Product Development: Moving from the Bench to the Clinic

Introduction to the Principles and Practice of Clinical Research

Indresh K. Srivastava, Ph.D.
Director,
Purification and Analytical Development
Vaccine Production Program Laboratory,
Vaccine Research Center
Product Development

- Overview of Product Development

- Costs of Product Development

- Product Development, Manufacture and Testing of Clinical Materials--VLP Based Vaccine for Chikungunya Virus

- GMP Facility and Environmental Monitoring

- Example: H5 Influenza Vaccine from Bench to Clinic
Product Development

- Overview of Product Development
- Costs of Product Development
- Product Development, Manufacture and Testing of Clinical Materials--VLP Based Vaccine for Chikungunya Virus
- GMP Facility and Environmental Monitoring
- Example: H5 Influenza Vaccine from Bench to Clinic
How are Products Selected for Development?

Financial value of the product if successful

= future revenue – cost of development

Probability of success

- Scientific
- Legal
- Engineering
- Business
Steps in Biological Product Development

- Candidates designed, created & evaluated
  - Lead candidate identified & validated
- IND enabling studies
- Process development
- CTM produced
- Demonstrated safety, immunogenicity and POC in phase 1 and 2 clinical studies
- CMC: scale-up and validation
- Confirmed safety, immunogenicity and efficacy of final dose / regimen;
  - Manufacturing consistency demonstrated
  - Product licensed

Discovery → Pre Clinical → PoC in Humans → Ph. III → Registration

Vaccine approved as development candidate? → Progress to clinical testing? → Advance to phase 3? → File BLA?

IND = Investigational New Drug Application;  BLA=Biologics License Application
Product Development Teams

• Product Development team is formed to direct development of clinical candidates
  • Includes members of all required functions: R, D, Reg, Clin, Manuf, PM...
  • Responsible for developing:
    • Target product profile
    • Overall development timelines
    • Budgets
## Example Target Product Profile

<table>
<thead>
<tr>
<th>Description</th>
<th>Prophylactic vaccine for AAA virus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Components</strong></td>
<td>Adenoviral vectored vaccine expressing GP from XX strain of virus. 2-dose vaccine regimen: 1&lt;sup&gt;st&lt;/sup&gt; dose rAdxx (prime) 2&lt;sup&gt;nd&lt;/sup&gt; dose rAdyy vaccine (boost)</td>
</tr>
<tr>
<td><strong>Indication</strong></td>
<td>For active immunization against disease caused by AAA virus acquired by either parenteral or mucosal exposure in persons X-Y age.</td>
</tr>
<tr>
<td><strong>Target Population</strong></td>
<td>Military  Healthcare workers  Individuals at risk of secondary exposure during a bioterrorist attack.  Travelers/ residents in endemic areas  Laboratory workers</td>
</tr>
<tr>
<td><strong>Efficacy</strong></td>
<td>&gt;80% of vaccinated individuals achieve correlates (titers?) that were protective in an animal model  Onset of protection by day 30 after the first dose  Duration of protection: 1 year after the last dose</td>
</tr>
<tr>
<td><strong>Safety &amp; Tolerability</strong></td>
<td>Generally safe and well tolerated  Mild systemic and local reactogenicity</td>
</tr>
<tr>
<td><strong>Dosage and Administration</strong></td>
<td>Xx Viral particles/ dose  IM injection  2 dose immunization regimen  0,1 month schedule</td>
</tr>
<tr>
<td><strong>Pharmaceutical Characteristics (how supplied, stability)</strong></td>
<td>Liquid, thimerosal-free, single dose vials, 1 ml, Storage: 2-8C  2 year stability</td>
</tr>
<tr>
<td><strong>Anticipated customers</strong></td>
<td>DoD: Stockpile ZZZ troop equivalent doses  Heathcare workers/ first responses teams  Post event  Veterinarians and Researchers  Foreign governments (Treaty partners)  Travel clinics  HHS</td>
</tr>
</tbody>
</table>
Product Development

- Overview of Product Development
- Costs of Product Development
- Product Development, Manufacture and Testing of Clinical Materials--VLP Based Vaccine for Chikungunya Virus
- GMP Facility and Environmental Monitoring
- Example: H5 Influenza Vaccine from Bench to Clinic
Drug Development Costs

• Data difficult to obtain
• Measurement of total cost
  • Out of pocket expenses
  • Risk adjusted cost of capital
• Basic Research Costs are Not Included
**EXHIBIT 3**
Capitalized Preclinical, Clinical, And Total Cost Per New Drug, In Millions Of 2000 Dollars

