PDA New England Chapter 2011

Development and Validation of a Potency Assay for a Recombinant Influenza Vaccine

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Protein Sciences CORPORATION

A Vaccine Company for the 21st Century

Company Overview

Protein Sciences CORPORATION

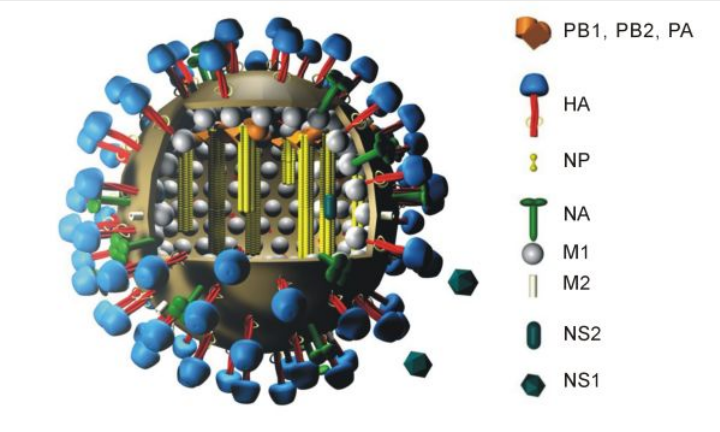
Mission: To save lives and improve health by effectively responding to our changing world with innovative vaccines and biopharmaceuticals

- Founded in 1983 (MicroGeneSys)
- Baculovirus/Recombinant Protein Production Technology
- On-Site GMP Manufacturing
- ~90 Employees

- FluBlok recombinant seasonal influenza vaccine
  - Supported by BARDA contract HHSO1002009000106C
- PanBlok recombinant pandemic influenza vaccine
  - Supported by BARDA contract HHSO1002009000106C

Influenza Viruses - Overview

Human Influenza



HA & NA Glycoprotein Spikes

- Enveloped virus
- Spike proteins bind target cell receptors
- Spikes are antigenic surfaces for immune system detection – changes lead to annual epidemics
- WHO select 3 strains annually for the vaccine/trivalent formulation
- HA = primary immunogenic component of influenza vaccines

U.S.- licensed Seasonal Influenza Vaccines - Routine Licensing Actions

Each year, one or more of the three vaccine strains may be replaced with a new strain

Each year, submission of a prior approval manufacturing supplement to an existing biologics license application (BLA) is required for annual influenza strain change

- "Strain change supplement"

Clinical Data:

- Inactivated vaccines: No clinical data
- Live attenuated: Limited clinical data

Production of influenza vaccine

Characteristics

- Trivalent vaccine: 2 A strains and 1 B strain
- Protection correlates with hemagglutinin (HA) antibodies

Production process:

Chicken Embryos

↓

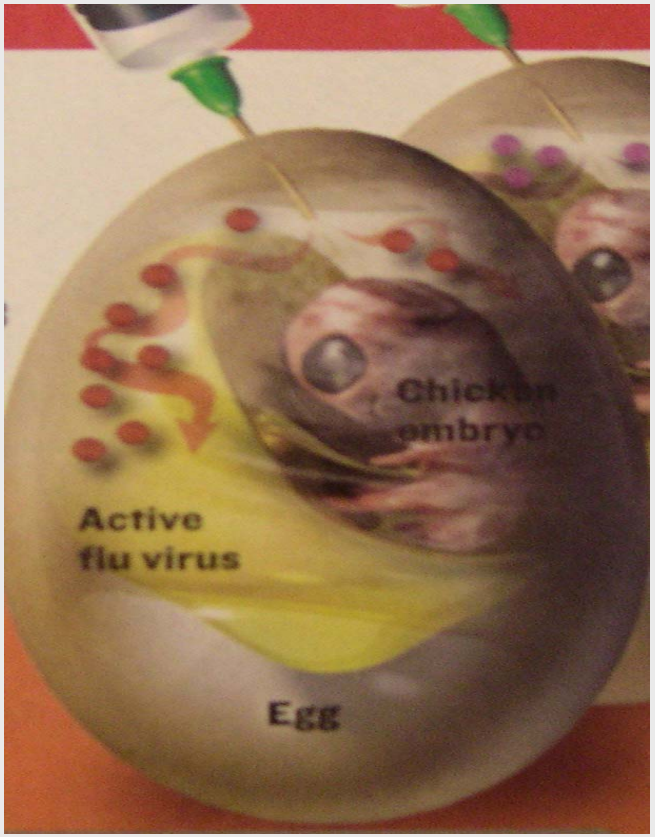
Isolation of Virus

↓

Kill Virus

↓

Isolate virus proteins



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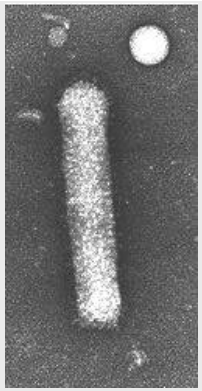
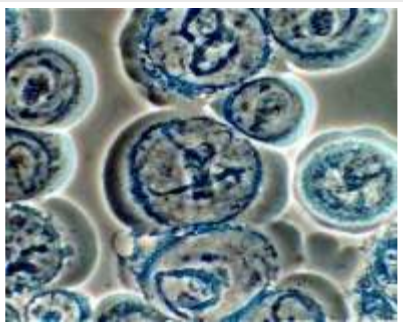
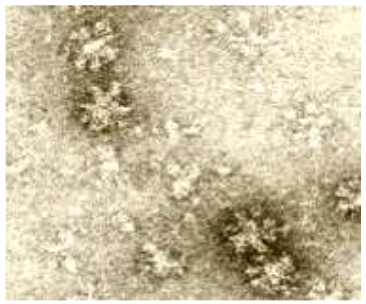
Technology Background BEVS

Baculovirus Expression Vector System (BEVS)



Engineer baculovirus with the gene of interest

Baculoviruses highly specific to insect cells

Powerful promoter generates high yield of protein of interest

Culture insect cells in a fermenter

Infect cells with engineered virus

Incubate infection for ~48 - 72 hours

Protein folding

Purify drug substance

Formulate into vaccine

Potency

Specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.

-- [21 CFR §600.3 (s)]

Necessary attributes for potency assays

Predictive of ***clinical benefit***

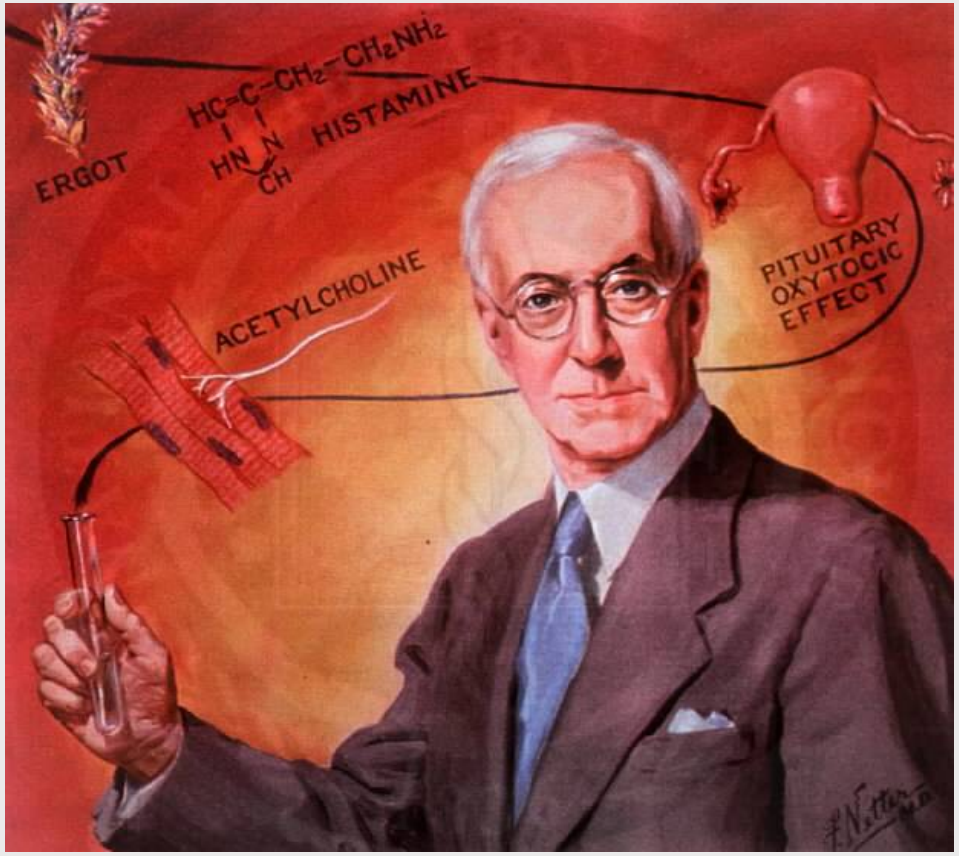
Possess characteristics that are amenable to ***validation***

