Development of a Multi-dose Formulation for Prevenar 13™

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PREVENAR IN THE DEVELOPING WORLD

Dr. Richard Sezibera, Rwanda's Minister of Health, administers the first official dose of Prevenar to 1-month-old Clementine in Rwanda's Eastern Province on Saturday, April 25, 2009.

- First NIP in a developing world country
- Donation for Rwanda and The Gambia
- First dose in child – Rwanda (April 2009), The Gambia (August 2009)

Trademark

INTRODUCTION

- Pneumococcal disease is the leading vaccine-preventable killer of young children worldwide according to the World health organization (WHO), killing between 800,000 to a million children a year. Ninety percent of these deaths occur in the developing world.
- Prevenar 13™ (Pneumococcal Polysaccharide Conjugate Vaccine [13-valent, adsorbed]) is a vaccine that has been approved for use in infants and young children in more than 48 countries. In addition, regulatory filings for Prevenar 13 for pediatric use are in advanced stages of review in various countries.
- Prevenar 13™ includes the seven serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) in Prevenar (Pneumococcal Saccharide Conjugated Vaccine, Adsorbed) plus six additional serotypes (1, 3, 5, 6A, 7F, and 19A). Together, these serotypes represent the most prevalent invasive disease causing strains in young children worldwide.
- Pfizer is partnering with the Global Alliance for Vaccines and Immunization (GAVI), and Pneumococcal Vaccines Accelerated Development and Introduction Plan (PneumoADIP) PneumoADIP and other public health partners to facilitate access to Prevenar and Prevenar 13™ with expanded serotype coverage to children in developing countries.
- Pfizer has signed a 10-year Provisional Supply Agreement to supply Prevenar 13™ for infants and young children in the world's poorest countries under the terms of the Advance Market Commitment (AMC) pilot project against pneumococcal disease.

OBJECTIVES

- Develop a preserved Prevenar 13™ formulation for distribution in the developing world (WHO Markets). Identify a suitable preservative
  - — Optimal concentration for preservative with safety record
  - — Stability of vaccine and preservative effectiveness
  - — Meets preservative effectiveness criteria for Developing world
- Preservative efficacy test
  - — Determination of antimicrobial effectiveness of vaccines (13vPnC) – containing preservatives
  - — Simulation of contamination events under worst conditions (e.g., single challenge by USP/EP or multiple challenge by WHO)
  - — Meets European Pharmacopeia 5.1.3 Criteria "B"
- Develop a 13vPnC primary and secondary package
  - — 4-dose/vial presentation in a multi-dose vial that minimizes storage space
- Good size for small hand transport within cold chain
  - — Transport to villages
  - — Refrigerator storage
- Advantages
  - — Lower price per dose
  - — Occupy less cold chain capacity

MATERIALS AND METHODS

- Formulations
  - — Multiple formulations of Prevenar 13™ were prepared in the presence and absence of different preservatives and analyzed for quality attributes such as total and bound antigenicity and protein, pH, appearance and preservative effectiveness
- Preservatives
  - — 2-Phenoxyethanol
  - — Phenol
  - — Meta-Cresol
  - — Methylparaben and Propylparaben
  - — Thimerosal (reference)

PRESERVATIVE EFFECTIVENESS TEST CRITERIA

Criteria	EP 5.1.3	USP <51>	WHO (Multi-challenge)
# of Challenge Organisms	4	4	4
Organisms	Bacteria, Mold & Yeast	Bacteria, Mold & Yeast	Bacteria
Challenged CFU/ mL	$10^5 – 10^6$	10^5 – 106	5 x 10^3
# of Challenges	Single challenge at t=0	Single challenge at t=0	Multiple challenges at t=0, 6h, 24h, 7d & 14d
Control – Preservative	Not required	Not required	Required
Sample storage after challenge	20 – 25 °C	20 – 25 °C	2 – 8 and 22 – 25 °C
Enumeration times	0, 6h, 24h, 7d, 14d & 28d	0, 7d, 14d & 28d	0, 6h, 24h, 7d, 14d & 28d

Acceptance criteria:

EP 5.1.3 Criteria B: 1 log reduction of bacteria in 24 hours, 3 logs in 7 days and 14 days for yeast and fungi.