<table>
<thead>
<tr>
<th>Millions of dollars</th>
<th>Hansen 1979</th>
<th>DiMasi 1991</th>
<th>DiMasi 2003</th>
<th>Pharmaprojects</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# Probability of Success

## EXHIBIT 2
### Average Phase Time And Clinical Capitalized Costs For Investigational Compounds

<table>
<thead>
<tr>
<th>Testing phase</th>
<th>Duration (months)</th>
<th>Mean cost(^a)</th>
<th>Expected cost(^a)</th>
<th>Total(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHG 1</td>
<td>DHG 2</td>
<td>Pharma-projects</td>
<td>DHG</td>
</tr>
<tr>
<td>Phase I</td>
<td>22</td>
<td>12</td>
<td>19</td>
<td>$31</td>
</tr>
<tr>
<td>Phase II</td>
<td>26</td>
<td>26</td>
<td>30</td>
<td>42</td>
</tr>
<tr>
<td>Phase III</td>
<td>31</td>
<td>34</td>
<td>30</td>
<td>119</td>
</tr>
</tbody>
</table>

Animal<br>Preclinical<br>Clinical<br>Mean cost<br>
10<br>10<br>3<br>3<br>335<br>467<br>$381<br>487


**NOTES:** DHG 1 is months to phase end; DHG 2 is months to start of next phase. The DHG new drug application (NDA) approval phase was estimated to be 18.2 months. Costs were capitalized at an 11 percent real discount rate. Pharmaprojects estimates used the DHG preclinical time of 52 months. The Pharmaprojects NDA approval phase was estimated to be 15.8 months.

\(^a\) Millions of 2000 dollars.

Health Affairs, 25, no. 2 (2006): 420-428
doi: 10.1377/hlthaff.25.2.420
Product Development

- Overview of Product Development
- Costs of Product Development
- Product Development, Manufacture and Testing of Clinical Materials--VLP Based Vaccine for Chikungunya Virus
- GMP Facility and Environmental Monitoring
- Example: H5 Influenza Vaccine from Bench to Clinic
Product Development: Why So Long and Expensive?

- Develop Process
- Production Facility
- Pre-clinical Safety Testing
- Clinical Trials

- FDA
Vaccine Development at the VRC

Immune Assessment

Development Cycle at the VRC

Clinical Trials

Research and Development

cGMP Production
**Vaccine Production Program**

**Goal:** Efficiently translate candidate research vaccines into materials for proof of concept clinical trials and enable advanced development and licensure by partners.

**Projects:** Flu, HIV, Ebola, Marburg, Alphaviruses

- Process Development
- Analytical Development
- cGMP Production
- Pre-clinical Safety
- Regulatory Science
Keys to Process Development

• Consistency

• Scalability

• Safety
  – Raw materials
  – Cell lines
  – Excipients

• Analytical methods
  – Product characterization
  – Characterization of residuals

• Formulation and Stability
  – Develop stable vaccine formulation
  – Perform stability studies to show that the vaccine is stable
Solutions to Some Product Safety Concerns

• Use of highly characterized cell lines rather than primary cells
• Validated manufacturing process
• Validated adventitious agent (bacterial and viral) clearance in the manufacturing process (where possible)
• Highly controlled raw materials
• Move to animal component-free raw materials
Development of VLP-Based Vaccine for Chikungunya Virus– A VRC Example
Chikungunya Disease

• Disease is caused by an alphavirus known as Chikungunya. This is a mosquito borne disease spread by Ades albopictus

• Similar to dengue fever, characterized by rash, high fever, and severe arthritis/arthralgia

• 1.6 - 6.7 million people living in Indian Ocean, India, Southeast Asia, Africa and limited areas of Europe

• Severe morbidity, low mortality (< 1%); excruciating pain/swelling of joints in fingers, wrists, and ankles can last for years

http://www.wellsphere.com/general-medicine-article/chikungunya/413213
### CHIKV VLPs

Chikungunya genome

<table>
<thead>
<tr>
<th>NS1</th>
<th>NS2</th>
<th>NS3</th>
<th>NS4</th>
<th>C</th>
<th>E3</th>
<th>E2</th>
<th>6K</th>
<th>E1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nonstructural</td>
<td>core</td>
<td>envelope</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**VLPs constructs:**

<table>
<thead>
<tr>
<th>C-Env</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
</tr>
</tbody>
</table>

