



# Vaccine Production: Strategies, Methods, and Challenges

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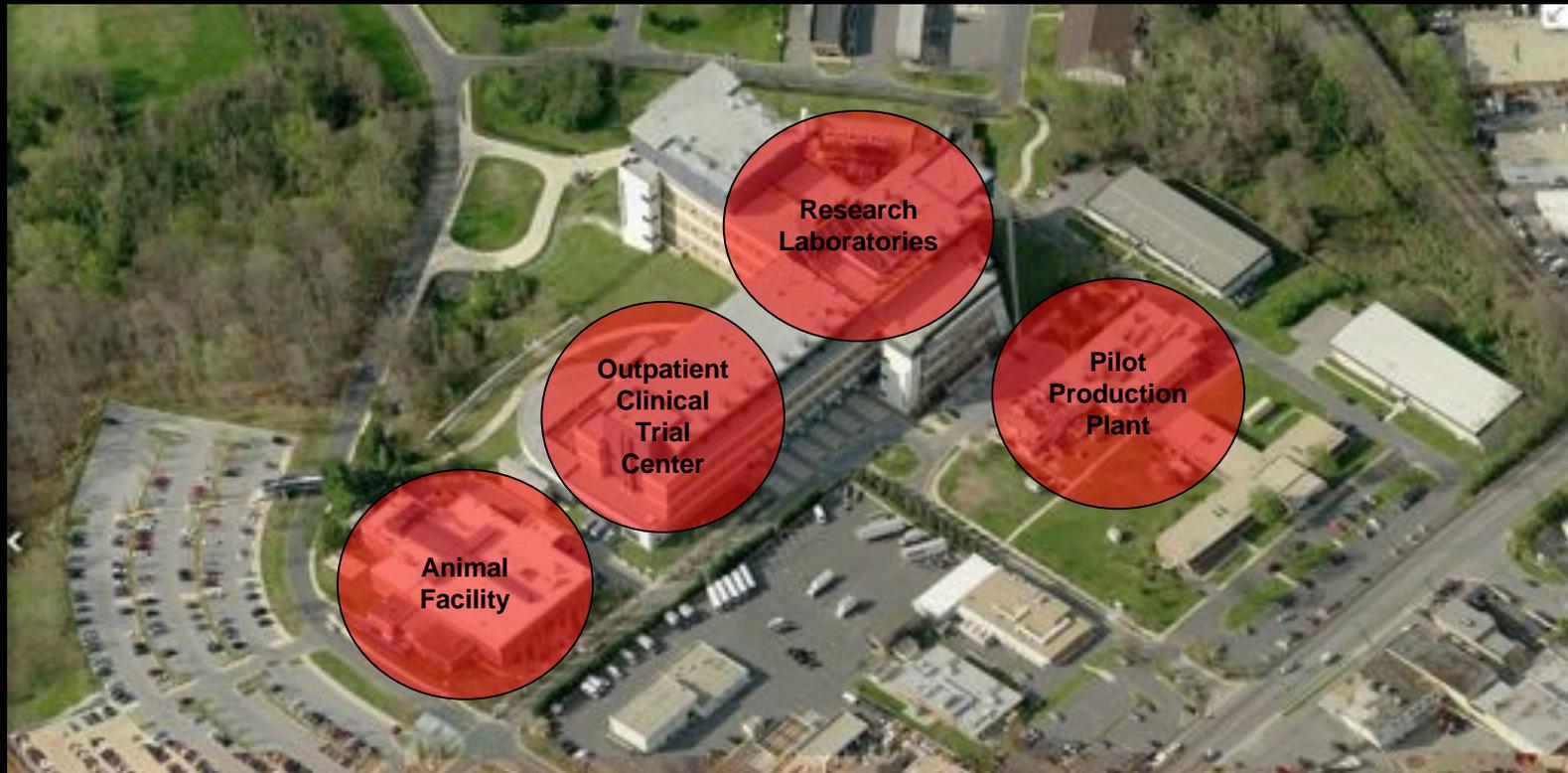
Johns Hopkins University  
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# Walter Reed Army Institute of Research



