

WKS Assays, Inc.

Horseradish Peroxidase
Immunoassays

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Chemical Engineering Senior Design Project

Overview

- I. Purpose**
- II. Immunoassay**
- III. Market**
- IV. Characteristics of Our Immunoassay**
- V. Design Options**
- VI. Fabrication**
- VII. FDA Approval**
- VIII. Financial Risk**

Objectives & Goals

- Produce a quality diagnostic test
- Provide leading immunoassays for bacterial detection
- Target rural hospital market initially
- **Make Money**

Purpose

Immunoassay

Market

Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk



Product

Immunoassay Kit

- Analytical test
- Targets specific molecule
- Results obtained by signal generation

Purpose

Immunoassay

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Why *S. aureus* immunoassay?

Staphylococcus aureus

- Major cause of hospital infections
- 8 common infections
- Can cause deadly complications
- Hospital Infections 4th leading cause of US deaths

Purpose

Immunoassay

Market

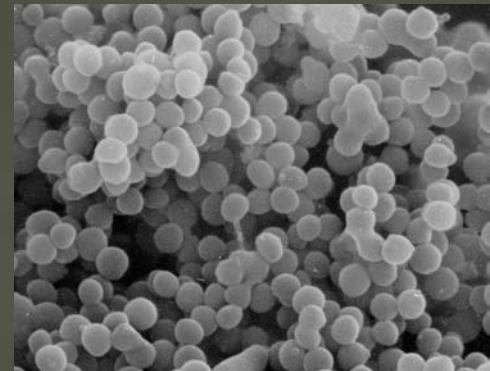
Characteristics

Design Options

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www.ulb.ac.be/sciences/biodic/ImBacterie2.html

Why *S. aureus* immunoassay?

Common Conditions

Purpose

Immunoassay

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Infection	Description
<i>Folliculitis</i>	inflammation of the follicles
<i>Boils</i>	painful, pus-filled inflammation of the skin and subcutaneous tissue
<i>Sties</i>	Inflammation of one or more sebaceous glands of an eyelid
<i>Impetigo</i>	a contagious skin infection
<i>Abscesses</i>	A localized collection of pus in part of the body, formed by tissue disintegration and surrounded by an inflamed area
<i>Staphylococcal pneumonia</i>	inflammation of the lungs
<i>Osteomyelitis</i>	onset after surgery
<i>Toxic shock syndrome</i>	acute infection associated with tampon use during menstruation

S. aureus immunoassay

- Early detection yields better treatment
- Sanitation control
- Can be modeled and adapted to other immunoassays

Purpose

Immunoassay

Market

Characteristics

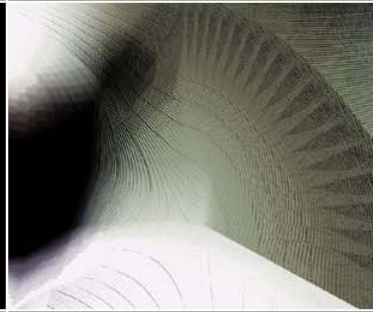
Design Options

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Immunoassay



Components

Antibody

- Immune system vesicle

Antigen

- Molecule being detected

Enzyme

- Oxidizing agent using in signal generation

Substrate

- Signal generator in conjunction with enzyme

Purpose

Immunoassay

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Background

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Immunoassay

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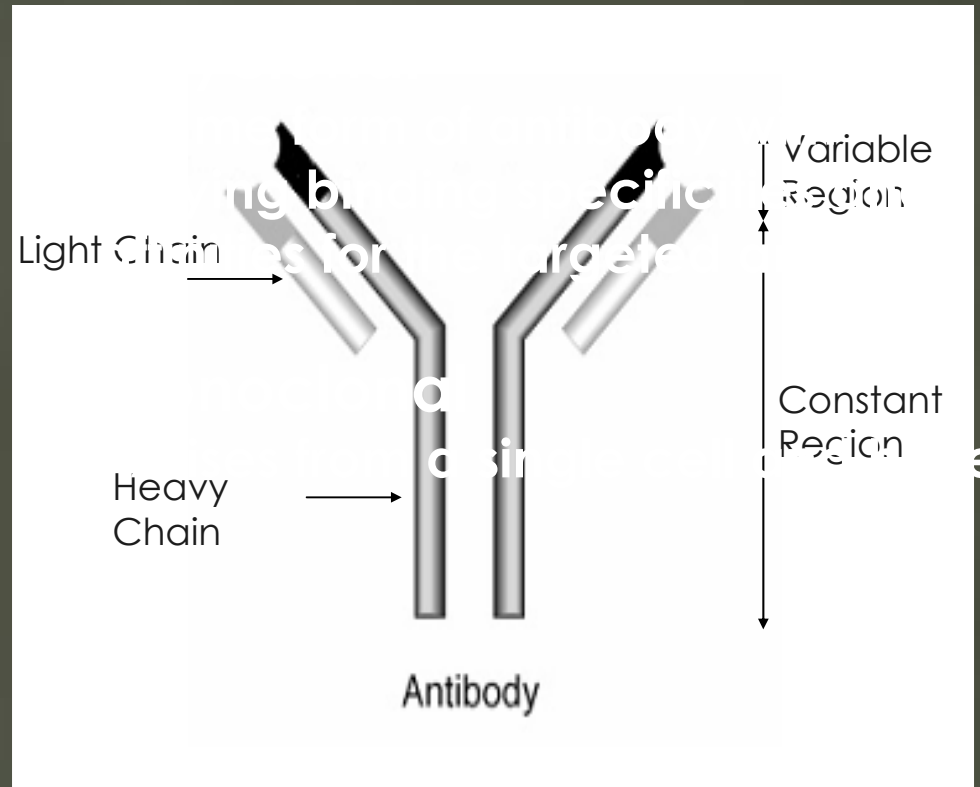
Design Options

Fabrication

FDA Approval

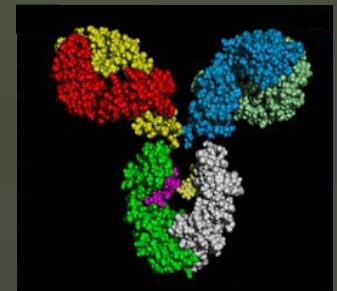
Financial Risk

- **Antibody**
 - Chains
 - Regions
 - Types
 - Polyclonal
 - Monoclonal



Background

- Which antibody for our assay?
 - Immunoglobulin G (IgG)
- Why IgG?
 - Widely available
 - Higher antigen affinity compared to other antibodies
- Structure of IgG



