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A Protein-Free Process and Product Protection System

Stephen Ward
Stabilitech Ltd., UK

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ECI Vaccine Technology IV 2012

Storage solutions

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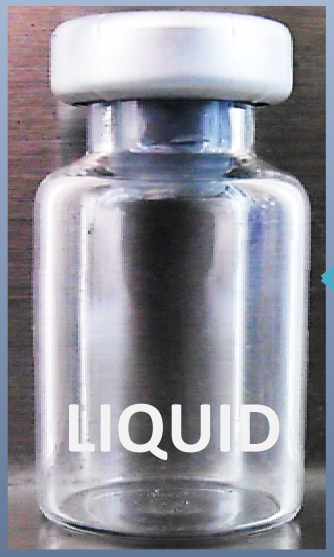

- Structural Stability
  - Physical temp, pH
  - Chemical oxidation, deamidation
- Colloidal Stability
  - Self association
  - Conformation Altered
  - Chemical Modification
  - Nucleation Mediated
  - Surface Associated

  (Aggregation)

- Freeze Stress
  - Physical shearing
  - pH Dependant structural damage
- Dessication
  - Dehydration

How do Excipients work

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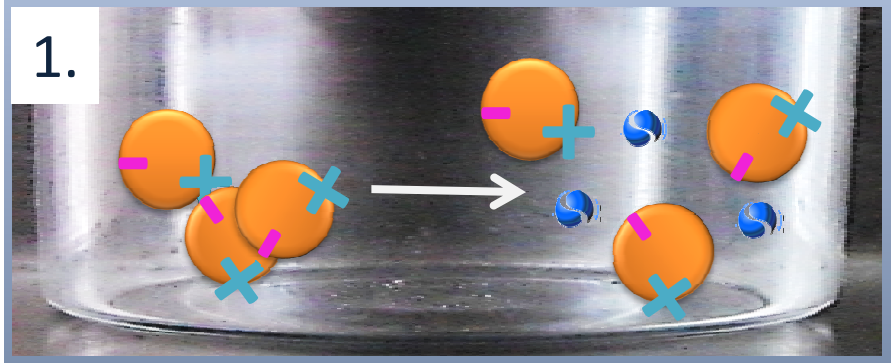
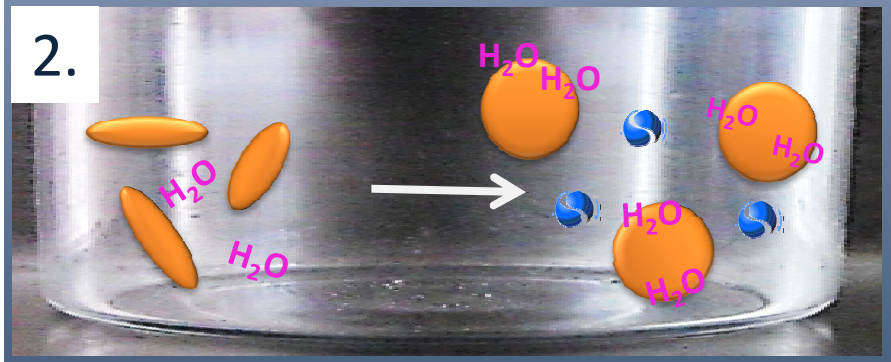
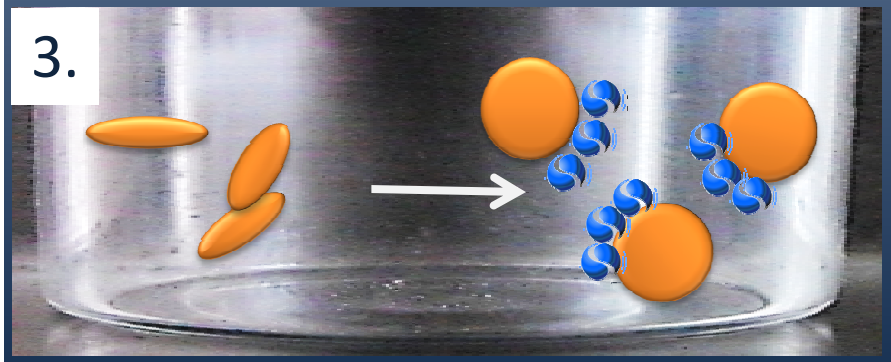


1. Charge screening – *screen ionic interactions*



2. Hydration – *preferentially excluded increasing hydration of molecule increases structural integrity (e.g. sugars)*



3. Chaperone molecules - *bind to the active molecule inhibiting aggregation*

3. Prevent denaturation – *inhibit intermediates forming(e.g. amino acids)*

Lyophilisation– Process Protection

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Freezing

Drying

- as liquid protection.

•Cryoprotection

- *Prevent protein interaction with ice crystals*
- *Reduces protein to protein interactions*
- *Alter melting temperature*

•Lyoprotection

- *Protect against dehydration*
- *Provide cake structure*

Lyophilisation– Product Protection

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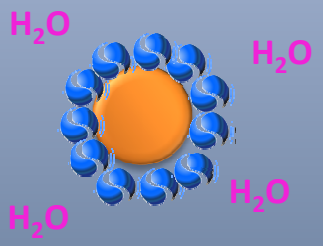
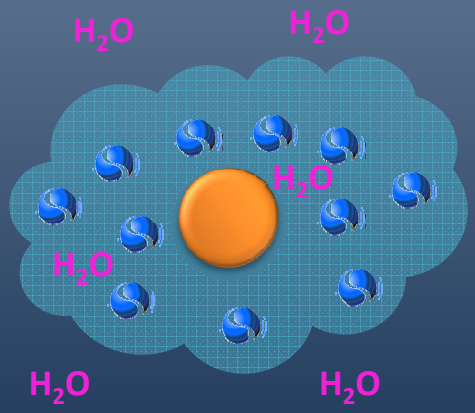


Water Replacement

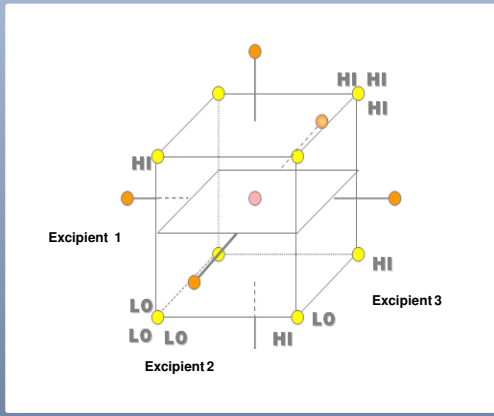
Excipients substitute for water maintaining hydration effect in the absence of water.



Vitrification

*Excipients form a viscous **amorphous** region around molecule preventing crystallisation and reducing rate of other degradation processes.*

Qbd Formulation Construction



Screening
Round 1
(Accelerated Stability)

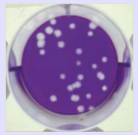
↓

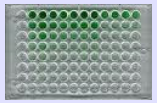
Optimisation
(Accelerated Stability)

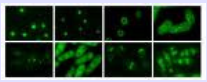
↓

Long-Term
(Real Time Stability)

Bioassays:


PLAQUE


ELISA


TCID50


POTENCY

Biophysical/structural Assays:


DLS


HPLC


DSC

Screening:

- Novel excipients are screened for efficacy in formulations

Optimisation:

- Successful candidates are further characterised and their formulation optimised.

Long-Term:

- Optimal formulations are tested in confirmational studies which define the efficacy of the formulation.

