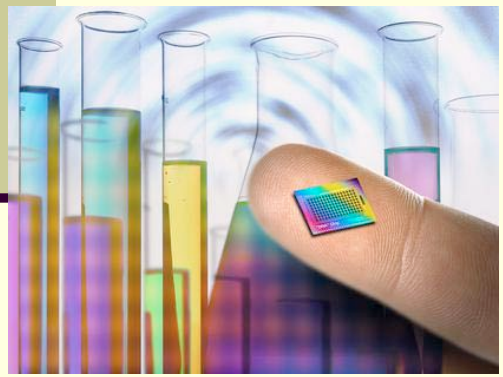
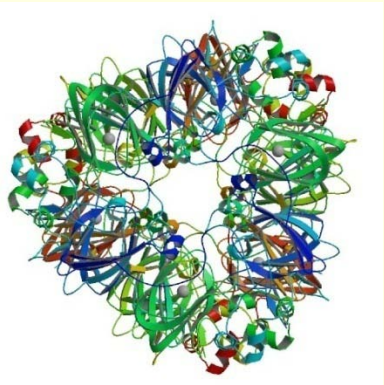
Detection of Food Allergens: Current Analytical Methods and Future Needs



Lauren S. Jackson, Ph.D.
Food and Drug Administration
National Center for Food Safety & Technology
6502 S. Archer Rd.
Summit-Argo, IL 60501



JIFSAN Advisory Council Spring Symposium
Wednesday, March 24, 2010

Presentation Outline

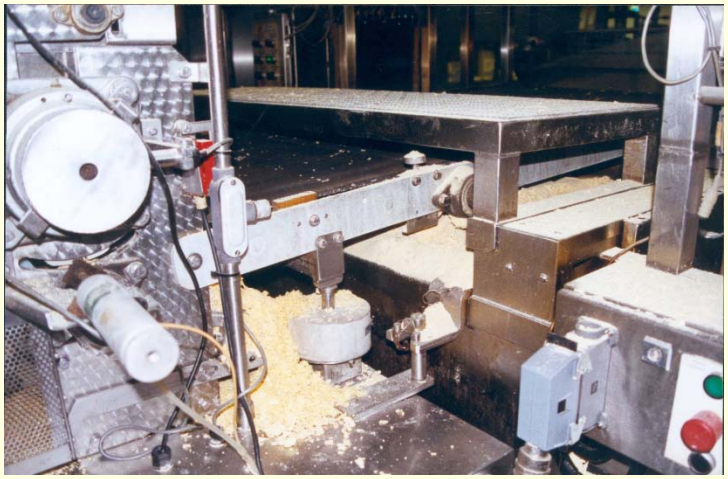
- Background
- Characteristics of food allergens
- Type of materials that are analyzed
- Methods for detection of allergens
  - Immunochemical methods
  - PCR
  - Mass spectrometry
  - Non-specific methods
- Methods in development
- Conclusions

Background

- Food allergies- immunological response to proteins
- Food allergies are a major health issue in industrialized countries
  - 10-12 million people in the U.S.
  - 30,000 emergency room visits due to food allergies
  - 150 deaths
- Prevalence is increasing
- Impact on society

Undeclared Allergens

- Strict avoidance is used by individuals with food allergies
- Food labels are used to indicate the intended presence or absence of allergens
- Undeclared allergens can be inadvertently introduced into a food
  - Ingredient/supplier changes
  - Labeling errors
  - Improper use of rework
  - Cross-contact

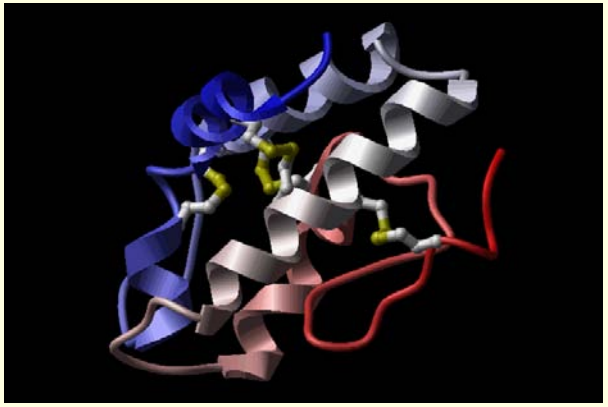


Why Test for Allergens?

- Consumer safety
- Ensure accuracy of food labeling
- Cleaning effectiveness
- Consumer complaints

Properties of Food Allergens

- Proteins
- Typically 10-80 kDa
- Most are water soluble
- No commonality in structure or amino acid sequence
- Multiple IgE binding sites
- Resistant to heat

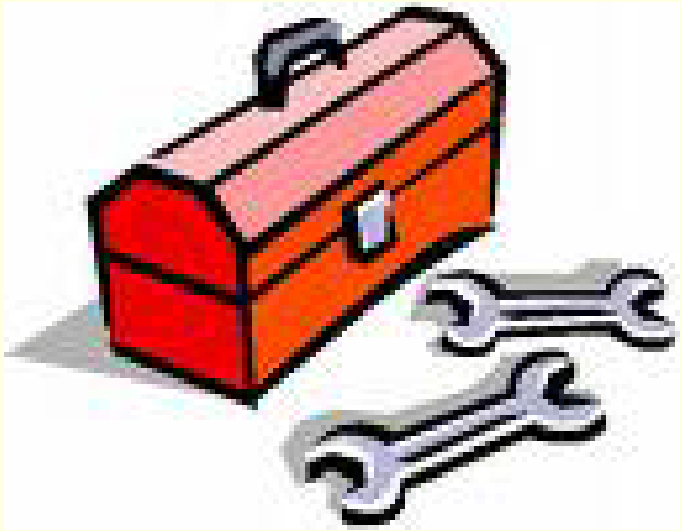


What Do We Test?