Precision sufficient to meet goal of potency assays, i.e., provide assurance that vaccine is safe and effective throughout the dating period

- Includes for use in stability studies
- Includes for use in the “bridge” between marketed and clinical trial materials

Stability indicating

Choosing “the right thing”



Nobel Laureate in Medicine, 1936

Every worker in biology must know the temptation to adopt a method because it measures something with reasonable precision, without waiting for conclusive evidence that what it measures is the right thing

- Sir Henry Dale

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Potency Assays and Vaccines: A Few Examples

Number of plaque forming units (e.g., mumps, measles, rubella, smallpox)

Number of colony forming units (e.g., S. typhi, TY21a)

SRID (e.g., flu vaccines)

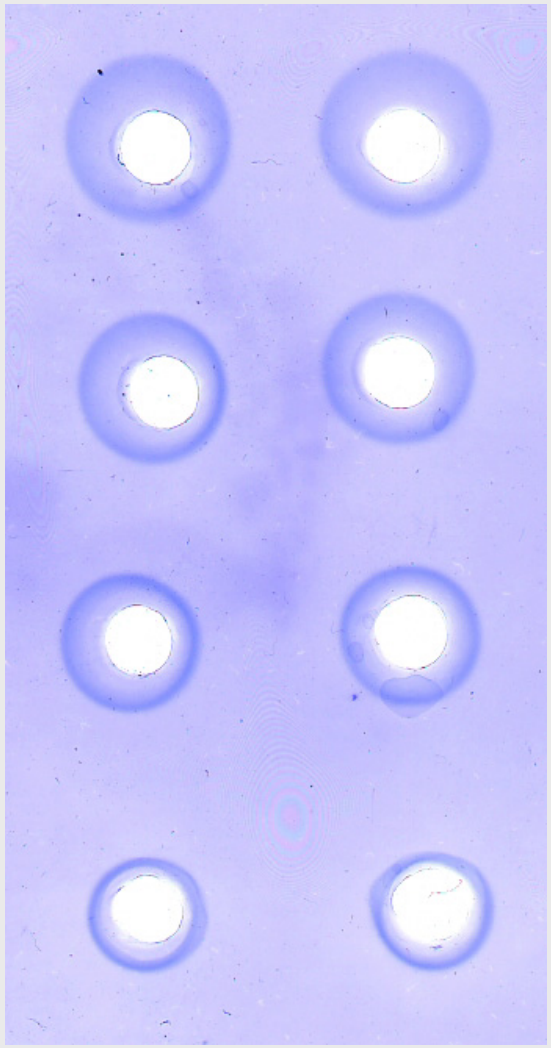
Epitope-based (e.g., HPV)

Chemical and Physical chemical characterization (e.g., polysaccharide and polysaccharide-protein conjugate vaccines)

Serological response in animals (e.g., diphtheria)

