

Optimal Vaccine Variables to Produce Antibodies Against Small Molecules

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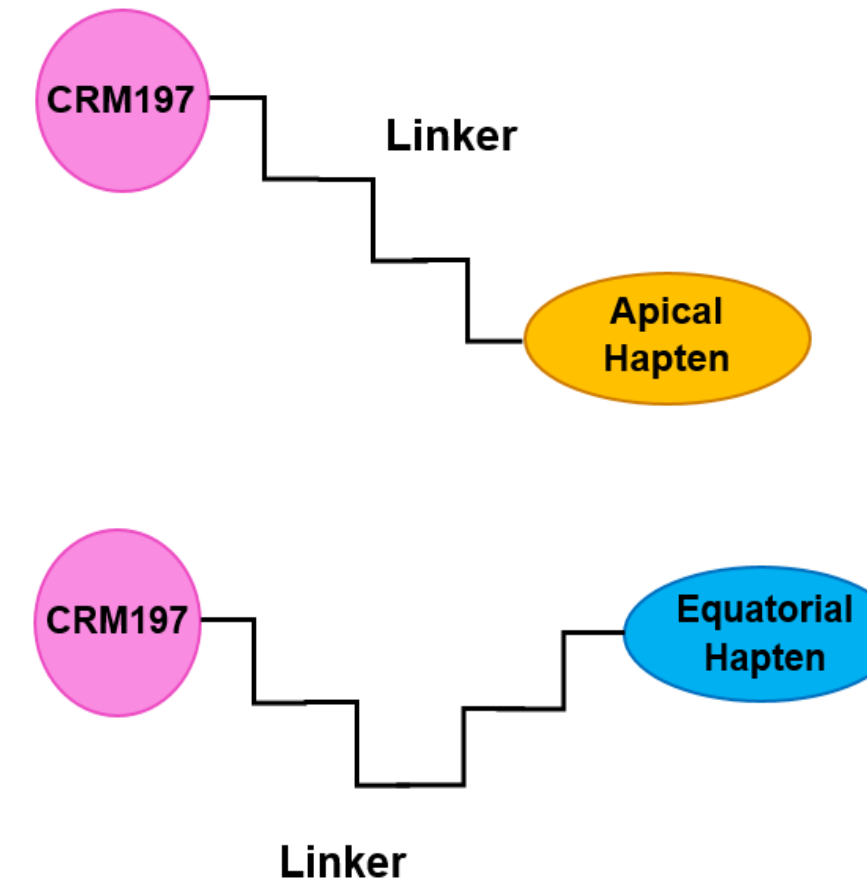


Introduction

- Small molecules are not immunogenic on their own – they require conjugation to a larger carrier protein to induce an immune response (called hapten-carrier conjugates)
- Several factors effect vaccine efficacy
 - Hapten structure and density
 - Linker design
 - Carrier protein selection
 - Adjuvant formulation
 - Vaccine delivery method and schedule



- Vaccine design:** CRM197 carrier protein conjugated to a small molecule (hapten). 68 possible combinations of the following parameters were administered to groups of 10 CD-1 male mice:
 - 9 linkers (varied in length and chemical structure)
 - 2 hapten structures (apical, equatorial)
 - 2 hapten densities (high, low)
 - 2 adjuvant formulations (AddaS03, AdjuPhos)



