Food allergen analysis, challenges and new trends

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27 DECEMBER, 2017
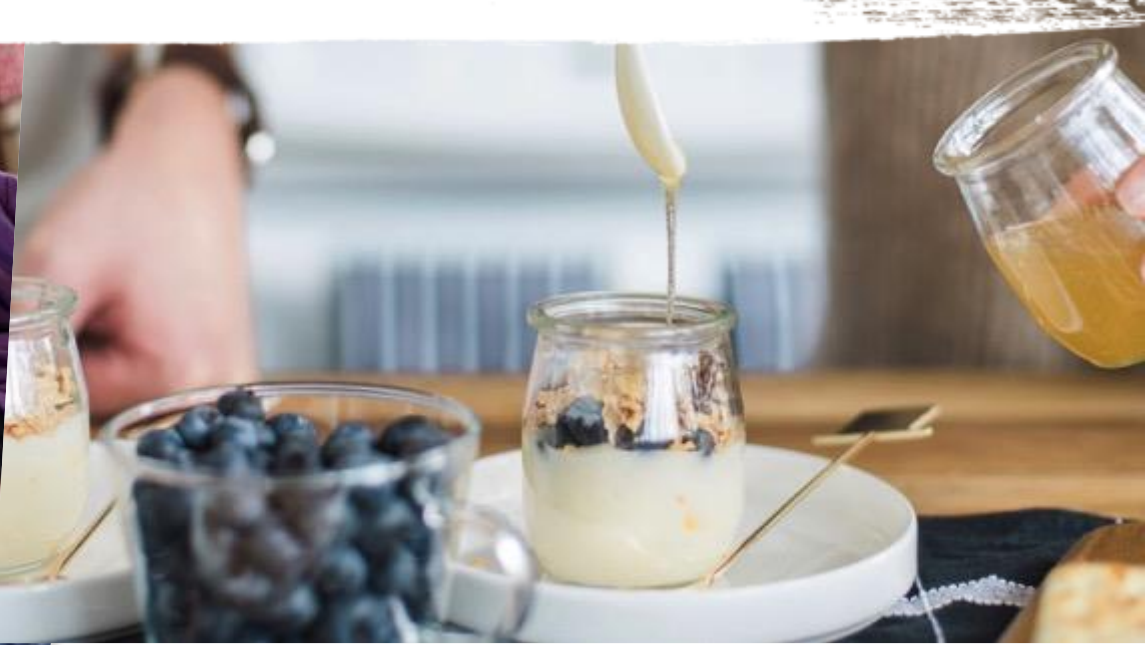
FOOD SAFETY

ANTICIPATE
PREVENT
& MANAGE

DANONE
ONE PLANET. ONE HEALTH

CONTENT 内容

A UNIQUE MISSION AND DUAL ECONOMIC AND SOCIAL PROJECT
一个使命，双重承诺

our mission

BRINGING HEALTH THROUGH FOOD TO AS MANY PEOPLE AS POSSIBLE

Our dual economic and social project
达能双重承诺：商业成功和社会进步

DANONE ONE PLANET. ONE HEALTH

OUR FOUR BUSINESS ACTIVITIES
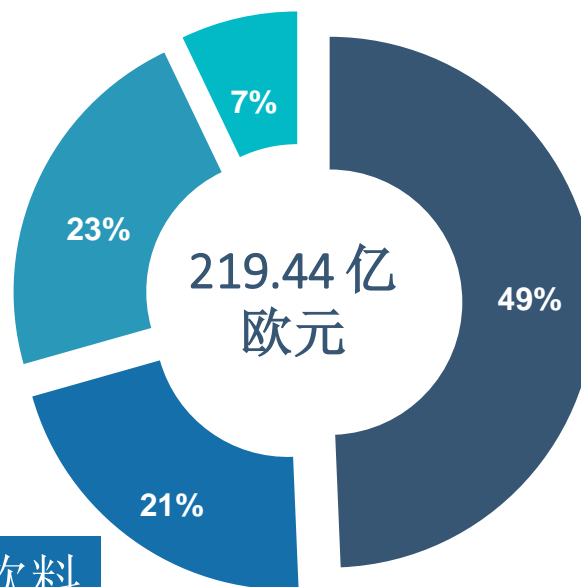
达能四大核心业务

医学营养品

16.18亿欧元

2016年同比增长7.4 %

生命早期营养品

50.17亿欧元

2016年同比增长3.5 %

219.44 亿
欧元

7%

23%

49%

21%

鲜乳制品 *

107.36亿欧元

2016年同比增长2.0 %

*为了反映达能最新的发展情况，包括整合
WhiteWave，自2017年二季度起鲜乳制品业务
被调整为基础乳制品和植物基产品业务

饮用水和饮料

45.74亿欧元

2016年同比增长2.9 %

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FOOD SAFETY & QUALITY:
A COMMON MISSION, CLEAR ACCOUNTABILITIES
食品安全和质量：共同的使命，清晰的职责

