



Medicines & Healthcare products  
Regulatory Agency

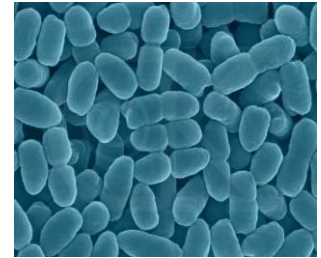
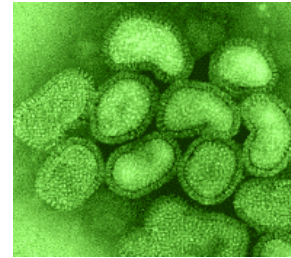
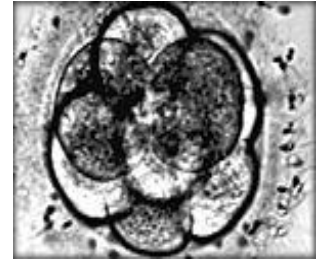
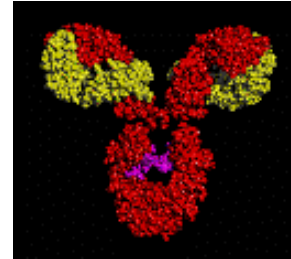
# Recent Developments in the QC of Vaccines

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# Biological Medicines: Why are they special?

- Made from biological sources
- Highly complex
- Must be measured by biological effect
- Special risks