Purpose

Immunoassay

Market

Characteristics

Design Options

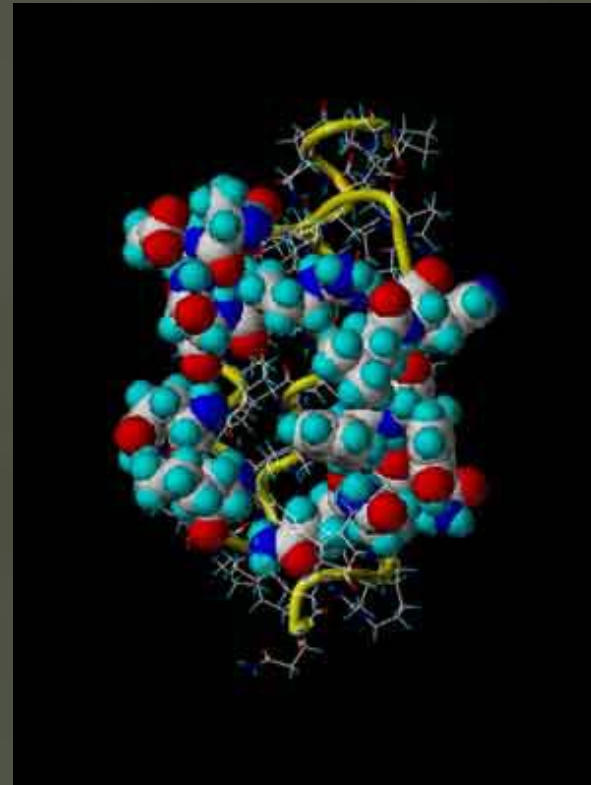
Fabrication

FDA Approval

Financial Risk

Background

- **Antigen — Protein A**
 - **Source** - *Staphylococcus aureus*



Purpose

Immunoassay

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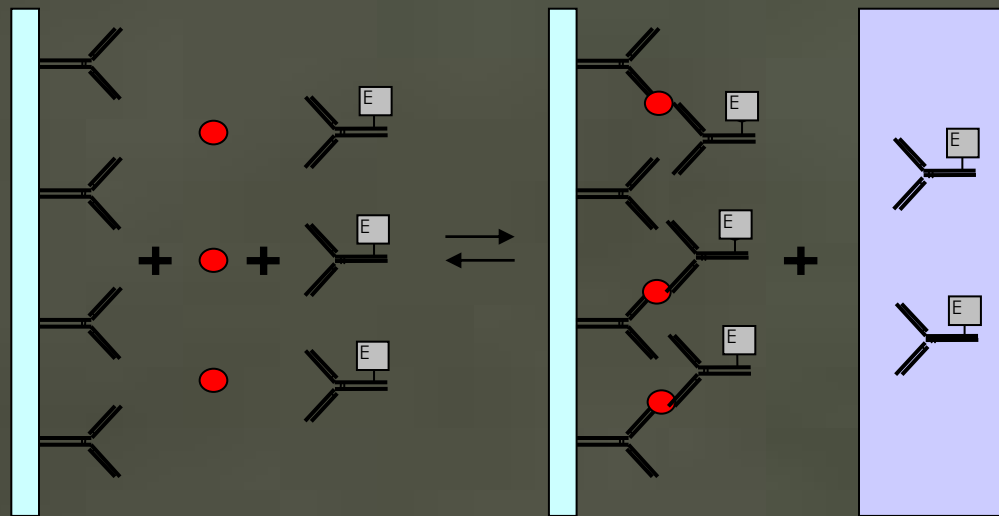
Fabrication

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Financial Risk

Background

- Immunoassays
 - Immunometric
 - Enzyme linked immunosorbent assay



Solid phase
coated with
antibody

Antigen

Enzyme
labeled
antibody

Wash to remove
unbound labeled
antibody

Purpose

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Background

o Immunoassays

Purpose

Immunoassay

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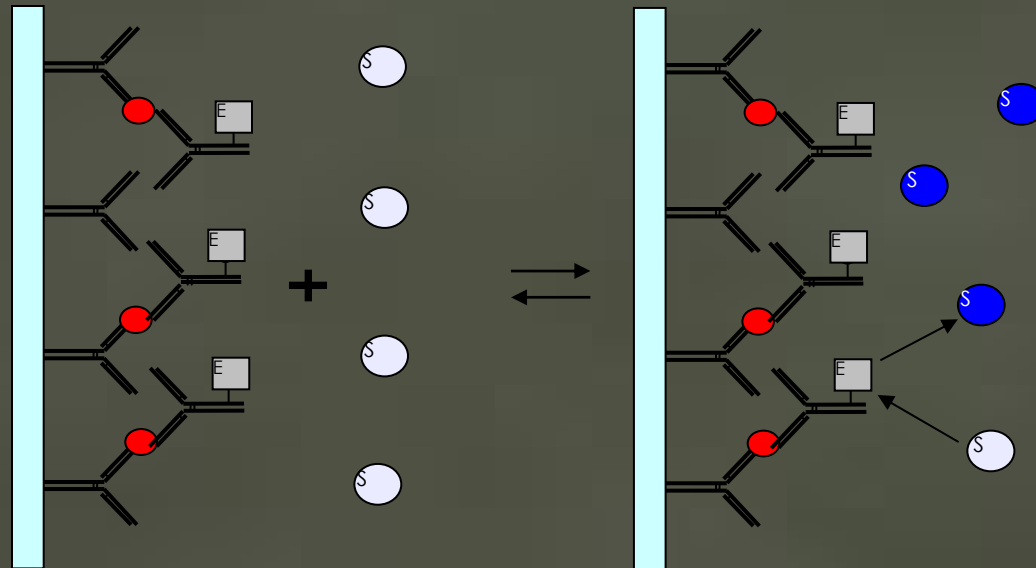
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Enzyme Catalyzed Reaction to Produce Signal

Immunoassay Operation

- Equilibrium test
- Approximately 3 hours

- Working Range
 - Pico-molar

A black rectangular box containing the text "WKS Assays, Inc." in a white, serif font.

WKS Assays, Inc.

○ Laboratory operation

Purpose

Immunoassay

Market

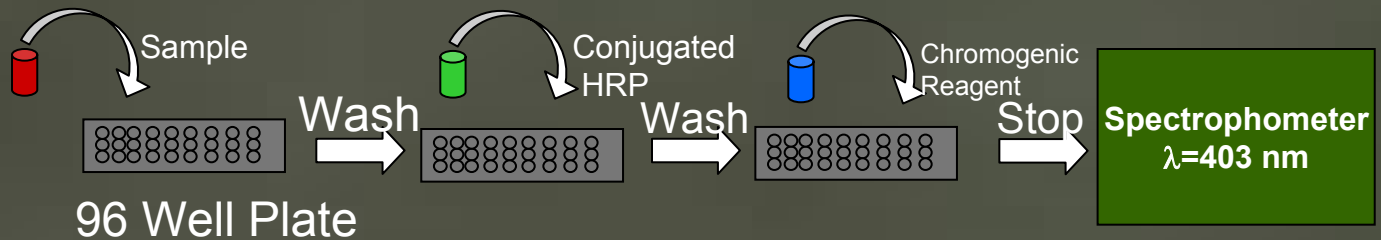
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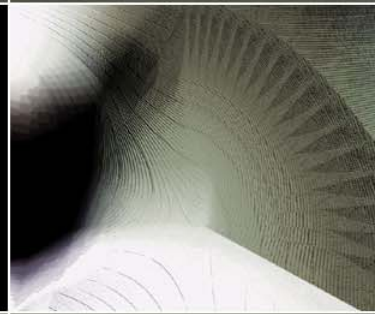
Fabrication

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Financial Risk



Market



Market

- Target market segment
 - Rural hospitals
- Why rural hospitals?
 - Untapped market
 - Technology — Cost barrier
 - Automated throughput vs. manual
 - Prohibitive capital cost



Purpose

Immunoassay

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Market

○ Rural Hospital Infection Rates

- 300,000 total infections annually in rural hospitals
- *S. aureus* responsible for approximately 76,000 infections
- 300,000 assays required to test all patients presenting symptoms