Successful Target Stabilisation

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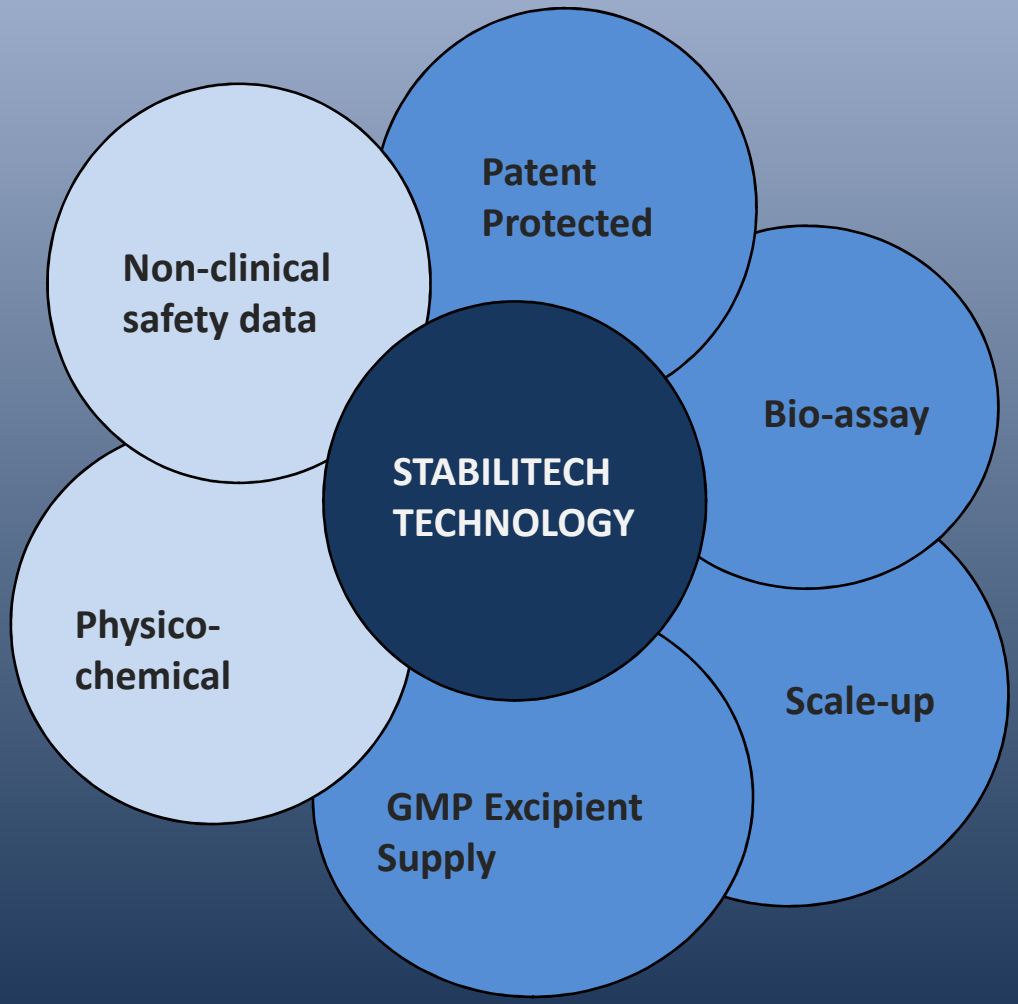
Live Adenovirus
Live Measles virus (Schwartz)
Live Foot-and-Mouth Disease
Inactivated Influenza (PR8)
Live Canine viruses
Live Modified Vaccinia Ankara
Therapeutic Antibodies
Therapeutic Fab's
Cytokines and growth factors
Alhydrogel adjuvanted vaccine

Supported Technology



Stabilitech Excipients

To prevent against the various degradation mechanisms stabilization mixes are formed from a panel of excipients

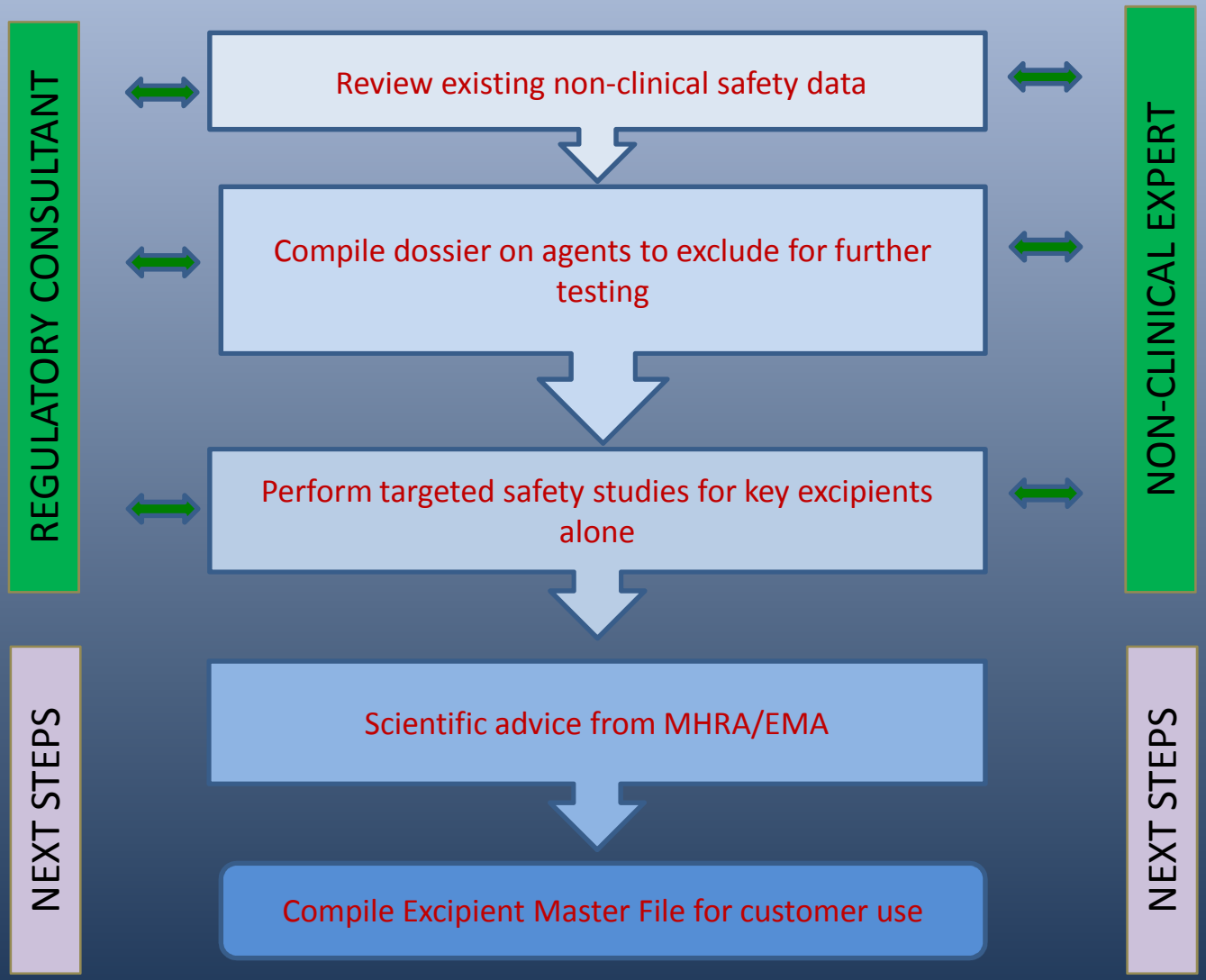
The base of the formulation is comprised of GRAS listed agents (sugar/sugar alcohol)

These are supplemented with small chemical reagents which have either:

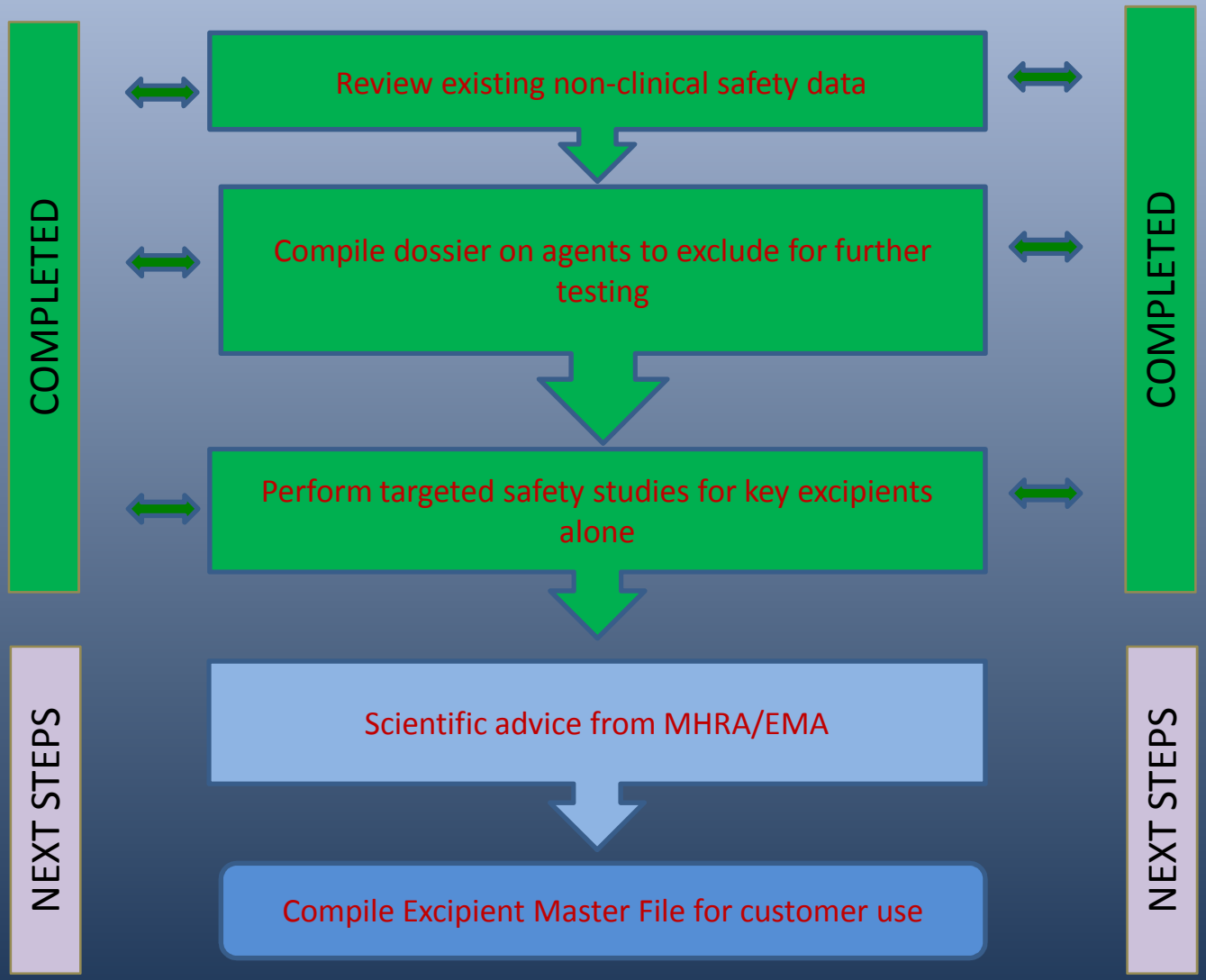
- Been used in clinical trials
- Been used in human Nutraceutical products

