

***We Stand Behind Our Results***

# Rapid Techniques for Allergen Detection

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Vancouver, BC

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# Agenda

Food Allergens:

What and How?

So What?

Tools for Protection?



# What Are Food Allergies ?

- Naturally occurring proteins
- Heat and processing resistant
- Resistant to extremes in pH
- Usually major proteins in food
- Foods can have 1 or many allergens
- No known cure...strict avoidance

# Food Allergy vs. Food Intolerance

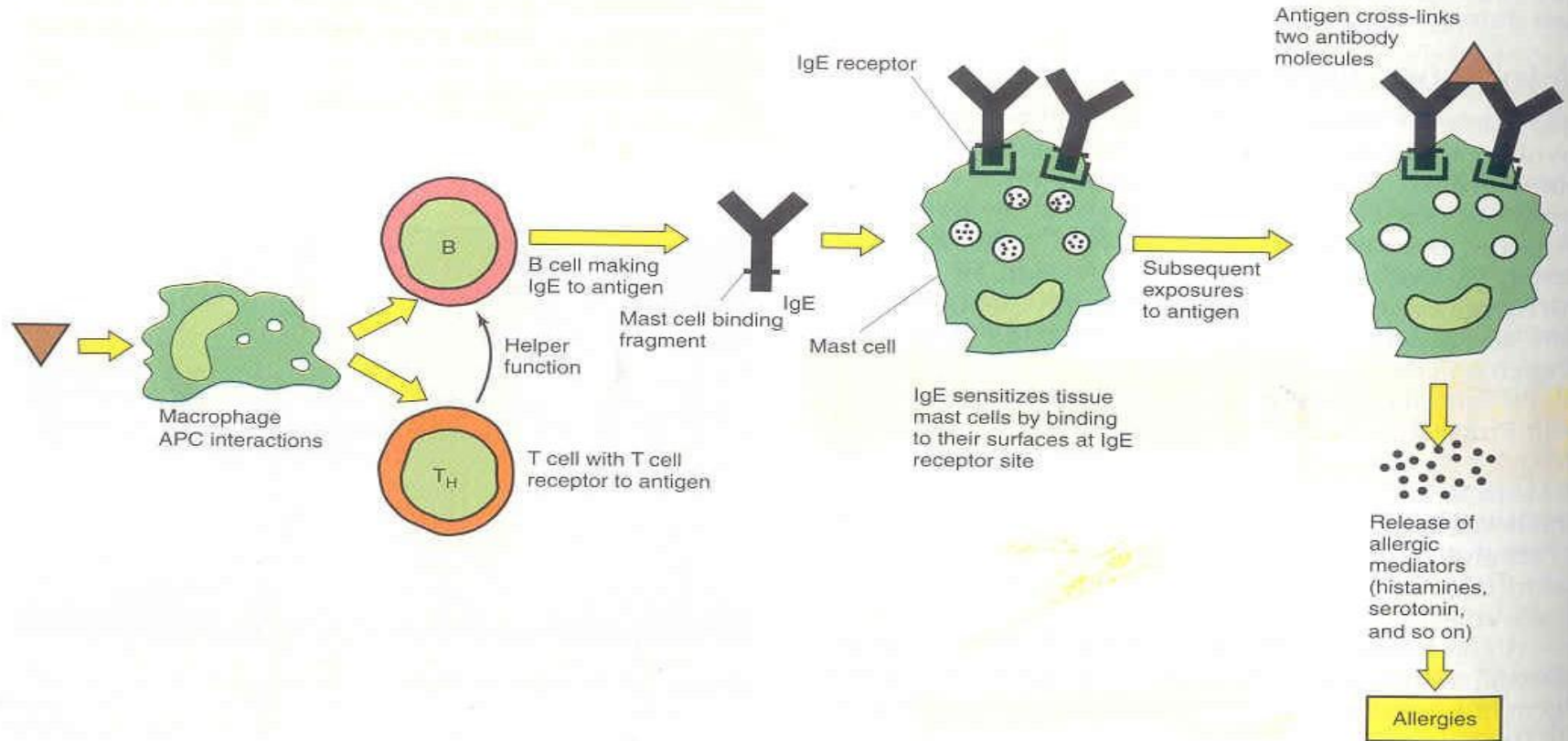
**Food Allergy:** IgE mediated immune response, triggers release of histamine, i.e. watering eyes, running nose, scratchy throat