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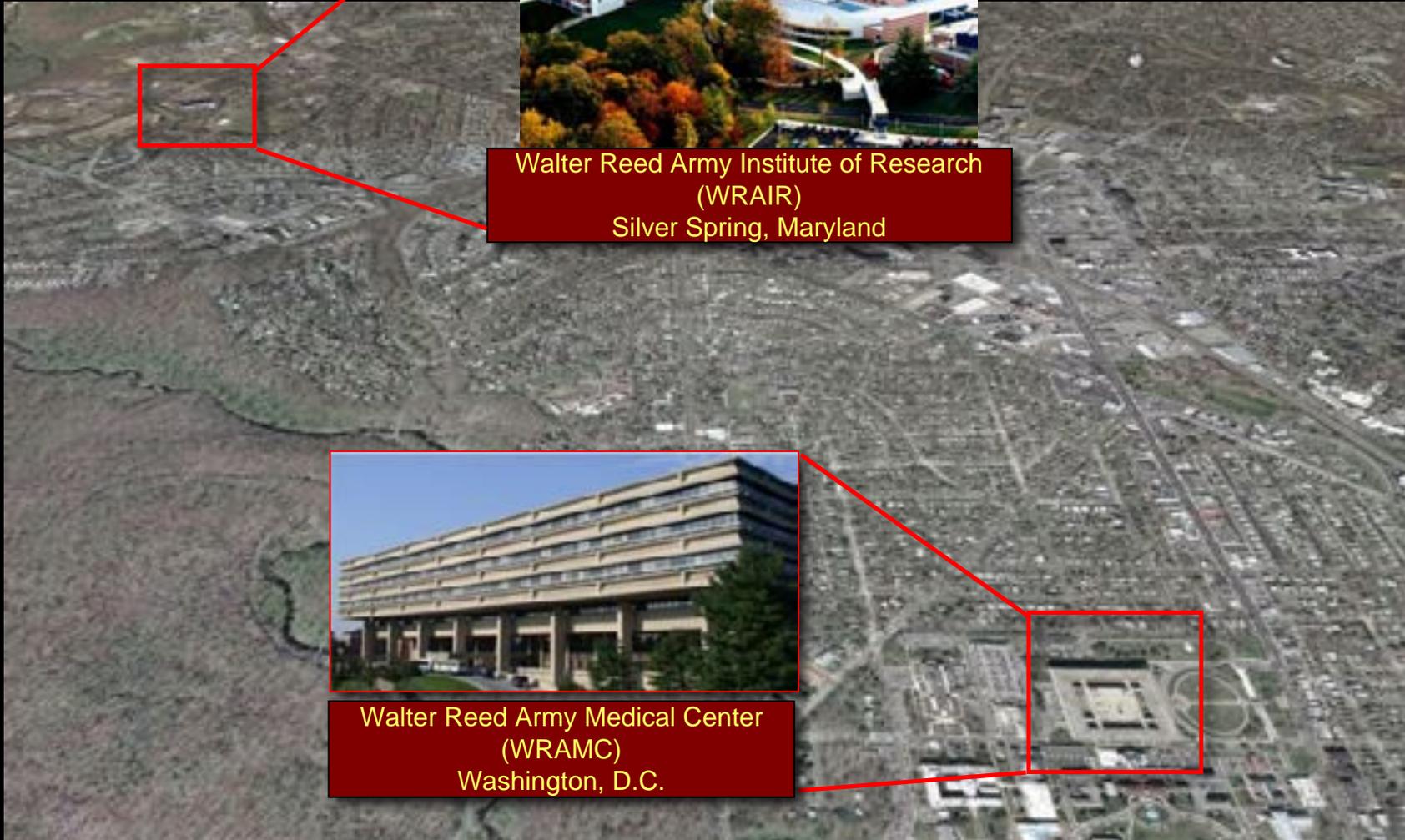
# WRAIR Campus: Vaccine Driven



# WRAIR and WRAMC



Walter Reed Army Institute of Research  
(WRAIR)  
Silver Spring, Maryland



Walter Reed Army Medical Center  
(WRAMC)  
Washington, D.C.

# Research Focus



- Focus on the development of a vaccine to prevent enteric infections in military troops
  - *Campylobacter* spp.
  - Enterotoxigenic *E. coli* (ETEC)
  - Norovirus
  - *Shigella* spp.
- Secondary focus is the deployment of successful enteric vaccines to developing countries, where the need is the greatest.

# Teaching Objectives



- By the end of this lecture, students will be able to:
  - Identify different vaccine categories and development technologies.
  - Describe the major historical events regarding smallpox vaccine production
  - Understand guidelines used for vaccine production
  - Understand future challenges for vaccine production

# Points to Review



- Vaccines do not protect against a pathogen but rather the immunological memory induced after vaccination protects against future encounters with a pathogen.
- Most vaccines are designed to protect against **DISEASE** and **NOT** against **INFECTION**.
- Most vaccines are **PROPHYLACTIC** but some newer vaccines may be used as a **THERAPUTIC**.

# Impact of Vaccines in the 20th Century



Vaccine-Preventable Disease	Peak Cases	Cases (2006)	% Reduction
Diphtheria	30,508	0	100.0
Measles	763,094	55	99.9
Mumps	212,932	6,584	95.9
Pertussis	265,269	15,631	92.2
Polio (acute)	42,033	0	100.0
Polio (paralytic)	21,269	0	100.0
Rubella	488,796	11	99.9
Congenital rubella	20,000	1	99.3
Smallpox	110,672	0	100.0