To further underscore the non-toxic nature of these excipients full GLP toxicological studies have been carried out (including immunotox) with no measurable toxic effects

Regulatory Support



Regulatory Support



GMP Supply of Excipients

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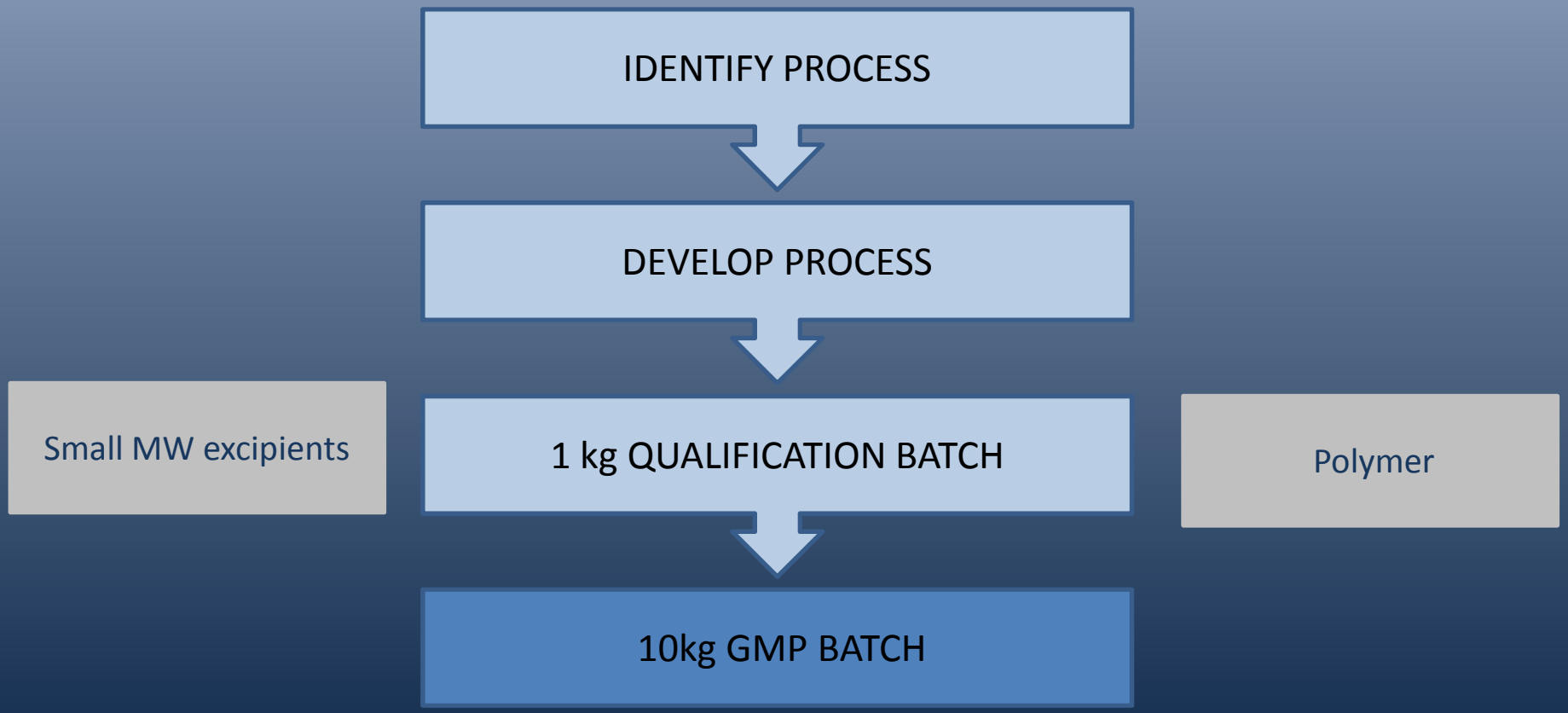
- Pharmacopeal excipients already widely available
- Lesser-used excipients being made by blue chip CMO in UK
  - MHRA and FDA Licence
- Second EU suppliers also identified



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CMC Scale-up of Technology

Stabilitech in-house capacity

400 x 2 ml DIN vials

0.5ml fill volume

Scale-up with CRO

1800 x 3ml vials

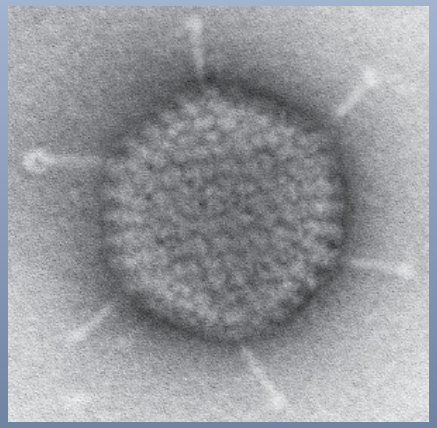
1.5ml fill volume

Stability Achievements

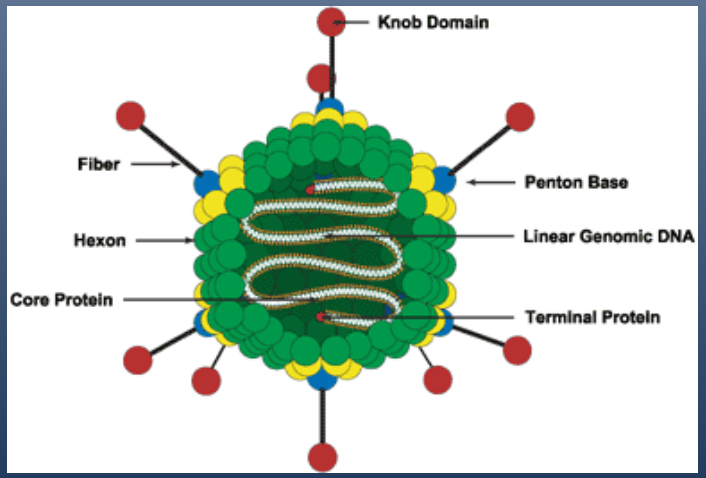
- **Protection against Production losses**
  - *Superior to established viral formulations*
  - *Allow Alum adjuvant to be frozen*
- **Protection against high and low temperature excursions**
  - *Significant improvement over industry gold-standard virus formulation*
- **Enhanced long-term stability**
  - *Markedly better than commercial vaccine at room temperature*

Adenovirus

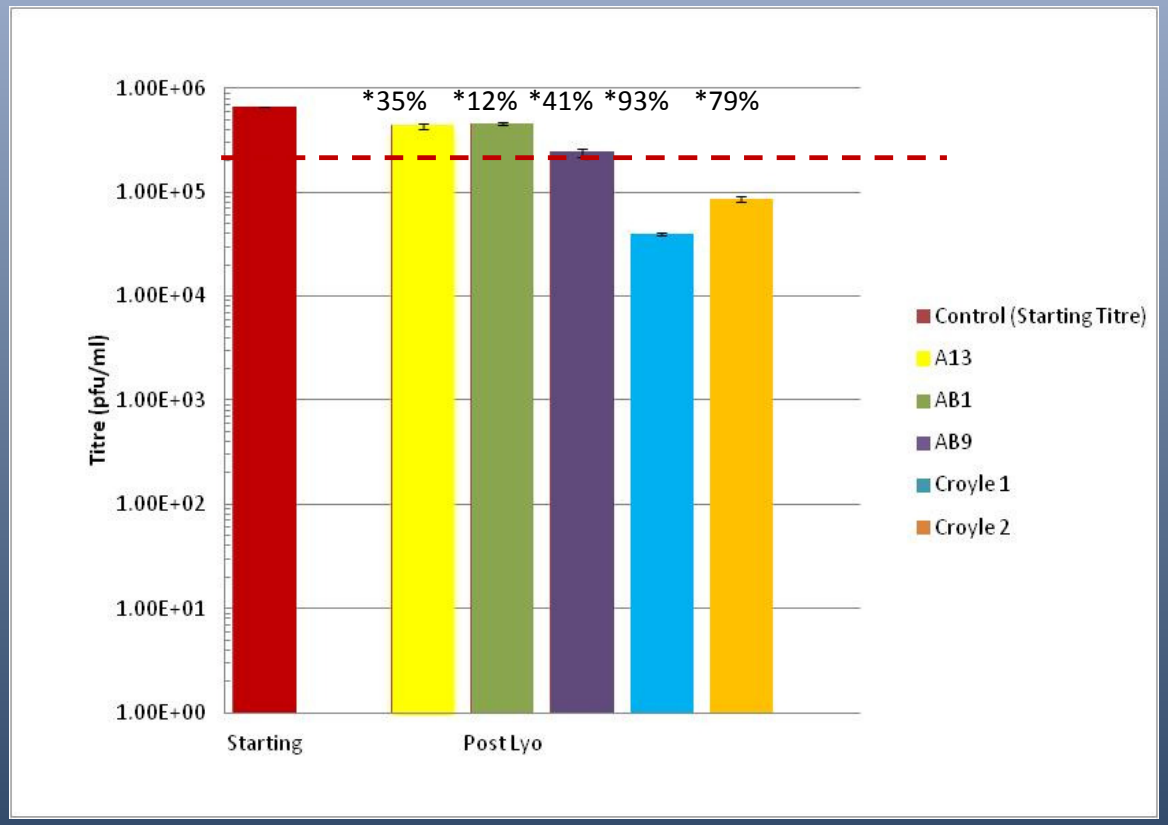
- Medium-sized
  - non-enveloped
  - double stranded DNA
- Vector in novel vaccines
- Gene therapy vehicle