Purpose

Immunoassay

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Market

○ Rural Hospital Trends

○ Recent growth in rural population

- 6% increase in hospital discharges between 1995 & 2000
- 11% growth in rural population between 1990 & 2000

○ Rural hospital discharges to increase

- Discharges expected to increase 10% between 2005 & 2014

Purpose

Immunoassay

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Market

- **Competition**

- Larger biotechnology companies
- Remel, Sure-View, Murel

- **Our Advantages**

- Quantitative Detection
- Shorter waiting time
- Does not require sample culturing

Purpose

Immunoassay

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Characteristics

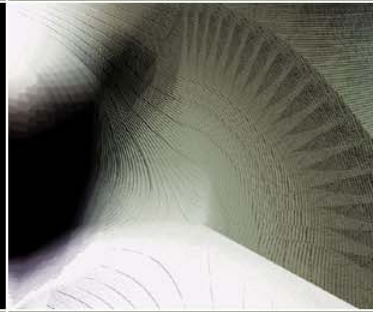
Design Options

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Immunoassay Characteristics



Reagents & Components

- **What's in the box?**
 - Two 96 - well plates
 - HRP-IgG conjugate solution
 - Wash buffer
 - TMB Substrate solution
 - Stop Solution
 - Standard Antigen Solution
 - Standard Dose Response Curve



Purpose

Immunoassay

Market

Characteristics

Design Options

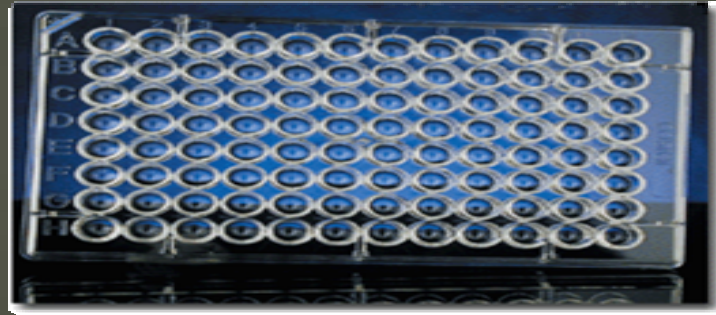
Fabrication

FDA Approval

Financial Risk

Reagents & Components

- **96 well plate**
 - Standard for use with ELISA
 - Allows for multiple sample testing
 - Polystyrene



- **IgG-HRP conjugate solution**
 - Signal generation component of assay

Purpose

Immunoassay

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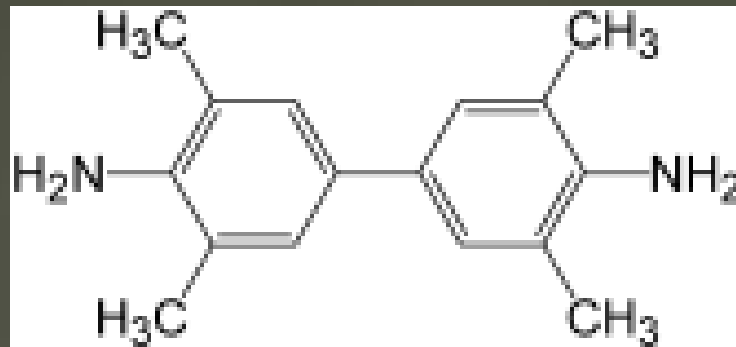
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Reagents & Components

- Wash buffer
 - TRIS buffered saline with 0.1% Tween
- Hydrogen peroxide
 - Oxidizing agent
- TMB Substrate solution
 - 3,3',5,5'-tetramethylbenzidine
 - Produces strong signal
 - Lacks mutagenic qualities



Purpose

Immunoassay

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Reagents & Components

- Assay buffer

- TRIS buffered saline with 1% BSA (Bovine Serum Albumin)

- Standard antigen solution

- Protein A antigen solution
- 4 concs. that span working range

Purpose

Immunoassay

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Reagents & Components

- Stop solution
 - 1M hydrochloric acid
- Standard dose response curve
 - Signal vs. antigen conc.
 - Conc. as low as 800 pg/mL

Purpose

Immunoassay

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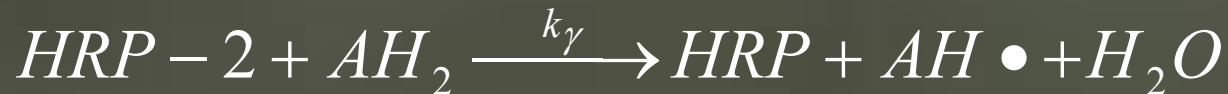
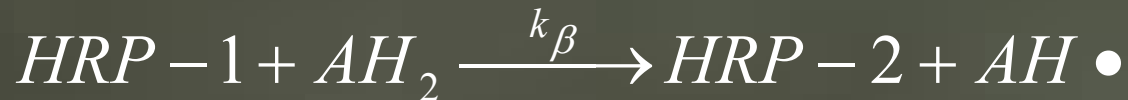
Financial Risk



HRP Kinetics

Enzyme Reaction

- HRP undergoes three reactions to generate signal



Color is generated from oxidized substrate radical

Purpose

Immunoassay

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Financial Risk

HRP Kinetics

- Stop solution will be added to the mix after 10 minutes.
- Spectrophotometer is used to measure the concentration of colored substrate in solution
- $\lambda=403$ nm

Purpose

Immunoassay

Market

Characteristics

Design Options

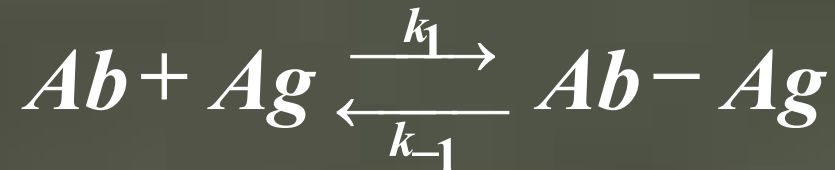
Fabrication

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Financial Risk

Kinetic Analysis

- Antibody-antigen binding
 - Surface adsorption kinetics



- Three binding reactions of interest
 - Antibody to surface
 - Antigen to antibody
 - Labeled antibody to antigen-antibody surface complex

Purpose

Immunoassay

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Fabrication

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Financial Risk

Kinetic Analysis

- Evaluation of rate constants
 - How?
- BIAcore s51

Purpose

Immunoassay

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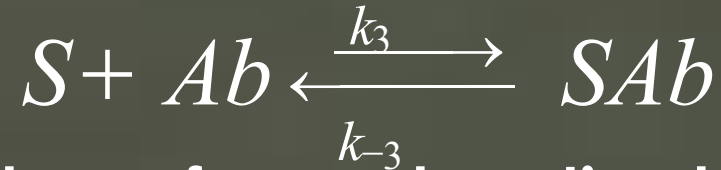
Financial Risk