**Food Intolerance:** Sensitivity to certain foods caused by a deficiency i.e. lactose intolerance

# How Food Allergens Work

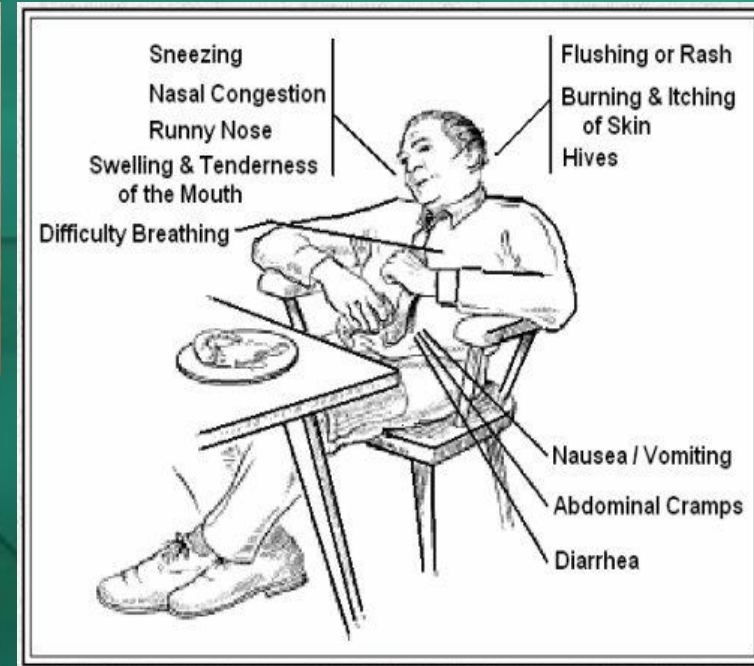
- Specific proteins stimulate immune response in two stages
  - A. Initial sensitization (no response)
  - B. Subsequent exposure (release of histamine)

# How Food Allergens Work



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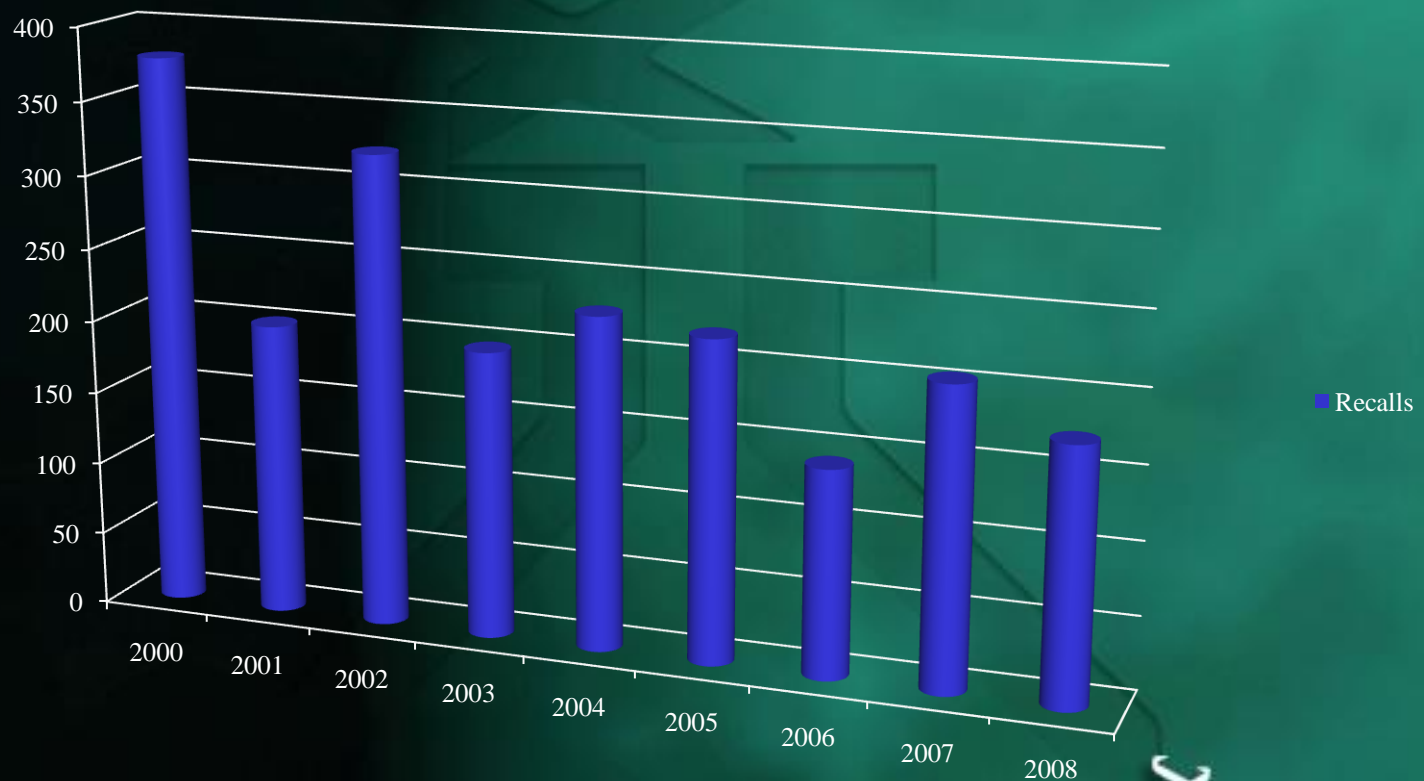
# How Food Allergens Work



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# Leading Cause of Food Recalls

**Food Allergen Recalls**



# Allergens of Concern

## United States

- Peanuts
- Soy
- Milk
- Eggs
- Fish
- Shellfish
- Tree Nuts
- Wheat

## Canada

- Peanuts
- Soy
- Milk
- Eggs
- Fish
- Shellfish
- Tree Nuts
- Wheat
- Sesame
- Sulfites