- Often an unstable product:
- Evans *et al*. (2004)
  - Liquid 4°C 2 year
- Croyle *et al*. (2001)
  - Lyophilised 4°C 1 year
- Stabilitech: liquid and Lyophilised



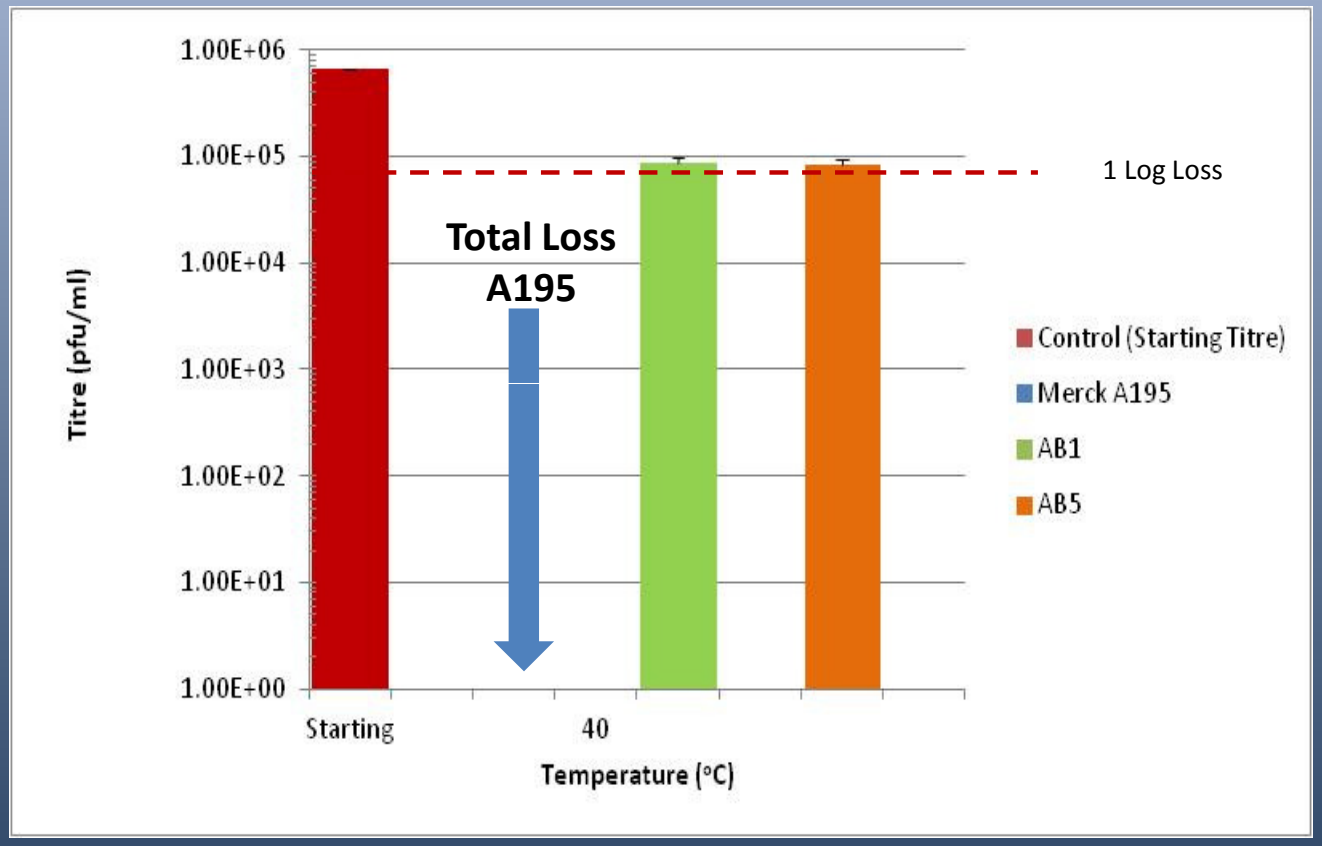
Adenovirus Protection against Process Loss



*Percentage Loss during lyophilisation

- GFP assay of formulations post lyophilisation.
- <0.5 log loss success target
- **Success criteria achieved with all formulations**
- **Out perform Best published formulation**

Liquid Protection of Adenovirus against Excursions: 40°C for 14 days



- GFP assay of liquid formulations after 40°C for 2 weeks
- **Total** loss in Merck A195 Buffer
- **Only a <1 log loss in AB1 and AB5**