USP: 1 log reduction in bacteria and no increase in yeast and fungi.

Analysis of preservative effectiveness:

Catalent Pharmaceutical Solutions, 160 Pharma Drive, Morrisville, N.C. 27560 performed preservative effectiveness tests, by single challenge or multi-challenge methods under Pfizer guidance.

INOCULATION SCHEME FOR SINGLE CHALLENGE STUDIES

Sample	Storage Temp (°C)	Inoculation Timing	Inoculation
Vaccine control Vaccine with Preservatives	22 - 25	T = 0	($10^5 – 10^6$/mL) *P.aeruginosa* *S.aureus* *E.coli* *B.subtilis* *No spike control*

INOCULATION SCHEME FOR MULTIPLE CHALLENGE STUDIES

Sample	Storage Temp (°C)	Inoculation Timing	Inoculation
Vaccine control Vaccine with Preservatives	2-8 22-25	0 6h 24h 7 days 14 days 28 days –	(5 x 10^3/mL) *P.aeruginosa* *S.aureus* *E.coli* *B.subtilis* *No spike control*

COMPARISON OF PRESERVATIVES

Preservative	0.5mL Dose Target	USP <51>	EP 5.1.3
			Fail
None	0	NA	Fail
None	0	NA	Fail
Phenol	0.25%	Pass	Fail
Meta-Cresol	0.03%	Pass	Fail
Methylparaben and Propylparaben	0.18% methylparaben 0.02% propylparaben	Pass	Fail
Reference control preservative			
Thimerosal	25µg Hg	Pass	Fail
Thimerosal	50µg Hg	Pass	Pass
Thimerosal	100µg Hg	Pass	Pass