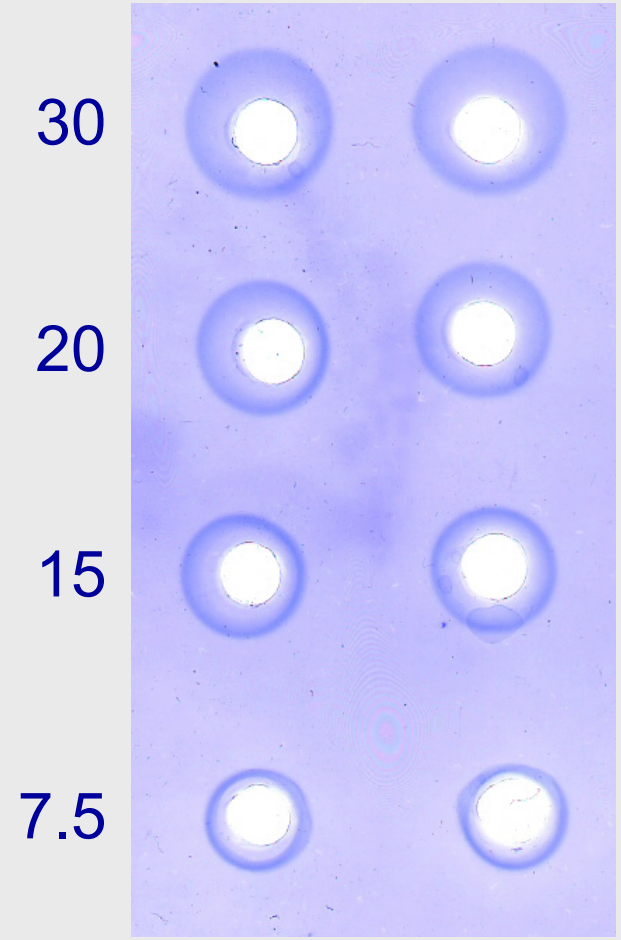
Animal protection against challenge (e.g., rabies, anthrax)

SRID (Single Radial Immunodiffusion) assay

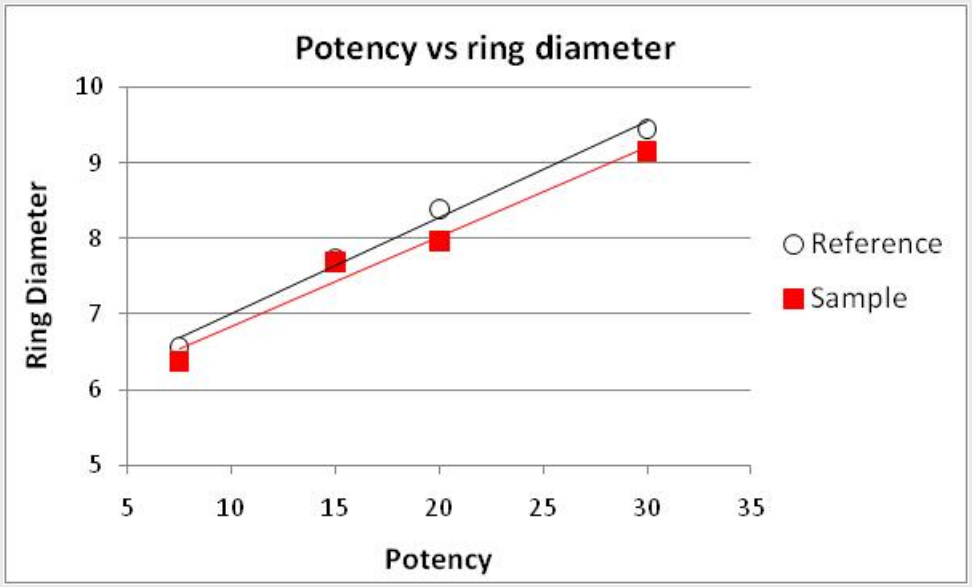


- In use since 1978 for determination of potency of all influenza viruses
- Antiserum against HA incorporated into agarose gels
- HA diluted to 30 µg/mL, treated with detergent, and added to wells in the gel
- HA diffuses radially and interacts with anti-HA antibodies
- Precipitin ring is formed
- Diameter of the ring corresponds to HA content (potency)
- Not an absolute measure; HA content is determined by comparison with a reference standard

SRID (Single Radial Immunodiffusion) assay



Dilution	Potency	Reference	Sample
1	30	9.45	9.15
1.5	20	8.39	7.97
2	15	7.73	7.69
4	7.5	6.57	6.38



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Adoption of CBER SRID Method

Original method at PSC used single sample preparation and linear regression to determine sample potency.