# Food Ingredient Issues

- Oils
- Enzymes
- Gums
- Flavors/Extracts
- Lecithin
- Lactose
- Starch
- Gelatin
- Processing Aids
- Colorings

# Why Monitor for Food Allergens ?

## 4 types of Risks

- 1) Clinical
- 2) Regulatory
- 3) Consumer
- 4) Business

# Types of Risk

- Clinical
  - Up to 8% of children, and up to 3% of adults in US have a food allergy
  - researchers estimate 50,000 emergency room visits, and 150-200 deaths per year from food-induced anaphylaxis
  - trace amounts (ppm) of the offending food will trigger reactions

## What is Tolerance Level ?

*Current available data indicate that it is not possible to set a limit to the amount of allergenic protein there must be in a food to elicit an allergic reaction*

# Types of Risk

- Regulatory

- FALCPA:
  - declaration of allergens on label
  - ingredients, except those exempt, but including incidental additives, flavors, colors, mixes and processing aids.
  - “enforcement discretion” on soy lecithin
  - define gluten-free as < 20 PPM “prohibited grains” (wheat, rye, barley)

# Types of Risk

- Consumer/Sales
  - dissatisfaction and overall loss of confidence
  - brand loyalty can be the enemy of the consumer
  - Proliferation of “May Contain...” type labels causing frustration and confusion to consumers

# Considerations When Using Precautionary Labeling

- Is the presence of allergen documented through visual inspection or analytical testing?
- Is the risk of presence of unavoidable?
- Is the allergen is present in some, but not all product?
- Is the presence of allergen is potentially hazardous?

# Types of Risk

- Business
  - increased costs due to down-time, change in product formulation and equipment change-over
  - loss of competitive edge in marketplace
  - Regulatory agencies will almost always investigate a facility that has had a recall

# “Hidden Risks”

- Recycled CIP and COP rinses
- Oils re-contaminated after refining
- Rework
- Labeling terms i.e. *non-dairy creamer*
- *Many others...*

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# Elements of an Allergen Control Plan

- Allergen Risk Assessment
- Engineering and System Design
- Scheduling
- Processing Controls
- Maintenance
- Sanitation/Change Over Cleaning
- Labeling/Packaging
- Consumer Complaint Systems
- Training
- Auditing/Verification



# Ingredients/Raw Materials

## Suppliers

- Ingredient suppliers should be evaluated and audited routinely for *Allergen Control Plans* and *Allergen Labeling Controls*
- *Certificates of Analysis* are provided, kept current and reviewed, and contain full disclosure of all ingredients used
  - Prior notification of any formulation changes are provided by suppliers and kept on file
    - Confirmation that unlabelled allergens are not in formulated multi-component ingredients i.e. spice blends



# Ingredients/Raw Materials

## Storage

- Incoming ingredients are clearly labeled with allergens
- Ingredients are given a specific lot # and tracked through system
- Designated storage areas for allergenic ingredients where feasible
  - If designated receiving area is not feasible, store allergenic ingredients below non-allergenic ingredients
- Use a color-coding system for totes and bins, and stickers for pallets and bulk ingredients



**Contains  
Allergen  
Ingredients**



# Equipment/Engineering

- Dead legs or dead spots can be areas that hold previous production
- Review equipment annually, with documentation, for dead spots, rough surfaces and voids
- Equipment should allow for thorough cleaning and be accessible for inspection
- Avoid a situation where product flow may allow for cross-contamination i.e valve clusters, layered conveyors
- Evaluate air handling system to insure it's not a source for contamination



# Operations/Processing

## Scheduling

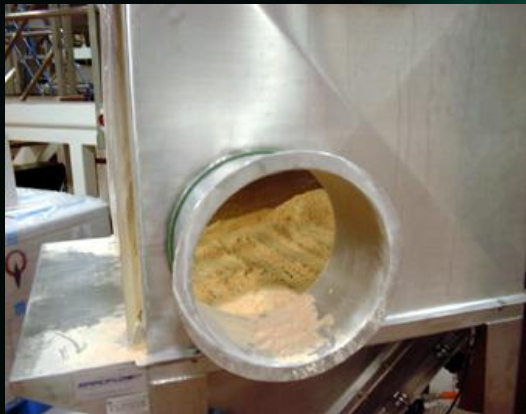
- Introduce allergenic components into the process as late as possible
- Whenever possible, avoid manufacturing an allergenic product prior to a non-allergenic product
- Develop an *Allergen Matrix* or changeover grid to identify necessary sanitation and scheduling practices
  - If using re-work, make sure to follow *Exact-into-Exact* philosophy
- Where possible, schedule long runs of allergenic product, and allow for full allergen clean-up when complete



# Operations/Processing

## Practice

- Do not allow the use of single-service items such as tray-liners to be reused
- When sampling the in-process product, be certain the sampling device is cleaned & sanitized appropriately between products
  - Double-check formula during scale-up
- Physical barriers such as shield covers and catch pans should be positioned to prevent spillage and cross-contamination



# Labeling/Packaging

- Should have a label SOP to insure all necessary steps are taken for accuracy
  - All formula changes are properly documented and synchronized between labeling and manufacturing
  - All minor ingredients and processing aids that contain allergenic protein are properly reflected on the label
- Precautionary labeling, if used, is justified, not overused, and not a replacement for proper GMP's