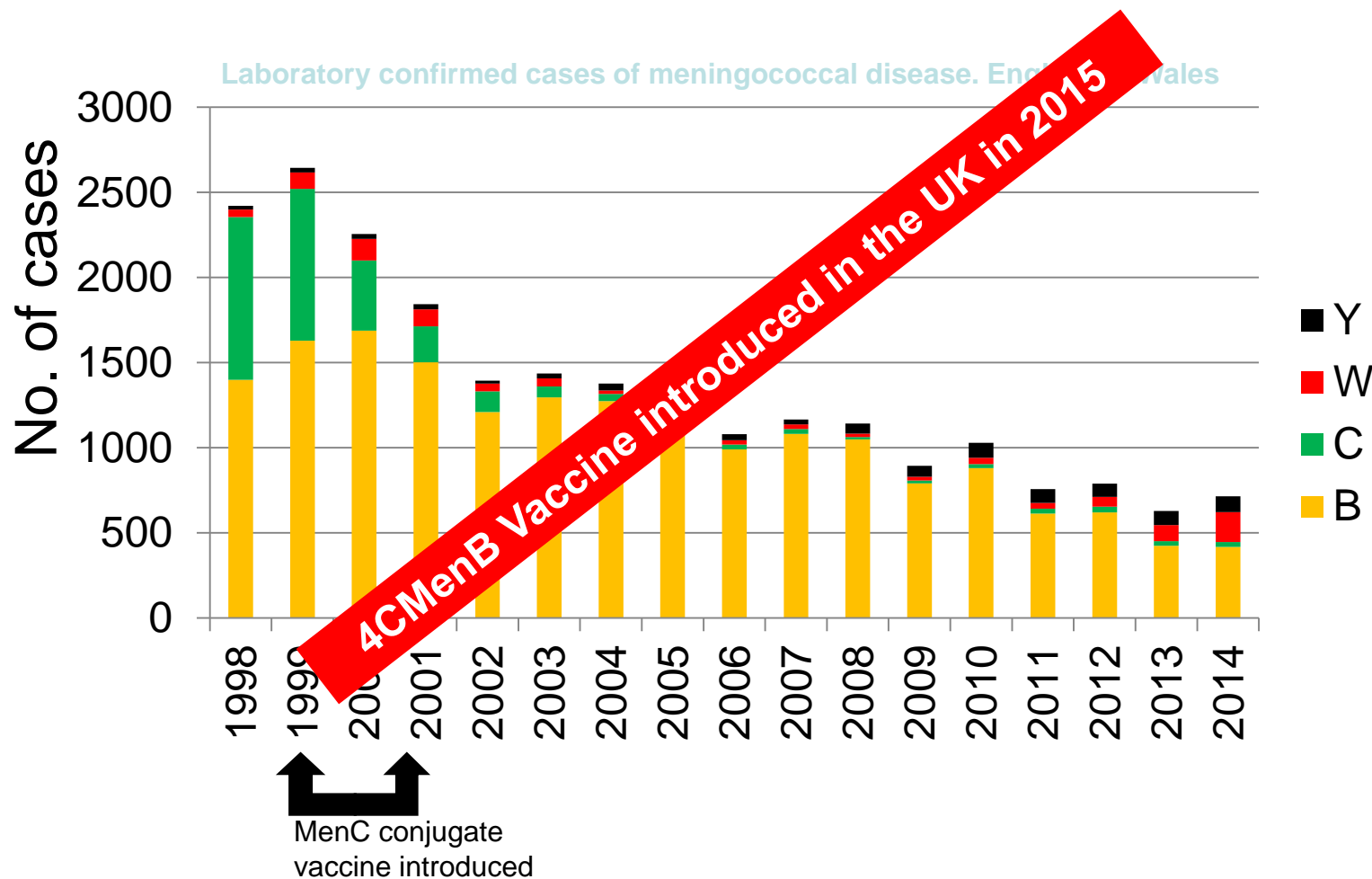


# Vaccine life cycle

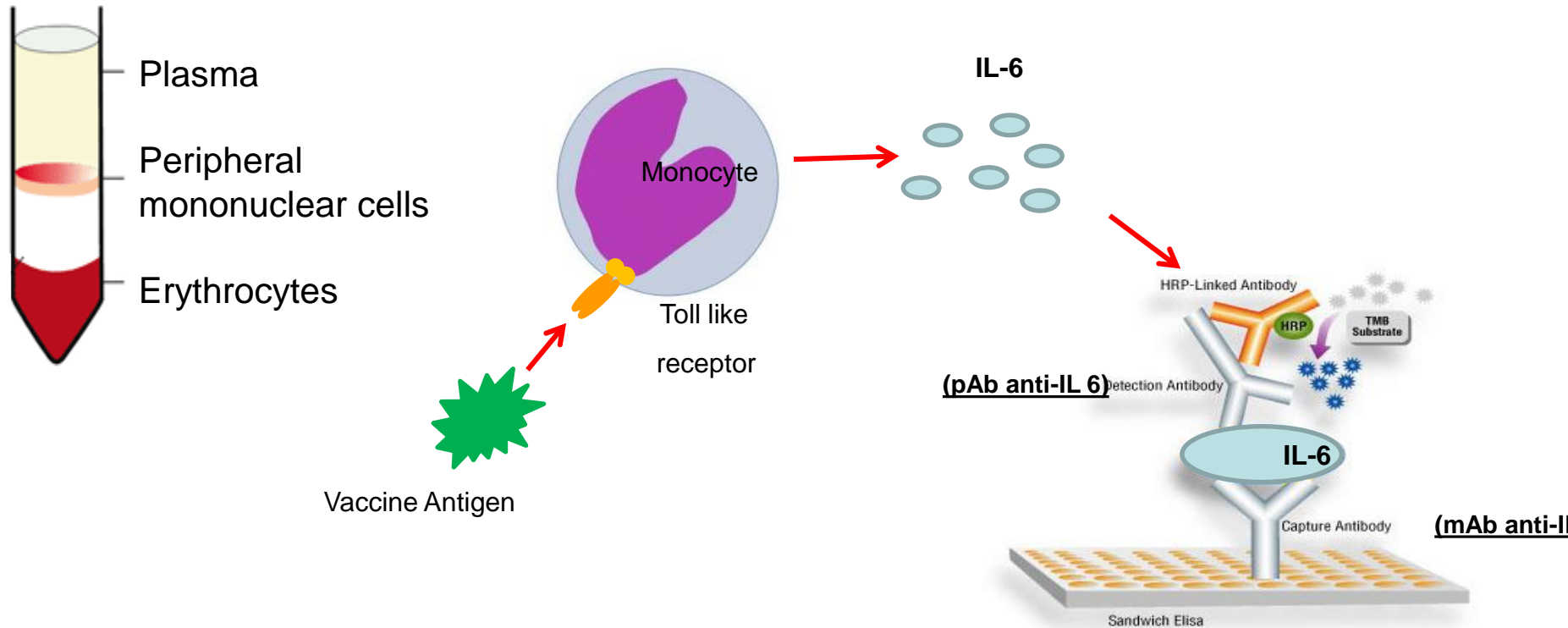


- Need to be able to change analytical methods but inherent inertia once specifications have been set
- But there drivers for implementing novel analytical approaches