# Impact of Vaccines



# Why Vaccines are Different than Drugs



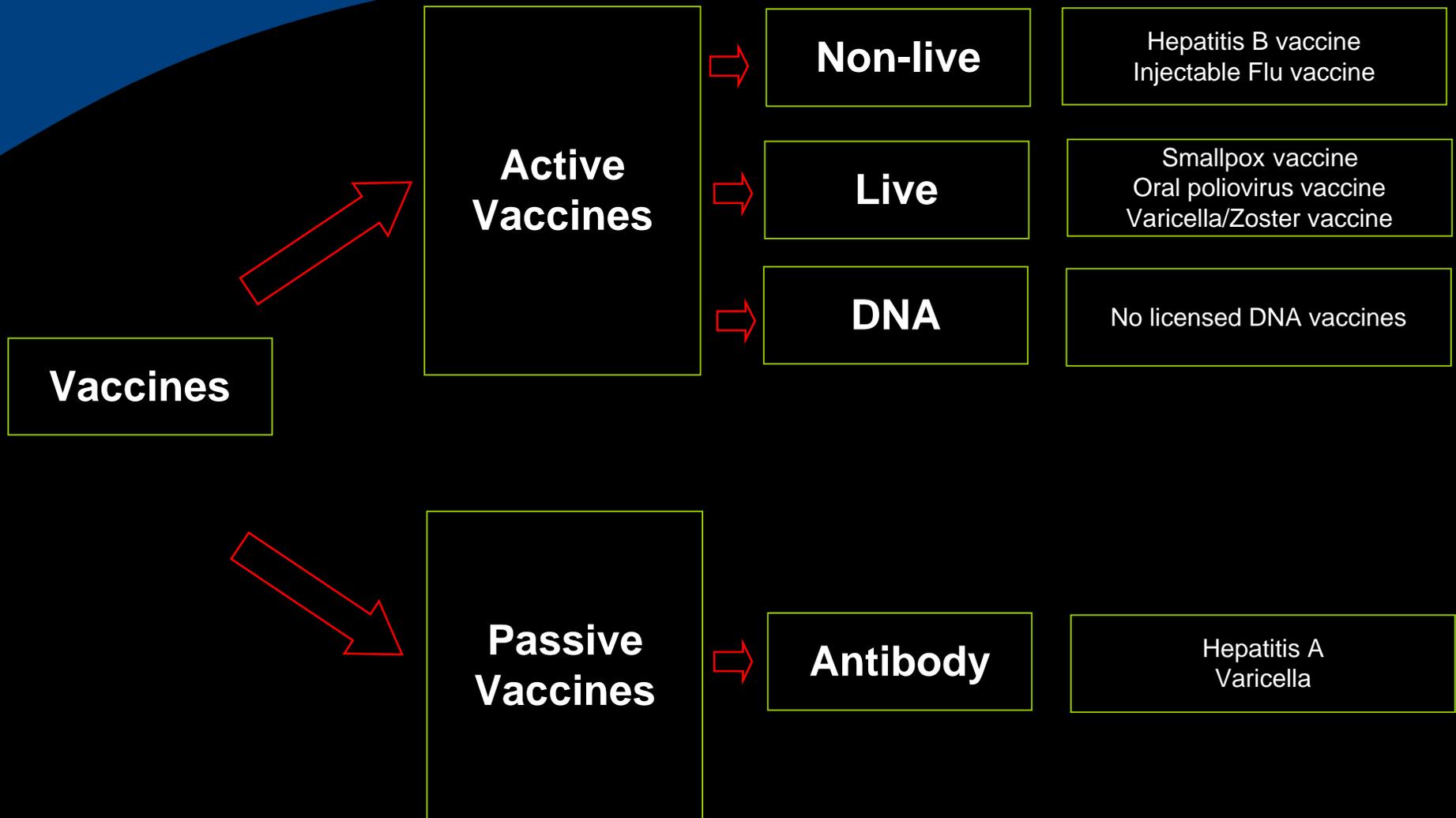
- Given to healthy people; high safety required
- Larger governmental role
- Low efficacy unacceptable
- Often used in infants
- Given once or a few times
- Manufacturing larger part of cost

# Vaccine Categories



- There are two broad categories of vaccines:
- **ACTIVE Vaccines**
  - Vaccines that induce an immune response in an immunized person.
  - Induce immunological memory.
- **PASSIVE Vaccines**
  - Administration of immune effectors (antibodies) with specificity for a pathogen.
  - The immune response is NOT generated in the immunized person and therefore NO immunological memory.
  - Offer limited protection for a short period of time.

# Vaccine Categories



# Vaccine Technologies



## Live Vaccines

- Viral
- Bacterial
- Recombinant virus
- Recombinant viral vector
- Recombinant bacterial
- Recombinant bacterial vector

## Non-Live Vaccines

- Whole Pathogen
- Protein-based
- Peptide-based
- Polysaccharide-based
- Anti-idiotypic antibodies