100 nm
CHIKV VLP Structure and Molecular Weight

• 3 proteins
  • E1 and E2 ~ 50kD each
  • C ~ 30kD
• 240 copies of each protein/VLP
• Protein MW ~ 31,200,000
• Additional lipid membrane
Development of VLP-Based Vaccine for Chikungunya Virus
Product Development of a Chikungunya VLP-Based Vaccine

Upstream Process Development
Cell Culture
CHIKV VLP Technology

Phase I

• Mammalian Cell Substrate
  – GMP serum-free suspension cell line (VRC-293) derived from HEK-293 cell line
• PEI transient transfection technology
• Shake-flask based upstream process
• All assays and process developed for Phase I manufacturing

Phase II

• Develop Scaled-up Processes
• Develop Inducible Cell Line
Cell Culture Process

1. Thaw Vial of VRC-293 Cells
2. Expand Cells in Shake Flasks
3. Expand in Bioreactor(s)
4. Concentrate Cells
5. Media Exchange into Transfection Media
6. Transfect with PEI:DNA
7. Dilute to Final Production Concentration with Growth Media
8. Feed as Required for Production
9. Harvest Cell Culture Fluid
# Upstream Transfection Process Optimization

## Multiple DOE Studies Performed to Determine Operating Space

<table>
<thead>
<tr>
<th>Process Parameter</th>
<th>Range Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Transfection Cell Density (2 days pre-trans)</td>
<td>0.5 – 2.5 x 10^6 cells/mL</td>
</tr>
<tr>
<td>Residual Growth Media at Transfection</td>
<td>0 – 100%</td>
</tr>
<tr>
<td>DNA Concentration</td>
<td>5 - 25 mg/L @ transfection</td>
</tr>
<tr>
<td>PEI:DNA Ratio</td>
<td>1:1 - 3:1</td>
</tr>
<tr>
<td>Hold Times Prior to Transfection</td>
<td>0 – 90 minutes</td>
</tr>
<tr>
<td>Transfection Time</td>
<td>1 – 18 hours</td>
</tr>
<tr>
<td>Post-Transfection Cell Density</td>
<td>2 - 10 x 10^6 cells/mL</td>
</tr>
<tr>
<td>Harvest Time</td>
<td>24 – 120+ hours</td>
</tr>
<tr>
<td>Medium Composition</td>
<td>Multiple vendors</td>
</tr>
<tr>
<td>Cell Line Stability</td>
<td>1-25 passages</td>
</tr>
</tbody>
</table>
CHIKV VLP Production

Production at 1L Shake Flask Scale

Ave = 107 mg/L
SD = 23 mg/L
Product Development of a Chikungunya VLP-Based Vaccine

Downstream Process Development
Downstream purification process for CHIKV VLP

1. Clarified supernatant
2. Filtration Protocol
3. Concentration 5X fold (UF)
4. Diafiltration against 5-volumes of 1XSP (DF)
5. Column Chromatography on QXL-Sepharose (Virus Licensed)
6. Pool Eluted Fractions from Step 1 Elution
7. Diafiltration against 5-volumes of Formulation Buffer
8. Final Filtration

- TFF Process
- Chromatography
# Downstream Process Optimization

## Multiple Studies Performed with Parameters to Determine Operating Space

<table>
<thead>
<tr>
<th>Process Parameter</th>
<th>Parameters Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvest Clarification Filtration</td>
<td>Filter, flow, pressure</td>
</tr>
<tr>
<td>UF</td>
<td>Porosity, shear, TMP, fold-concentration</td>
</tr>
<tr>
<td>DF</td>
<td>shear, TMP, DF volumes</td>
</tr>
<tr>
<td>Benzonase Treatment</td>
<td>Step location, conc, time, temp, MgCl2</td>
</tr>
<tr>
<td>Chromatography</td>
<td>Resin, load, volumes, elution...</td>
</tr>
<tr>
<td>Final DF</td>
<td>shear, TMP, DF volumes</td>
</tr>
<tr>
<td>Formulation</td>
<td>Buffer, pH, stabilizers...</td>
</tr>
</tbody>
</table>
Chromatographic Purification of CHIKV with On-Column Benzonase Treatment
Product Development of a Chikungunya VLP-Based Vaccine

Analytical Development
Analytical Development
Characterization of Vaccines (Safety)

Different sets of assays are required for different purposes

Analytical Assay
In Process analysis
Product characterization
Product release

- Residual Host Cell DNA
  - PicoGreen Assay
  - Agarose gel electrophoresis
  - Capillary Electrophoresis
- Capture ELISA
- Western – ID
- Bioburden
- Endotoxin
- Host cell protein (ELISA)
- SDS-PAGE
- Residual Benzonase