Linkers

Gly4	PAS1	Gly4PASGly4
Gly8	PAS2	PEG4
Gly12	PAS3	PEG6

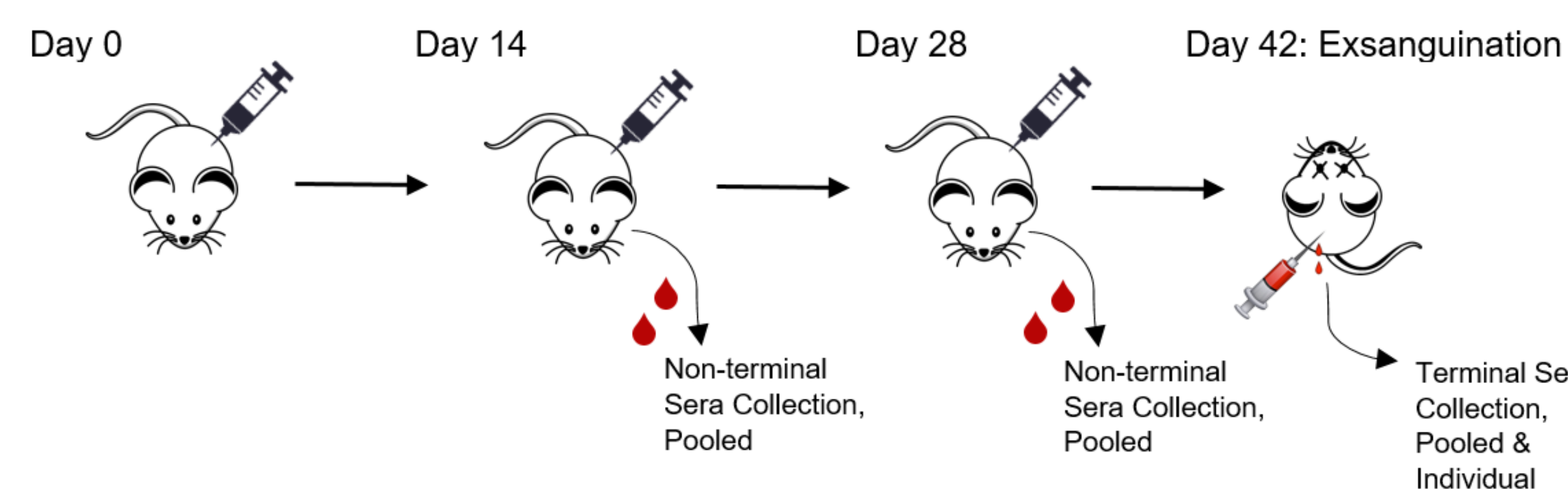
- ELISA design:** Standard ELISA methods were used. A BSA carrier protein conjugated to the same hapten as given in the vaccine was used to detect the presence of anti-hapten antibodies. Both pooled and individual animal sera were evaluated.



- Combinations of parameters were down-selected to determine the optimal vaccine variables to induce antibodies against small molecules in CD-1 male mice

In Vivo Vaccination

- 68 Groups consisting of 10 CD-1 male mice injected subcutaneously with vaccine combinations on **Day 0, Day 14, and Day 28**
 - 100µl injections on all three days, each containing 18µg of hapten