Kinetic Analysis

Plate Antibody Binding

- Binding reaction of antibody to surface



- First order surface adsorption kinetics
- Adsorption and Desorption reactions considered

$$\text{Adsorption} = r_{AD} = k_3 C_{Ab} C_{v,S}$$

$$\text{Desorption} = r_{DES} = k_{-3} C_{SAb}$$

$$\text{Equilibrium} = K_3 = \frac{k_3}{k_{-3}}$$

$$\text{Netrate} = r_{AD} - r_{DES} = k_3 C_{Ab} C_{v,S} - k_{-3} C_{SAb}$$

Purpose

Immunoassay

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Design Options

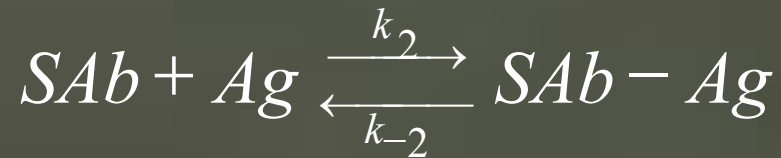
Fabrication

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Financial Risk

Kinetic Analysis

○ Binding of antigen to antibody



$$Adsorption = r_{AD} = k_2 C_{v,SAb} C_{Ag}$$

$$Desorption = r_{DES} = k_{-2} C_{SAb-Ag}$$

$$Equilibrium = K_2 = \frac{k_2}{k_{-2}}$$

$$Net\ rate = r_{AD} - r_{DES} = k_2 C_{v,SAb} C_{Ag} - k_{-2} C_{SAb-Ag}$$

Purpose

Immunoassay

Market

Characteristics

Design Options

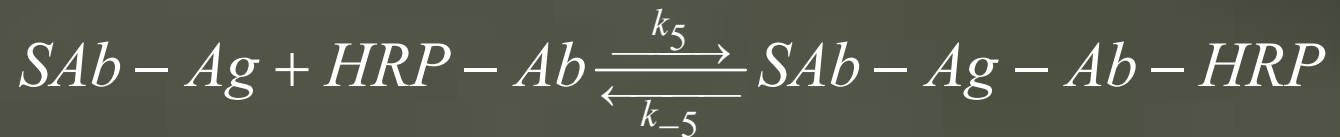
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Financial Risk

Kinetic Analysis

○ Surface complex binding



$$Adsorption = k_5 C_{HRP-Ab} C_{v,SAb-Ag}$$

$$Desorption = k_{-5} C_{SAb-Ag-Ab-HRP}$$

$$Equilibrium = \frac{k_5}{k_{-5}} = K_5$$

$$Netrate = k_5 \left[C_{HRP-Ab} C_{v,SAb=Ag} - \frac{C_{SAb-Ag=Ab-HRP}}{K_5} \right]$$

Purpose

Immunoassay

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Kinetic Analysis

Equilibrium interaction model

$$r_{SAb-Ag} = k_2 \left[C_{Ag} C_{v,SAb} - \frac{C_{SAb-Ag}}{K_2} \right]$$

$$r = k_1 C_{HRP-Ab} C_{v,SAb-Ag} - \frac{C_{SAb-Ag-Ab-HRP}}{K_1}$$

$$r_{bind.} = k_1 \left[C_{SAb} C_{HRP-Ab-Ag} - \frac{C_{SAb-Ag-Ab-HRP}}{K_1} \right]$$

Purpose

Immunoassay

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Kinetic Analysis

- Assume Adsorption & Desorption rates are equal to zero

- Solve for unknown concentrations

$$C_{SAb-Ag} = K_1 (C_{Ag} C_{v,SAb})$$

$$C_{Ag} = C_{Ag,0} - C_{SAb-Ag} - C_{SAb-Ag-Ab-HRP} - C_{HRP-Ab-Ag}$$

$$C_{SAb-Ag} = K_1 \left[(C_{Ag,0} - C_{SAb-Ag} - C_{SAb-Ag-Ab-HRP} - C_{HRP-Ab-Ag}) C_{v,SAb} \right]$$

- Initial antigen concentration is known
- Initial surface antibody conc. is known

Purpose

Immunoassay

Market

Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk

Kinetic Analysis

- **System of 3 equations**
 - Solved using Polymath™
- **Conc. of surface antibody-antigen complex critical variable**
- **Strength of signal is defined by this concentration**

Purpose

Immunoassay

Market

Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk

Kinetic Analysis

- Dose response curve
 - Indicates antigen concentration
 - Curve asymptotes as surface antibody concentration is approached

Purpose

Immunoassay

Market

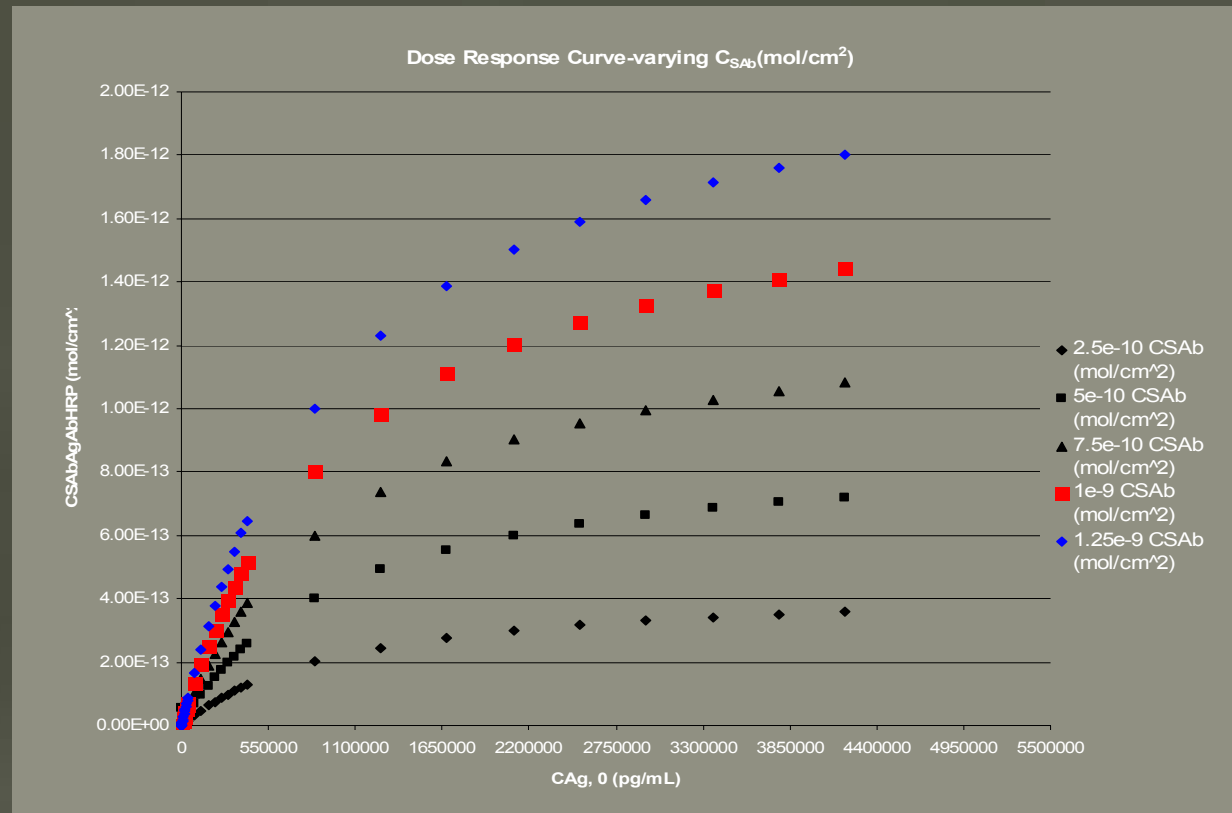
Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk



Sensitivity

- Assay sensitivity
 - How low can we go?
- Determined by surface complex concentration
 - Minimum complex conc. = 1.8×10^{-15} mol/cm²