BRINGING HEALTH THROUGH FOOD TO AS MANY PEOPLE AS POSSIBLE

The **complementarity** of two fields ensuring **Danone's uniqueness** and key to achieve Danone's mission

FOOD SAFETY

a **scientific** approach

QUALITY

consumer driven

"Food Safety First"

Outstanding products and experience

No compromise on Food Safety

Ingredients, processes, traceability are under control to design, produce and deliver safe foods

Convenient offer, great tasting products with sustainable quality, responsible communication, sustainable and responsible production schemes

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ALLERGEN AND ALLERGY

致敏原和过敏

Allergen

Antigen that produces an abnormally vigorous immune response.
致敏原：能够诱发机体发生过敏反应的抗原物质（GB/T 23779-2009）

Allergy is an abnormal immune response to food.
过敏：指少部分特应性体质的个体在与过敏原（如食物、花粉和药物等）接触后诱发的异常免疫应答，引起相应的临床症状（如皮疹、呼吸道症状、胃肠道症状、过敏性休克等）

ALLERGENIC FOODS

食品过敏

The Big-8: Milk, Eggs, Fish, Crustacean Shellfish, Tree Nuts, Peanuts, Wheat, Soya

Other Priority Allergens: Buckwheat, Celery, Lupin, Molluscan Shellfish, Mustard, Sesame

Major allergenic foods
Account for about 90% of all food allergies

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FOOD ALLERGEN MANAGEMENT
食品致敏原管理

➢ World widely exist

➢ No cure found yet

➢ Attention paid since 1990s
   自上世纪90年代以来逐渐受到重视

➢ CODEX list of 8 types of food allergens in 1999
   1999年CODEX修订标签标准，列出8种主要致敏原

➢ Food allergen management led by governments
   各个国家和地区开始政府主导的致敏原管理

Avoidance is the only choice
只能依靠避免摄入含有致敏原的食物来防止

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FOOD ALLERGENS - INTERNATIONAL REGULATORY CHART
食品过敏原-国际法规

致敏原（包括某种或某类食物及其制品）	Codex	美国	加拿大	EU	英国	瑞士	俄罗斯，哈萨克斯坦，白俄罗斯	土耳其	澳新	中国（包括香港）	日本	韩国	马来西亚	阿根廷	智利	墨西哥	巴西	南非
含有麸质的谷物（如小麦、黑麦、大麦、燕麦、斯佩耳特小麦或它们的杂交品系）	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
甲壳纲类动物	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
软体动物			√	√	√	√	√	√	√	√	√	√	√					√
蛋类	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
鱼类	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
花生	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
大豆	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
乳	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
坚果	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
芝麻籽			√	√	√	√	√	√	√	√								√
芥末			√	√	√	√	√	√						√				
芹菜				√	√	√	√	√										
羽扇豆				√	√	√	√	√										
荞麦											√	√						
牛肉											√	√						
鸡肉											√	√						
猪肉											√	√						
蘑菇											√	√						
苹果											√							
香蕉											√							
猕猴桃																		
柑橘类											√							
桃子											√	√						
西红柿											√	√						
薯蓣属植物											√							
天然乳胶																	√	
阿斯巴甜								√										
亚硫酸盐 >10mg/kg	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√		√

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FOOD ALLERGEN MANAGEMENT

食品过敏原管理

Consumers who are allergic to things (population)
仅保护存在食物过敏的消费者（人群）

Known food allergen (hazards)
仅针对已知致敏原（危害）

Two ways (contamination pathways, possibility)
致敏原通过两种途径进入食物（污染途径、可能性）

As ingredient 作为配料添加入食品中
Unintended presence 作为非有意添加的物质存在于食品中

管理措施：标示致敏原 labelling

Cross contamination
交叉污染等

- Farm 农田
- Transportation 运输过程
- Storage 储存过程 storage
- Manufacture, process, packaging 生产、加工、包装过程

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Food allergen labeling requirements in China
我国标签标准的致敏原标示要求 GB7718-2011

4.4.3 致敏物质 food allergen

4.4.3.1 以下食品及其制品可能导致过敏反应，如果用作配料，宜在配料表中使用易辨识的名称，或在配料表邻近位置加以提示：ingredients list or contain statement, **voluntary**

a）含有麸质的谷物及其制品（如小麦、黑麦、大麦、燕麦、斯佩耳特小麦或它们的杂交品系）；Cereal containing gluten

b）甲壳纲类动物及其制品（如虾、龙虾、蟹等）；crustacean

c）鱼类及其制品；fish

d）蛋类及其制品；eggs

e）花生及其制品；peanuts

f）大豆及其制品；soybeans

g）乳及乳制品（包括乳糖）；milk

h）坚果及其果仁类制品。nuts

4.4.3.2 如加工过程中可能带入上述食品或其制品，宜在配料表临近位置加以提示。May contain statement, **voluntary**

Regulation on implementation of food safety law (draft)
食品安全法实施条例（征求意见）

第八十一条 食品配料含有可能导致过敏反应的物质的，食品生产者应当在配料表中予以标注。 Food ingredients which may cause allergy should be labeled

