TEST REPORT

TEST REPORT NUMBER:	**B41-Stablepharma-2016**
SAMPLE TYPE: (nature of material)	**Tetanus toxoid vaccine**
LOT/BATCH/BULK NUMBER OR OTHER IDENTIFIER:	**Sample V** – Liquid aluminium adsorbed tetanus vaccine **Sample S** – Stablevax syringe loaded with a dried aluminium adsorbed tetanus vaccine **Sample C** – Stablevax syringe loaded with dried vaccine excipients only (no tetanus antigen)

CLIENT NAME: Stablepharma Ltd NIBSC CONTRACT No: 10857

CLIENT ADDRESS: 1 Queen Square, Bath, BA1 2HA

TEST METHOD(S): Immunogenicity (anti-tetanus antibody response), Antigen content

RELEVANT METHOD IDENTIFIER(S): In-house method based on SOPs BACT/SEROL and BACT/DT-ELISA (immunogenicity) and BACT/DT-ANTIGEN (antigen content)

DATE OF SAMPLE RECEIPT: 05 May 2016

SAMPLE CONDITION ON RECEIPT:
Delete as appropriate:
~~Freeze dried/Frozen/~~Chilled/~~Room temperature~~
Samples suitable~~/unsuitable~~ for testing
Unsuitable for testing because: N/A

REPORT COMPLETED BY:
Signed: L.Coombes Name: Laura Coombes Position: Scientist Date 09 Sep 16
(print name) (print job title)

REPORT AUTHORISED BY:
Signed: Name: PAUL STICKING Position: DT Area Study Director Date 26 SEP 2016
(print name) (print job title)

World Health Organization

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Introduction

NIBSC was supplied with samples of tetanus toxoid vaccine in liquid form and also dried in a sponge matrix inside a disposable syringe (stablevax syringe). The syringes were loaded with the same amount of tetanus antigen (2.5 Lf) and aluminium phosphate adjuvant (0.17 mg Al^{3+}) as that found in a single human dose (0.5 ml) of the liquid vaccine. As a negative control, stablevax syringes were also prepared containing the same amount of adjuvant but with no tetanus antigen present. An immunogenicity study in guinea pigs was performed to determine whether the stabilised, dried tetanus vaccine preparation gave the same antibody response as the fresh liquid vaccine. In addition to the immunogenicity study, the antigenicity of the tetanus toxoid in the stablevax syringe was assessed by measuring the total antigen content and degree of adsorption using an in-house monoclonal antibody capture ELISA assay.

Part 1: Immunogenicity study

1.1 Study design

Groups of 10 female guinea pigs (GPs) were immunised subcutaneously with a single dose of vaccine as shown in Table 1. Animals were bled prior to immunisation (day -1) and then again 4 and 8 weeks post immunisation. The serum was collected and analysed for anti-tetanus antibody titres.

Table 1: Summary of vaccine samples

Code	Group	Formulations	Procedure
V	Positive control	Liquid aluminium phosphate-adsorbed tetanus vaccine	Liquid vaccine drawn up into a normal syringe. GPs immunised with 0.5 ml each
S	Experimental group	Stablevax syringes containing dried aluminium phosphate-adsorbed tetanus vaccine.	0.6 ml water drawn up into a stablevax syringe to rehydrate the sponge. GPs immunised with all of the sample by pressing plunger down and fully compressing the sponge
C	Negative control	Stablevax syringes containing dried excipients but no tetanus antigen	

1.2. *Measurement of antibody titres*

Antibody levels in the guinea pig serum samples were determined using an in-house ELISA assay. Briefly, samples were titrated alongside a guinea pig reference serum (NIBSC 98/572, 3.5 IU/ml tetanus antitoxin) on plates coated with tetanus toxoid. Bound antibody was detected with an anti-guinea pig conjugate antibody followed by substrate. The amount of antibody in the test sample was calculated relative to the reference serum using parallel line analysis.

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1.3. Results

As expected, there was no measurable antibody response in any of the pre-immune serum samples. The guinea pigs elicited a comparable antibody response against the liquid tetanus vaccine (V) and the dried stablevax tetanus vaccine (S) at both 4 and 8 week time points. No antibody titre could be determined for one animal in the group immunised with sample S when tested at a 1/1000 starting dilution, and it is likely that this was due to a miss injection. The antibody response for both groups was more variable (as expressed by the Geometric Coefficient of Variation, GCV) at the 8 week time point than at the 4 week time point, and in general the titre for the same animal had gone down. No response was observed at either time point for the negative control sample (C) when tested a 1/10 dilution. A summary of the results is shown in Table 2 and Figure 1.