Purpose

Immunoassay

Market

Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk

Sensitivity

- Signal strength increases with surface antibody concentration
- Optimize cost of surface antibody using this plot
- Use minimum amount of surface antibody that can generate signal

Purpose

Immunoassay

Market

Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk

Sensitivity

Purpose

Immunoassay

Market

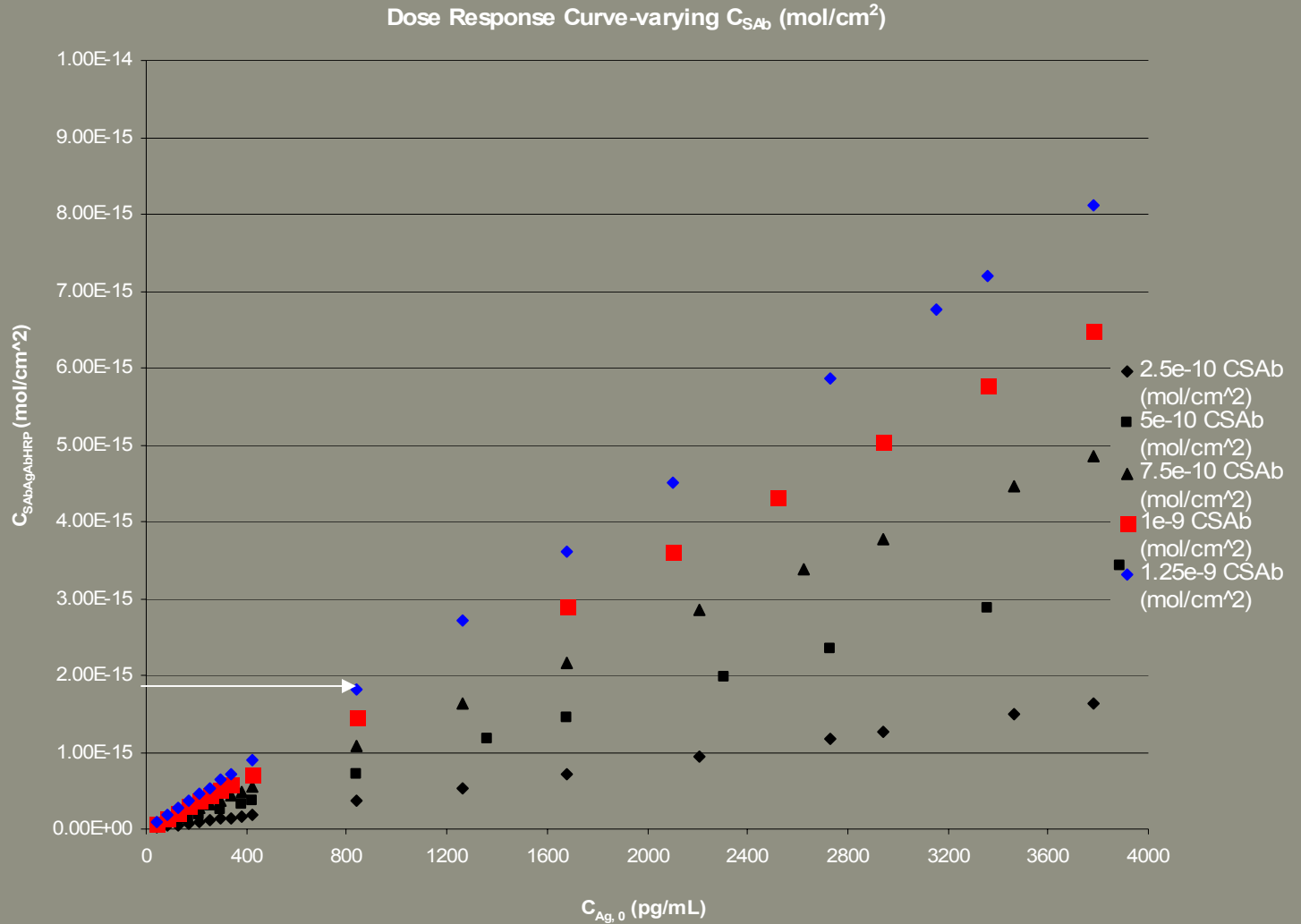
Characteristics

Design Options

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Sensitivity

- Dose response curve is only used in linear range
- Linear range= 800 pg/mL – 4000 pg/mL

Purpose

Immunoassay

Market

Characteristics

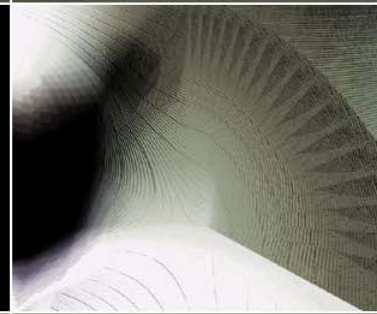
Design Options

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Design Options

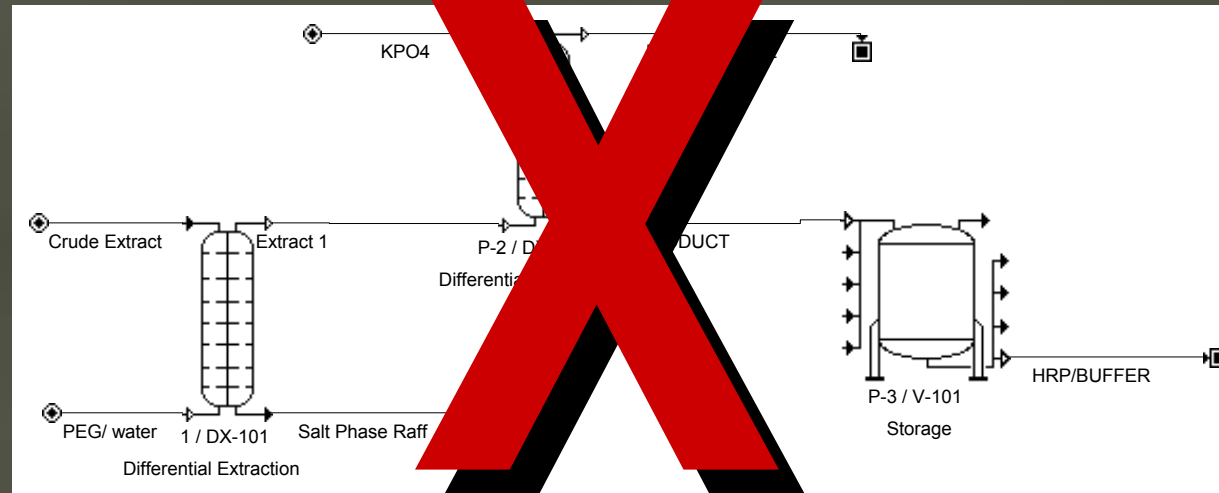


Evaluated Processes

Horseradish Peroxidase (HRP) Extraction

- 2 Differential Extractors
- Biphasic Salt Solution
 - Polyethylene Glycol (PEG) & KPO_4
- Reagent Cost \approx \$20,000
- FCI \approx \$150,000

Amount: 1.24kg
Activity: 117U/mL



Modeled using
SuperPro Designer 4.7

Purpose

Immunoassay

Market

Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk

Evaluated Processes

- **Cost Comparison of Produced and Purchased HRP**

- **Purchase HRP**

- Annual requirement: 5 g/yr
- Cost: \$15,000/yr

- **Produce HRP**

- Approximate Labor cost: \$35,000/yr

Purpose

Immunoassay

Market

Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk

Evaluated Processes

HRP Extraction *continued*

- Decided not to produce
- Purchase HRP
 - Lyophilized
 - Small amounts needed
 - Efficient for small company
 - \$266 per 100 mg (Sigma)

Purpose

Immunoassay

Market

Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk

Fabrication



Plate Preparation

Polystyrene Plate Steps

1. Fill wells with IgG coating solution
2. Incubate for 18 hours
3. Remove coating solution. Wash.
4. Fill wells with 2nd coating solution
5. Incubate for 30 minutes.
6. Remove coating solution. Wash, and dry for 24 hours.