ELISA on Day 42 Pooled Sera

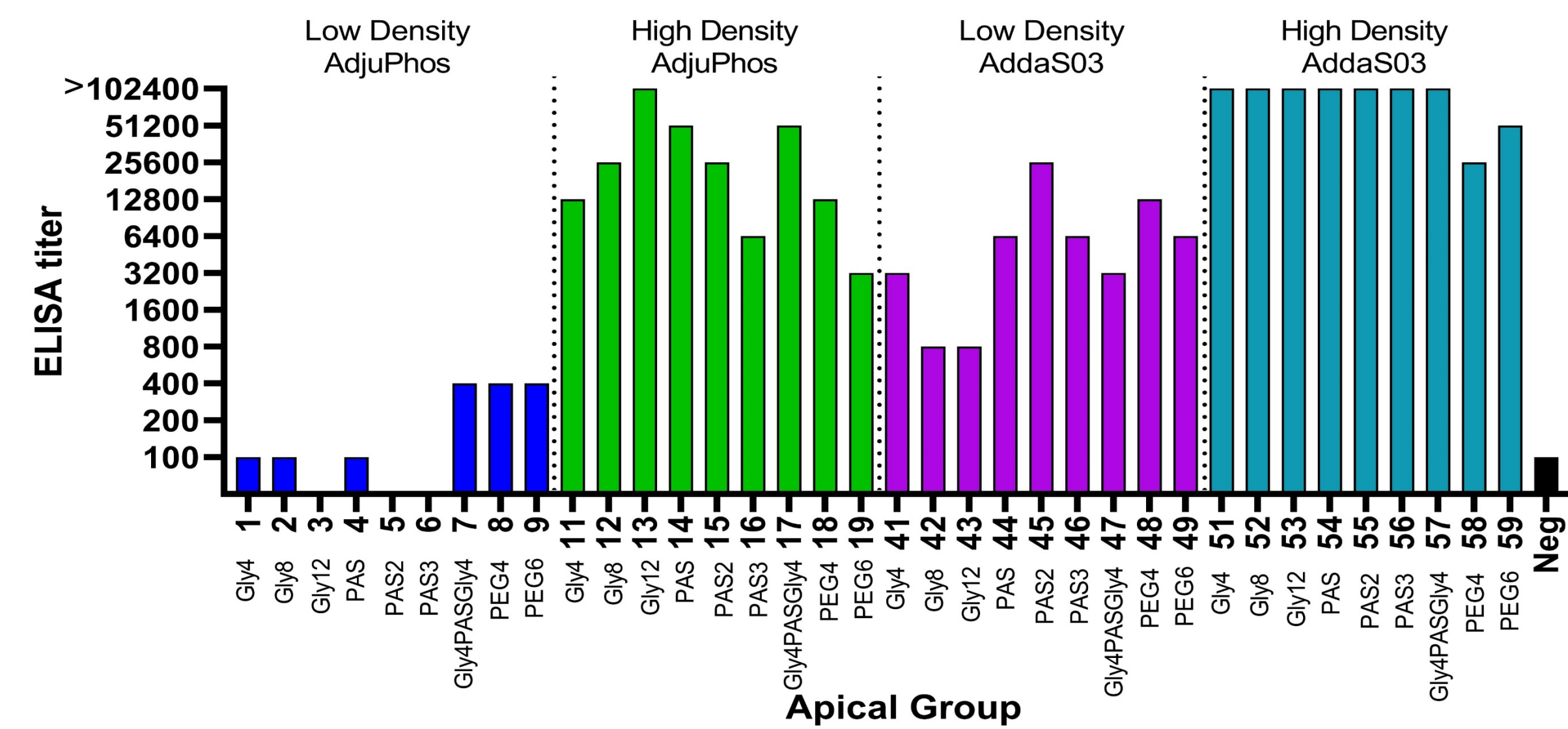


Figure 1 ELISA titer for anti-hapten antibodies in pooled sera from apical groups collected on study day 42. The ELISA titer is defined as the last sera dilution which is 3x the plate blank control wells.