- CD Spectroscopy
  - Dynamic Light Scattering (DLS)
  - Differential scanning calorimetric analysis
  - Zeta potential
  - Lipid analysis
  - Reverse Phase HPLC
  - Tryptophan fluorescence spectroscopy
  - Glycosylation analysis
  - Profiling and sequence analysis
  - TEM
  - Mass Spec
A. Purity and Identity of CHIKV VLPs

A. SDS-PAGE Analysis

B. Western Blot

Conclusions:

a) Purified CHIKV VLP to >95% purity,

b) Capsid, E1 and E2 were recognized by anti-CHIKV polyclonal antibody. Minor bands at 100, and 200 kDa were detected.

Janel Holland-Linn and Xin Wang
D. Homogeneity of CHIKV VLP Product

Particle size by Dynamic Light Scattering (DLS) Technique

Size distribution

Polydispersity Index

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Diameter</th>
<th>Polydispersity Index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENG 06 Dec 10</td>
<td>66.6</td>
<td>0.03</td>
</tr>
<tr>
<td>10-263</td>
<td>67.7</td>
<td>0.05</td>
</tr>
<tr>
<td>10-273</td>
<td>67.1</td>
<td>0.03</td>
</tr>
<tr>
<td>10-274</td>
<td>67.6</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Conclusions:
The average size of the CHIKV VLP particle is 63-67 nm. The low PDI indicates that purified VLPs are homogenous, and consistent in size.

Martha Till and Emnet Yitbarek
Characterization of Impurities in CHIKV VLPs Product

Analytical Assay Development
- Characterization - Product
- Characterization - Impurities

- Residual Host Cell DNA
  - PicoGreen Assay
  - Agarose gel electrophoresis
  - Capillary Electrophoresis
- RNA
- Bioburden
- Endotoxin
- Host cell protein (ELISA)
- Residuals

- CD Spectroscopy
- Dynamic Light Scattering (DLS)
- Differential scanning calorimetric analysis
- Zeta potential
- Lipid analysis
- Reverse Phase HPLC
- Tryptophan fluorescence spectroscopy
- Glycosylation analysis
- TEM
- Mass Spec
- Size exclusion HPLC
- Reverse Phase HPLC
- ELISA
- Western – ID
- SDS-PAGE

**FDA Guidance:** The concentration of HCD in the drug substance should be $<10 \text{ ng/dose}$
The size of the HCD in the drug substance should be $<200 \text{ bp.}$
## Specification for CHIKV VLP Bulk Product

### Results of the testing of CHIKV VLP Bulk Product

<table>
<thead>
<tr>
<th>Assay Name</th>
<th>SOP No.</th>
<th>Test Code</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance, USP&lt;1&gt;</td>
<td>TM-103</td>
<td>TM-103A</td>
<td>Clear to slightly hazy solution, some small white or translucent particles may be visible</td>
</tr>
<tr>
<td>pH, USP&lt;791&gt;</td>
<td>TM-006</td>
<td>TM-006A</td>
<td>6.2 - 8.2</td>
</tr>
<tr>
<td>VLP Immunoreactivity by ELISA¹</td>
<td>TM-143</td>
<td>TM-143A</td>
<td>Report Result</td>
</tr>
<tr>
<td>Protein Concentration by Micro BCA¹</td>
<td>TM-147</td>
<td>TM-147A</td>
<td>40 ± 10 µg/mL</td>
</tr>
<tr>
<td>Ratio of Immunoreactivity (ELISA) to Protein Concentration (Micro BCA)</td>
<td>TM-143</td>
<td>TM-143</td>
<td>Report Result</td>
</tr>
<tr>
<td>Purity by SDS-PAGE</td>
<td>TM-148</td>
<td>TM-148A</td>
<td>Report Result</td>
</tr>
<tr>
<td>Identification by Western Blot</td>
<td>TM-142</td>
<td>TM-142A</td>
<td>Conforms to Reference</td>
</tr>
<tr>
<td>General Safety, 21CFR 610.11</td>
<td>WuXi Apptec 30003</td>
<td>OTL-G</td>
<td>Negative</td>
</tr>
<tr>
<td>Volume in Container, USP&lt;1&gt;</td>
<td>TM-104</td>
<td>TM-104A</td>
<td>Recoverable volume ≥ 0.5 mL</td>
</tr>
<tr>
<td>Endotoxin ² by Chromogenic LAL, USP&lt;85&gt;</td>
<td>TM-009 or TM-112</td>
<td>TM-009A or TM-112A</td>
<td>≤ 10 EU/40 µg VLP</td>
</tr>
<tr>
<td>Sterility, 21CFR610.12</td>
<td>Catalent Pharma TTP-SZJ-M0001 or Lancaster Labs 8266</td>
<td>TM-XXXD</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Product Development