None of the above preservatives were suitable with Prevenar 13™

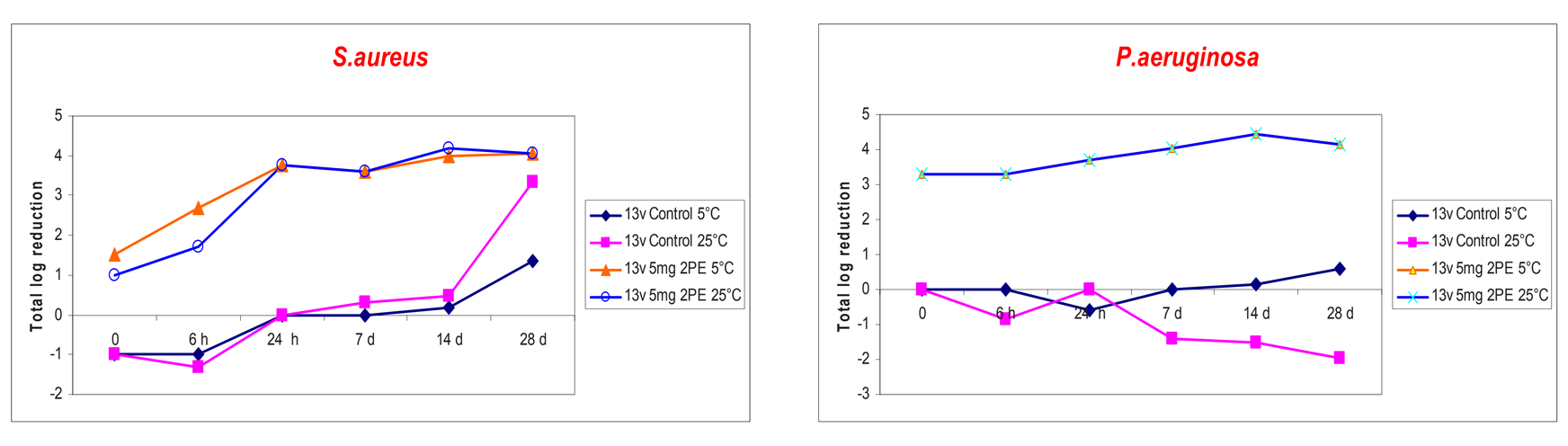
PRESERVATIVE EFFECTIVENESS OF 2-PE IS DOSE DEPENDANT

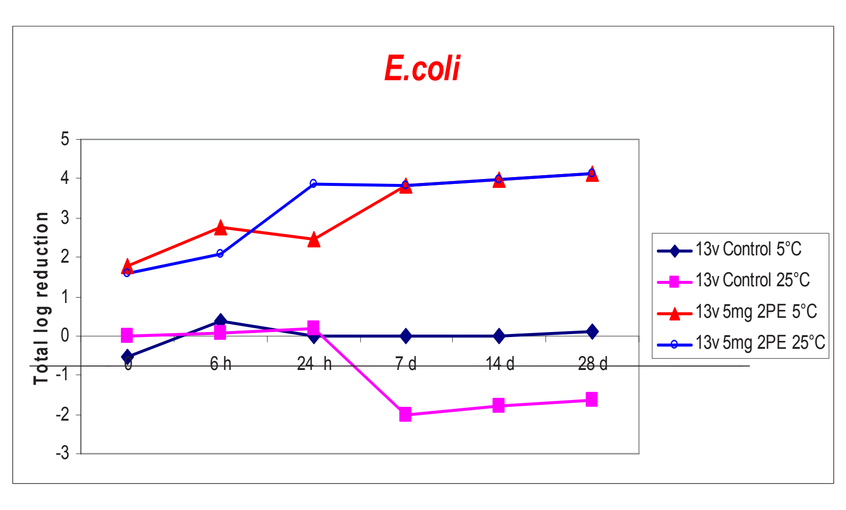
0.5mL Dose Target	USP <51>	EP 5.1.3
		Fail
2.5mg	NA	Fail
3.5mg	NA	Pass
4.5mg	NA	Pass
5.0mg	NA	Pass
5.0mg	Pass	Pass
5.0mg	NA	Pass
5.5mg	NA	Pass
6.0mg	NA	Pass
6.5mg	NA	Pass
7.0mg	NA	Pass
7.5mg	NA	Pass
7.5mg	NA	Pass
8.0mg	NA	Pass
10.0mg	Pass	Pass

A minimum concentration of 3.5mg 2-PE is required to demonstrate consistent preservative effectiveness