- Ingredients or raw materials
- End product testing
- Environmental samples
- Cleaning
  - Food-contact surfaces
  - Rinse-water
  - Push through materials (salt, sugar, first product off line)

Allergen Detection "Toolbox"

- **Immunochemical methods**
  - ELISA
  - Lateral flow devices (dipsticks)
  - Multiplex
- **DNA-based methods**
  - PCR
  - Multiplex
- **Mass spectrometry**
- **Generic/non-specific (cleaning)**
  - Protein
  - ATP
  - Visual inspection
- Other methods

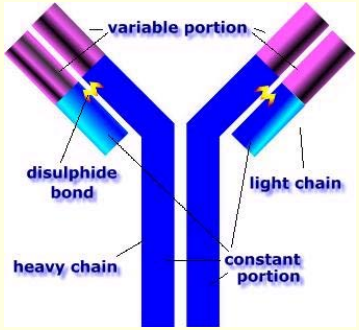


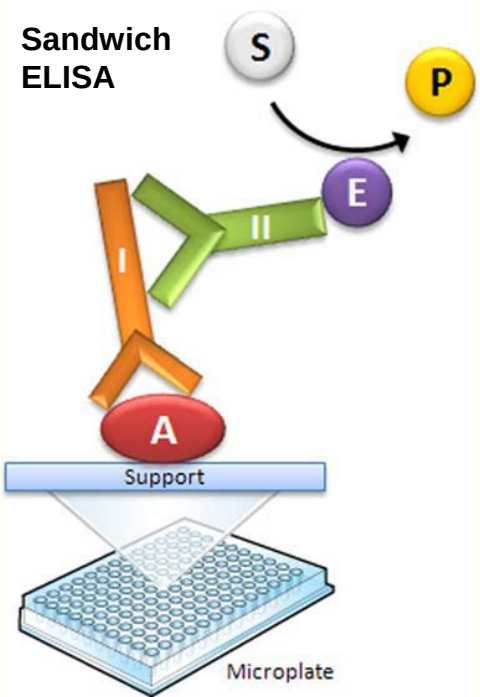
Choice of Allergen Detection Method

- Purpose
- Type of sample
- Food matrix
- Processing effects
- Turn-around time
- Availability of equipment/cost

Enzyme-Linked Immunoassay (ELISA)

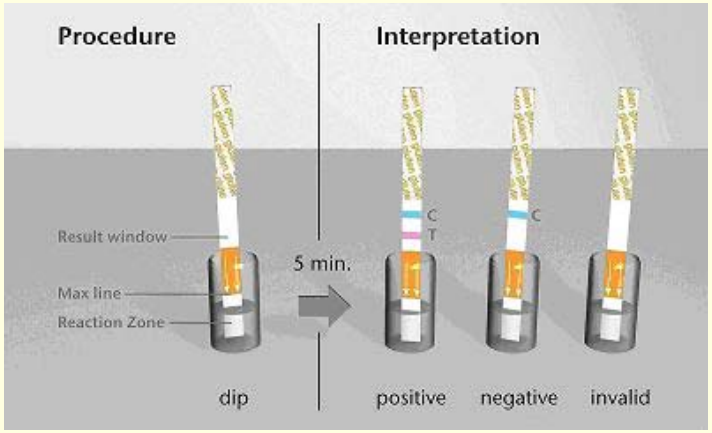
- Antibody-based detection of allergenic protein or other protein in food
- Available in well and lateral flow formats
- Sandwich format most common
- Analysis take between 1 – 2 h to complete
- Quantitative or qualitative
- Kits available for most of the 8 major allergens
- Used for ingredients, finished products, cleaning fluids, swabs, environmental samples





Lateral Flow Devices (LFD) and Dipstick Tests

- Qualitative (can be semi-quantitative with reader)
- Available for many allergenic foods
- Typically used for environmental sampling, cleaning verification, screening of foods
- True measurement of presence of allergenic food
- Rapid
- Sensitive (DL~ 5 ppm)

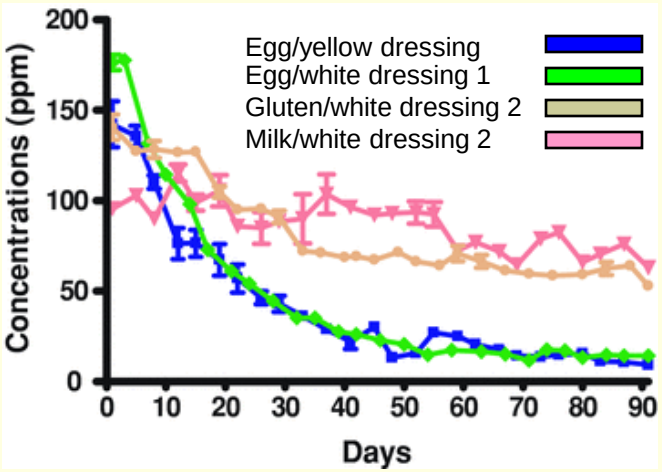


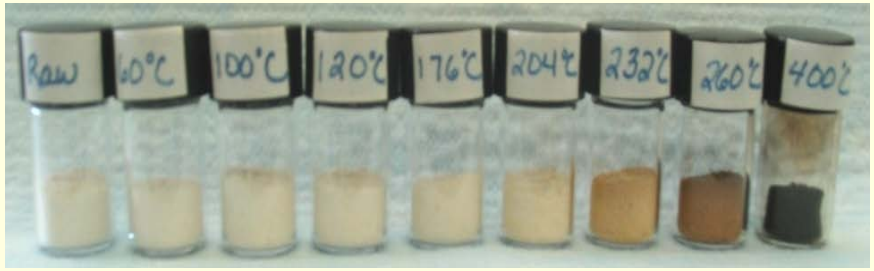
Advantages of ELISA

- Sensitive (ppm range)
- Quantitative or semi-quantitative
- Measure amount of offending food component (i.e. proteins)
- Antibody can detect allergenic proteins or marker protein in food
- Fairly rapid
- Equipment needs are minor (plate reader)
- Skill level = low to medium