Table 2: Anti-tetanus antibody titres at 4 and 8 weeks post immunisation

	Anti-tetanus antibody titres (IU/ml)					
	Sample V		Sample S		Sample C	
Individual animal	Week 4	Week 8	Week 4	Week 8	Week 4	Week 8
1	12.00	9.20	14.49	12.00	<0.0001	<0.0001
2	4.87	3.06	10.97	6.66	<0.0001	<0.0001
3	20.78	18.52	13.59	13.61	<0.0001	<0.0001
4	15.57	13.92	<0.1	<0.1	<0.0001	<0.0001
5	7.62	4.30	14.27	12.03	<0.0001	<0.0001
6	12.59	9.89	14.39	21.38	<0.0001	<0.0001
7	16.53	30.33	15.54	10.67	<0.0001	<0.0001
8	24.48	16.98	9.19	5.25	<0.0001	<0.0001
9	6.74	4.09	7.90	6.27	<0.0001	<0.0001
10	8.98	6.91	6.30	3.08	<0.0001	<0.0001
Geomean (IU/ml)	**11.6**	**9.2**	**11.4**	**8.8**	-	-
GCV (%)	68	111	38	80	-	-

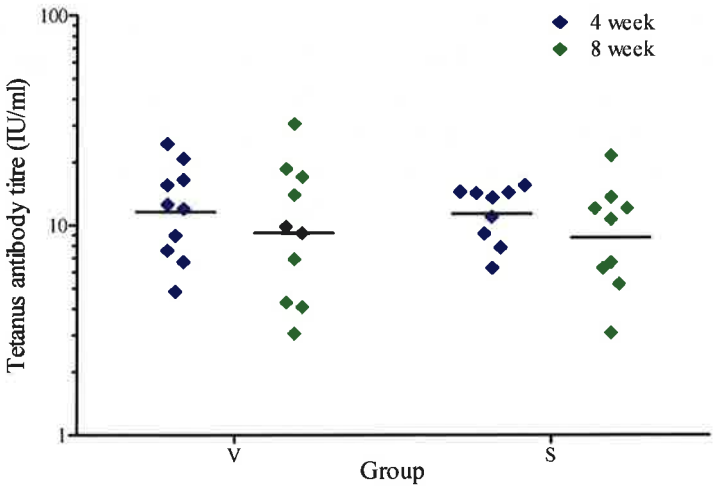

Figure 1: Tetanus antibody levels (IU/ml) for individual animals immunised with either sample V or sample S at 4 week (blue diamond) and 8 week (green diamond) time points. The geometric mean of each group is shown by a horizontal line.

Part 2: Antigenicity

2.1. Preparation of vaccine samples

Stablevax syringes S and C were rehydrated with 0.6 ml water for approximately 10-30 seconds before expelling the sample into a tube. The sponge for sample S was then washed, up to 2 times, by taking up a further 0.6 ml and expelling the sample into a separate tube. To test for total antigen content, the vaccine samples (V and the expelled samples from C and S) were desorbed by incubating overnight at +37°C with an equal volume of 20% w/v sodium citrate. Following incubation the samples were centrifuged and the supernatant collected. To measure the non-adsorbed antigen content a portion of the adsorbed vaccine sample was centrifuged to pellet the adjuvant and the supernatant retained for testing.

2.2 Measurement of antigen content

Samples were tested for tetanus antigen using a monoclonal antibody capture ELISA. Briefly, samples were titrated alongside a toxoid reference (WHO IS 04/150, 690 Lf/ml) on 96 well plates coated with an anti-tetanus monoclonal antibody (directed against the C fragment of the heavy chain). Bound toxoid was detected by successive incubations with polyclonal antibody against the toxoid, peroxidase-labelled antibody, and substrate. The amount of antigen in the test sample was calculated relative to the reference toxoid using parallel line analysis.