Prevenar 13™ is stable in the presence of 2-PE

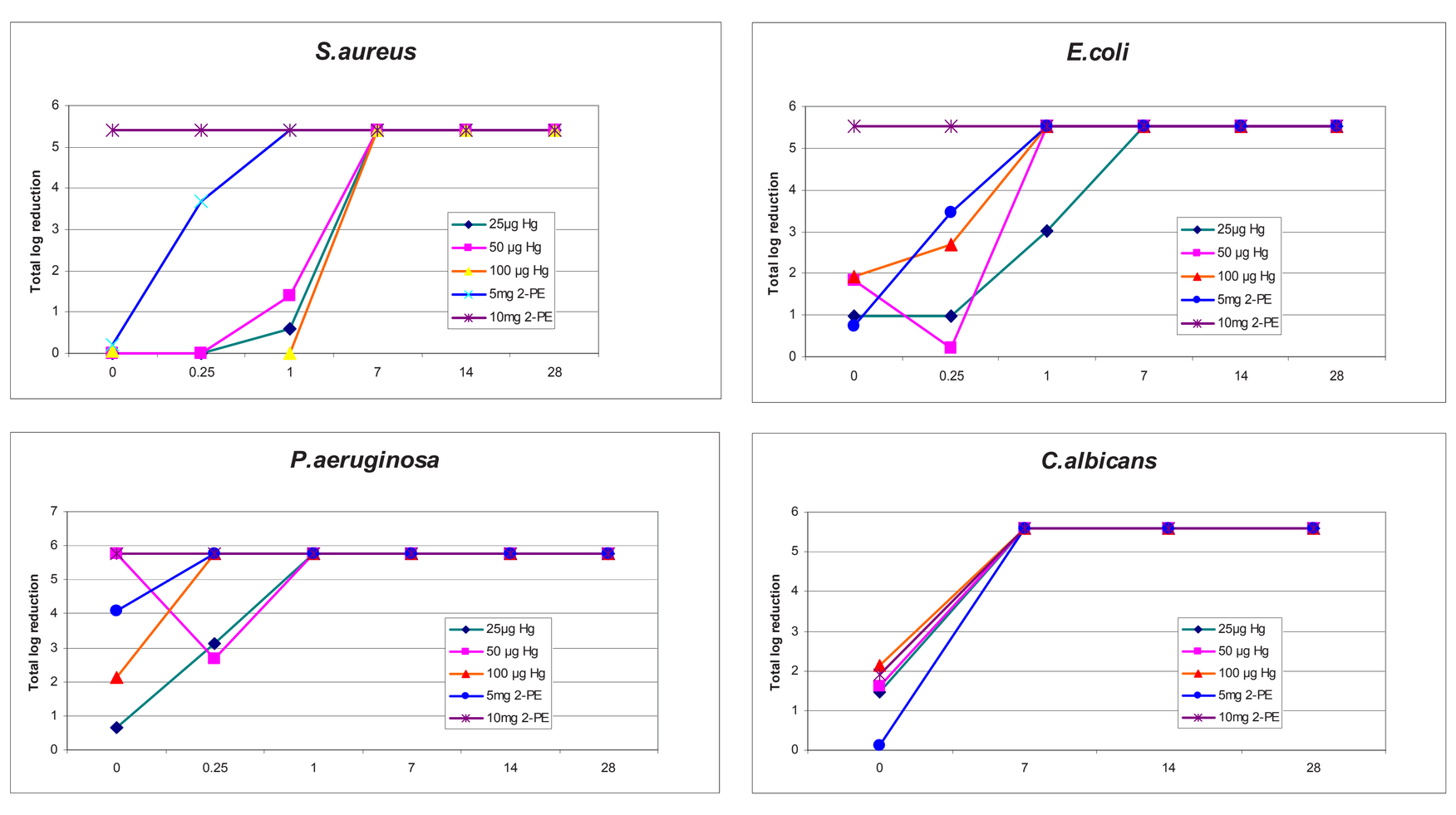
PREVENAR 13™ WITH 2-PE MEETS BOTH SINGLE AND MULTI-CHALLENGE PRESERVATIVE EFFECTIVENESS TEST

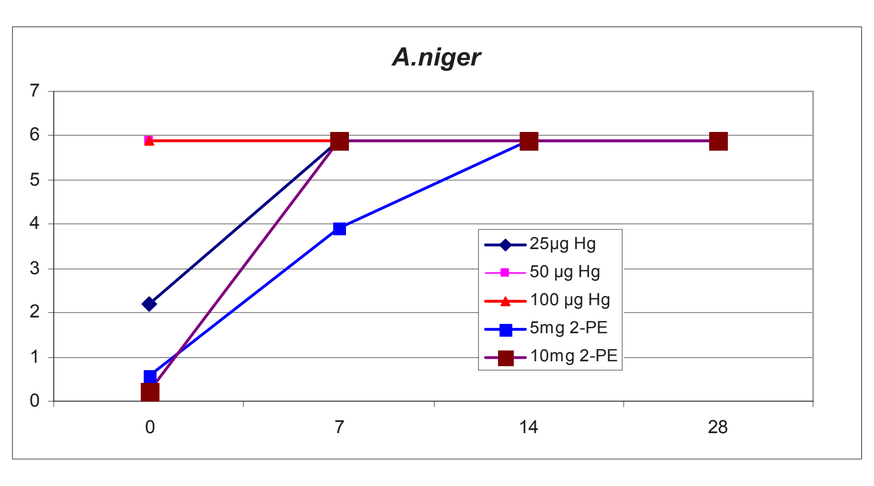




Vaccine formulation	Control Prevnar 13™	Prevenar 13 ™ with 5mg dose 2-PE
Single challenge EP 5.1.3	*Fail*	*Pass*
Multi-challenge	*Fail*	*Pass*