WHO: VVM 14

Liquid Stability of Adenovirus: up to 25°C for 6 Months

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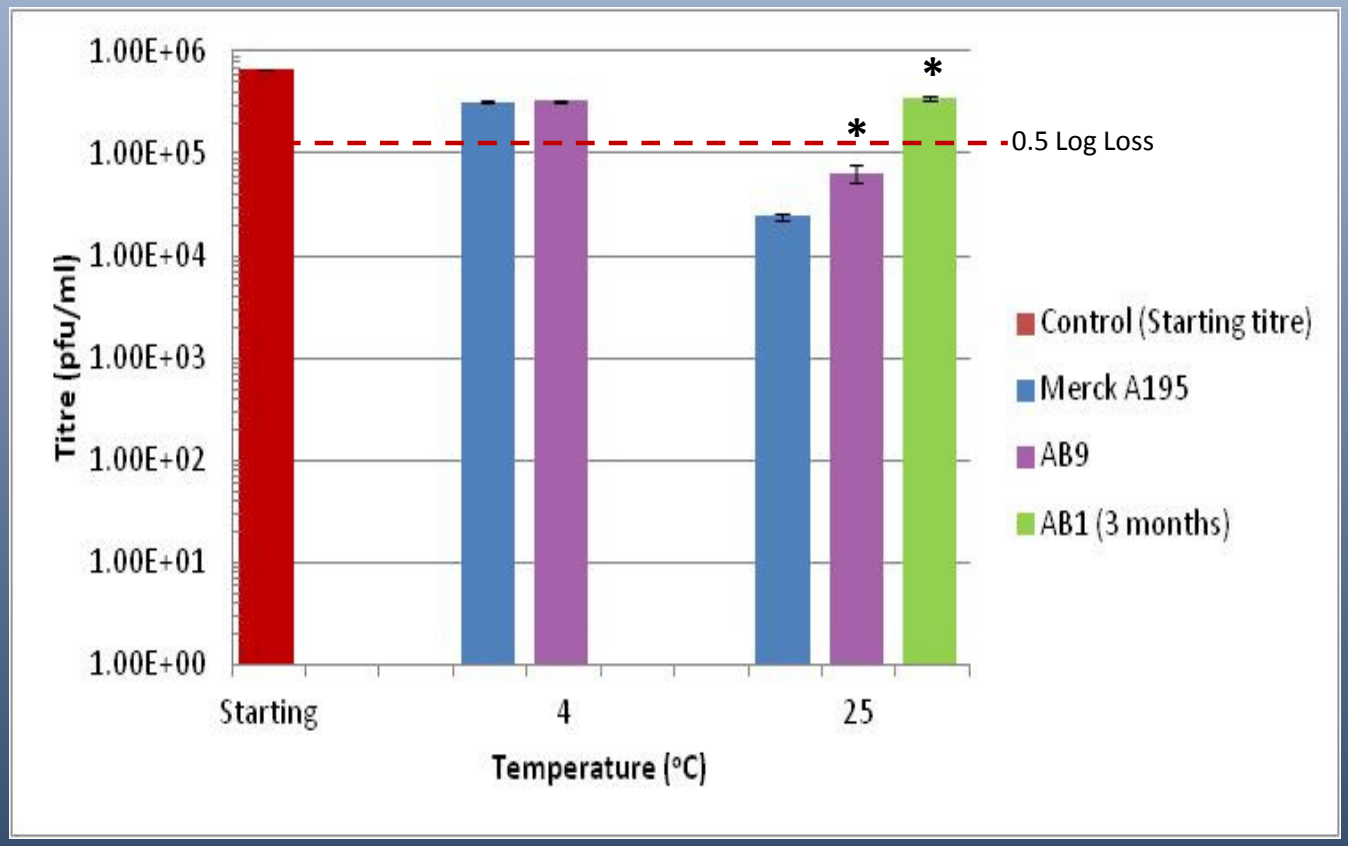
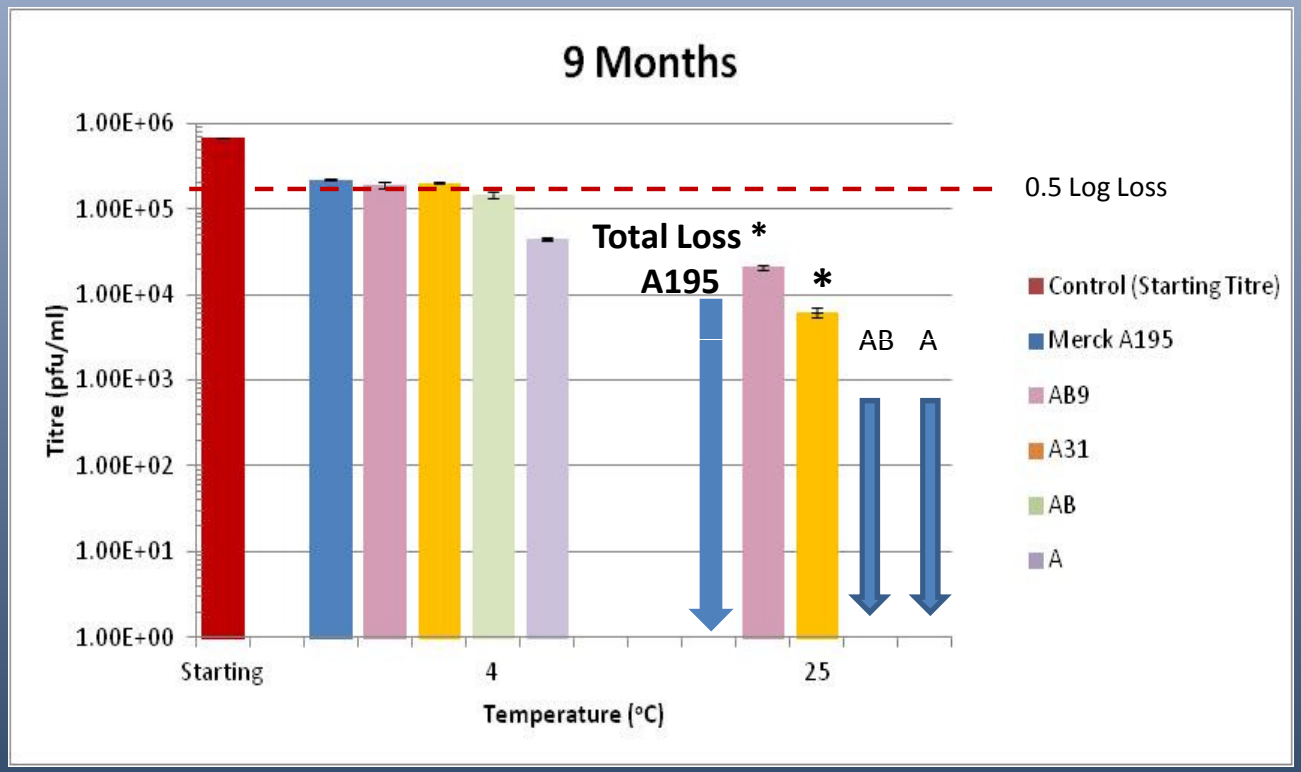


*** Significant improvement to Merck A195 Buffer at 95% confidence interval**

- GFP assay of liquid formulations after storage at 4°C and 25°C for 6 months
- <0.5 log loss in Merck A195 Buffer at 4°C, and 1.5 log loss at 25°C
- **Significant ($p<0.05$) enhancement of protection at 25°C compared to A195**
- **AB1 Improved formulation performing well at 3 months so far**
- **2 year study ongoing**

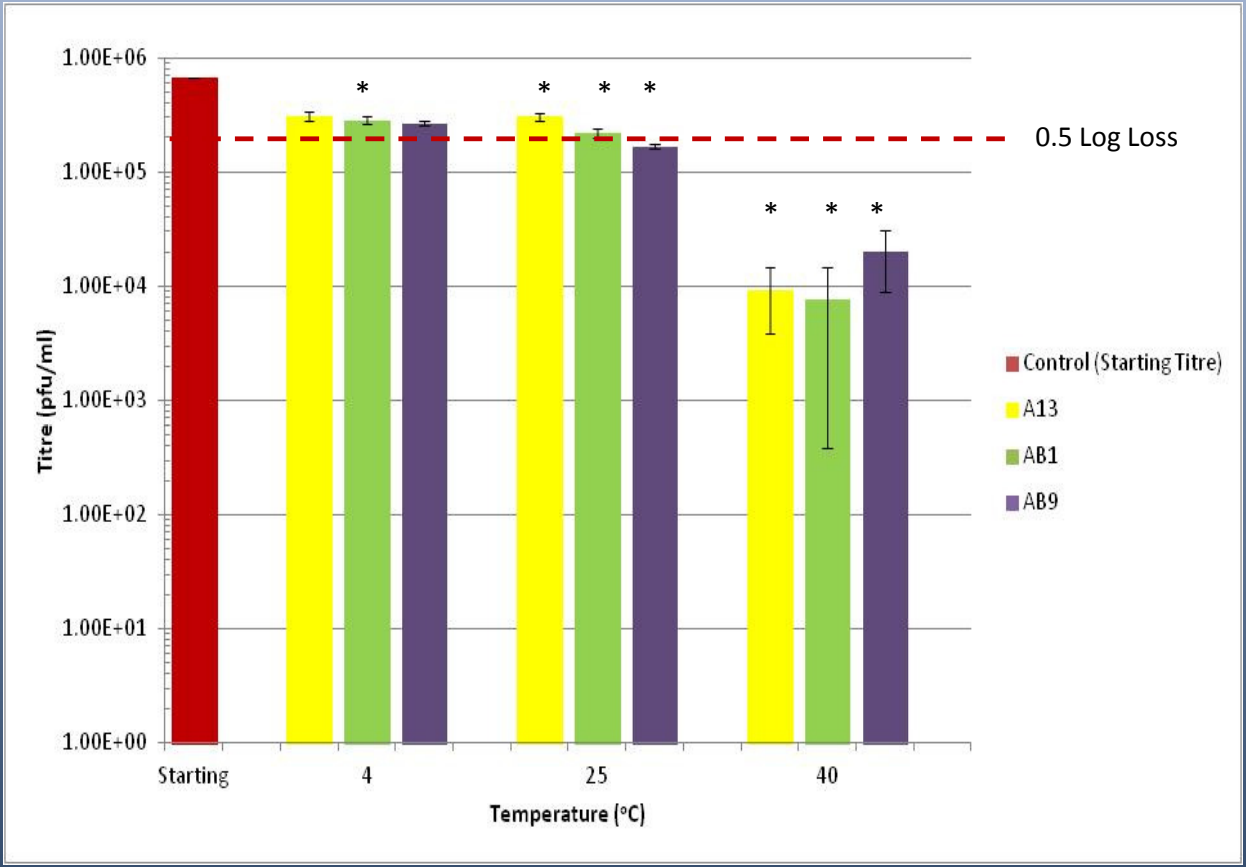
Liquid Stability of Adenovirus: up to 25°C for 9 Months



*** Significant improvement to Merck A195 Buffer at 95% confidence interval**

- GFP assay of liquid formulations after storage at 4°C and 25°C for 9 months
- Total loss in Merck A195 Buffer at 25°C
- **Significant ($p<0.05$) protection with AB9 and A31 compared to Merck A195**
- **Opimised sugar-component only A and AB insufficient at ambient temperatures**
- **2 year study ongoing**

Lyophilised Stability of Adenovirus: up to 40°C for 6 Months

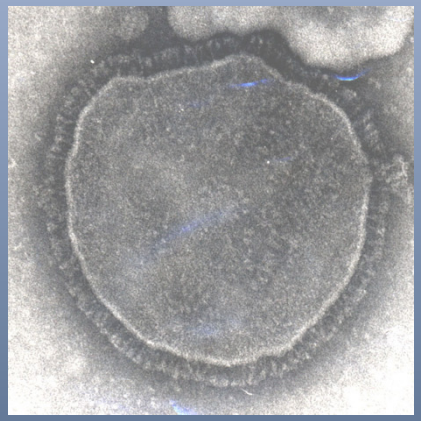


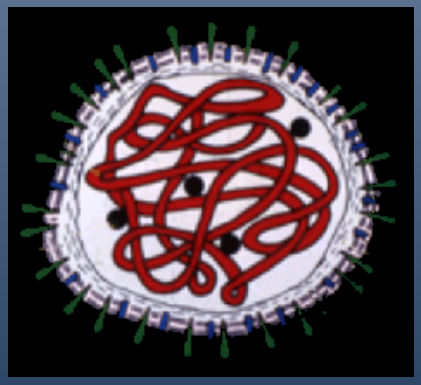
*** Significant difference to post lyophilised at 95% confidence interval**