  - Replacement of *in vivo* assays with *in vitro* or cell-based alternatives
  - Implementation of new technological approaches for existing products

# Meningococcal disease in the UK



# Monocyte Activation Test (MAT) NIBSC



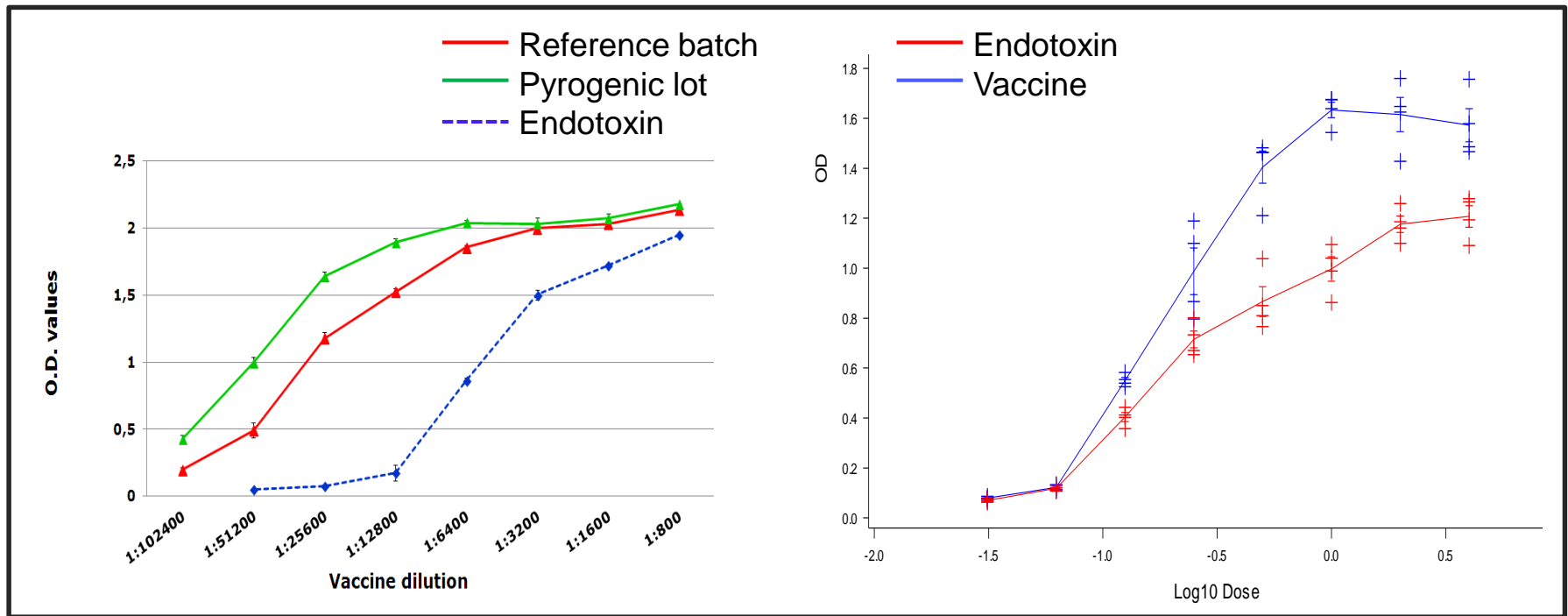
1. Blood taken from 4 donors. PBMCs isolated by density gradient centrifugation