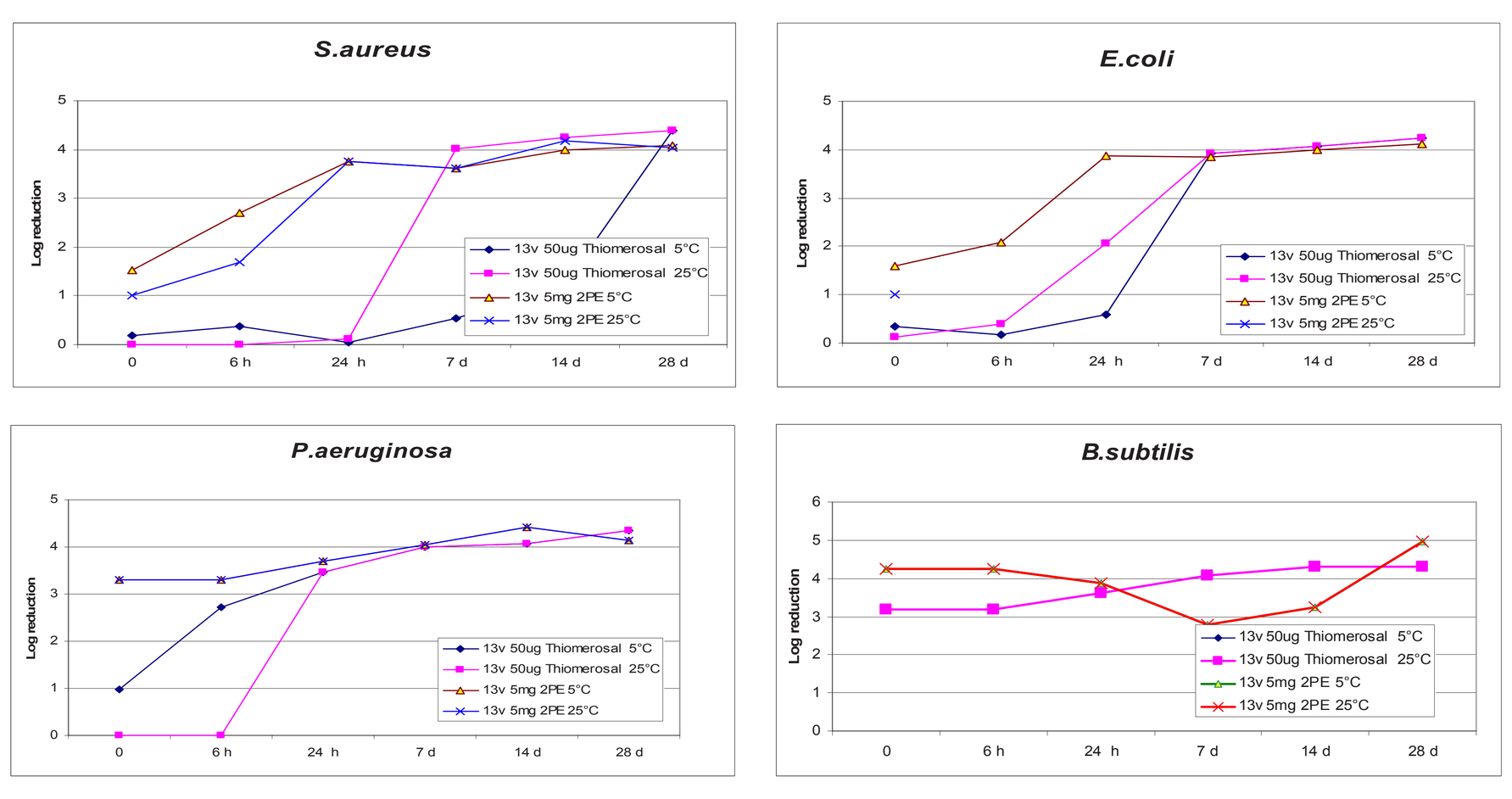
2-PE IS MORE EFFECTIVE THAN THIMEROSAL IN INHIBITING GROWTH PARTICULARLY *S.AUREUS*