- Overview of Product Development
- Costs of Product Development
- Product Development, Manufacture and Testing of Clinical Materials--VLP Based Vaccine for Chikungunya Virus
- GMP Facility and Environmental Monitoring
- Example: H5 Influenza Vaccine from Bench to Clinic
Manufacture of Products for Clinical Trials

Good Manufacturing Practices (GMPs)

- FDA Regulations – apply to process and facility
- Philosophy of cGMP
  - Document/approve exactly what you’re going to do
  - Document/approve exactly what you did
  - Review all work to ensure that what you did is exactly what you said you would do
- Paramount concern is safety of clinical subject
Facility Design and GMP
Environmental Considerations
Engineering Controls

- **HVAC design**
  - Air handlers
  - Airlock setup and room pressurizations
  - Room classifications
  - Hepa filtered air

- **Water for Injection (WFI) system**

- **Liquid and solid biowaste systems**

- **Unidirectional flow building layout**
Airlock Setup and Room Pressurizations

- Separate air handlers for each area

- Separation between production areas is maintained by a system of negative pressure airlocks protecting both entry and return corridors

- All airlock functions and room pressure differentials are individually monitored and alarmed
GMP – Protect Product from Contamination
Solution: Unidirectional Flow/Segregated Manufacturing Areas
Utilities

- Flexible delivery of 19 utility systems
- Approximately 9 miles of pipe
- Over 180 miles of cable
How Clean is a GMP Cleanroom and How Do We Keep it That Way?

Environmental Monitoring

(or should the 5 second rule apply?)
Purpose of Environmental Monitoring

- Monitor critical processes within the pharmaceutical and biotechnology industries.
- Determine the microbial and particulate content of cleanroom air and surfaces.
- Highlight conditions contributing to excessive microbial & particulate levels due to ineffective cleaning, or personnel/equipment issues (trending).
- Alert to conditions exceeding classifications
- Pro-active tool for Quality Assurance
To be monitored

• Non-viable airborne particulates
• Viable airborne particulates
• Viable surface bound particulates on cleanroom surfaces and personnel

• Contamination Sources:
  • People ~75%
  • Ventilation ~15%
  • Room Structure ~5%
  • Equipment ~5%
<table>
<thead>
<tr>
<th>Critical Environment Classification</th>
<th>Concentration (particles/meter$^3$) ( \geq ) Size Shown</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS 209E ISO 14644-1</td>
<td>0.1um 0.2um 0.3um 0.5um 1.0um 5.0um</td>
</tr>
<tr>
<td>N/A 1</td>
<td>10 2</td>
</tr>
<tr>
<td>N/A 2</td>
<td>100 24 10 4</td>
</tr>
<tr>
<td>1 3</td>
<td>1,000 237 102 35 8</td>
</tr>
<tr>
<td>10 4</td>
<td>10,000 2,370 1,020 352 83</td>
</tr>
<tr>
<td>100 5</td>
<td>100,000 23,700 10,200 3,520 832 29</td>
</tr>
<tr>
<td>1,000 6</td>
<td>1e6 237,000 102,000 35,200 8,320 293</td>
</tr>
<tr>
<td>10,000 7</td>
<td>352,000 83,200 2,930</td>
</tr>
<tr>
<td>100,000 8</td>
<td>3.52e6 832,000 29,300</td>
</tr>
<tr>
<td>N/A 9</td>
<td>3.53e7 8.32e6 293,000</td>
</tr>
</tbody>
</table>
Counting Particles

• **Particle Counter** (for measurement of non-viable airborne particles)
  – Uses a calibrated laser particle counter

• **Settling Plates** (for measurement of viable airborne particulates)
  – Uses active settle plates and/or air sampler

• **RODAC Plates** (for measurement of viable, surface-bound particles)
  – Uses agar plates with agar above the edge of the plate
A Real Life Example
## Comparison of GMP and non-GMP Areas