# Sanitation

## Process

- Identify all equipment, conveyors and food contact surfaces that require cleaning after an allergen run
- Be sure to include splash zones, indirect product contact surfaces, utensils, employees and sampling equipment in the SSOP
  - On pre-op inspections, make sure you specify allergen check points
- Focus on hard-to-clean areas, including valves, dead spots, pumps corners, welds and angles
  - Consider using color-coded cleaning utensils



# FDA “Guidance on Inspections...”

*www.fda.gov/ICECI/Inspections/InspectionGuides*

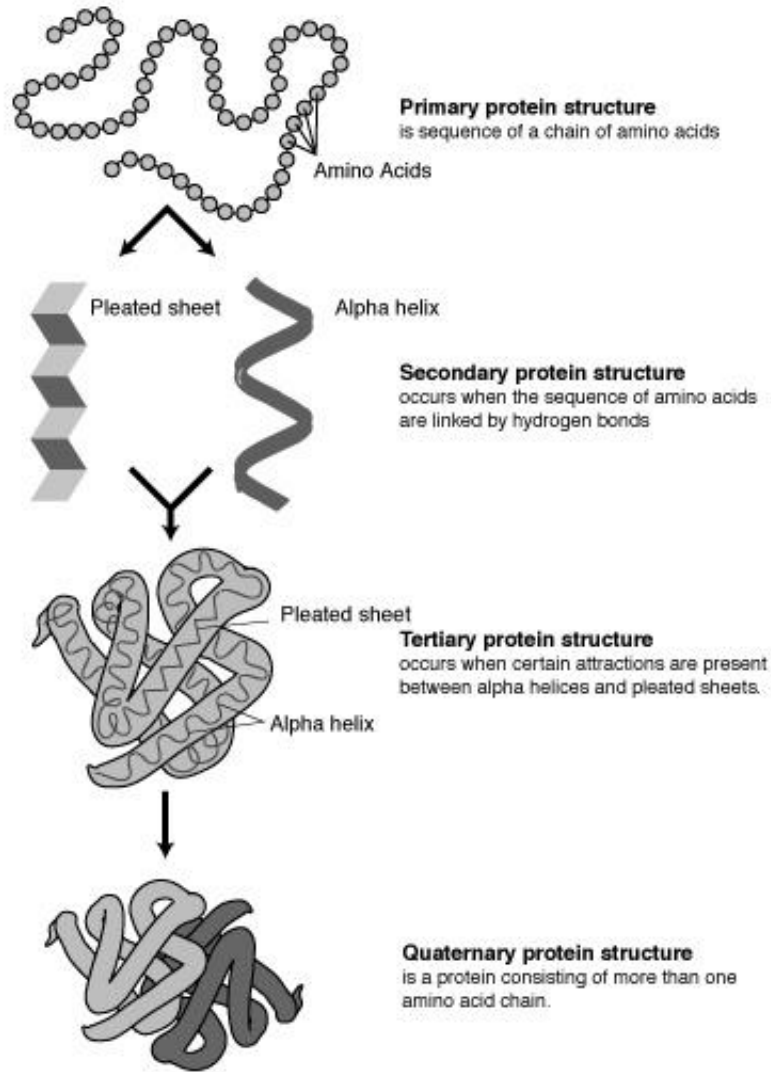
- Product Development
- Receiving
- Equipment
- Processing
- Final Product Testing
- Labeling