	USP <51>	EU Ph 5.6 (5.1.3) Criteria "B"
Thimerosal 25µg Hg	Pass	Pass
Thimerosal 50µg Hg	Pass	Pass
Thimerosal 100µg Hg	Pass	Pass
5 mg 2-PE	Pass	Pass
8 months RT	ND	Pass
10 mg 2-PE	Pass	Pass
8 months RT	ND	Pass

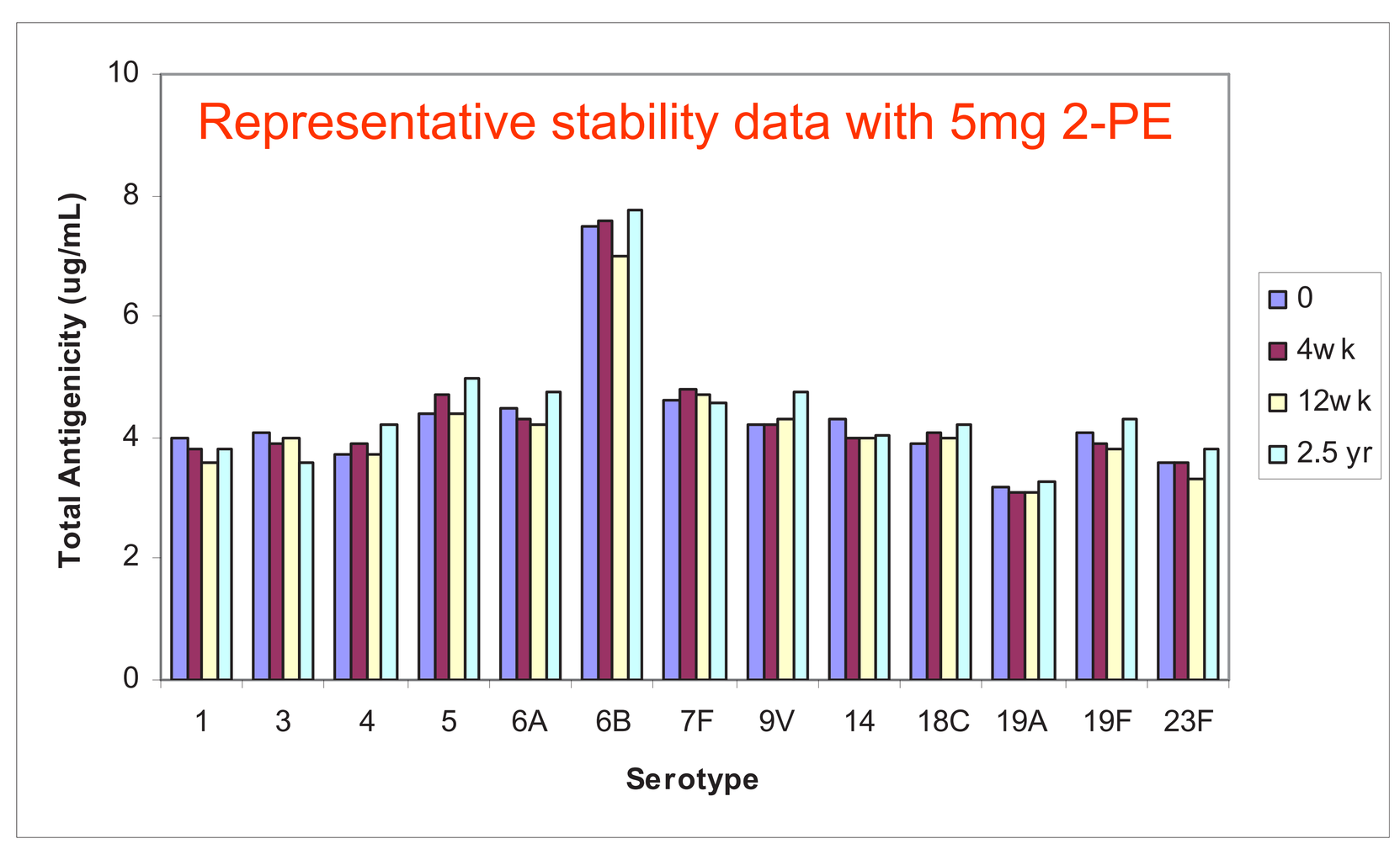
2-PE IS MORE EFFECTIVE THAN THIMEROSAL IN MULTI CHALLENGE TEST



2-PE IS MORE EFFECTIVE THAN THIMEROSAL IN MULTI CHALLENGE TEST

Preservative	0.5mL Dose Target	Multi-challenge	Single challenge