FOOD ALLERGEN MANAGEMENT

食品过敏原管理

Regulatory Threshold

Methods-based, safety assessment based,
risk assessment-based, statutorily derived

By FDA threshold working group

FOOD ALLERGEN MANAGEMENT 食品致敏原管理

TO ENSURE THAT DANONE <u>CREATES</u>, <u>MANUFACTURES</u> AND <u>DELIVERS</u> ITS PRODUCTS <u>EVERYDAY</u> WITH NO COMPROMISE ON FOOD SAFETY BY STRICTLY FOLLOWING THE DANONE COMPLIANCE POLICY AND ANTICIPATING EMERGING RISKS
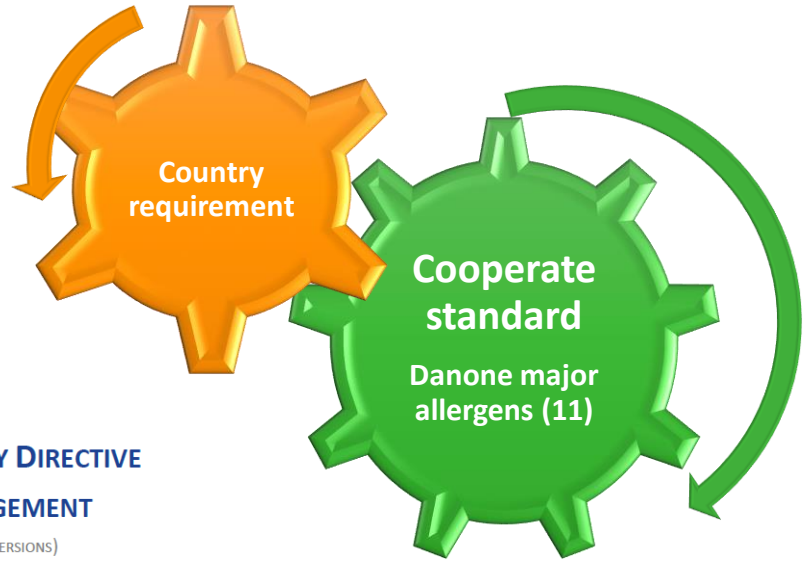
Anticipate
Prevent
& Manage

Food Safety@DANONE

DANONE FOOD SAFETY DIRECTIVE

ALLERGEN MANAGEMENT

(SUPERSEDES ALL PREVIOUS VERSIONS)

Country requirement

Cooperate standard

Danone major allergens (11)

ANALYTICAL TECHNIC – CONTROL CHECK FOR FOOD ALLERGEN 控制检测

- Incoming check of raw ingredient
- Labelling Final product
- Process control Cleaning validation
- Working station

"Free from…"

Free from ingredient
No cross contamination
Can not be detected

- Intentional addition of allergens
- Cross contamination

FOOD ALLERGEN ANALYSIS食品过敏原检测技术

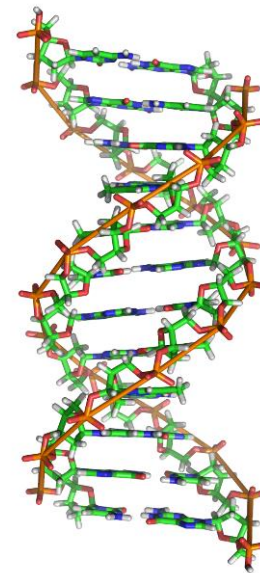DETECTION AND QUANTIFICATION OF ALLERGEN TRACES IN FOOD PRODUCTS

What are we testing for ?

True food allergies are an immunological response to specific food proteins, target proteins or the genetic material that produce the proteins (DNA)

C-terminus

Alpha-lactalbumin

α-乳白蛋白

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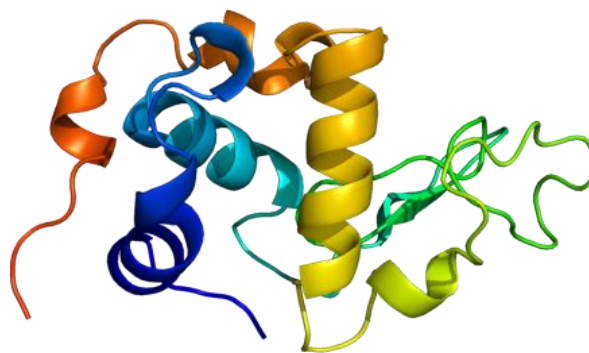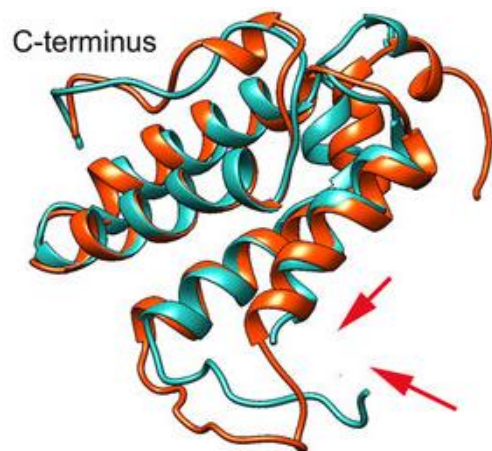
FOOD ALLERGEN TESTING TECHNIQUES 食品过敏原检测技术
PROTEIN-BASED METHODS

Enzyme-Linked Immunosorbnent Assay (ELISA)
酶联接免疫吸附剂测定

Receptor-based methods
Widely used for allergen detection, based on the interaction between specific **antibodies** and an **antigen** (food allergen).