2.3. Results

The results of two separate assays are shown in Table 3 (assay 1) and Table 4 (assay 2). For the first assay, the stablevax syringes were rehydrated for about 10 seconds only. As expected, there was no tetanus antigen detected in sample C. For sample S, tetanus antigen was measured in both the initial sample from the rehydrated sponge and the 1st wash step. In total the syringe was found to contain approximately 2.8 Lf/ml of tetanus antigen (1.7 Lf per syringe when the volume used to rehydrate the sponge has been taken into account). In comparison, sample V was found to contain approximately 2.9 Lf/ml (1.5 Lf per 0.5 ml SHD). Unlike sample V which was highly adsorbed, sample S contained a significant amount of non-adsorbed antigen.

For the second assay, three of the stablevax syringes containing tetanus antigen were tested to look at variability between syringes. The time allowed for the sponge to rehydrate was increased to about 30 seconds to try and reduce the amount of antigen left on the sponge. Results showed a lower proportion of tetanus antigen in the wash step compared to the first assay. The three different syringes tested were very comparable, and showed a similar result to that observed in assay 1. A summary of the data for stablevax sample S from both assays is shown in Figure 2.

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Table 3: Tetanus antigen and degree of adsorption measured in assay 1

Sample	Tetanus antigen Lf/ml (± 95% cl) Total	Non-adsorbed	% adsorbed
V sample 1	2.89 (2.71-3.09)	< 0.05	> 98
S syringe 1	1.95 (1.82-2.08)	1.18 (1.10-1.26)	39
S (syringe 1) wash 1	0.81 (0.74-0.87)	-	-
S (syringe 1) wash 2	< 0.1	-	-
C	< 0.1	<0.05	-

Table 4: Tetanus antigen and degree of adsorption measured in assay 2

Sample		Tetanus antigen Lf/ml (± 95% cl) Total	Non-adsorbed	% adsorbed
V sample 2		2.81 (2.64-2.99)	0.08 (0.07-0.08)	97
S	Syringe 2	1.62 (1.53-1.73)	0.72 (0.69-0.76)	56
	Syringe 3	1.46 (1.38-1.55)	0.93 (0.88-0.98)	36
	Syringe 4	1.68 (1.58-1.78)	0.92 (0.88-0.97)	45
S wash 1	Syringe 2	0.31 (0.30-0.33)	-	-
	Syringe 3	0.49 (0.46-0.51)	-	-
	Syringe 4	0.38 (0.36-0.40)	-	-

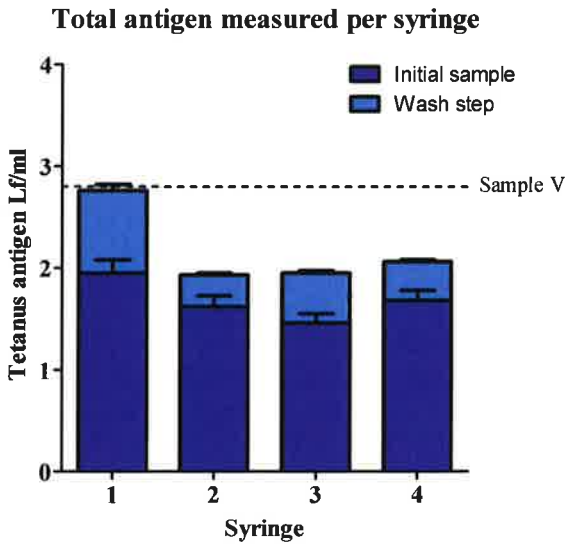

Figure 2: Summary of tetanus antigen measured in the dried stablevax tetanus vaccine. Data shows individual results (Lf/ml) ± 95% cl for each syringe tested. As a comparison the average result (n=2) for the positive control sample (V) is indicated by a dashed horizontal line.

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Conclusion

In this study the stablevax tetanus vaccine was found to induce the same level of antibody response as the positive control vaccine. One guinea pig in the group immunised with the stablevax vaccine did not give a measurable response, however non-responders are occasionally observed in these types of studies, and it is unlikely that this is linked to the vaccine sample. Based on results of the antigen assay, the immunising dose for both groups was likely to be very comparable (within 1 Lf). Some of the antigen may have remained in sponge when performing the immunisations, however the syringes were rehydrated for at least 30 seconds prior to use to try and maximise the antigen recovery. The antigen results also showed a higher percentage of non-adsorbed antigen in the stablevax vaccine sample compared to the positive control vaccine, but this does not appear to have affected the immune response.

REPORT AUTHORISED BY:

Signed: PAS Name: PAUL STICKINGS Position: DT Area Study Director Date 26 SEP 2016

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