

[1]Jariwala AR, et al. Potency testing for the experimental Na-GST-1 hookworm vaccine. Expert Rev Vaccines 2010;9:1219–1230.

[2]European Pharmacopoeia Commission. Statistical analysis of results of biological assays and tests. In: European Pharmacopoeia, 6th edition. Council of Europe, Strasbourg, France, 571–600 (2008).

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