CBER prefers harmonization of SRID method.

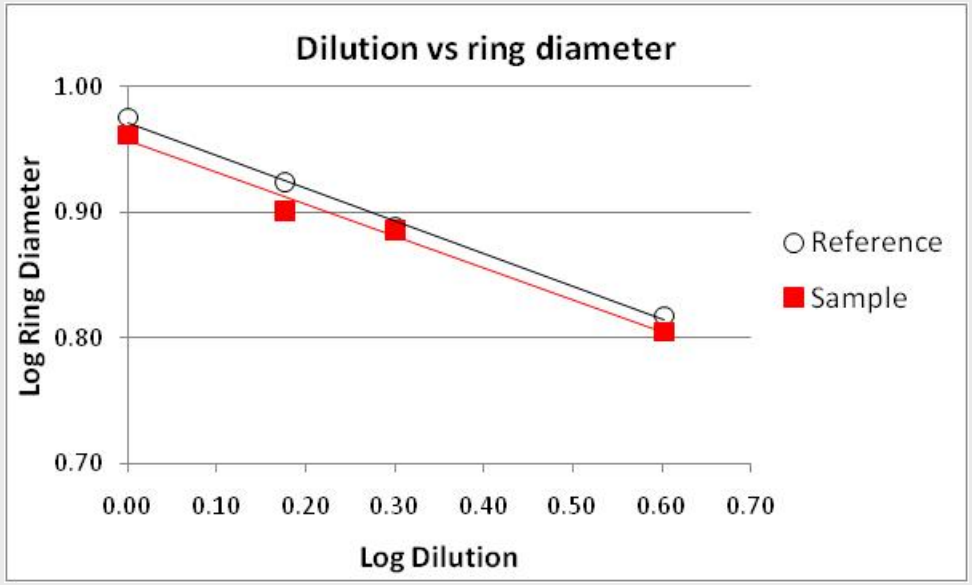
- Ensure consistent product quality
- Expedite release

Transitioned to CBER's method in 2008

- Multiple sample preparations within each test to capture inherent variability
- Mechanical differences in test performance
- Parallel line analysis

SRID (Single Radial Immunodiffusion) assay

Dilution	Reference	Sample
0.00	0.98	0.96
0.18	0.92	0.90
0.30	0.89	0.89
0.60	0.82	0.80



Assay Validity

- Linearity: $r \geq 0.95$
- Parallelism: Student's t test (compare slopes) less than 4.604
- Relative potency of sample must be 24-36 (0.67 to 1.2 relative to reference)
- Standard deviation between potency of preps within test (differs for DS and DP)

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Optimization of CBER SRID Method