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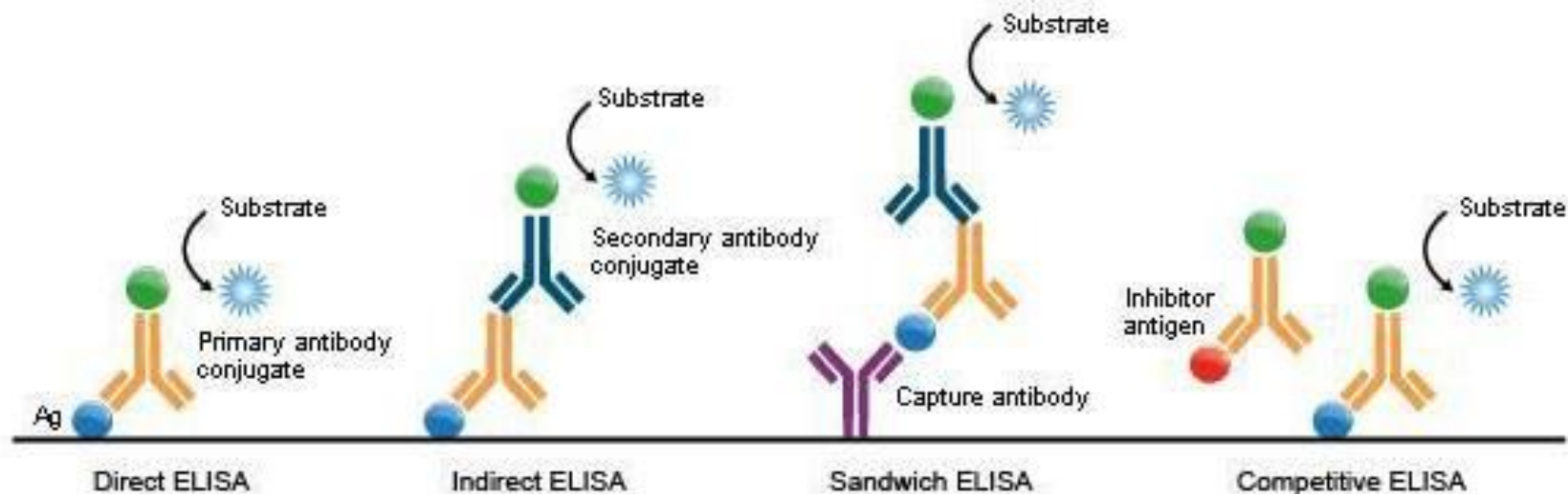
ELISA
PROS AND CONS

PROS
- ✓ Sensitive (ppm range)
- ✓ Quantitative or semi-quantitative
- ✓ Measure amount of allergen target (i.e. proteins)
- ✓ Antibody can detect allergenic proteins or marker protein in a wide number of different matrices
- ✓ Fairly rapid
- ✓ Equipment needs are minor (plate reader)
- ✓ Skill level = low to medium

CONS
- ✓ 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

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VALIDATION OF ELISA METHOD
ELISA 方法验证