2. PBMCs incubated 16-24 hours with vaccine

3. Levels of IL-6 in the culture supernatant are measured in a capture ELISA

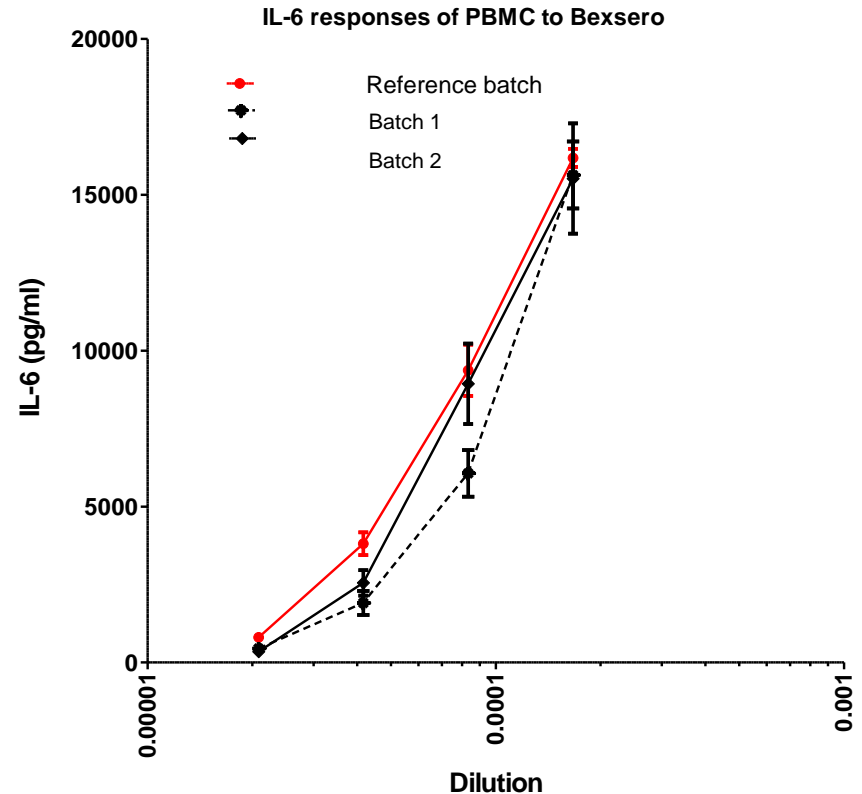
# EP Methods

- Method A: Quantitative test comparing preparation to endotoxin dose response curve ✗
- Method B: Semi-quantitative test comparing preparation to standard endotoxin ✗
- Method C: Reference lot comparison comparing preparation to a reference lot of material ✓