None	0	Fail	Fail
Thimerosal reference	50µg Hg	Fail	Pass
Thimerosal reference	25µg Hg	Fail	Fail
Site Control	50µg Hg	Fail	Pass
2-PE	5.0mg	Pass	Pass
2-PE/0.1mg Formalin	7.5mg	Pass	Pass
EP criteria 5.1.3 Criteria "B"			

LONG TERM STABILITY OF PREVENAR 13™ CONTAINING 2-PE

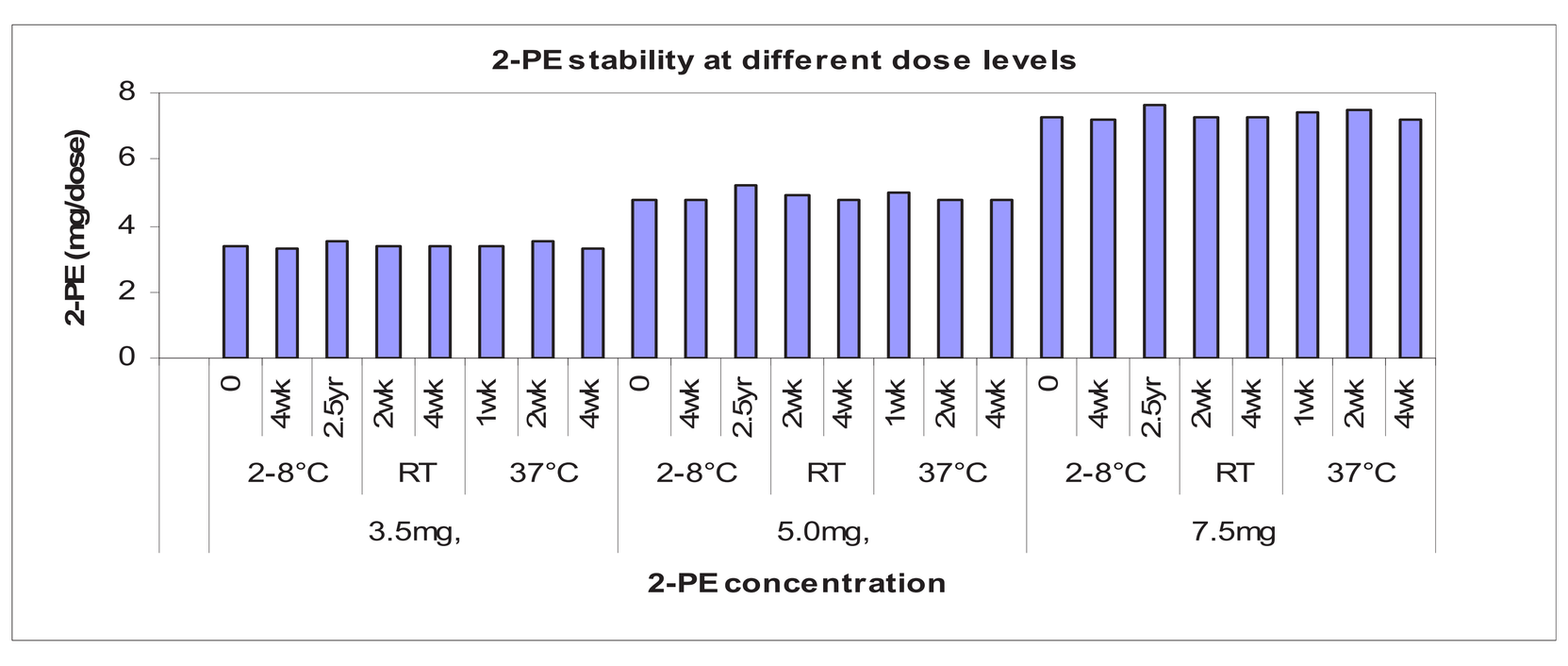


Representative data demonstrates long term stability measured as total antigenicity of each individual serotype in Prevenar 13™.