- GFP assay of lyophilised formulations after storage at 4°C, 25°C and 40°C for 6 months
- <0.5 log loss success target
- **Less than half log loss of Adenovirus at 4°C and 25°C**
- **Recoverable virus even at 40°C**
  - Less than two log loss of Adenovirus at 40°C

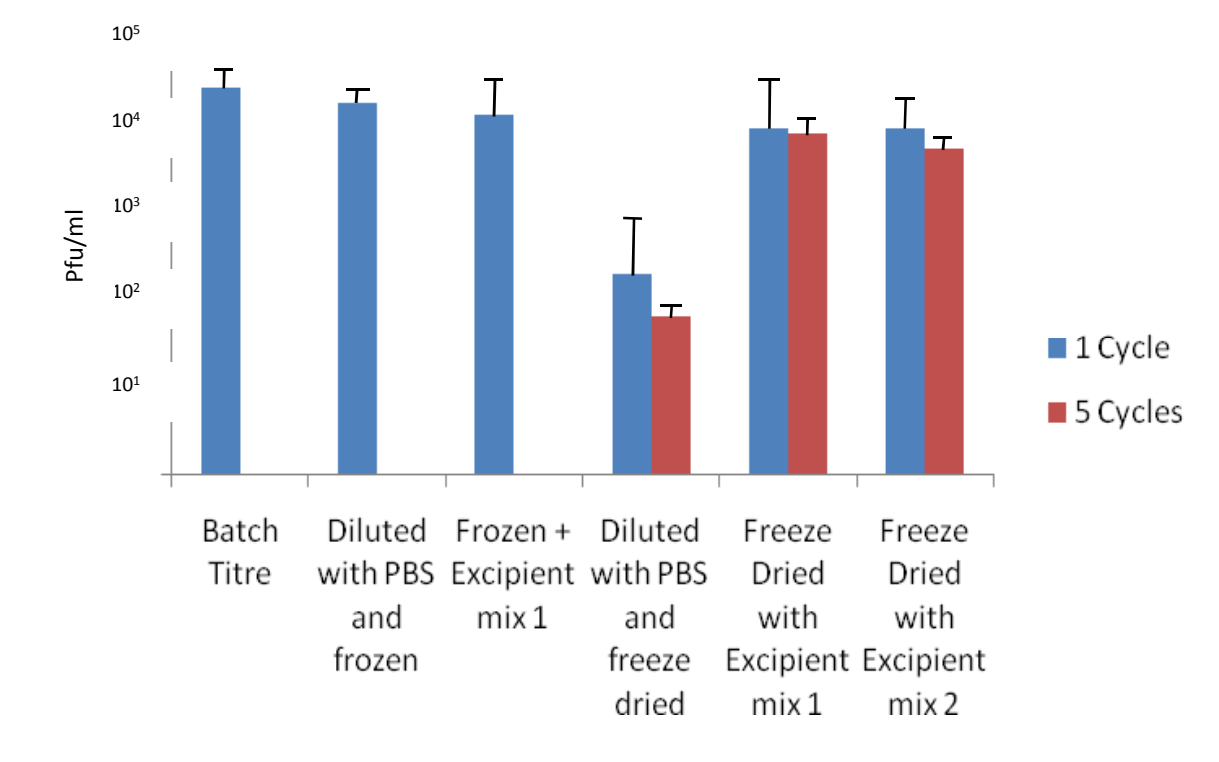
Measles Virus

- Enveloped virus
- Genus Morbillivirus in the family Paramyxoviridae.
- Widely regarded, after polio, as one of the most temperature sensitive vaccines
- Continuing need for thermally stable vaccine for developing world





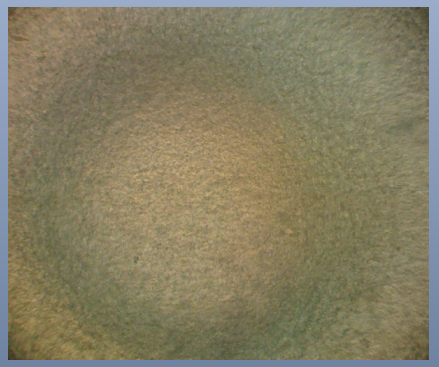
Lyophilised Protection of Measles: repeated Freeze-Thaw



- Repeated cycling from -20ºC (20 hours) to +37ºC (4 hours).
- **Excipients protect against severe temperature excursions**

Alhydrogel™ Freeze Protection

- Loss of Alhydrogel™ structure:
  - Lyophilisation not previously possible
  - Accidental freezing during cold chain
- Real problem if vaccine antigen temperature sensitive
- Solutions such as:
  - Separate alum and antigen containers
  - Complex cold chain management
  - Administration complexity



Normal

Post Freeze

Alhydrogel™ Lyo Protection

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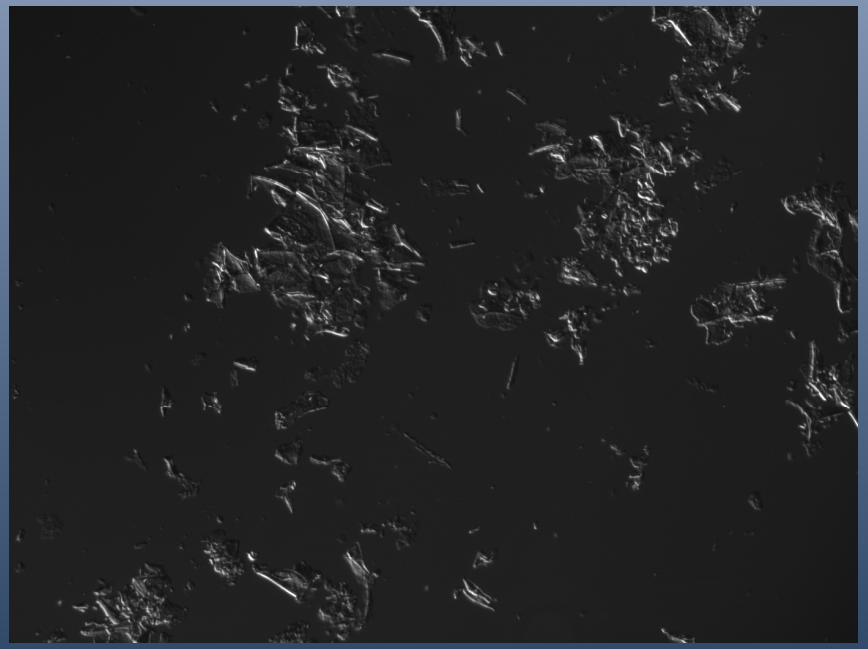
Lyophilised Formulation B