### Cleanroom Areas

<table>
<thead>
<tr>
<th>Site</th>
<th>Bacteria</th>
<th>Mold</th>
<th>Total</th>
<th>Site</th>
<th>Bacteria</th>
<th>Mold</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbial Active Air Monitoring</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corridor A</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>Office</td>
<td>32</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>Inoculum B</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Café</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><strong>Microbial Surface Monitoring</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corridor A Wall I</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Office Wall I</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Corridor A Wall II</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Office Wall II</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Corridor A Door to A/L</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Office Door</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Corridor A Floor</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Office Desk</td>
<td>TNTC</td>
<td>0</td>
<td>TNTC</td>
</tr>
<tr>
<td>Inoculum B Worktable I</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Office Phone (handle)</td>
<td>79</td>
<td>0</td>
<td>79</td>
</tr>
<tr>
<td>Inoculum B Worktable II</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Office Computer Mouse</td>
<td>46</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td><strong>Microbial Passive Air Monitoring</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corridor A (2 hr exposure)</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>Office (2 hour exposure)</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Inoculum B (2 hr exposure)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Café (2 hour exposure)</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

### Administration Area

<table>
<thead>
<tr>
<th>Site</th>
<th>Bacteria</th>
<th>Mold</th>
<th>Total</th>
<th>Site</th>
<th>Bacteria</th>
<th>Mold</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-Viable Particulate Monitoring</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corridor A</td>
<td>237</td>
<td></td>
<td></td>
<td>Office</td>
<td>33138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculum B</td>
<td>34</td>
<td></td>
<td></td>
<td>Café</td>
<td>31644</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Personnel Gowning Process

1. Sterile gloves
2. Mask
3. Hood
4. Gown
5. Boots
6. Second pair of sterile gloves
   (IPA is used between each step)
Over 1 million pages of QA documentation to date in validated electronic document control system
Product Development

- Overview of Product Development
- Costs of Product Development
- Product Development, Manufacture and Testing of Clinical Materials--VLP Based Vaccine for Chikungunya Virus
- GMP Facility and Environmental Monitoring
- Example: H5 Influenza Vaccine from Bench to Clinic
Swine-Origin Influenza A (A/California/04/2009 (H1N1))
DNA Vaccine Development Timeline

2009

Mid March 2009
La Gloria, Veracruz Mexico 60% population sickened by Respiratory illness

Late March 2009
First case in USA

April 1, 2009

April 25, 2009
VRC received the flu sequence

May 28, 2009
Plasmid Received at July 21, 2009 Pilot Plant

May 20, 2009
First mice studies initiated at VRC

May 15, 2009
First isolate completely sequenced (A/California/04/2009 (H1N1))

May 15, 2009
2nd Gen Codon optimized plasmids received at VRC

Jun 4, 2009
VRC Vaccine Pilot Plant Releases Vaccine

Jun 9, 2009
cGMP Bulk Complete

Jun 10, 2009
cGMP Filled Drug Product Complete

Aug 5, 2009
FDA Release of VRC Product

Aug 24, 2009
Influenza Phase I Clinical Trial Initiated at VRC/NIAID

Jun 21, 2009
Plasmid Received at Pilot Plant

Jul 21, 2009
VRC Vaccine Pilot Plant Releases Vaccine

Aug 5, 2009
cGMP MC Complete

Aug 5, 2009
cGMP MC Complete

Jun 10, 2009
cGMP Filled Drug Product Complete
Conclusions

- Product Development is multi-disciplinary
- Industry estimates average drug development requires 8-10 years and $800 million (year 2000 dollars)
- Economics drive the selection of drug candidates
- FDA establishes strict rules for the manufacture (cGMP), animal testing (GLP) and clinical evaluation (GCP) of new drug products
Vaccine Production

VPPL
Gretchen Schieber
Himadri Bhattacharya

Joshua Merrit
James Lee
Diane Wycuff
Jacob Demirji
David Berlinger
Ranjana Srivastava

Indresh Srivastava
Ya-chen Chang
Ying Shi
Mridul Ghosh
Xin Wang
Janel Holland-Linn
Shayla Perkins

Regulatory
Jane Halpern
Rebecca Sheets
Michelle Conan-Cibotti

VCMP
Criss Tarr
John Madsen
Paul Mutolo
Matt Westerman
Doug Cooper
Barbara Brooks
Patricia Marshall
Phillip Ramsey
Martha Till

Everyone At the VPP

VRC
Gary Nabel
John Mascola
Richard Koup
Barney Graham
Robert Seder
Judy Stein
Srini Rao
Abe Mittelman
Bob Bailer
Mario Roederer