Appendix M: Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices

Although there are a number of documents published on method validation (1, 2) which target analytical methods in general, and there are numerous publications on validation of ELISA methods for pesticides, these documents do not address specific areas of concern for food allergen analysis, such as reference materials, spiking methods, or choice of matrixes. In the absence of a universally recognized reference standard for food allergen the study design and data would be subject to scrutiny before acceptance by the AOAC or other authority.
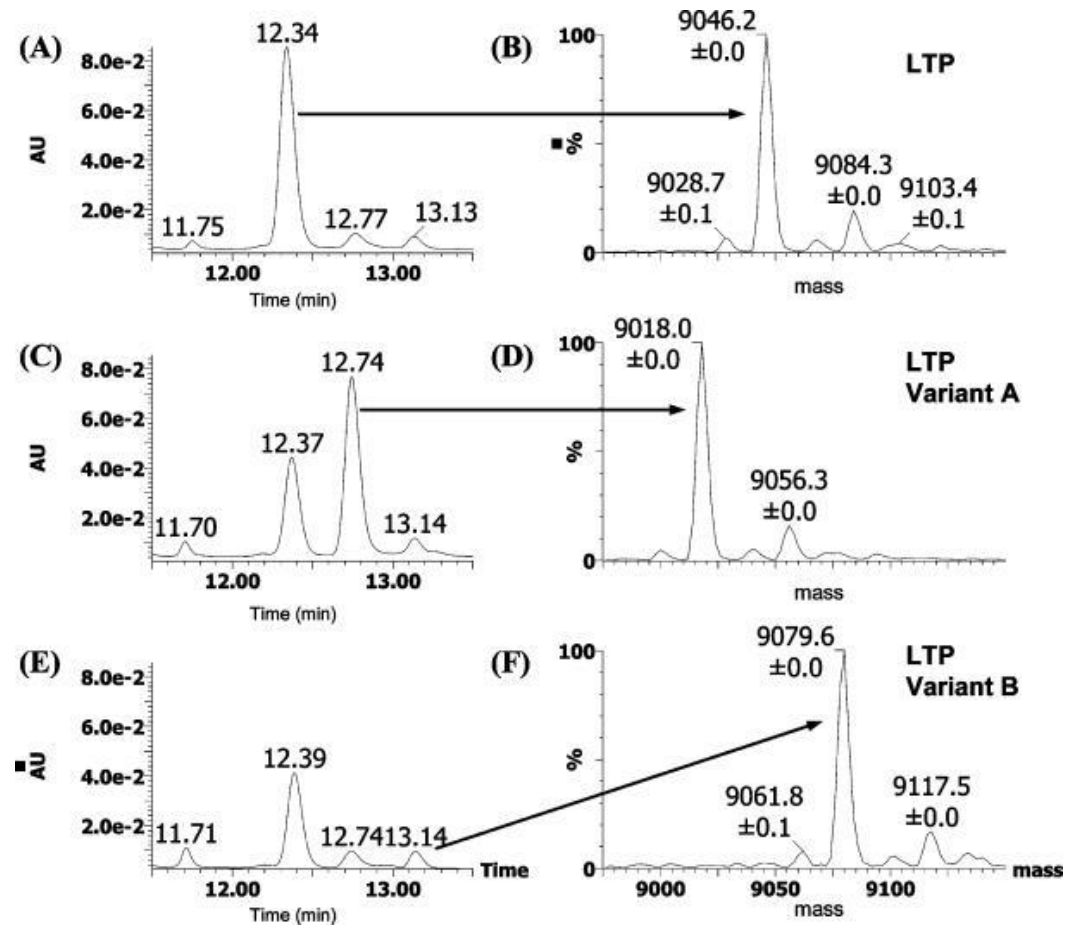
Methods for detecting various food allergens have been available for a number of years. Many of these methods use ELISA-based techniques to detect specific protein markers in food matrixes. The detection of food allergens by ELISA is a unique analytical procedure characterized by the recognition and binding

Table 1. Method performance requirements

Parameter	Target allergen			
	Whole egg	Milk	Peanut	Hazelnut
Analytical range, ppm	10–1000	10–1000	10–1000	10–1000
MQL[a], ppm[b]	≤5	≤10	≤10	≤10
MDL[a], ppm[b]	≤1.65	≤3	≤3	≤3
Recovery, %	60–120	60–120	60–120	60–120
RSD$_r$, %	≤20	≤20	≤20	≤20
RSD$_R$, %	≤30	≤30	≤30	≤30

[a] Definitions for MQL and MDL provided in section 4.
[b] Reported as ppm of the target allergen in food commodity, i.e., 25 ppm of "whole egg" in cookies.

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FOOD ALLERGEN ANALYSIS
PROTEIN-BASED METHODS

- Chromatographic Techniques

- Liquid chromatography-Mass Spectrometry

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FOOD ALLERGEN ANALYSIS 食品过敏原分析
PROTEIN BASED

Liquid chromatography-
Mass Spectrometry

Martina Koeberl, Dean Clarke, and
Andreas L. Lopata. J. Proteome
Res. 2014.