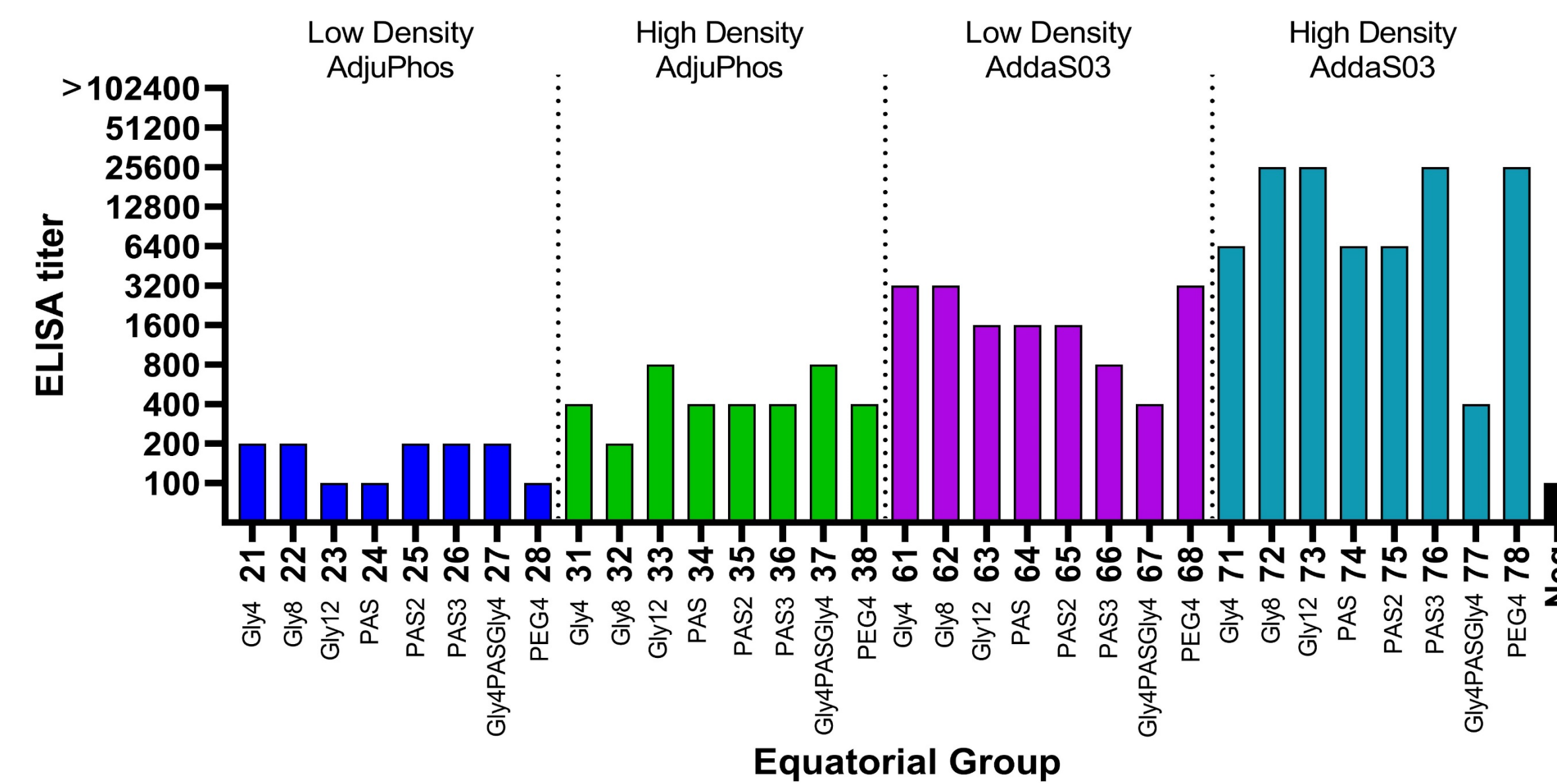


Figure 2 ELISA titer for anti-hapten antibodies in pooled sera from equatorial groups collected on study day 42. The ELISA titer is defined as the last sera dilution which is 3x the plate blank control wells.