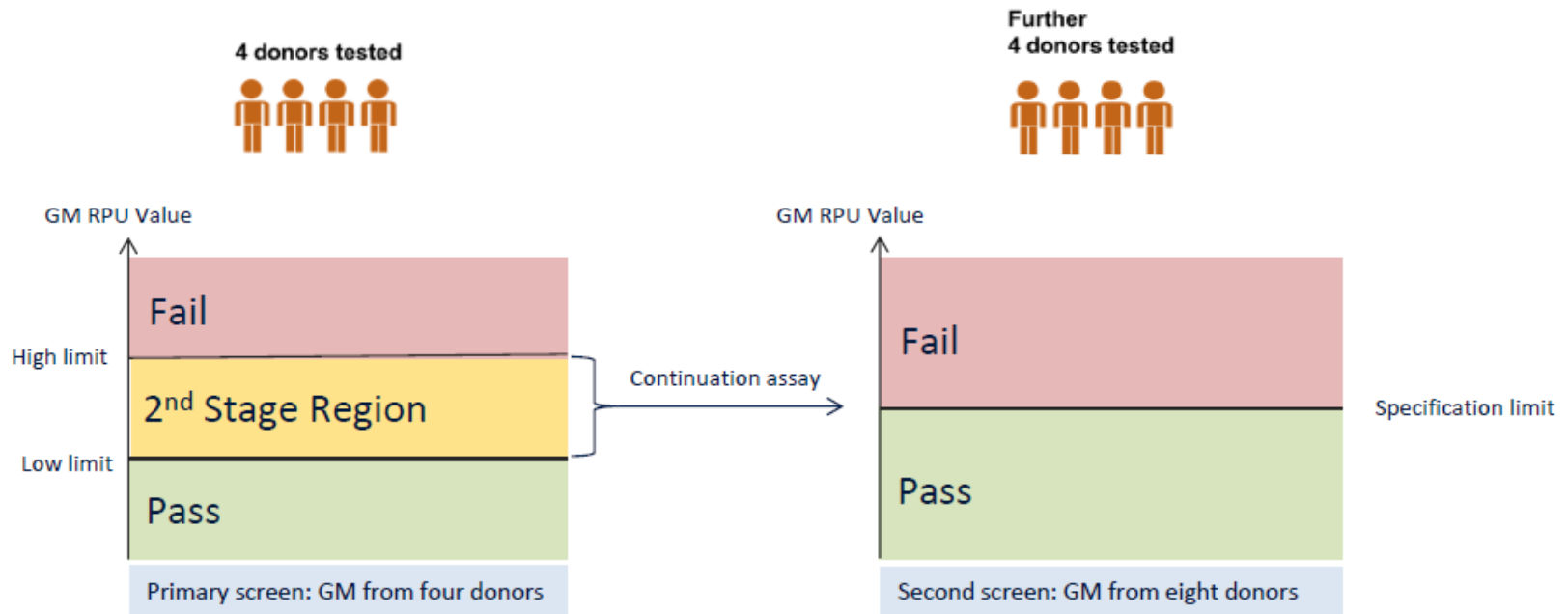


# Modifications to Method C

- Parallel line analysis to express pyrogenicity of test batch to reference batch as 'relative potency' or ratio.
- Reference batch is used to define quantitative measure of pyrogenicity, not to set an acceptable upper limit.



# Monocyte Activation Test for inherently pyrogenic vaccines



**Two stage screening approach using geometric mean relative pyrogen unit values from four (or eight if required) donors**

Specification limit is based on the results (RPU) from a large number of batches: geometric mean  $\pm$  3SD



# Reducing the animal use in testing pertussis vaccines

Pertussis toxin in its detoxified form (PTd) is an important antigen present in both ACVs and WCVs.

The histamine sensitization test (HIST) is the current regulatory required test for batch release of pertussis vaccines of both pertussis mono-component and pertussis containing combination vaccines.

## **HIST test:**

- Lethal challenge procedure
- Large variations in test performance and difficulty in standardisation
- This normally leads to repeated tests caused by invalid assays, a frequent occurrence with this procedure
- There is an urgent need to develop alternatives to the HIST

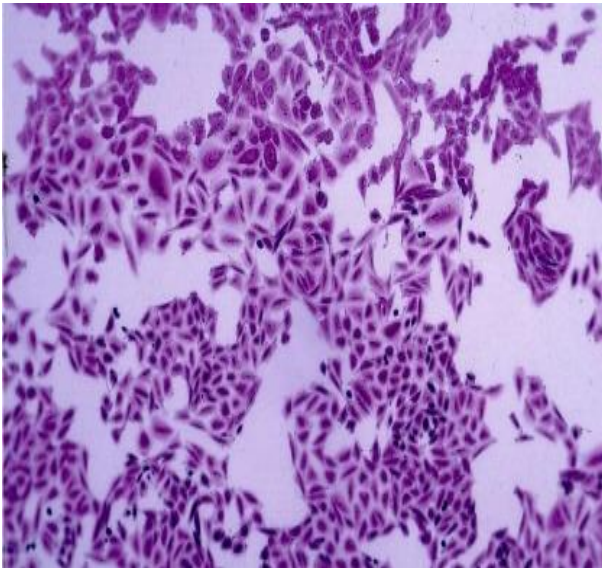
## **Alternatives to HIST**

- CHO cell clustering assay
- Enzyme coupled-HPLC assay
- Carbohydrate-binding assay