## DNA Vaccines

- “Naked” DNA
- Facilitated DNA
- Viral Delivery
- Bacterial Delivery

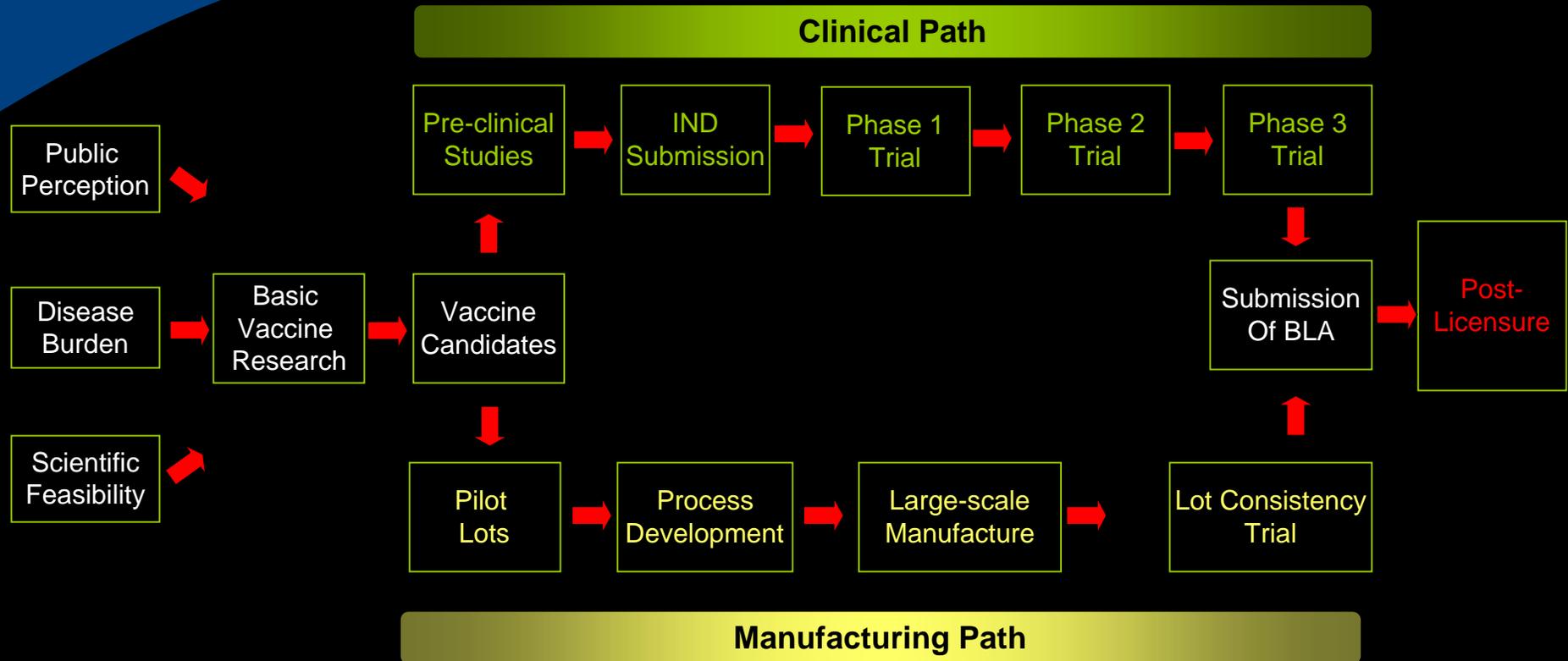
## Antibodies

- Polyclonal antibodies
- Monoclonal antibodies
- Natural Human
- Recombinant Human
- Recombinant Humanized