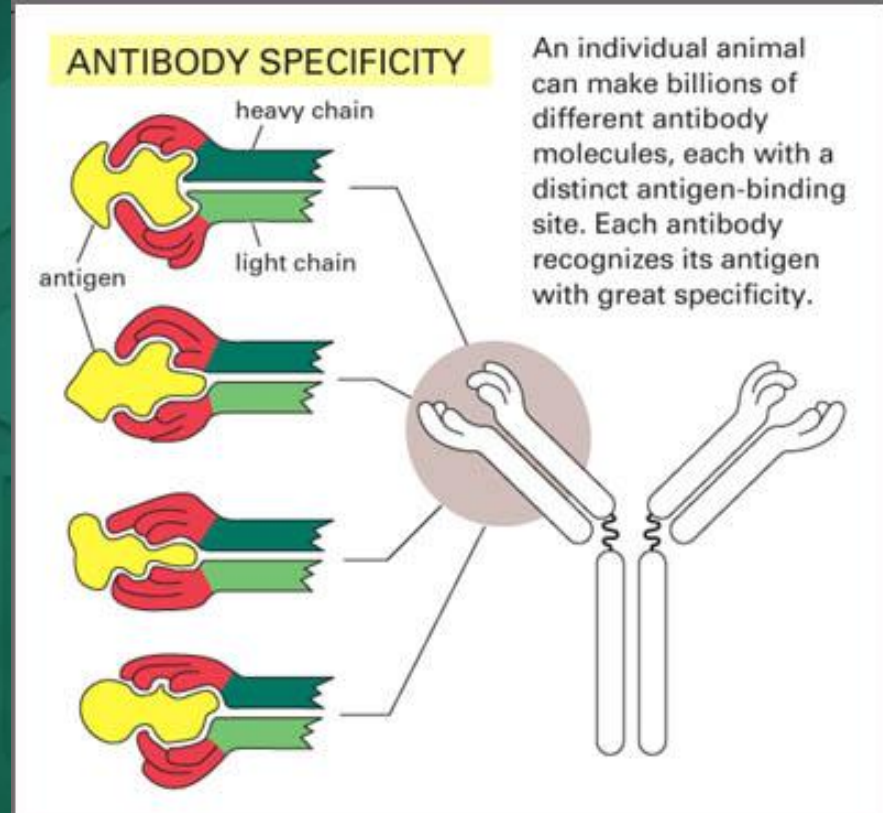
# Neogen and Food Allergens

- Neogen joined FARRP in 1997 and collaborated on the industry's first test kit for peanut allergen
- FARRP – Food Allergy Research and Resource Program, University of Nebraska
- FARRP is globally recognized in the food industry as a trusted advisor on allergen issues
- Later collaborations on development of rapid test kits for peanut, milk, egg, almond, hazelnut, gluten and soya

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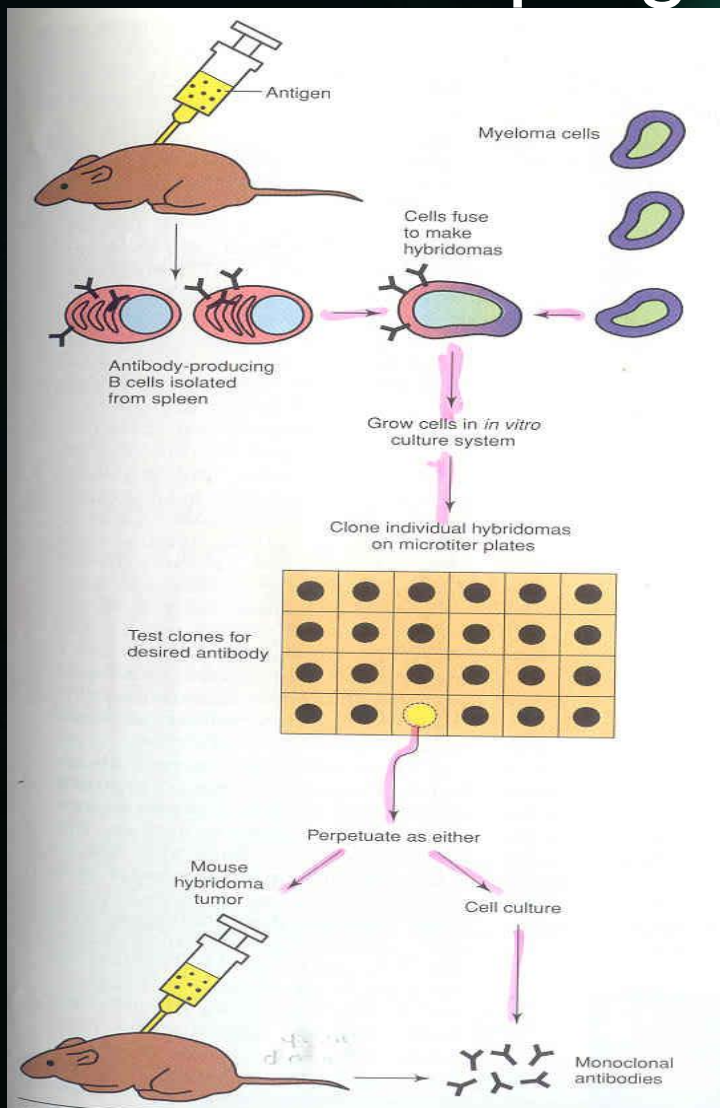
# Designing a Suitable Antibody



## Fermented and Hydrolyzed Proteins: Known Limitations

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# Developing the Right Antibody



Monoclonal or Polyclonal

Cooked or Raw

Rabbits, Sheep, Goats, Mice

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# Quantitation and Screening



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# *Reveal 3-D* Products



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# Allergen Specific Tests



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## ***Reveal 3-D™* Components**

### **Components provided**

(from top left to bottom right):

- **Extraction buffer sachet**
- **Sample tube & cap**
- **RAPID 3-D test device**
- **swab**



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# Reveal 3-D Specification

<b>Kit Format</b>	Lateral Flow Device, 3-D Technology, no equipment
<b>Limit of Detection</b>	Low ppm levels of food residue
<b>Suitable for Testing</b>	environmental swabs and rinse solutions
<b>Samples per kit</b>	10 samples or swabs
<b>Total test time</b>	<10 min per sample including extraction