Purpose

Immunoassay

Market

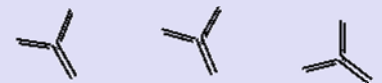
Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk



Manufacturing

Equipment

- 3 major pieces
 - Sterilization
 - Bottling
 - Labeling

Purpose

Immunoassay

Market

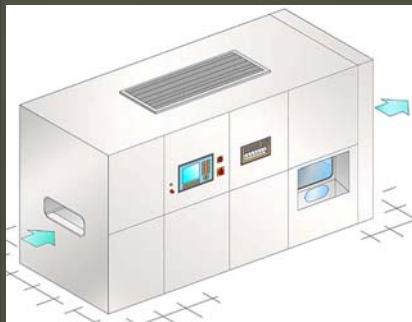
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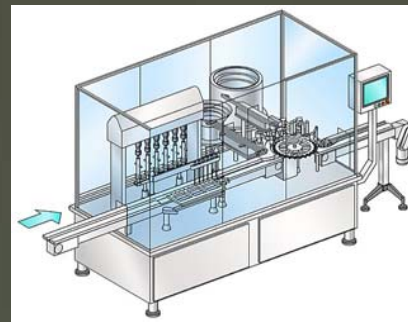
Fabrication

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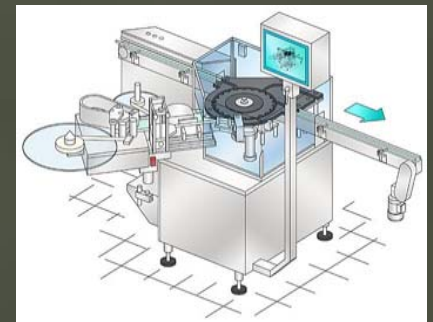
Financial Risk



STS Series



KFVG x212



HEA 105

Packaging

Shipping Box

- 6"x6"x4" Indestructo Mailing

Gray Convoluted Foam

- 8"x6"x2"

Yearly Packaging Costs
\$24,370



Purpose

Immunoassay

Market

Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk

Quality Control

- Random quality audits

- 2.5% of each assay batch

- Verification of working range

- Error greater than 5% audit

- 2.5% of tested assays discard batch

Purpose

Immunoassay

Market

Characteristics

Design Options

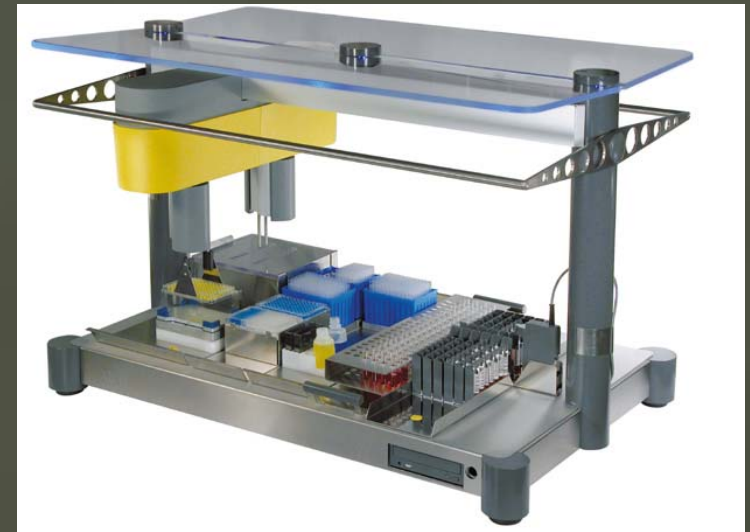
Fabrication

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Quality Control

- Apparatus
 - Xiril™ X100 combination liquid handler and plate reader
 - Higher test throughput
 - Less chance of human error



Purpose

Immunoassay

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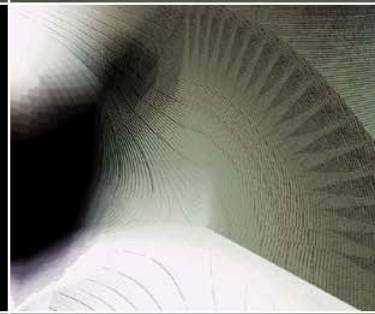
Design Options

Fabrication

FDA Approval

Financial Risk

FDA Approval



Why FDA Approval

Food & Drug Administration

- Government organization

- Protects Consumers

- Promotes Public Health

- Regulates Medical Devices

- Approval

- Require for US sales

- Quality product endorsement

Purpose

Immunoassay

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FDA Testing Phases

Purpose

Immunoassay

Market

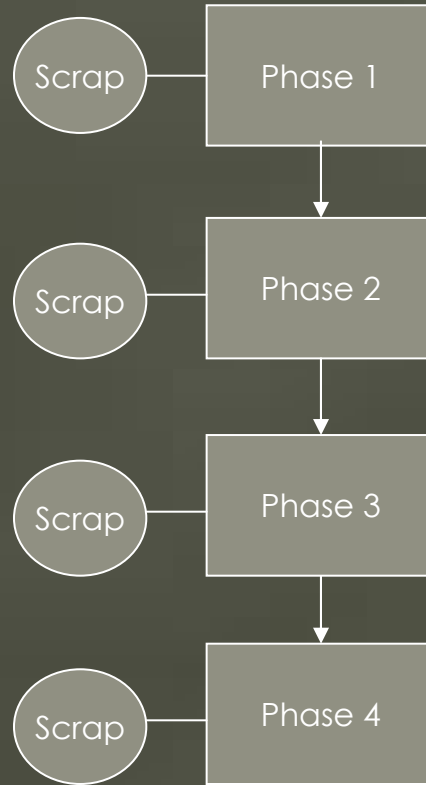
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Accuracy and Reproducibility Test

Human Serum Testing

Chemical Inhibition Testing

Quality Control

FDA Testing Decisions

- How much pre FDA testing should we do?
 - Should we perform many tests in the same phase, or just a few?
 - After what phase should we enter the FDA approval process?
- To answer these questions we developed a novel model of the FDA approval process.