**Active**

**Passive**

# Vaccine Development Paradigm

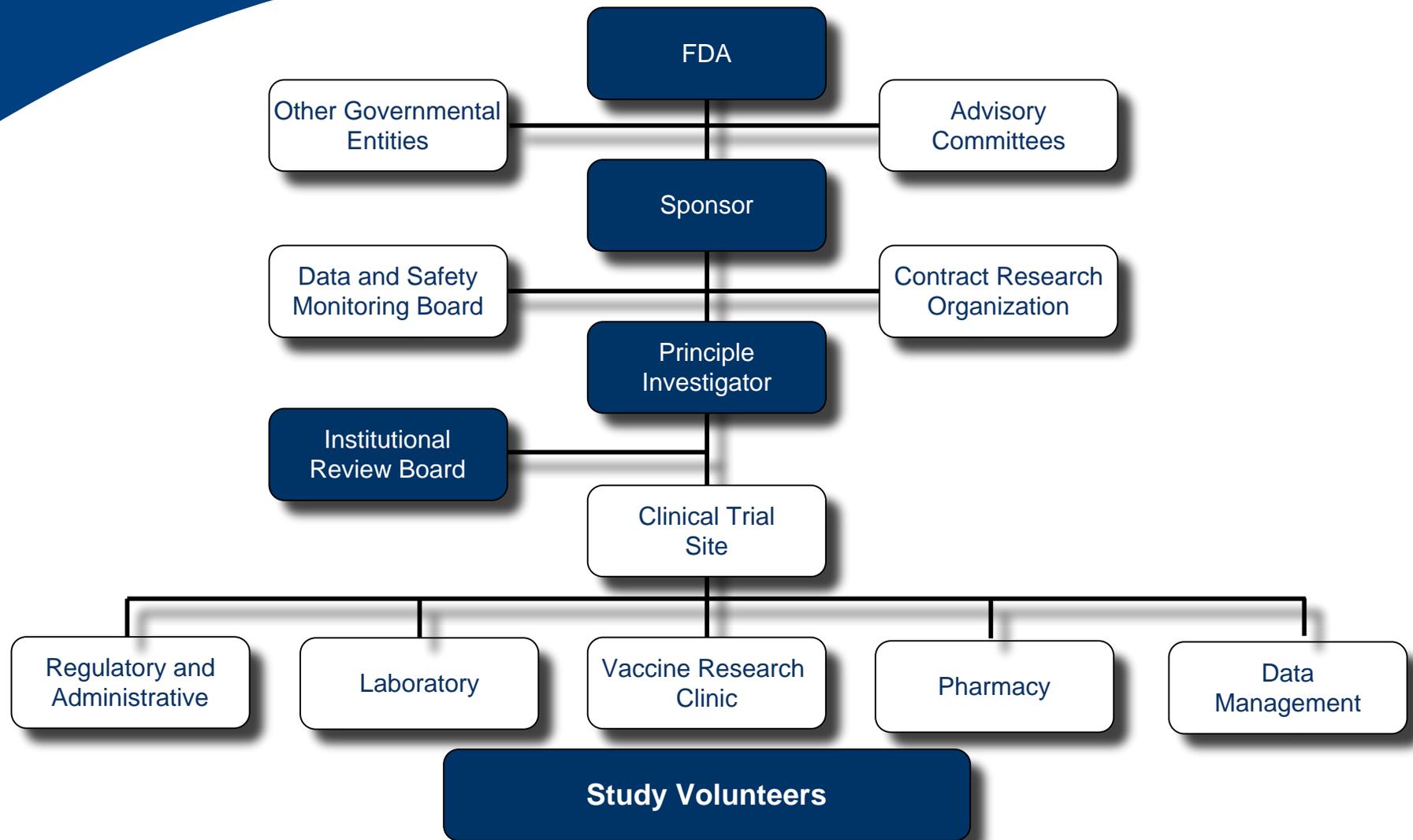


# Phases of Clinical Vaccine Development



Phase	Major Objectives	Typical Number of Subjects	Type of Subjects	Comments
1	<ul style="list-style-type: none"> <li>• Safety</li> <li>• Tolerability</li> <li>• Preliminary immunogenicity</li> </ul>	20-80	Healthy adults	<ul style="list-style-type: none"> <li>• Dose escalation</li> <li>• First introduction of vaccine into humans</li> </ul>
2	<ul style="list-style-type: none"> <li>• Safety</li> <li>• Immunogenicity</li> </ul>	100s	Healthy adults followed by target population	<ul style="list-style-type: none"> <li>• Dosage levels</li> <li>• Number of doses</li> <li>• Route</li> <li>• Schedule</li> </ul>
3	<ul style="list-style-type: none"> <li>• Safety</li> <li>• Immunogenicity</li> <li>• Efficacy</li> </ul>	100s to 1000s	At-risk population	<ul style="list-style-type: none"> <li>• Immune response determination</li> </ul>
4	<ul style="list-style-type: none"> <li>• Safety</li> <li>• Duration of protection</li> <li>• Efficacy in other populations</li> </ul>	1000s to 100,000s	Vaccine recipients	<ul style="list-style-type: none"> <li>• Post-marketing studies</li> </ul>

# Organizational structure of a vaccine clinical trial



# Minimal Timing of Vaccine Development



Phase	Minimum Time
Preclinical	2 years
Phase 1	1 year
Phase 2	2-3 years
Phase 3	3 years
Licensing	1 year
Phase 4	3 years

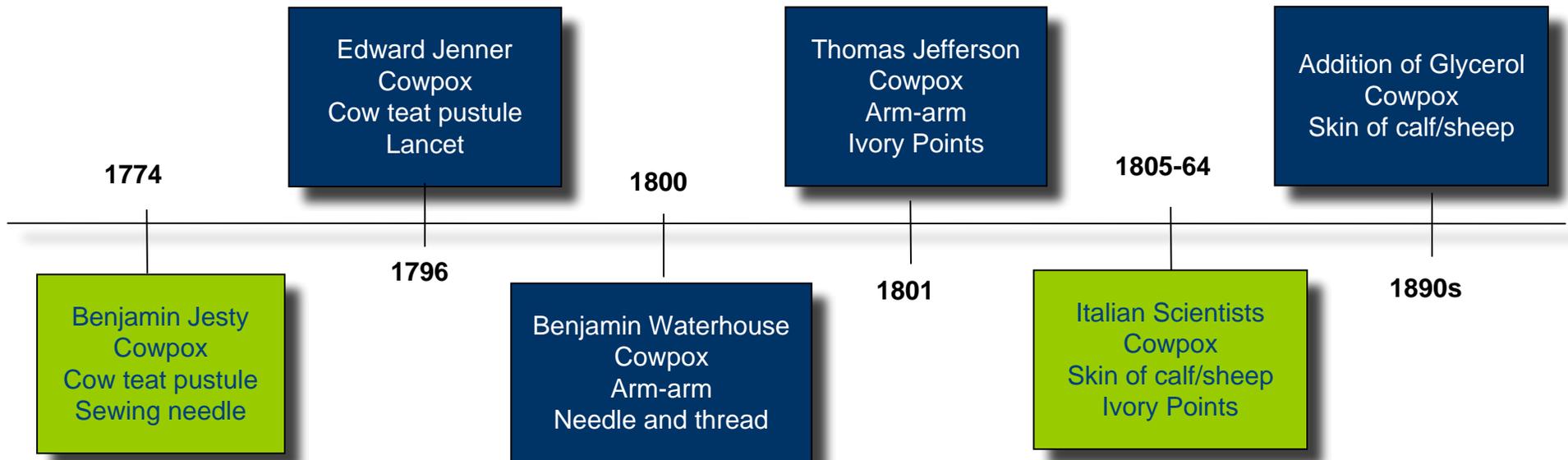


10 yrs minimum  
15 yrs median



# Evolution of vaccine production: The smallpox paradigm

# Evolution of Smallpox Vaccine Production



# Jenner's Lancets



# Benjamin Jesty



- Cattle farmer who introduced cowpox to protect against smallpox
- Jesty took his family to a cow at a farm that had cowpox, and using a darning needle, transferred pustular material from the cow by scratching their arms.