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# Reveal 3-D Procedure

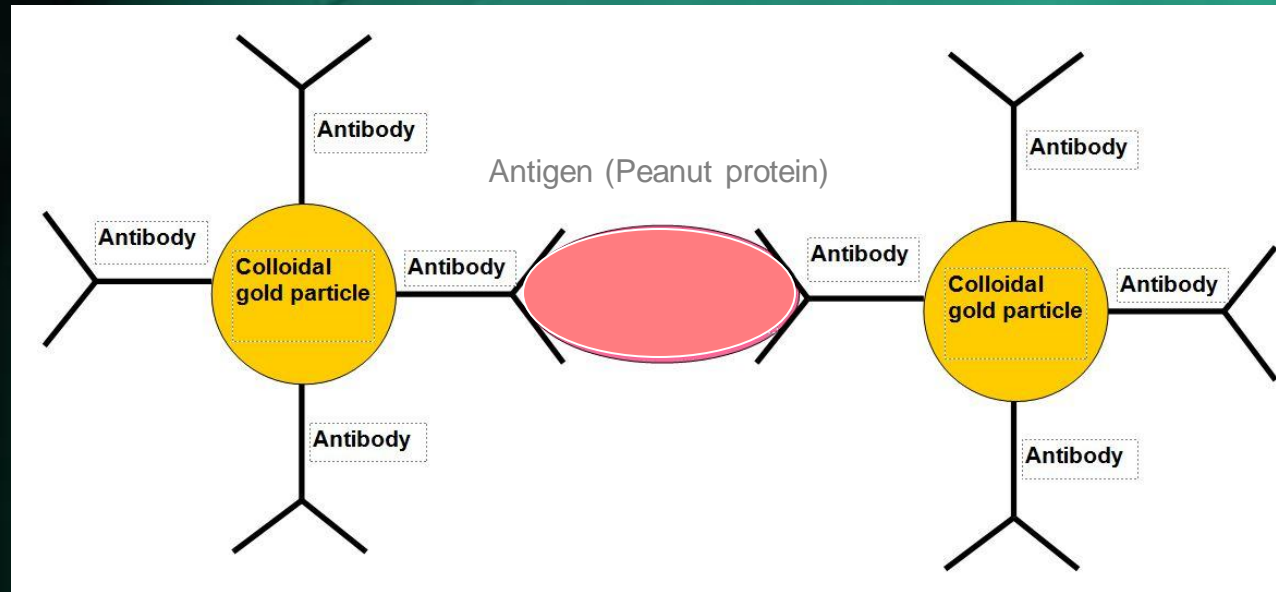
Surface swabs



- Ensure samples are as homogenous as possible
- Avoid cross contamination during sample preparation
- Ensure assay steps are followed as outlined in insert

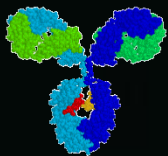
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- Flows across line of more antibody-coated colloidal gold
- Antibody captures target



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# Reveal 3-D™ Test Schematic



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# *Reveal 3-D™ Test - Positive Result*

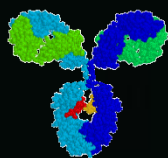


Sample



Result Window

T                      O                      C  
Test                      Overload                      Control



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# *Reveal 3-D™ Test - Negative Result*



Sample

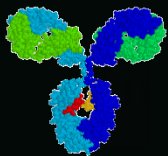


Result Window

T  
Test

O  
Overload

C  
Control

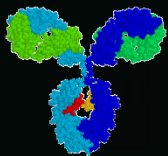
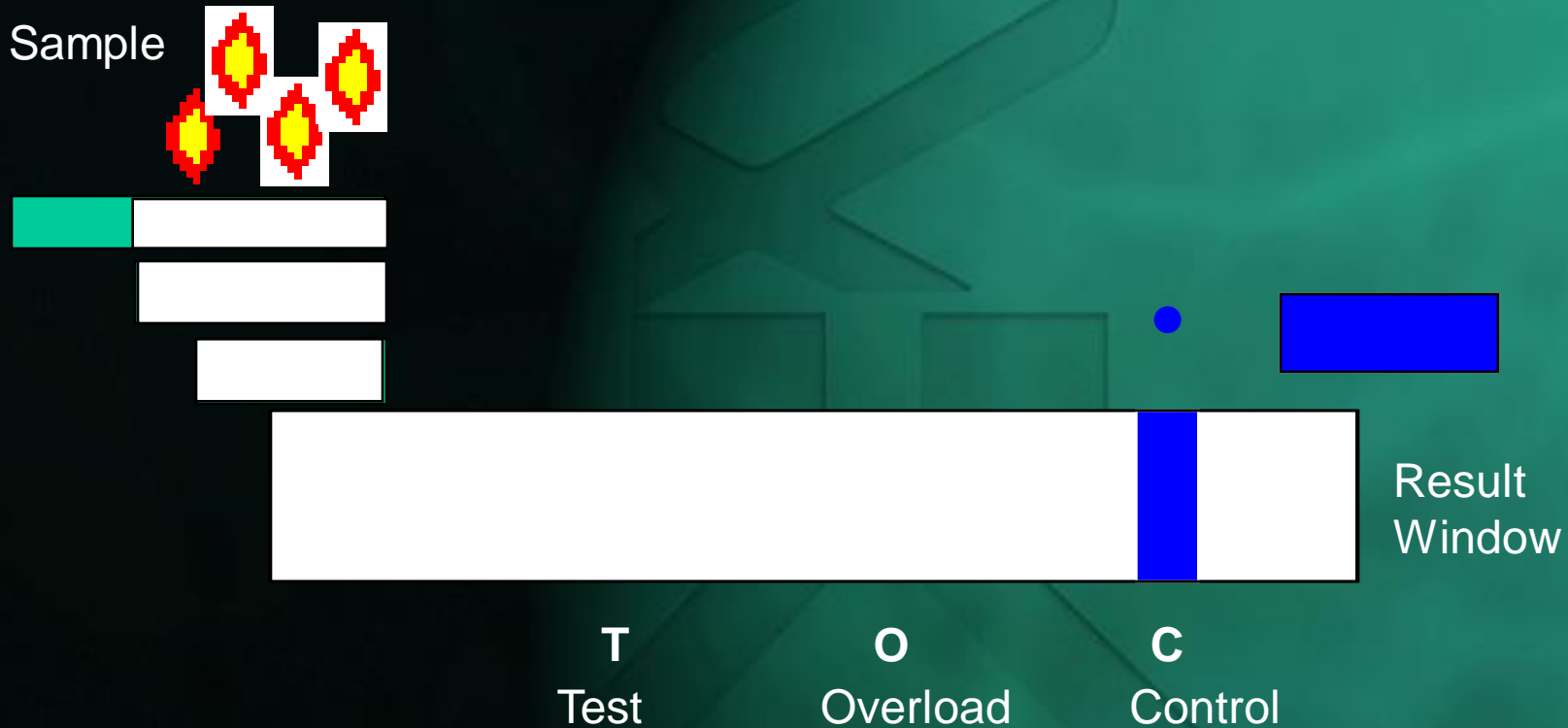