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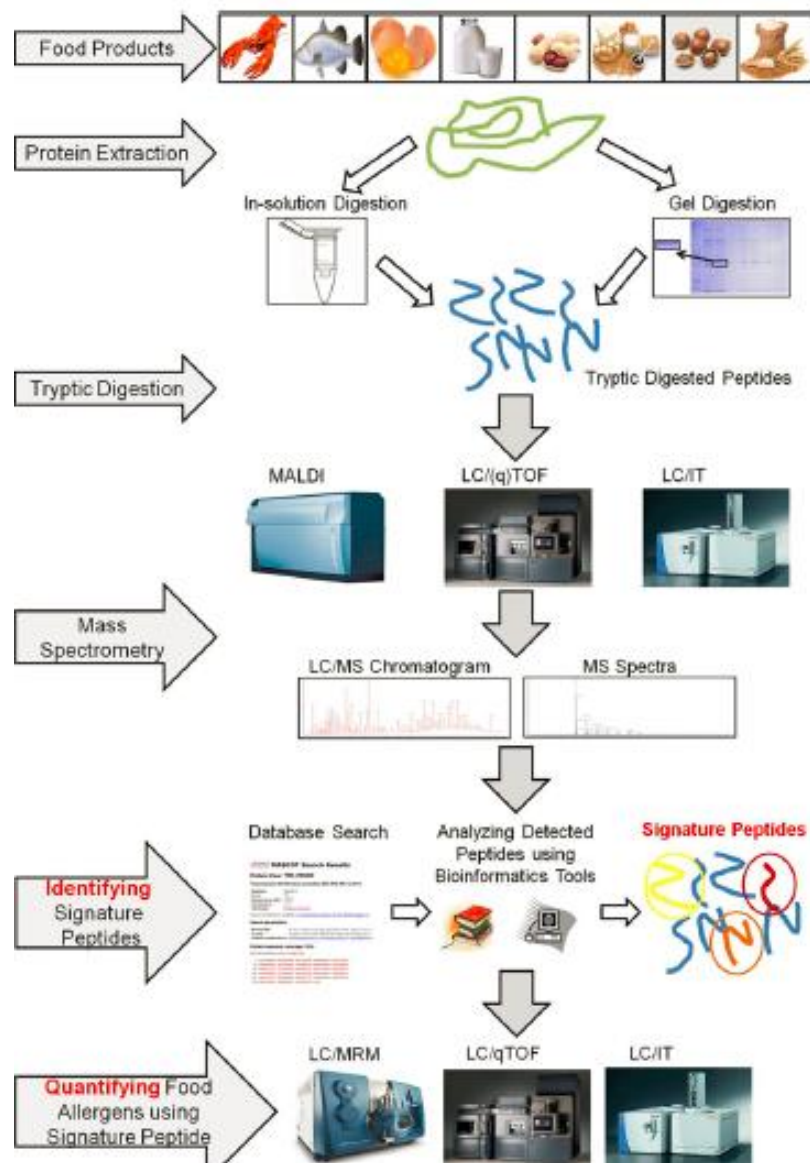
LC/MS/MS
PROS & CONS

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

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

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COMPARISON BETWEEN IMMUNOLOGICAL AND CHEMICAL METHOD FOR ALLERGEN ANALYSIS

免疫学与化学方法比较

Comparison between immunological and chemical methods for allergen analysis

Food matrix containing allergen

|	Antibody based			MS based		
	ELISA	**Immunoblot**		**MALDI**	**qTOF/IT**	**MRM**
Extraction Buffer	Strong detergents	Strong detergents		Weak buffers	Weak buffers	Weak buffers
Treatment	No	No		Yes/No	Digestion	Digestion
Detection Method	Spectrometry	Visual		Mass spectrometry	Mass spectrometry	Mass spectrometry
Allergens can be analyzed	One	One		Multiple	Multiple	Multiple
Standard required	Yes (internal kit standard)	No		No	Yes/No	Yes
Cross Reactivity	Yes	Yes		No	No	No
Species Specificity	No	No		Yes	Yes	Yes
Can be standardized	No	No		Yes	Yes	Yes
Results comparable	No	No		Yes	Yes	Yes
Time	Time consuming	Time consuming		Fast analysis	Fast analysis	Fast analysis
Cost	Medium	Low		Medium	Medium	Medium
Quantification	LOD (lowest 0.2ppm) LOQ (lowest 0.3ppm)	Semi quantitative		Semi quantitative	LOD (lowest 0.06ppm) LOQ (lowest 3.7ppm)	LOD (lowest 0.001ppm) LOQ (lowest 0.01ppm)

MS not for routine analysis

Martina Koeberl, Dean Clarke, and Andreas L. Lopata. J. Proteome Res. 2014.