Limitations of ELISA

- Some training required/adherence to instructions
- Sampling important
- Extraction and immunoreactivity important
- Food matrix important
  - Polyphenols
  - Oils
  - pH
  - Processing





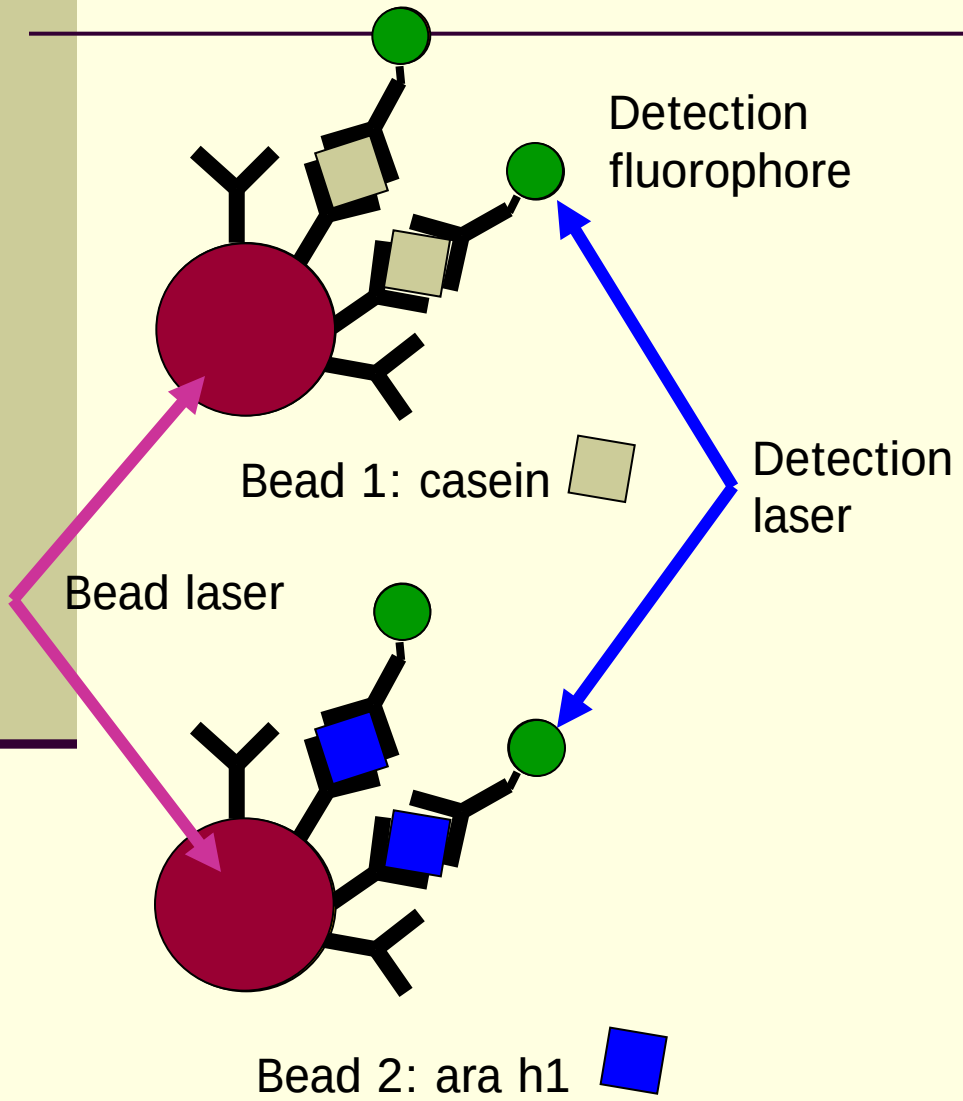
From: Taylor et al. 2009, JFS; 74(5):T46-50

Limitations of ELISA

- Cross-reactivity
- Need to understand what kit detects (e.g. some milk kits detect casein while other detect whey proteins)
- Values obtained from kits do not agree
- **Lack of reference materials**
- **Need to do “in house” validation of ELISA**

Immunochemical Methods of the Future:

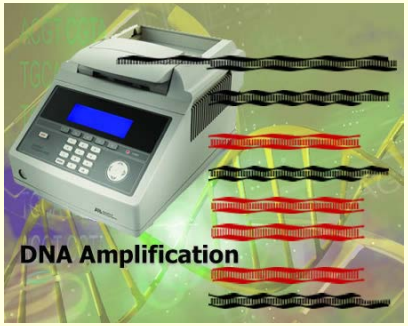
Multiplexed approaches for detecting protein allergens- (Bioplex) Eric Garber (FDA/CFSAN) and coworkers