Similar stability was observed with formulations containing 2-PE concentration ranging from 3.5 and 7.5mg per dose for total antigenicity, protein, pH and appearance

Agitation stress studies demonstrated compatibility with chosen vial/stoppers

LONG TERM STABILITY OF 2-PE



2-PE in a Prevenar 13™ formulation is stable for 2.5 years at 2-8°C

PRESERVATIVE EFFECTIVENESS IS MAINTAINED AFTER STORAGE OF PREVENAR 13™ WITH 2-PE

Dose 2-PE/0.5mL	Sample time point Storage temp.	EP 5.1.3
0	0	Fail
0	37°C 1m	Fail
0	2.5yrs at 2-8°C	Fail
3.5mg	0	Pass
3.5mg	37°C 1m	Pass
3.5mg	2.5yrs at 2-8°C	Pass
5.0mg	0	Pass
5.0mg	37°C 1m	Pass
5.0mg	2.5yrs at 2-8°C	Pass
7.5mg	0	Pass
7.5mg	37°C 1m	Pass
7.5mg	2.5yrs at 2-8°C	Pass

Formulations consistently meet the preservative effectiveness criteria

CONCLUSIONS

- 2-phenoxyethanol at a target concentration of 5.0 mg per dose is recommended for a multi-dose formulation of Prevenar 13™
  - — Demonstrated storage temperature (2-8°C) stability and accelerated short term accelerated temperature stability for both antigen (all thirteen serotypes) and preservative (2-PE)
  - — Consistently meets preservative effectiveness single dose (EP 5.1.3 "B" criteria)
  - — Demonstrated that the formulation meets EP 5.1.3 "B" criteria in a multi challenge study
  - — This concentration has been used in two infant based vaccines Engerix and Twinrix from GSK and has been demonstrated to be safe.
- Thimerosal is not an effective preservative compared to 2-PE for Prevenar 13
  - — Demonstrated slower killing of *S.aureus*
  - — Slow reduction of EC and SA viability in samples containing thimerosal
  - — In multi-challenge tests the rate of killing in the first 24 hours is faster with 2-PE
- The data support the use of 2-PE as a more effective preservative with the potential to replace thimerosal, the most commonly used preservative in multi-dose vaccine formulations.

REFERENCES

- "Pneumococcal vaccines. WHO position paper". *Relevé épidémiologique hebdomadaire / Section d'hygiène du Secrétariat de la Société des Nations = Weekly epidemiological record / Health Section of the Secretariat of the League of Nations* **74** (23): 177–83. June 1999. PMID 10437429.
- World Health Organization (WHO). The use of opened multi-dose vials of vaccine in subsequent immunization sessions. WHO policy statement: Document WHO/V&B/00.09, 2000 [on line]. Available from URL: http://www.who.int/vaccines-documents/DocsPDF99/www9924.pdf pp. 1 –9
- Single-dose versus multi-dose vaccine vials for immunization programmes in developing countries. Bulletin of the World Health Organization, 2003 by John S. Lloyd, Paul K. Drain, Carib M. Nelson
- WHO study protocol nº 2 on preservative efficacy in multi dose vaccine. (2006) Antimicrobial effectiveness testing in multi-dose vials of vaccine. Protocol Part I and II, WHO Geneva.
- The European Pharmacopoeia Supplement 6.8 to the 6th Edition (2010), Chapter 5.1.3 Efficacy of Antimicrobial Preservation, In *The European Pharmacopoeia* published by the Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM), Strasbourg Cedex, France, pp. 5129 -5130.
- United States Pharmacopoeia 32, Rockville (MD) Chapter 51 (2009).
- http://www.who.int/wer/pdf/1999/wer/7423.pdf

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