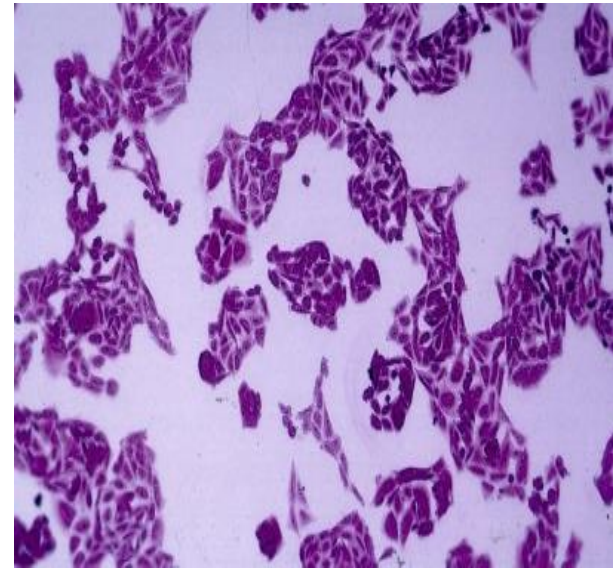
# CHO cell assay

Chinese Hamster Ovary (CHO) cells are sensitive to pertussis toxin

No clustering - negative



Clustering - positive



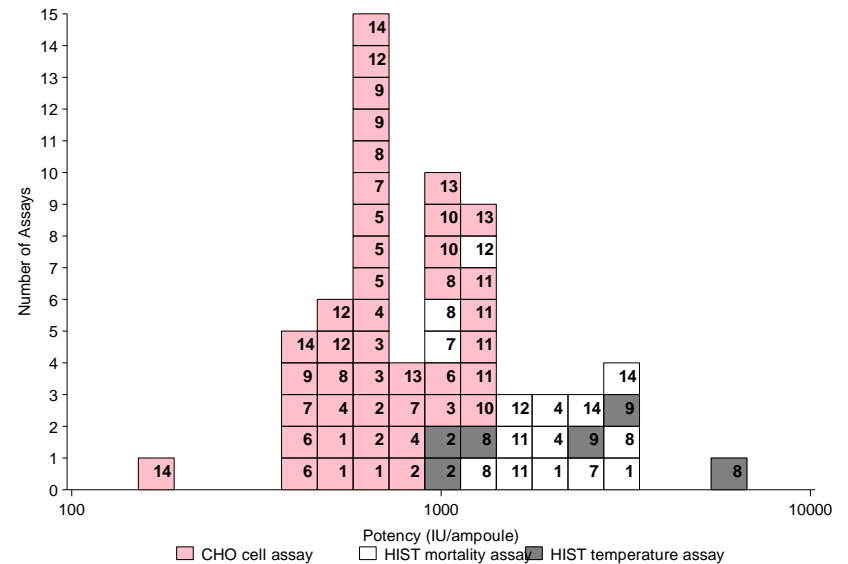
Collaborative study organised

To calibrate the activity of the candidate material with for the the Second International Standard for Pertussis Toxin for HIST and CHO

To assess the possibility of assigning unitage for different *in-vitro* assay systems