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# Reveal 3-D™ Test - Overload Result



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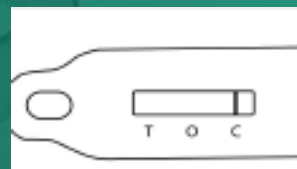
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# Reveal 3-D Test Results

for clear decision making

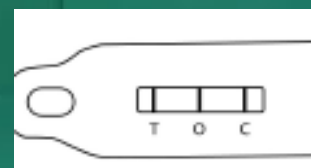


From Left to Right:  
Negative; Positive; High Positive



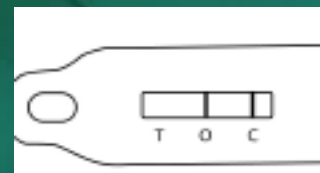
HIGH  
POSITIVE

No line at O, faint or line  
absent at T (Overload)



POSITIVE

Any intensity of line at T  
- Above detection limit



NEGATIVE

No line at T  
- Undetectable



# Sample Preparation

Sample (5 grams)+ 125 ml PBS (60 °C)



Shake in water bath for 15 min at 60 °C



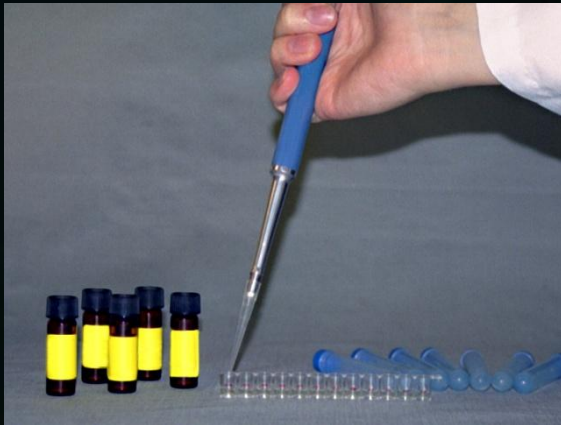
Filter/Settle/Centrifuge



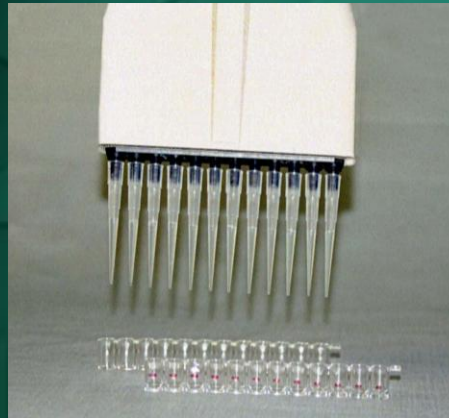
Run Test Kit  
(30 min)

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# Veratox Food Allergens



Extract samples prior to running assay. Add controls and samples to appropriate wells.



Transfer to antibody wells and incubate 10 minutes.



Wash wells with wash buffer solution.

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# Veratox Food Allergens



Add conjugate to all wells and incubate 10 minutes.



Wash wells with wash buffer solution.



Add substrate and incubate 10 minutes.