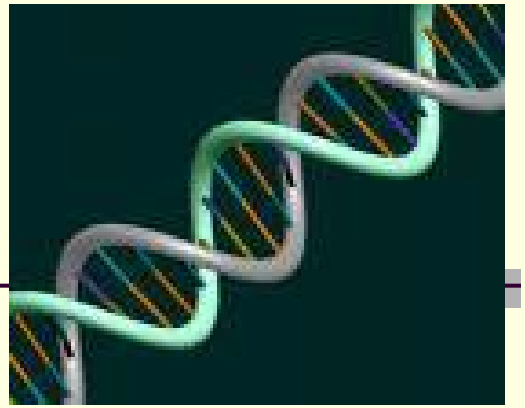
- Beads are labeled with antibodies for selected antigens
- Beads are mixed and exposed to antigens for binding
- Second antigen-specific labeled antibody is added for detection
- Beads flow through optical path of two lasers
- First laser identifies bead by color; correlated with specific antigen
- Second laser determines whether recognition event has occurred and how many

Polymerase Chain Reaction (PCR)

- Detects DNA sequences indicative of allergenic species
- Based on heat stable DNA polymerase amplifies DNA fragment
- Kits available for milk, peanut, soy, walnut, hazelnut, fish, crustaceans
- Useful in cases where ELISAs are not available or results questionable (e.g. hydrolyzed proteins)
- Good method for verifying ELISA or immunochemical assay results
- Equipment are becoming more common
- Very specific
- High throughput
- Multi-screening (multiplex) potential



PCR- Pitfalls

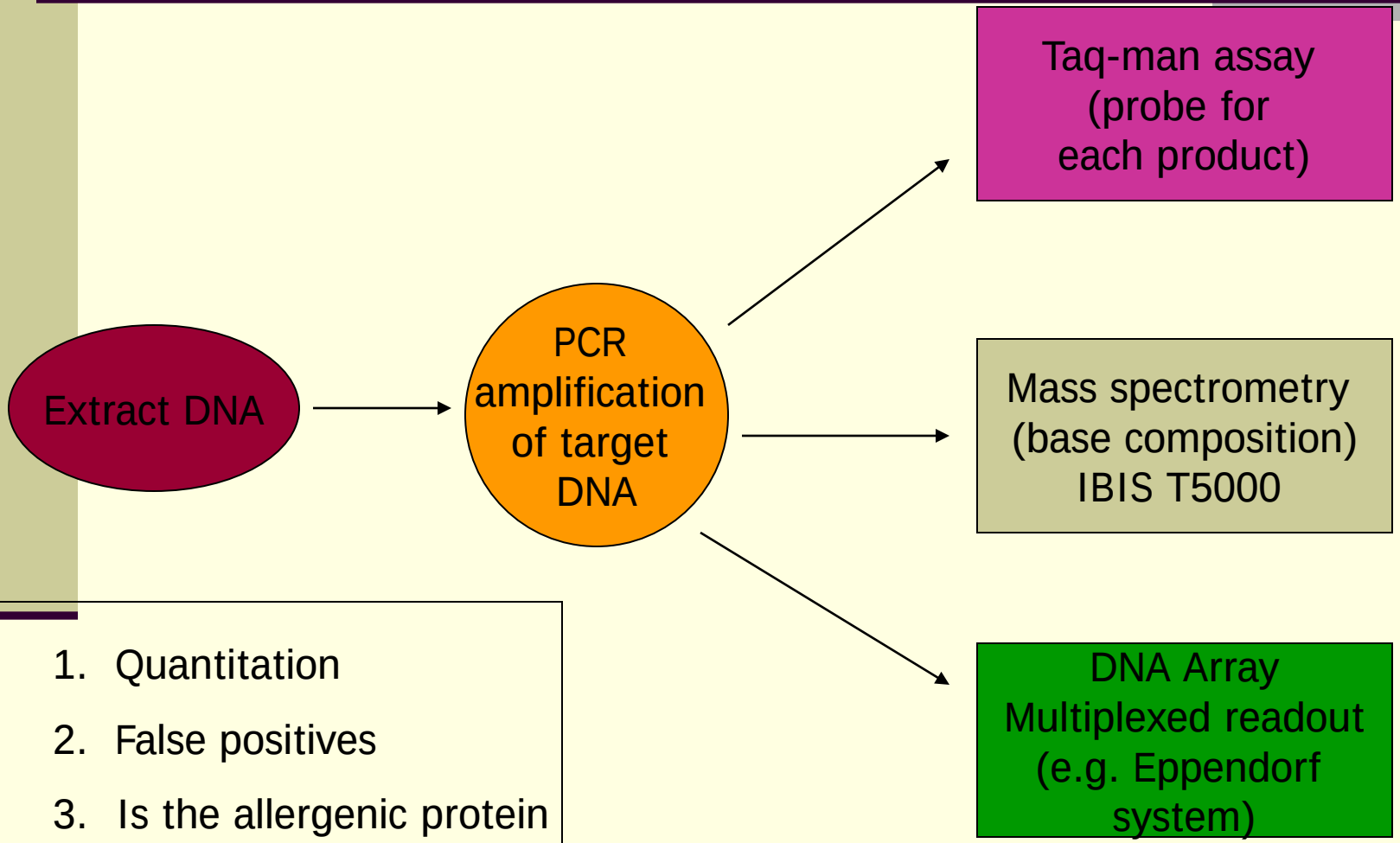


- Detect DNA not protein
- Qualitative
- Sample preparation and analysis require skill
- Cross-contamination possibilities
- Equipment expensive and not available in all labs
- Absence of DNA does not indicate absence of protein

The Future of PCR-Based Analysis:

Multiplexed approaches for detecting allergen DNA

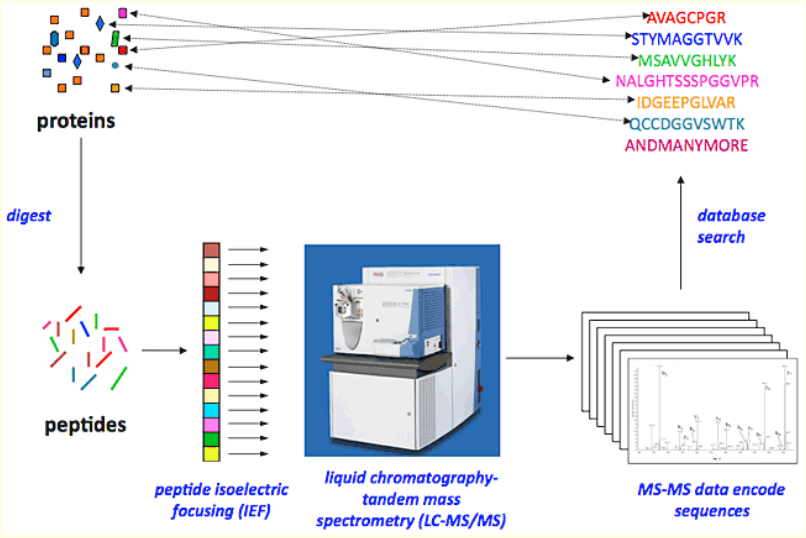
Eric Garber (FDA/CFSAN) and coworkers



1. Quantitation
2. False positives
3. Is the allergenic protein expressed?

Mass Spectrometry

- Detects proteins and peptides
- Involves extraction, cleanup, ionization, separation of ionized protein/peptide, detection
- High degree of sensitivity and resolving power
- Provides protein composition, structure and sequence information
- Protein detection and confirmation in single run
- Peptides detection and quantification easier



Ara h1 as a Marker for Peanut in Foods

Callahan et al. (FDA/CFSAN)