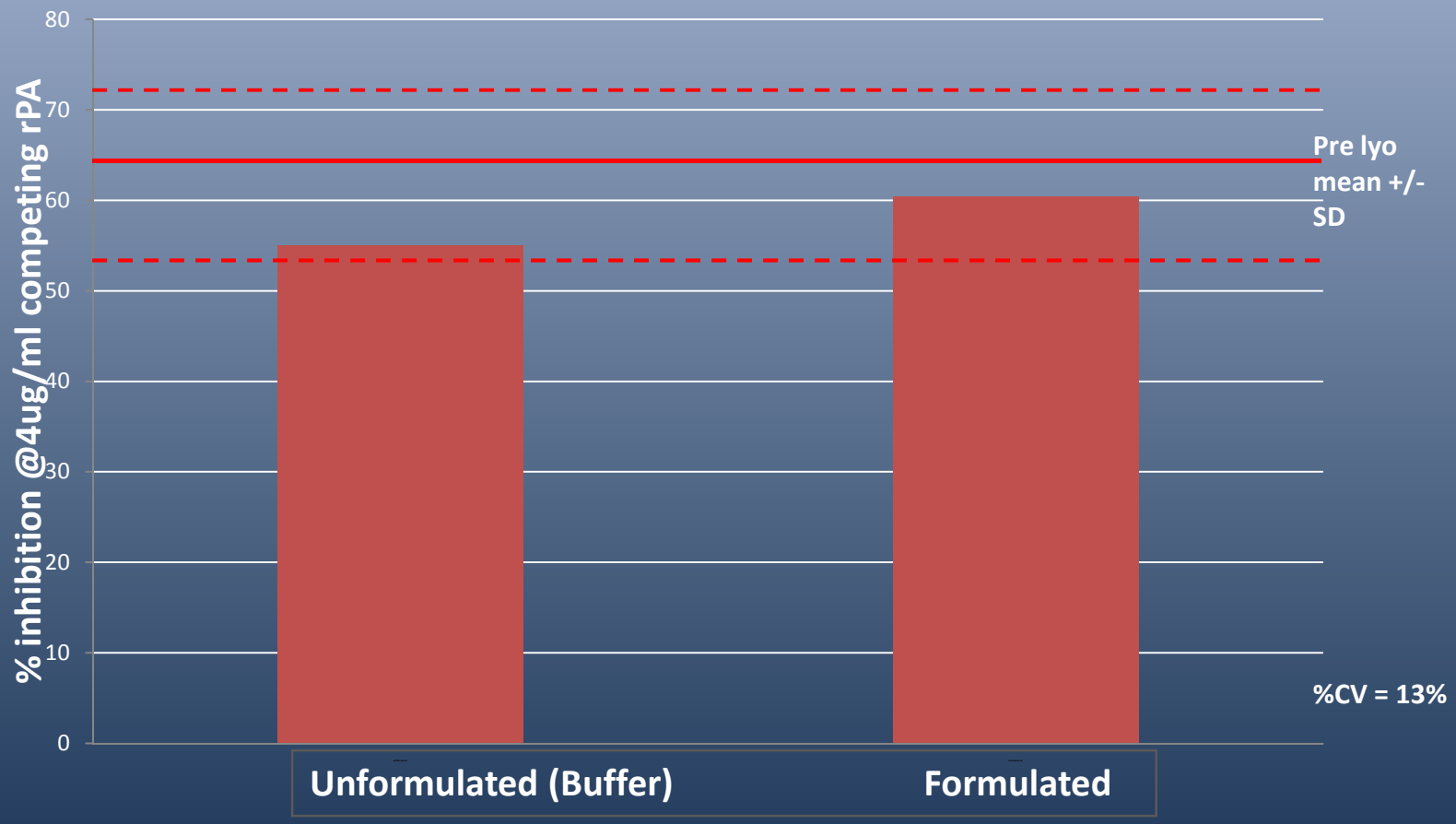
- Structural retention 90%

Lyophilised HEPES control



- Structural retention 7%

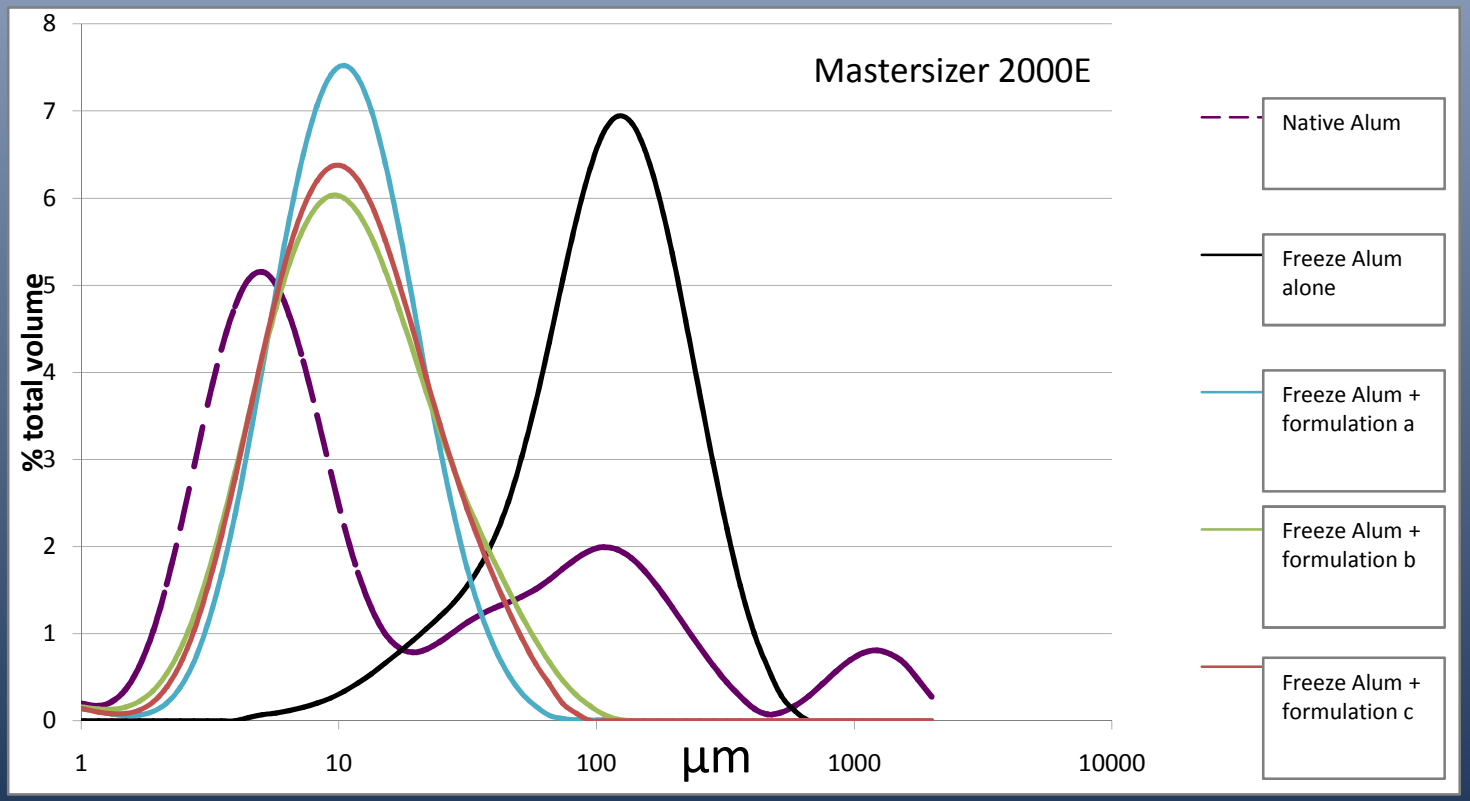
Lyophilised: Protection of antigen (rPA) adsorbed to Alhydrogel™



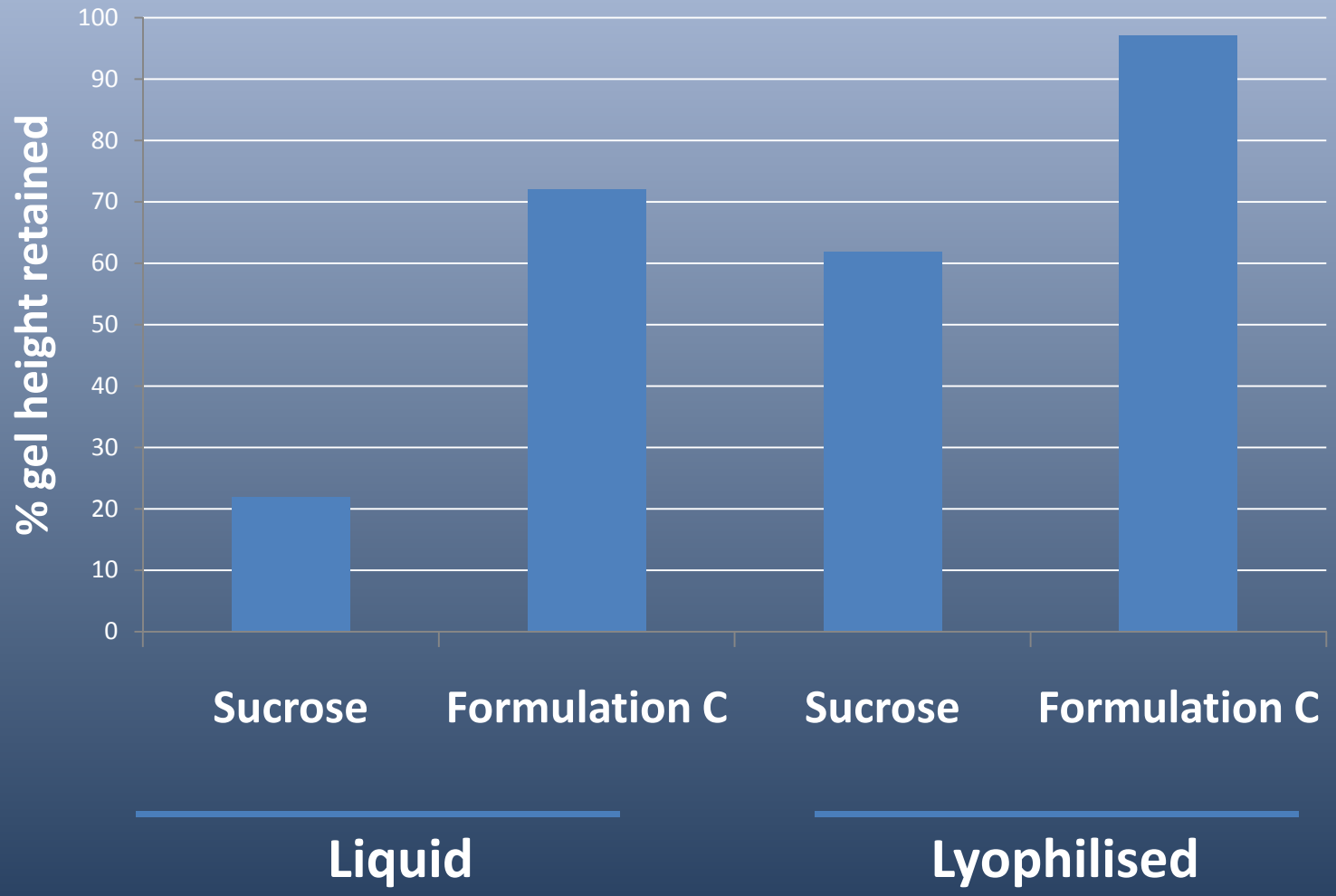
Labile antigen also protected along with Alhydrogel