# Edward Jenner



- Dr. Jenner is credited with first vaccination.
- Inoculated 8 year old servant boy named Phipps
- Intentional variolation of the boy failed, demonstrating efficacy.
- Named the product vaccine (from Latin meaning “of cows”)
- Later Pasteur named the process of immunizing “vaccination”, in honor of Jenner



Image credit: <http://www.sciencemuseum.org.uk/>

# Early Vaccination Tools



Ivory points used for immunization



Pad placed over vaccine site to reduce infection and vaccine spread

# Early Vaccination Tools

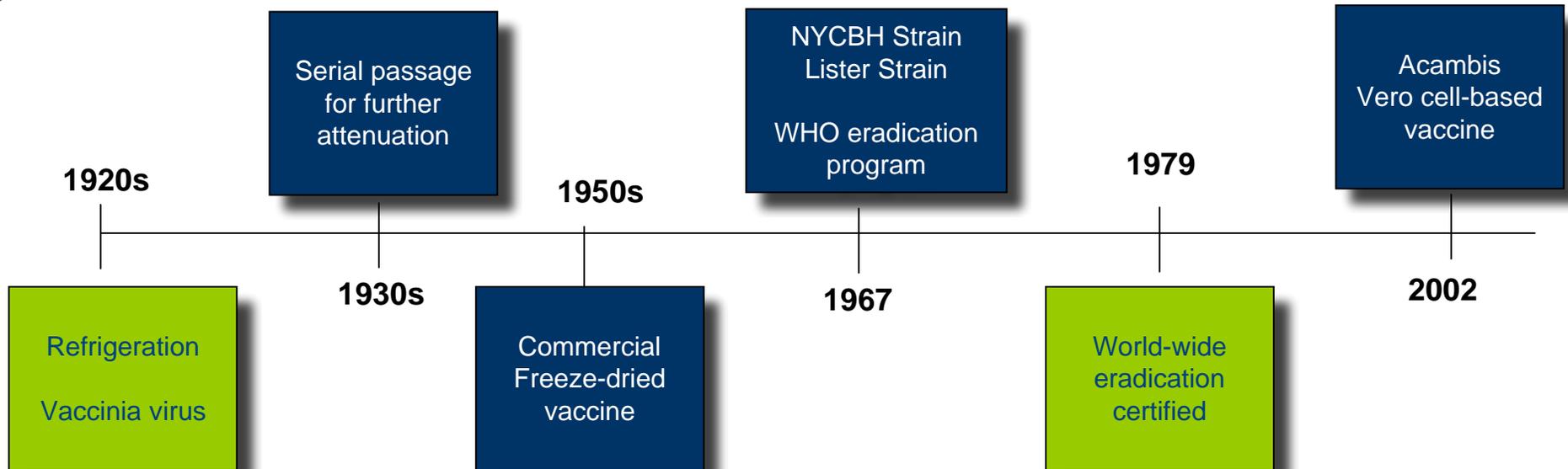


Vaccination lancet was used for giving smallpox vaccines by scarification.



Specially designed lancet for scraping smallpox pustules into the skin

# Evolution of Smallpox Vaccine Production



# Freeze-dried Smallpox vaccine



Freeze-dried vaccine and bifurcated needles for immunization

# Inoculation guns



- Used to vaccinate large numbers of people very quickly
- Vaccine was forced through the skin at high pressures
- Eventually replaced in favor of simple bifurcated needles
- Would later be withdrawn because of concerns about cross infection

# Current smallpox vaccines

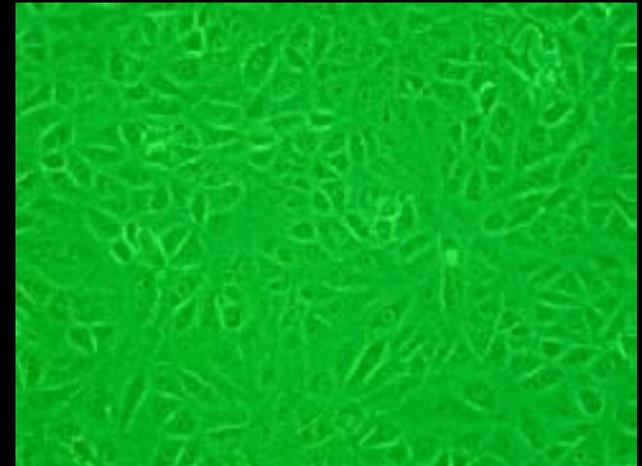


- There are two licensed smallpox vaccines:
  - **Dryvax** (Wyeth Laboratories, Inc.)
    - Freeze-dried from skin of calves
  - **ACAM2000** (Acambis, Inc.)
    - Freeze-dried from cell culture
- Both vaccines are administered by scarification to the deltoid muscle or the posterior aspect of the arm over the triceps muscle.
- Vaccinia Immune Globulin, Intravenous (VIGIV) (Cangene Corporation) is used to treat rare serious complications of smallpox vaccination.