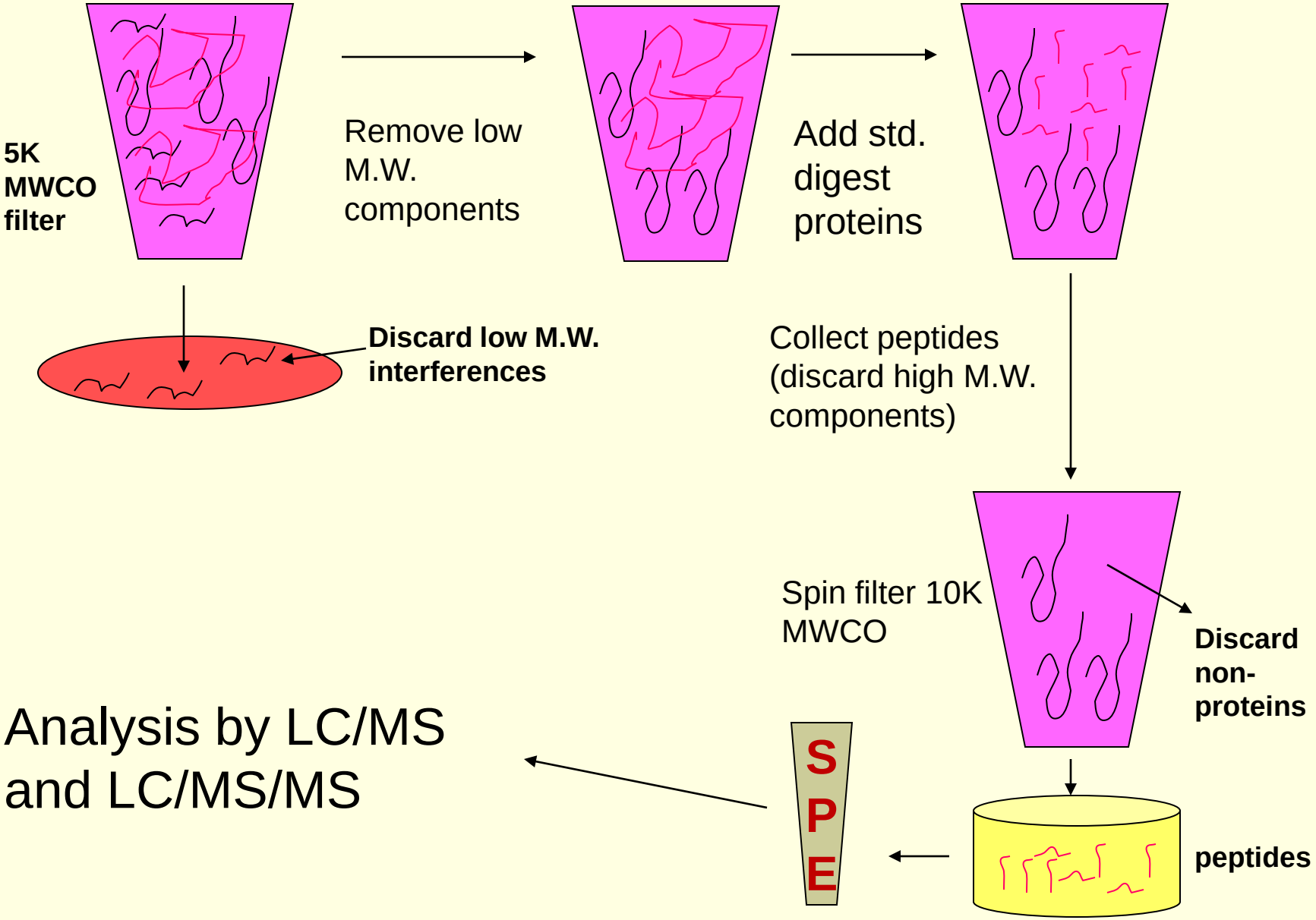
- 68 kDa vicilin seed storage protein

MRGRVSPLMLLLGILVLASVSATQAKSPYRKTENPCAQRCLQSCQQEP
DDLKQKACESRCTKLEYDPRCVYDTGATNQRHPPGERTRGRQPGDYD
DDRRQPRREEGGRWGPAEPRERERREEDWRQPREDWRRPSHQQPRK
IRPEGREGEQEWGTPGSEVREETSR**NNPFYFPSR**RFSTRYGNQNGRI
RVLQRFDQRSKQFQNLQNHRIVQIEARPNTLVLPKHADADNILVIQQGQ
ATVTVANGNNRK**SFNLDEGHALR**IPSGFISYILNRHDNQNLRVAKISMPV
NTPGQFEDFFPASSRDQSSYLQGFSR**NTLEAAFNAEFNEIR**RVLLEEN
AGGEQEERGQRRRSTRSSDNEGVIVKVSKEHVQELTKHAKSVSKKGS
EEEDITNPINLRDGEPDLSNNFGRLFEVKPDKKNPQLQDLDMMLTCVEI
KEGALMLPHFNSKAMVIVVVNKGTGNLELVAVRKEQQQRRREQEWEE
EEEDEEEEGSNREVRRYTARLKEGDVFIMPAAHPVAINASSELHLLGFGI
NAENNHR**IFLAGDKDNVIDQIEK**QAK**DLAFPGSGEQVEK**LIKNQRESHF
VSARPQSQSPSSPEKEDQEEENQGGKGPLLSILKAFN