Protection of Alhydrogel™ against Freeze-Thaw

- Particle size distribution of alum (0.26%) before and after freezing.
- Addition of formulation prevents agglomeration
- Keeps mean at 10µm not allowing it to slide to 100µm



Protection of Alhydrogel™ against Repeated Freeze-Thaw



- Significant improvement in protection when excipient added to base sugar alone

Liquid Stability of rPA adsorbed to Alhydrogel™ at 25°C for 3 weeks

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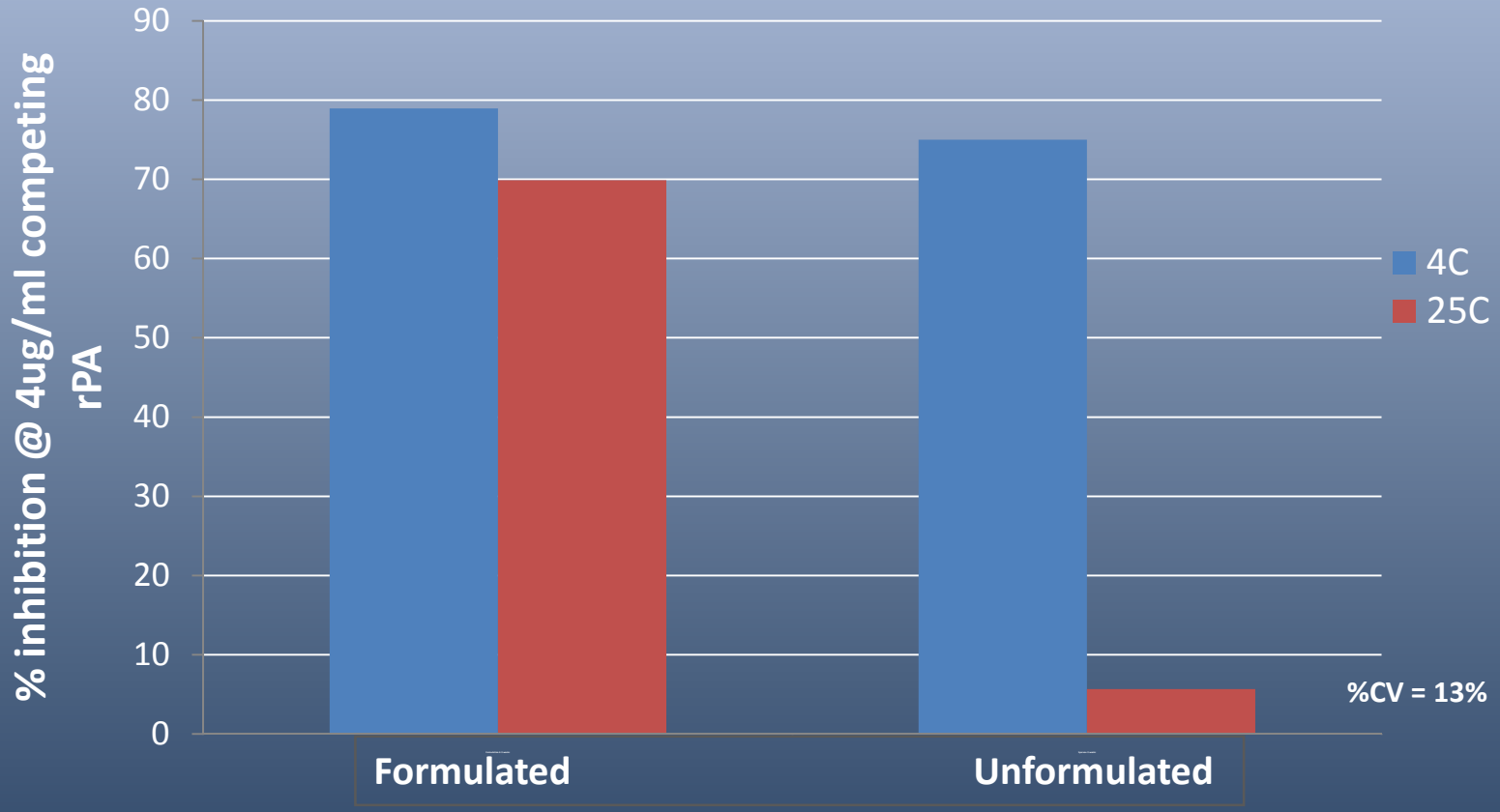
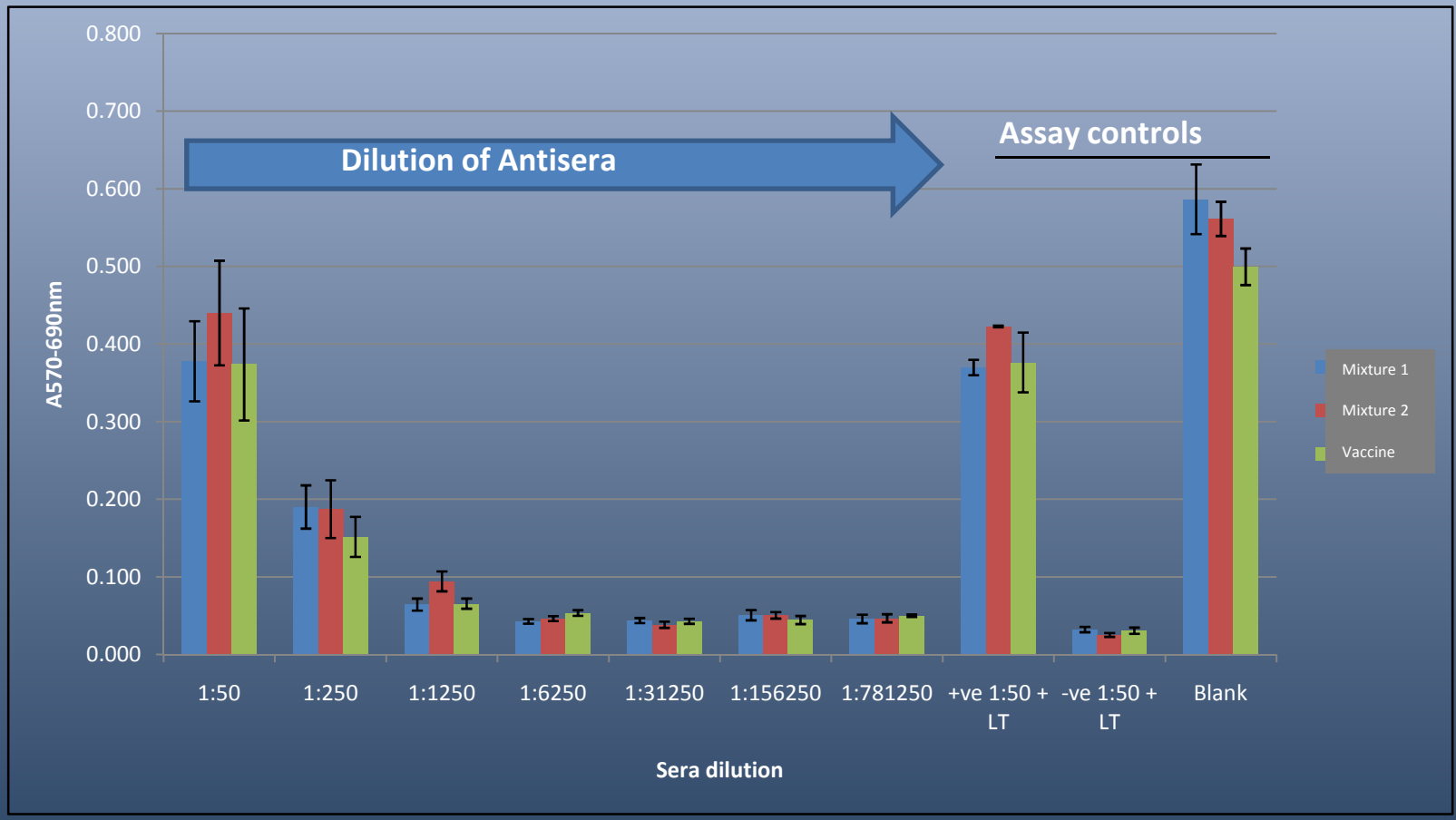


- **Significant improvement in protection to antigen adsorbed to alum when excipient added**

Toxin Neutralising Antibody Levels in Rabbits Vaccinated with rPA Adsorbed to Alhydrogel™



1 way ANOVA 1:50 p=0.72 1:250 p=0.62

- **EQUAL level of neutralising immunity when formulation added to vaccine**

Technology Conclusions

- **Safe and Compliant technology**
  - Wide vaccine-type utility
  - Commonly used Alum adjuvant
- **Protects fragile material during processing**
  - Significant COG impact
  - Prevents future stability losses
- **Excellent product protection**
  - Prevents freeze and high temperature excursion losses
- **Widens distribution and administration options**
  - Potential for cold-chain removal itself
- **Novel intellectual property position**
- **In vivo toxicology showed no adverse reaction**

Stabilitech

Delighted to speak to you about potential feasibility studies to evaluate our Technology with your vaccine

stephen@stabilitech.com

www.stabilitech.com