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# Veratox Food Allergens



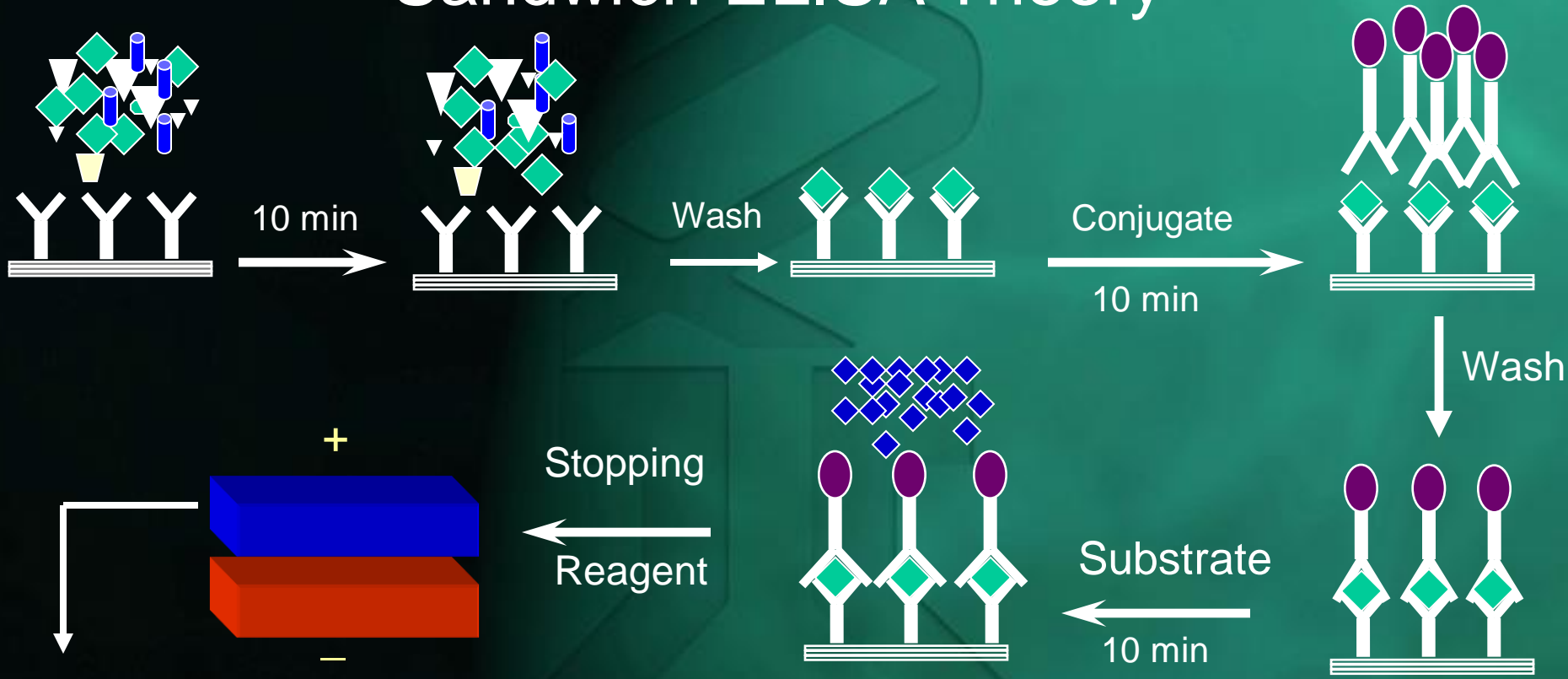
Add Red Stop.



Read results in a microwell reader.

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# Sandwich ELISA Theory



Read ( $OD_{650\text{ nm}}$ )



Capture Antibody

Allergens

Detector Antibody

Substrate

# Validation

- Once prototype is developed it must be validated
  - Cross Reactivity
  - Limit of Detection
  - Inter and Intra Assay Variability
  - Recovery
  - Stability
  - Food Trials (various processed conditions)
  - Alpha and Beta Testing

# The Role of Rapid Test Kits

- Become “Standard of Care” in food industry
- A valuable tool, but know what to do with results.
- Understand power of final results and have a corrective action plan in place.
- Most not checking final product.

# Limitations of Test Kits

*All antibody-based tests*

- Hydrolyzed proteins
  - Example: HVP, hydrolyzed egg protein
- Fermentation substrates
  - Examples: guar gums, xanthan gums, starter cultures, soy sauce
- Processing aids
  - Examples: lecithin, enzymes

*Proteins from these products are generally not detectable on the test kits. However, allergenicity can remain.*

# Know What You're Detecting

## Neogen Test Kit

Peanut

Egg

Milk

Almond

Hazelnut

Gliadin

Processed Soy

## Detection

Total peanut protein

Raw and cooked egg whites

Casein and whey proteins

Total almond protein

Total hazelnut protein

Prolamins (gliadin, secalin, hordein)

*Most* soy ingredients

# Industry Usage of Test Kits

## High Frequency

- Manufacturing issue diagnosis
- Sanitation validation

## Medium Frequency

- Allergenic ingredient determination
- Consumer complaint sample

## Low Frequency

- Routine finished product testing
- Supplier ingredient verification



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# Tools to simplify your HACCP

- Validation
- Verification
- Corrective actions
- Training
- Trouble-shooting



**HACCP**

Risk  
Analysis





Corporate Headquarters  
Food Safety Division, Acumedia  
LANSING, MICHIGAN

Animal Safety Division  
LEXINGTON, KENTUCKY

Hacco, Inc.  
RANDOLPH, WISCONSIN

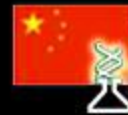


Neogen Europe  
AYR, SCOTLAND

Neogen Latin America  
Mexico City, Mexico



Neogen do Brazil  
Indaiatuba, Brazil



Manufacturing Facilities  
SHANGHAI, CHINA

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***Thank You !***

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