Purpose

Immunoassay

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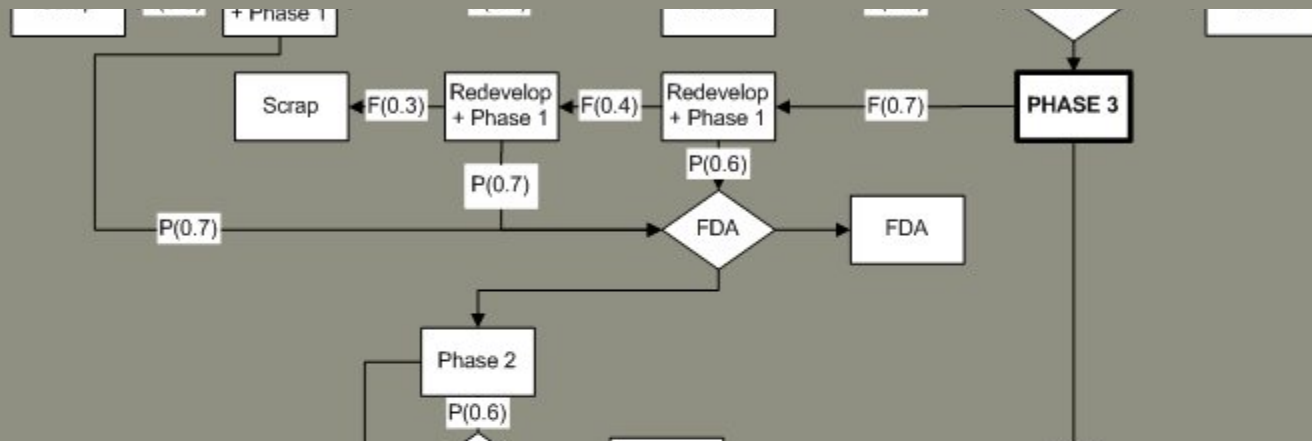
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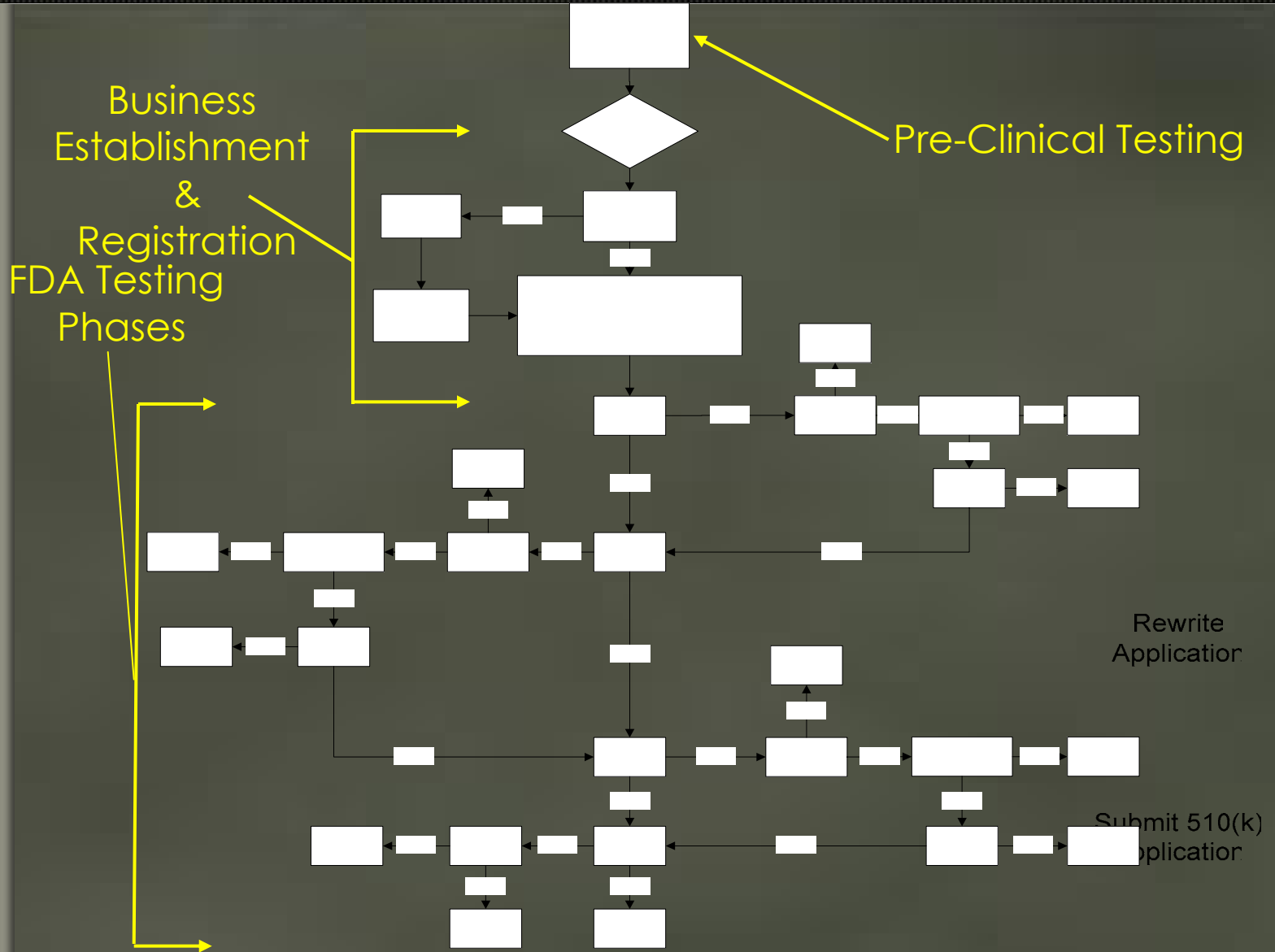
Financial Risk



Phase 3: Chemical Inhibition Testing

Phase 4: Quality Control

FDA Approval Outline



Purpose

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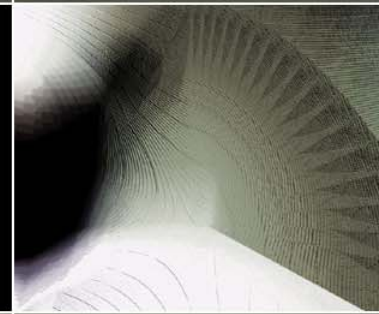
Design Options

Fabrication

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Financial Risk

Financial Risk



Stochastic Modeling

○ Evaluated Design Variables

Purpose

Immunoassay

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Option	Number of Tests	FDA
1	Phase 1 & 2 1000 Phase 3 & 4 500	Phase 4
2	Phase 1 & 2 1000 Phase 3 & 4 500	Phase 3
3	Phase 1 & 2 500 Phase 3 & 4 250	Phase 4
4	Phase 1 & 2 500 Phase 3 & 4 250	Phase 3

Stochastic Modeling

- Evaluate all possible pathways in the FDA model for each design variable to determine:

- Cost

- Time

- NPW

Purpose

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Stochastic Modeling

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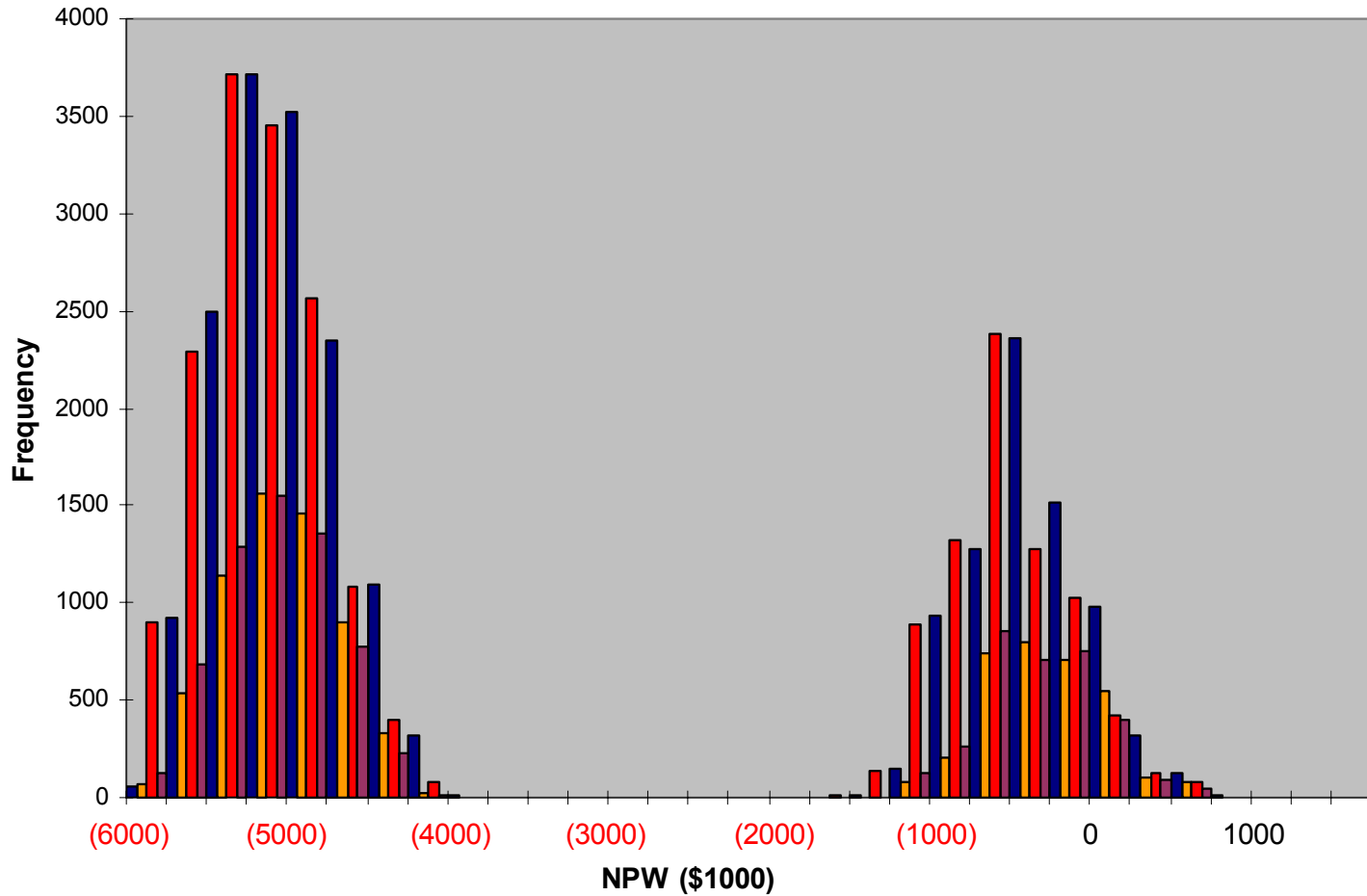
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Stochastic Modeling

○ Risk Assessment

Purpose

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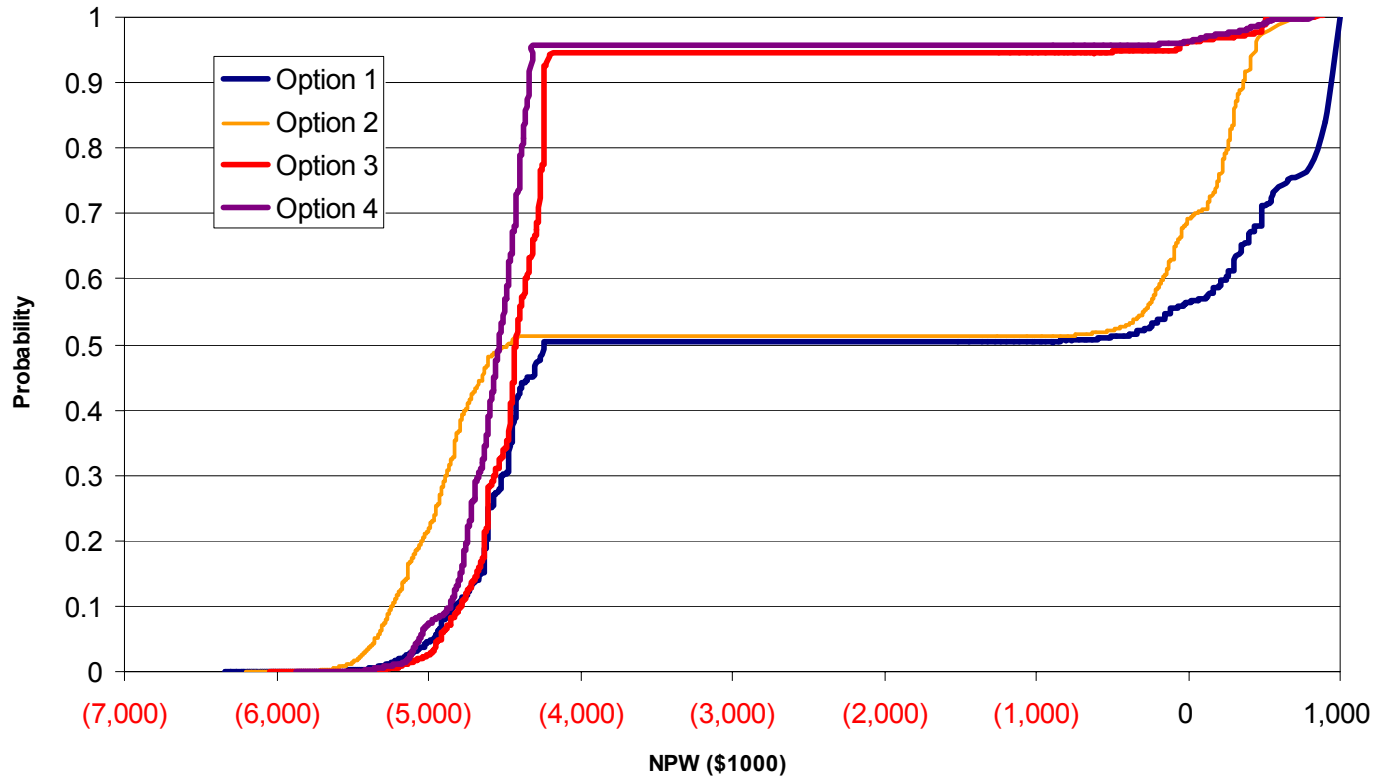
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Stochastic Modeling

- **Option 1 gave the best results**
 - **Thorough testing through all phases reduces the uncertainty in the FDA approval process**
 - **Estimated testing cost: \$1.8 Million**
 - **Pre FDA Testing: \$1 Million**
 - **FDA Testing: \$800,000**
 - **Estimated time: 1.7 years**

Purpose

Immunoassay

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Financial Risk

Breakdown

- **Estimated Annual Sales**
 - 250,000 Immunoassay Kits
 - \$125 per kit
 - Total Sales: \$31 Million
- **Total Product Cost**
 - \$30 million
- **Total Capital Investment**
 - \$5.8 Million
- **Estimated NPW**
 - -\$2 Million



Purpose

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Questions

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Market

Characteristics

Design Options

Fabrication

FDA Approval

Financial Risk

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