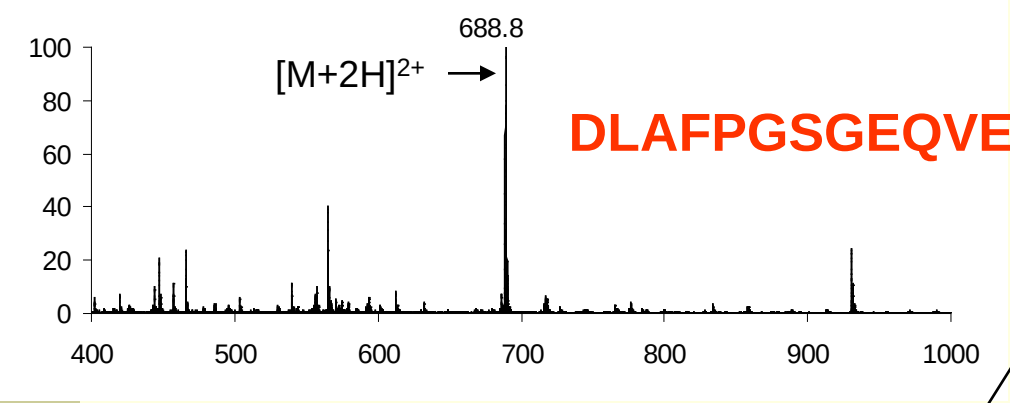
606.7 $[M+3H]^{3+}$

686.8 $[M+2H]^{2+}$

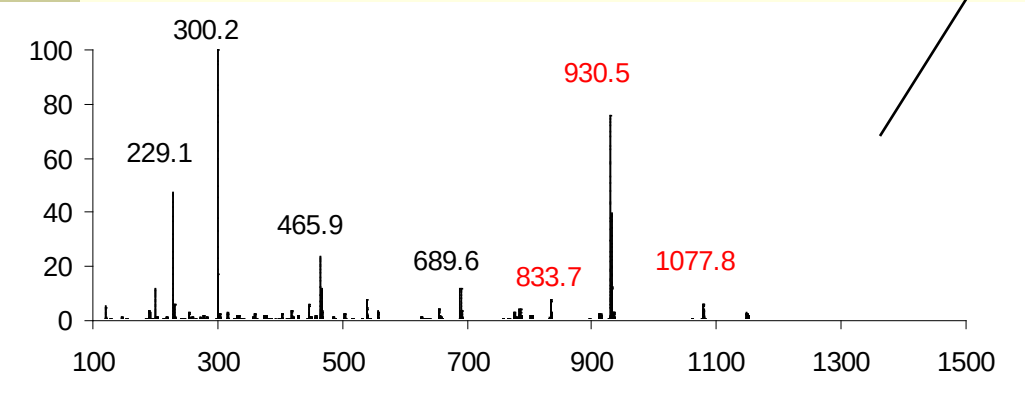
General Sample Preparation



Low-ppm confirmation of Ara h1 in chocolate

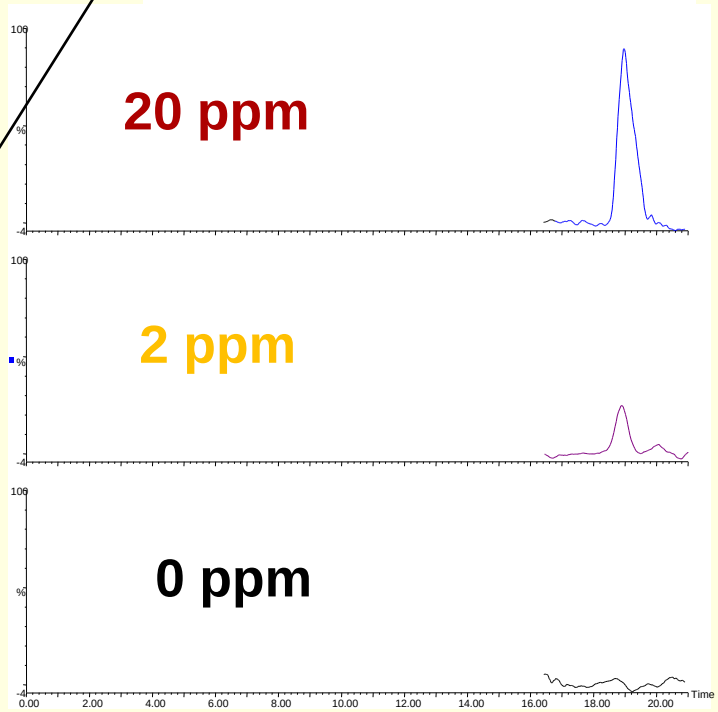


DLAFPGSGEQVEK



Multiple Reaction Monitoring (MRM)

Sum of 229.1, 300.2, 930.5



Mass Spectrometry

Advantages

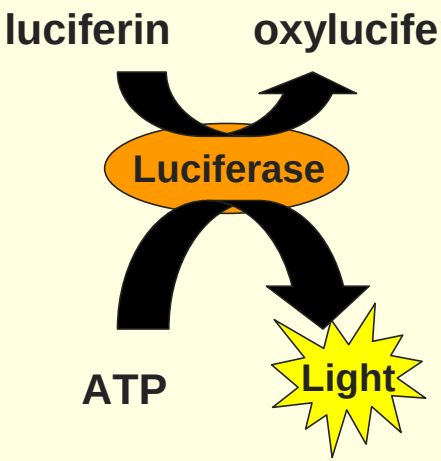
- Absolute identification and quantification of allergens
- Highly sensitive
- Excellent confirmatory method

Limitations

- Requires high level of expertise
- High cost of equipment
- Time consuming
- Laborious
- Extraction and cleanup needed
- Not useful for routine analyses

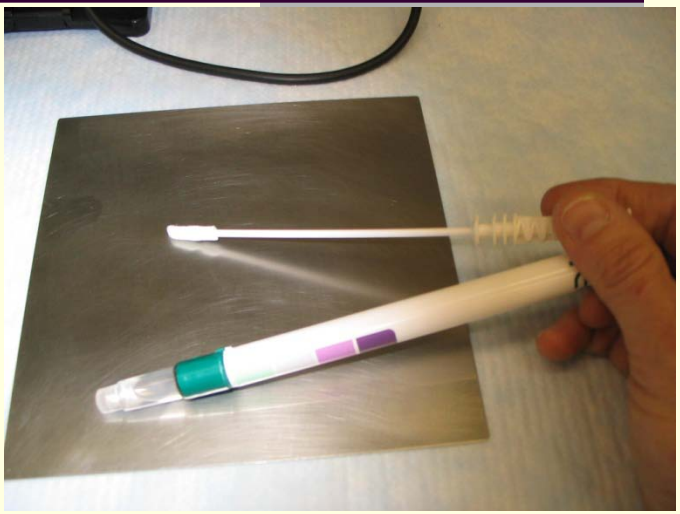
Non-Specific Methods: ATP

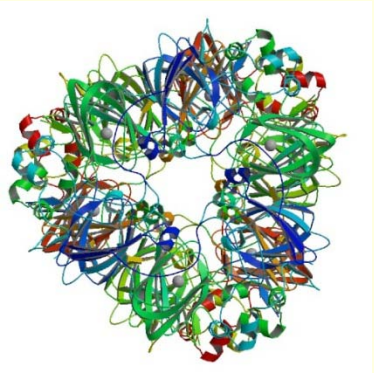
- **Sanitation effectiveness**
- **Detects ATP from biological sources**
- **Conventional ATP swabs - hygiene**
- **Sensitive ATP swabs – detect presence of food soils**
- **Advantages**
  - Rapid (< 30 sec)
  - Less expensive than ELISA
  - Test can be performed on site (‘real time’)
- **Disadvantages**
  - Limited applicability (wet-cleaned surfaces)
  - May pick up ATP from water supply
  - Measures presence of ATP, not allergenic food
  - May be difficult to detect some food soils
  - Need to determine background ATP levels at facility



Non-Specific Methods: Total Protein

- **Cleaning effectiveness**
- **Different companies and formats available**
- **Advantages**
  - Rapid (< 5 min)
  - Less expensive than ELISA
  - Measures protein
- **Disadvantages**
  - Measures all proteins, not only from allergenic food
  - ???