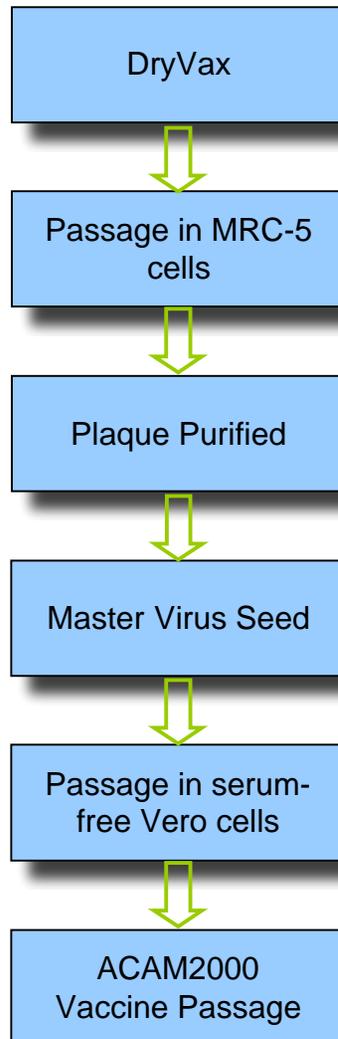
# ACAM2000



- Smallpox vaccine (Wyeth) FDA approved in 2007
- Contains live vaccinia virus (cloned from Dryvax; New York City Board of Health Strain)
- Propagated in African Green monkey kidney epithelial cells (Vero cells)
- **Inactive ingredients:**
  - 6-8 mM HEPES (pH 6.5-7.5)
  - 2% human serum albumin USP
  - 0.5 - 0.7% sodium chloride USP
  - 5% mannitol USP
  - trace amounts neomycin and polymyxin B



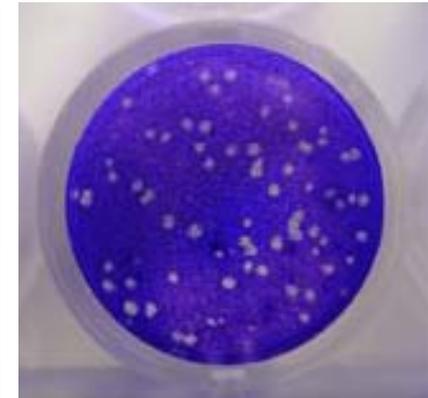
# ACAM2000 Production



Cell culture: Viral passage

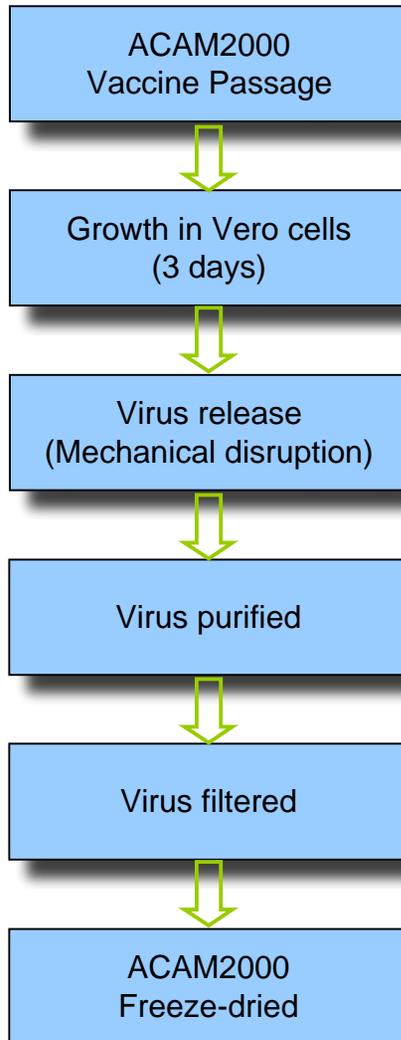


Plaque Negative



Plaque Positive

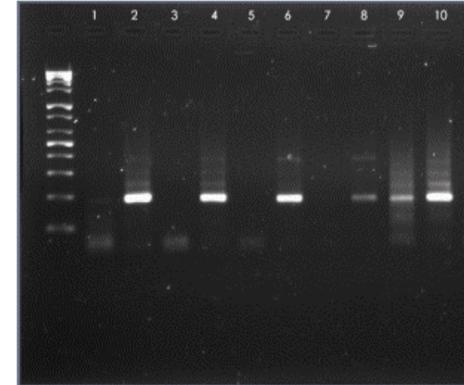
# ACAM2000 Production



# ACAM2000 Qualification



- Determine plaque-forming units
- Screen for adventitious agents (PCR)
  - Bacteria
  - Fungi
  - Mycoplasma
  - Viruses
- Neurovirulence in suckling mice
- Residual Vero cell DNA
- Immunogenicity and protection in mice and rhesus macaques
- Clinical trial testing



Polymerase chain reaction (PCR)



# Current vaccine manufacturing

# Current Good Manufacturing Practices (cGMP)



- Products destined for human testing and use must be made under cGMP
- The guidelines are covered under Section 501(B) of the 1938 Food, Drug, and Cosmetic Act (21USC351)
- GMP's are enforced in the United States by the US Food and Drug Administration (FDA)
- In general cGMP regulations require:
  - Define and follow conditions to produce a safe, pure, and potent product
  - Define controls to monitor the production
  - Validation and documentation of each manufacturing step

# Regulatory Definitions - (21 CFR 600.3)



## Safety

Relative freedom from harmful effect to persons affected, directly or indirectly, by a product when prudently administered...