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DNA BASED METHOD
REAL PCR

targeting a segment of the gene coding for the allergenic or other proteins of interest and amplifying only this DNA fragment to make it detectable.
Extensively used for allergen detection because of their ease in application using kits.
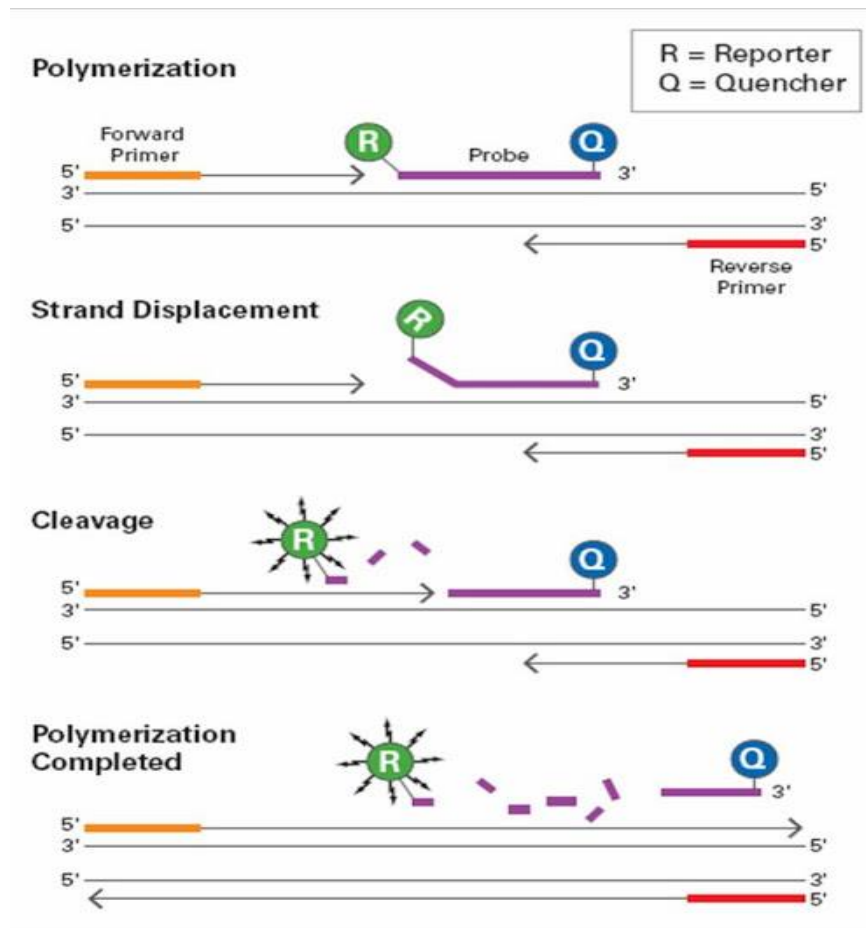
Critical point
- Design of primers and probe is important for specificity
- Sample prep
- Cross contamination and target template
- Baseline and Threshold definition
- Efficiency
- DNA extraction

PCR
PROS & CONS

+
- ✓ The fact that PCR detects the extremely stable DNA molecule might be an advantage when analyzing highly processed food.
- ✓ Useful in cases where ELISAs are not available (fish), reliable (celery) or results questionable (e.g. hydrolyzed proteins)
- ✓ Good method for verifying ELISA or immunochemical assay results
- ✓ Specific if well design
- ✓ High throughput
- ✓ Multi-screening (multiplex) potential PC

-
- ✓ Detect DNA not protein
- ✓ Qualitative
- ✓ Sample preparation and analysis require skill
- ✓ Cross-contamination possibilities.
- ✓ Target template can be a source of contamination : DNA templates are more troublesome as contaminants
- ✓ Absence/Presence of DNA does not indicate absence/presence of protein

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CHALLENGES TO FOOD ALLERGEN DETECTION
食品过敏原分析方法的挑战

Only few of the known allergens are characterized and available in pure form
仅有少数的过敏原获得纯化及其特性被充分认知

Highly purified proteins are not representative for food samples
实物样品中不存在高纯度的过敏原蛋白

There are no reference methods for allergens in food
没有官方的过敏原检测方法

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CHALLENGES TO FOOD ALLERGEN DETECTION
食品分析方法的挑战

➢ **Incomparable Results with Commercial Kits**
No general agreement on the expression of reporting unit
ELISA/ LCMS

➢ **Matrix effect**

Interaction of the analyte with matrix, hinder extraction
Coextraction of matrix proteins bind with antibody. False positive

➢ **Processing**

Denatured, millard reaction, partial hydrolysis

➢ **Extractability**

Various processing impairs solubility of allergen
~75%, 80% extractability yield decrease after thermal processing

Validate method for individual allergens and various matrices

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NEW TRENDS IN THE QUANTIFICATION OF FOOD ALLERGENS
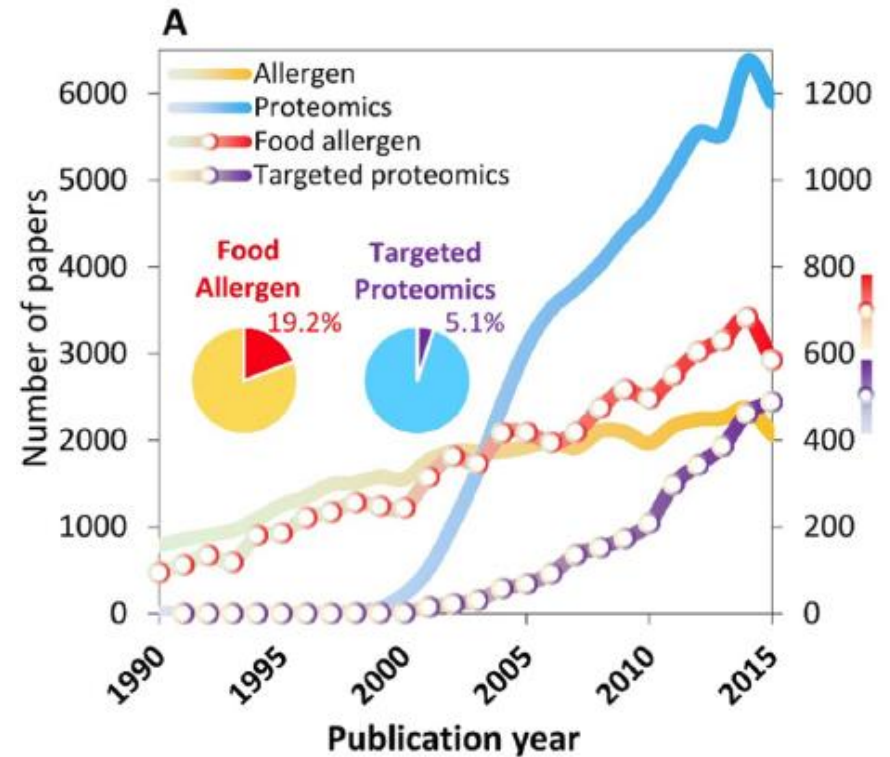
食品过敏原定量检测新趋势