Detection of Soy Products in Solution

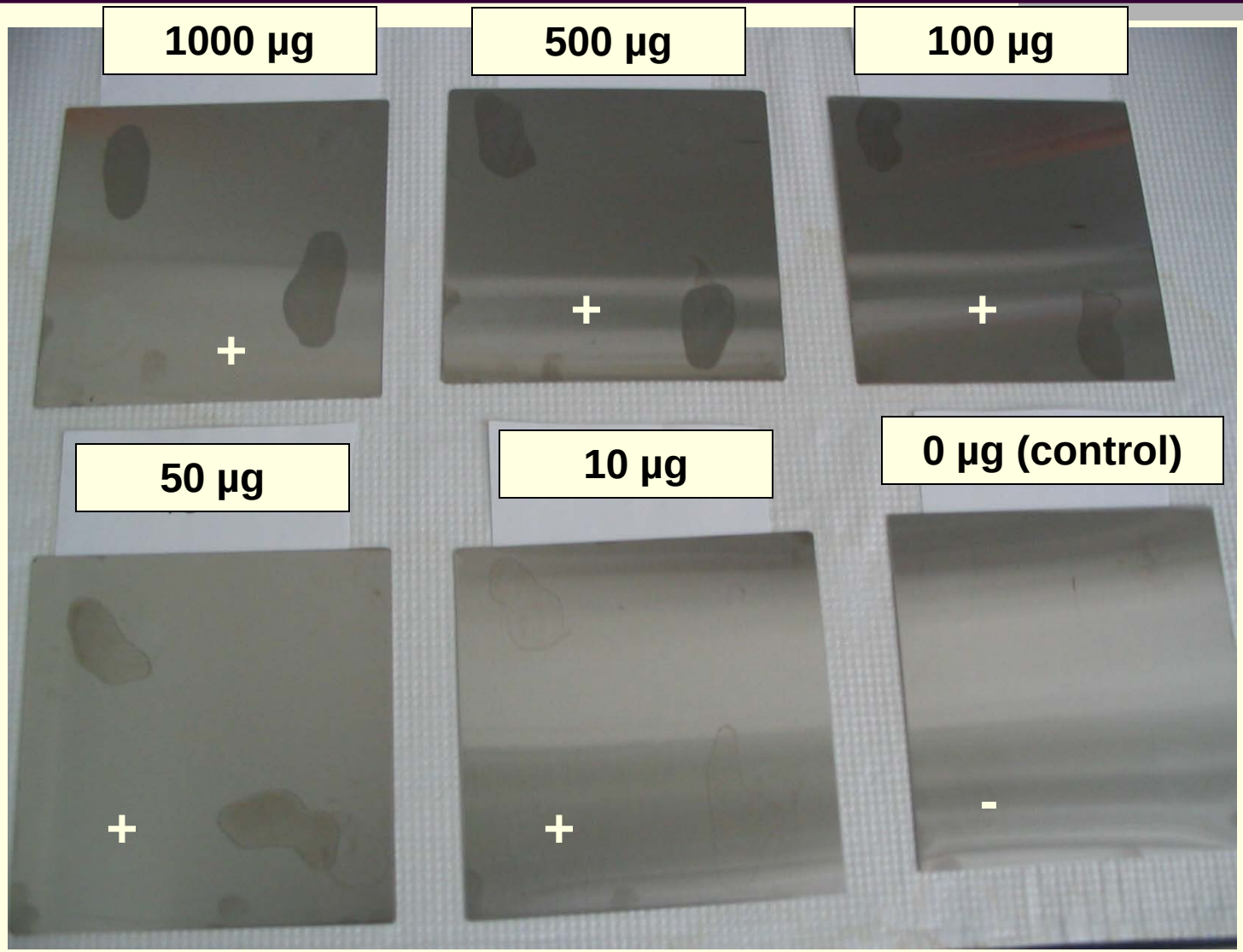
Soy Product	Method of Detection	Amount of soy product in solution (µg/mL)					
		0	100	250	500	1000	2500
Soy flour	ELISA 1	-	+	+	+	+	+
	ELISA 2	-	+	+	+	+	+
	Conventional ATP	-	+	+	+	+	+
	Sensitive ATP	-	+	+	+	+	+
	Total protein	-	+	+	+	+	+
Soy milk	ELISA 1	-	-	-	-	-	-
	ELISA 2	-	-	-	-	+	+
	Sensitive ATP	-	-	-	-	-	+
	Conventional ATP	-	-	-	-	-	-
	Total protein	-	+	+	+	+	+
Soy-based Infant formula	ELISA 1	-	-	-	-	-	-
	ELISA 2	-	-	-	-	-	-
	Sensitive ATP	-	-	-	+	+	+
	Conventional ATP	-	-	-	-	-	-
	Total protein	-	+	+	+	+	+

Visual Inspection

- Most common method for validating/verifying cleaning procedures
- First step in determining if equipment is clean
- Points for inspection
  - Flat surfaces
  - Difficult to clean areas
  - Areas above processing zone
- **Advantages**
  - Does not require lab equipment/inexpensive
  - Rapid
- **Disadvantages**
  - Depends on accessibility, lighting, surface, etc.
  - Limited to accessible equipment
  - Does visually clean = allergen clean?

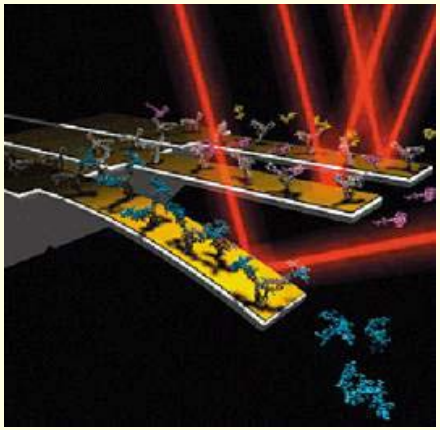
Examples of “Visibly Dirty” Surfaces

Visual Inspection- Milk on Stainless Steel Plates



Methods in Development

- **Multiplex DNA and immunochemical methods**
- **LC-MS and LC-MS/MS**
- **Spectroscopic methods**
  - Mid-IR fiber optics
  - Real time
  - Can be used to detect different organic analytes
- **Biosensors**
  - Receptor and transducer that results in optical signal
  - Surface Plasmon Resonance (SPR)
  - Real-time, fast, automated



Conclusions

- Many tools are available for detection of allergens or allergenic foods
- Immunochemical methods the most common
- Choice of method depend on specific use, type of food matrix, and other factors
- Need to conduct “in-house” validation
- More than one method may be needed
- More work is needed to understand the chemical properties of food allergens- better extraction and detection
- There is a need for reference standards so that methods can be evaluated and compared