Comparison with existing method

Evaluation of assay parameters

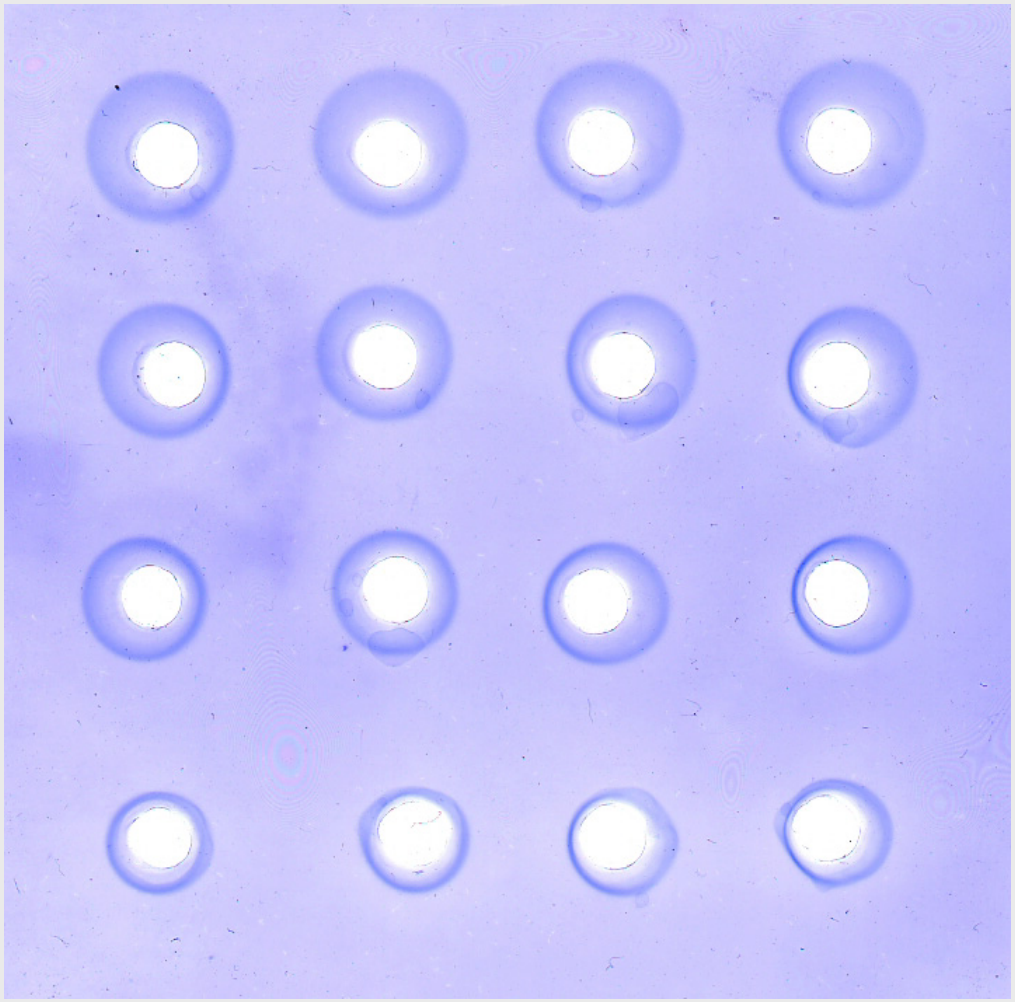
- Gel drying temperature
- Stain comparison
- Linear range
- Incubation time
- Detergent concentration

Visit to CBER laboratory

Ongoing program to optimize method and reduce variability

SRID Assay Validation

- Specificity
- Linearity
- Precision
- Accuracy
- Range



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Linearity

Performed for three strains using FluBlok

Extended range (36 µg/mL to 6 µg/mL)

Criteria: R > 0.95

All strains passed

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Precision

- Performed for three strains using FluBlok
- Fill/finish contractor vs. PSC
- Each group analyzed same batch three times (five sample preparations per analysis)
- Results calculated as per monovalent method (using all 5 preps) and trivalent method (using first 3 preps) for each analysis
- Criteria: %CV ≤ 12.5% (n=6)

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Accuracy

Performed for three strains using FluBlok® and a monovalent batch

Sample spiked with reference

Three levels: 80% (24µg/mL), 100% (30µg/mL), and 120% (36µg/mL)

Calculations

- Trivalent samples calculated using both calculation types
- Monovalent samples calculated using monovalent calculations only

Criteria: 80 – 120% recovery at each level

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CBER Influenza Potency Reagents

Only CBER Authorized Reagents shall be Used to test potency of Vaccines Marketed in the US.

Authorized Reagents will be produced by CBER or adopted by CBER for use. CBER will collaborate in the calibration of adopted reagents.

CBER will verify availability and acceptable performance of compatible authorized reagents with each manufacturer's vaccine product.