ELISA

➢ Use of antibodies raised against modified proteins
➢ Use of antibodies raised against stable proteins

And LC/MS

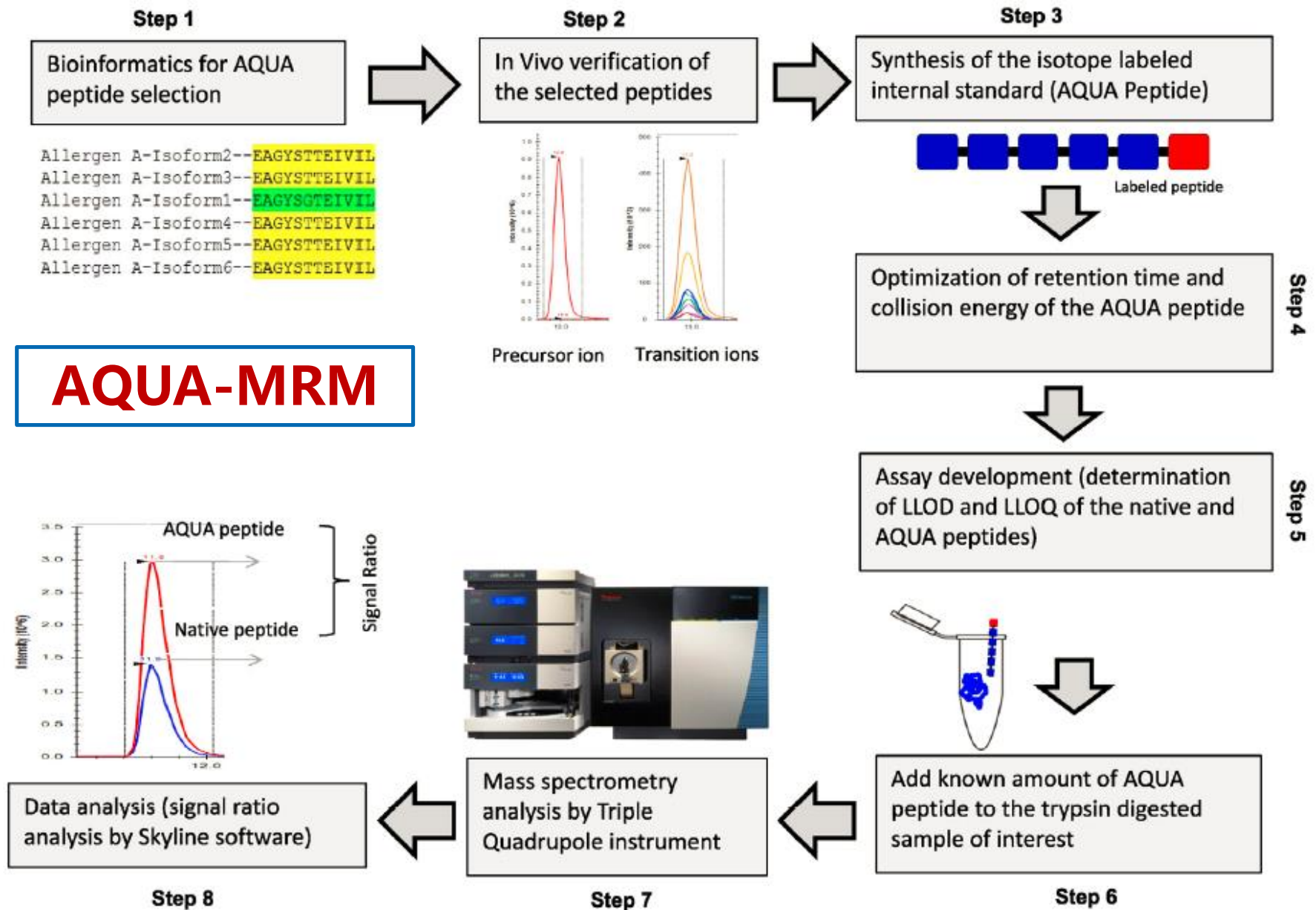Use of stable peptides as analytical target

Multiallergen Methods

Targeted proteomics

Nagib Ahsan, et al. Journal of Proteomics. 2016.

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Targeted proteomics in quantification of food allergens

靶向蛋白质组学在食物过敏原定量中的应用

AQUA: Absolute quantitation

Nagib Ahsan, et al. Journal of Proteomics. 2016.

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