# Collaborative study

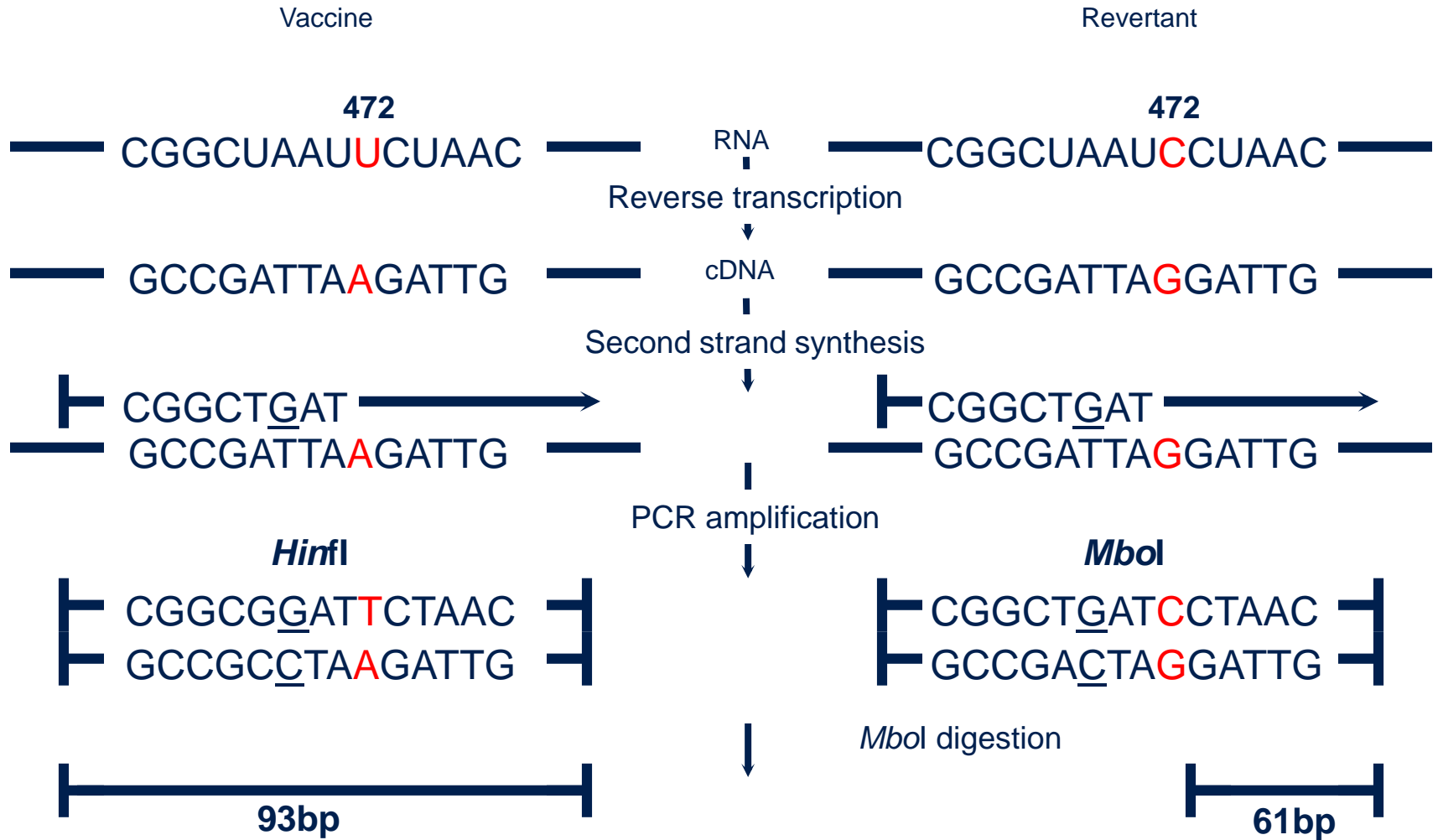
- 14 laboratories took part in collaborative study
  - 11 labs performed HIST
  - 14 labs performed CHO
- Candidate material has demonstrated suitable biological activity and stability in both HIST and CHO to be established as the 2<sup>nd</sup> International Standard for Pertussis Toxin
- Proposed unitage agreed with participants 1,813 IU/ampoule for Histamine sensitisation assay
- 680 IU/ampoule for CHO cell clustering assay



Histogram showing the distribution of individual assays potency estimates for 15/126 relative to JN1H-5 (numbers in boxes indicate laboratory code)

# The MAPREC Test

Mutant Analysis by PCR and Restriction Enzyme Cleavage (MAPREC) for type 1, 2, 3 OPV  
Complex, technically challenging procedure (e.g. Type 3)



# Deep sequencing

- Sensitive without the use of isotopes
- Generates information about mutations at all locations in the viral genome