## Purity

Relative freedom from extraneous matter in the finished product...

## 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.

# cGMP guidelines



- To ensure consistency and compliance with specifications all processes are:
  - Clearly defined
  - Controlled
  - Validated
  - Changes to the process are evaluated
- Instructions and procedures are written in clear and unambiguous language.
- Operators are trained to carry out and document procedures.



# cGMP guidelines



- Record keeping:
  - Made manually or by instruments.
  - Demonstrate that all the steps required by the defined procedures and instructions were in fact taken.
  - Ensure quantity and quality of the drug was as expected.
  - Deviations are investigated and documented.
  - Enable the complete history of a batch to be traced
  - Are retained in a comprehensible and accessible form.



# cGMP guidelines

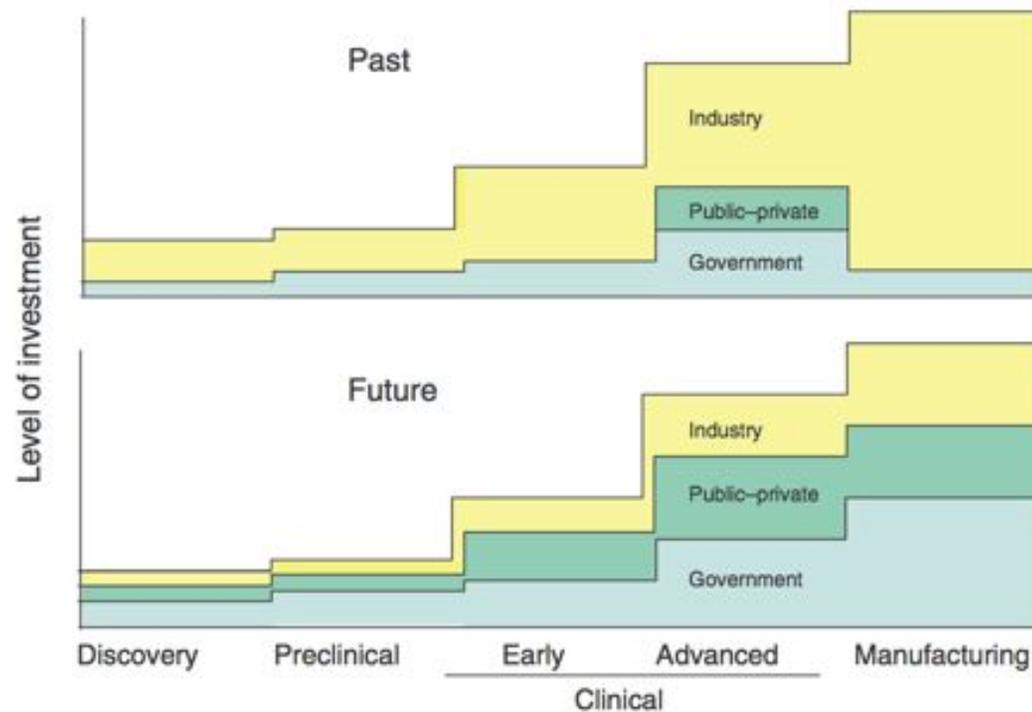


- A system is available for recalling any batch of drug from sale or supply.
- Complaints about marketed drugs are examined, the causes of quality defects are investigated, and appropriate measures are taken with respect to the defective drugs and to prevent recurrence.



# Challenges for the future

# Changing Roles in Vaccine Development



- In the past, industry bore the burden of clinical assessment and manufacturing
- In the future, the final phases of vaccine testing with likely be shared among several groups and more equally.

# Flu Vaccine (1950s technology still in use)



- The two types of influenza vaccines licensed in the US utilize eggs to generate the virus used for immunization.
- Slow growing viral strains and manufacturing issues can delay the production of seasonal and H1N1 flu vaccines.
- Such delays undermines the vaccine campaigns and the public's perception of vaccines



# Egg vs Cell Culture



## Egg

- Process takes 6-9 months to produce enough vaccine
- Virus kills the chicken embryos before the virus can grow
- Need millions of high quality eggs (pathogen free)
- More cost efficient
- Egg allergies
- Viruses grown in eggs are antigenically distinct from original clinical isolates

## Cell Culture

- Faster turnover time to produce a vaccine.
- Can be scaled up in times of emergency
- Higher costs for fermentors
- Vero and MDCK cells support virus growth and isolation
- Viruses grown in culture more closely represent original clinical isolates
- Regulatory issues need to be addressed

# Vaccine Critics



Vaccination has never been without its critics. In this cartoon from 1802, the British satirist James Gillray implied that vaccination caused people to become part cow.