ELISA Individual Mice Sera Data

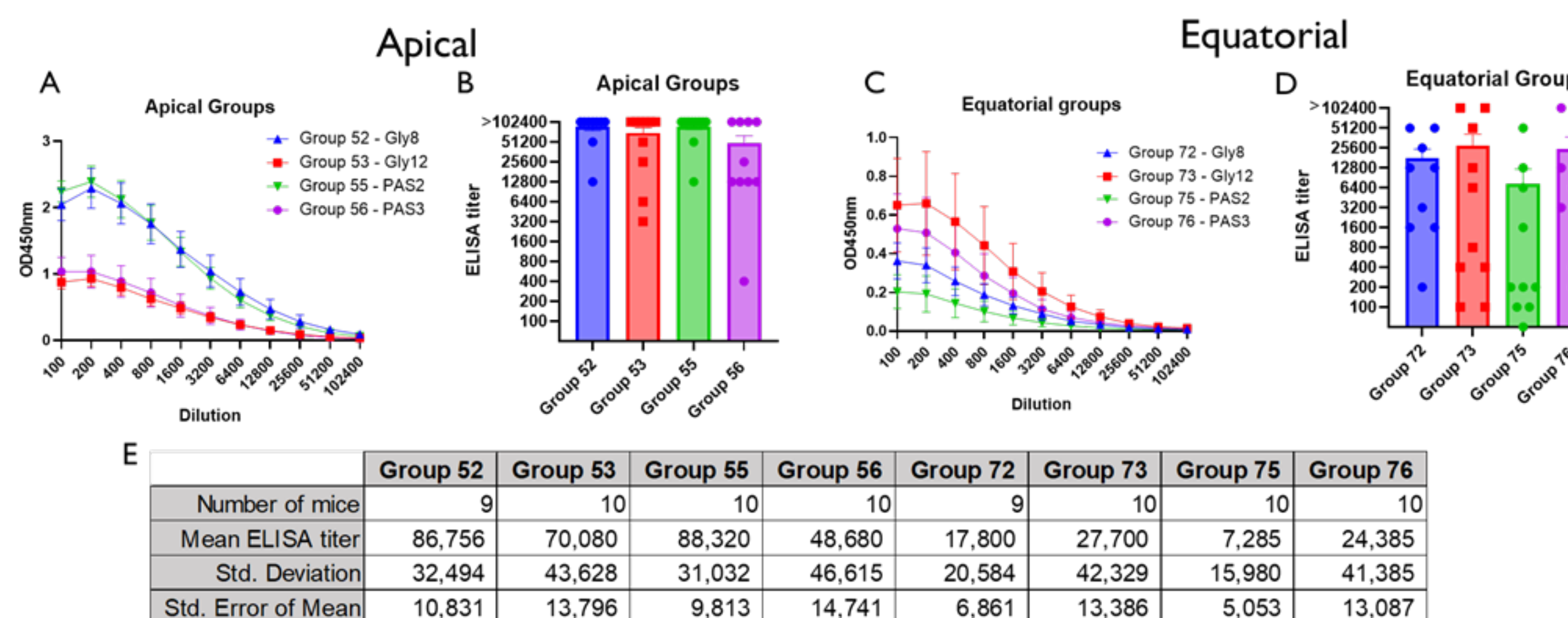


Figure 3 ELISA titer for anti-hapten antibodies for individual mice sera from apical and equatorial groups collected on study day 42. The ELISA titer is defined as the last sera dilution which is 3x the plate blank control wells. Data are presented as the mean with standard error (SEM).

Rank	Linker	Adjuvant	Density	Group	Apical or Equatorial	ELISA titer	Std. Error of Mean	Group	Apical or Equatorial	ELISA titer	Std. Error of Mean
1	Gly8	AddaS03	High	52	Apical	86,756	10,831	72	Equatorial	17,800	6,861
2	PAS3	AddaS03	High	56	Apical	48,680	14,741	76	Equatorial	24,385	13,087
3	Gly12	AddaS03	High	53	Apical	70,080	13,796	73	Equatorial	27,700	13,386
4	PEG4	AddaS03	High	58	Apical	25,600	N/A	78	Equatorial	25,600	N/A

Table 1 Ranking of the four paired groups in which both apical and equatorial groups had a specific anti-hapten ELISA titer response of at least 1:10,000 dilution above baseline (go/no-go criteria). ELISA titers with standard error reported were determined from individual animal responses, while ELISA titers without standard error reported were determined from pooled sera.

Discussion

Pooled Sera Data

- Apical and equatorial groups had the strongest responses with high hapten density and AddaS03 adjuvant
- For apical groups with high density and AddaS03, seven groups showed ELISA titers >1:102,400
- For equatorial groups with high density and AddaS03, four groups showed ELISA titers >1:25,600

Individual Mice Data

- Linkers Gly8, Gly12, PAS2, and PAS3 were selected for further examination in individual sera
- Apical groups: Gly8 and PAS2 had strongest average titer and lowest standard error
- Equatorial groups: Gly12 and PAS3 had strongest average titer, but a high standard error
- Gly8, PAS3, Gly12, and PEG4 were ranked based on their ELISA titer strength and standard error
- Based on this ranking: apical and equatorial haptens with high hapten density and AddaS03 adjuvant are the most optimal variables – the best linker is Gly8, followed by PAS3, Gly12, and finally PEG4**

Conclusion

- 68 groups of vaccinated mice were down-selected to determine the optimal vaccine variables to induce antibodies against small molecules
- Determined the optimal variables are apical and equatorial haptens with the Gly8 linker, high hapten density, and administered with the AddaS03 adjuvant
- Future studies for this vaccine will use these down-selected variables

References

Clementi ME, Condo SG, Giardina B, Marini S. 1991. Antibodies against small molecules. Ann Ist Super Sanita.1991;27(1):139-43. PMID: 1958021.

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