## Collaborative study

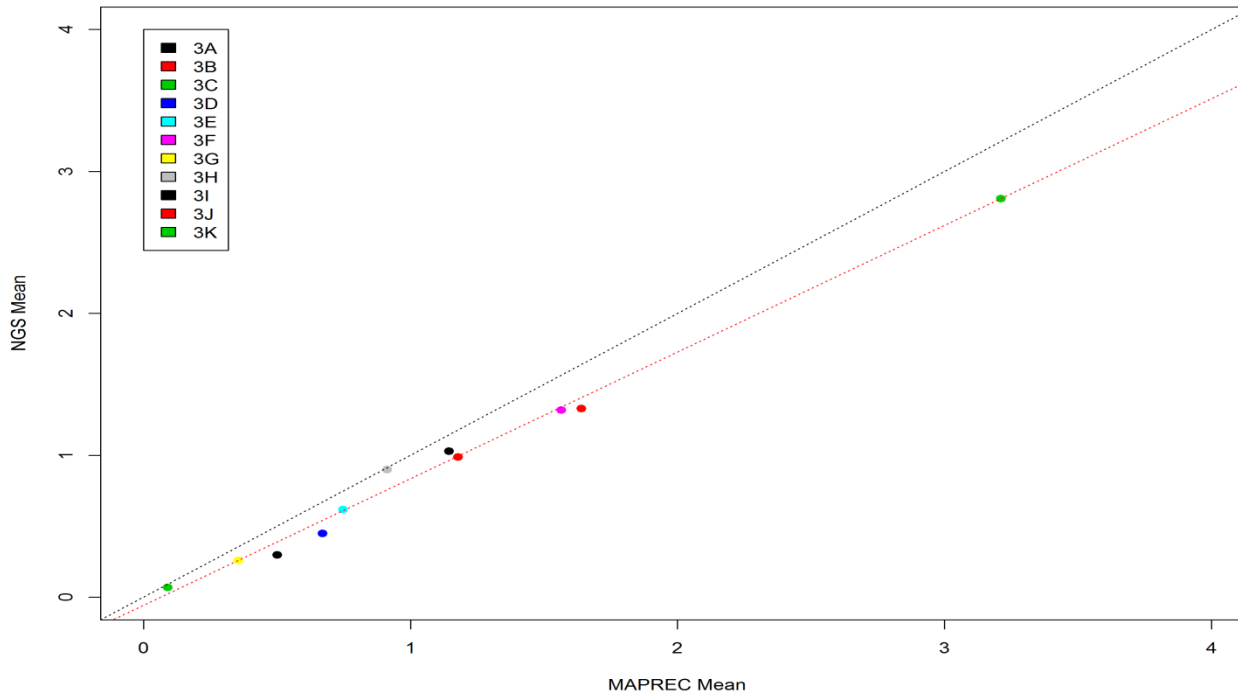
### Study Samples

- ❑ 11 samples with MAPREC, MNVT and TgmNVT data
- ❑ Including 6 MAPREC reagents (low and high reference mutants for each serotype)

### Study Method

- ❑ 8 laboratories
- ❑ NGS and bioinformatics according to in-house protocols with 5 replicate NGS determinations

# Deep sequencing vs. MAPREC



8 labs returned processed %472C  
6 labs returned processed %2493U  
8 labs returned raw FASTQ data  
7 labs returned methodology details

All raw data reanalysed by NIBSC  
bioinformatics department

Phase 1: type 3 472-C and 2493-U complete; report in progress

Phase 2: type 1 and 2 vaccines and evaluation of genetic consistency of mutational profiles in entire viral genome – analysis ongoing

# Development of new methods for vaccines – NIBSC activities

Monocyte activation test for pyrogenic vaccines ✓

Development of alternatives to the histamine assay for pertussis ✓

New Vi and Vi antiserum standards for typhoid vaccines

Genome Sequencing vs. MAPREC for polio vaccine ✓

Pseudotype virus neutralisation assays

Application of deep sequencing for adventitious agents

# Acknowledgements

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