CBER will make every effort to assure availability of reagents appropriate for all strains selected for production of vaccine.

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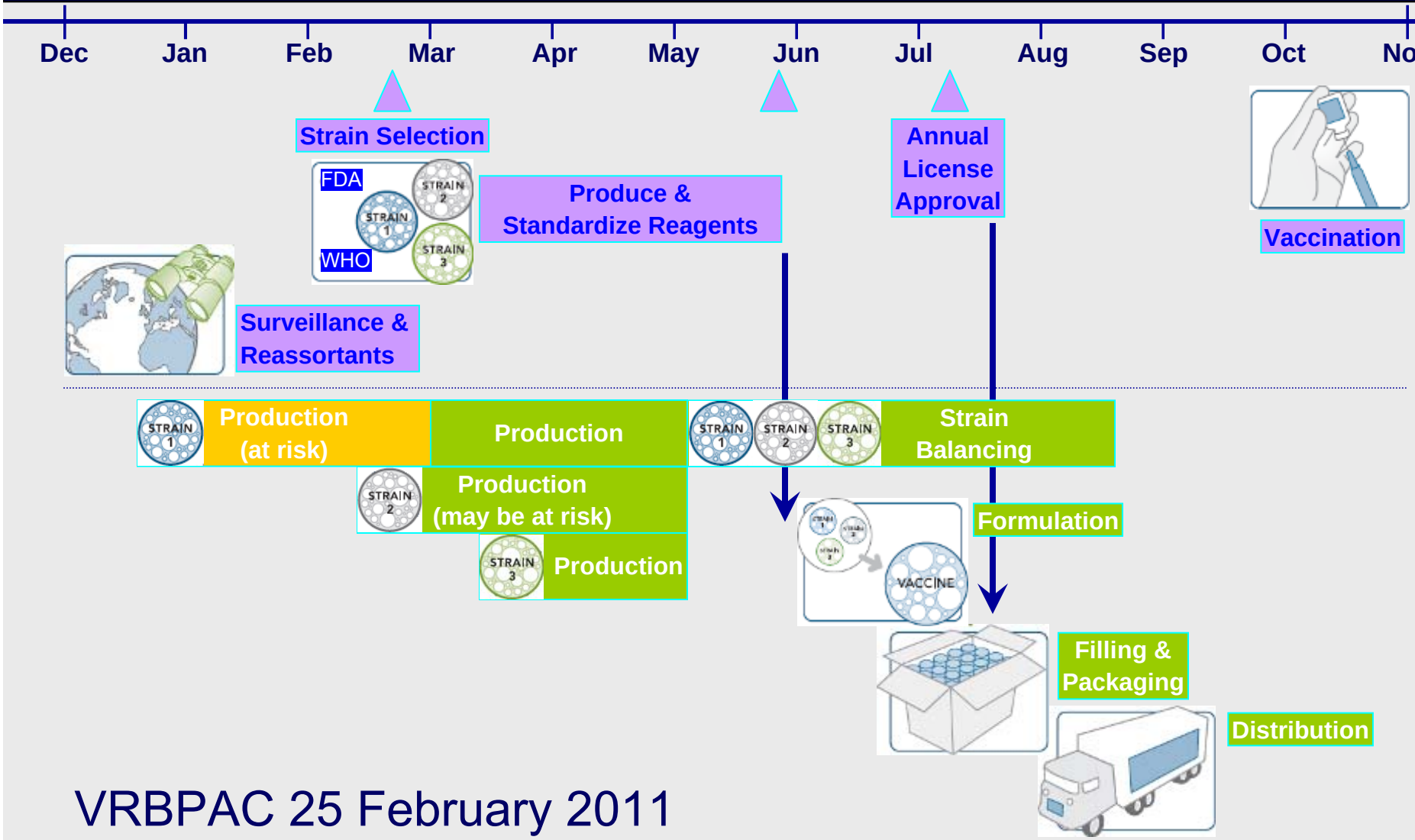
Influenza Vaccine Manufacturing Timeline



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