Gluten analysis and the challenges of consumer testing devices.

: Adrian Rogers
Speaker Disclosure

Speakers – Adrian Rogers

Employee of Romer Labs part of the Erber Group

Nothing Else to Disclose
Why Me?

**Adrian Rogers** has been with Romer Labs for 8 years in his role as a Senior Research Scientist. He is responsible for research and development within Romer's allergen competence centre based in the UK.

Before joining Romer Labs, Adrian was an R&D Scientist involved in the development of ELISA and Lateral Flow immunoassays for the detection of food allergens. Adrian is a microbiologist by training and has 17 years experience in the development of immunoassays, 15 years of which have been spent developing test kits for the detection of food allergens.

Over the years Adrian has been involved in a number of food allergy projects including EuroPrevall, an EU funded multidisciplinary integrated project which investigated the prevalence of food allergy across Europe. He is currently a member of the University of Manchester's Food and Health Network allergy cluster and co-ordinated Romer Labs’ contribution to the “Innovate UK Knowledge Transfer Project”, with the University of Manchester looking at improving soya allergen analysis.
Why Me?

Allergy B*ll*CKS
Overview

• Gluten Analysis the challenges
• Current Methods of Analysis
• Consumer testing devices Pros and cons
• Gluten Detective : Opinion
• Conclusions
CHALLENGES OF GLUTEN ANALYSIS

In general: quantitation by measurement of a signal (e.g. UV absorption) and comparison to a known substance (reference material)

Defined substance

alternariol

Asam et al., 2009

Defined substance ??

gluten

Asam et al., 2009
# Classification of Cereal Proteins

<table>
<thead>
<tr>
<th>Albumins</th>
<th>Globulins</th>
<th>Prolamins</th>
<th>Glutelins</th>
<th>Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>soluble in water</td>
<td>soluble in salt solution</td>
<td>soluble in aqueous alcohol</td>
<td>soluble after reduction of disulfide bonds</td>
<td>insoluble</td>
</tr>
<tr>
<td>metabolic proteins</td>
<td>storage proteins (gluten)</td>
<td></td>
<td></td>
<td>structural proteins</td>
</tr>
</tbody>
</table>

- **Wheat:** Gliadins + Glutenins
- **Rye:** Secalins
- **Barley:** Hordeins
- **Oats:** Avenins
- **Rice:** Oryzins
- **Maize:** Zeins
- **Sorghum:** Kafirins
- **Maize:** Zeins
CHALLENGES OF GLUTEN ANALYSIS

Highly complex signal: gluten is a mixture of hundreds of proteins

- Variability of gluten: cultivar, climate, soil, origin, primary structures
- Gluten: prolamins (soluble in aqueous alcohols) + glutelins (soluble only after reduction of disulfide bonds)

RP-HPLC

**prolamins**

**glutelins**
CHALLENGES OF GLUTEN ANALYSIS

- Gluten: complex mixture of 100+ proteins
- Variability (due to genetics and environment)
- Protein modifications during processing

Patients react individually
- to different proteins and epitopes
- with variable sensitivities

- Selection of relevant target epitopes and antibodies
- Protein polymorphism
- Sample preparation
- Trace analysis in complex food matrices
GLUTENFREE PRODUCTS: LEGISLATION

International: Codex Alimentarius

Threshold for gluten in gluten-free products: **20 mg/kg** of the product

- Gluten analysis: immunologic or other method
- Antibody should react with protein fractions that are toxic to persons intolerant to gluten
- Validated methods calibrated against a certified reference material
- Limit of detection: ≤ 10 mg gluten/kg
- Method for gluten determination: ELISA R5 Mendez Method

Mandatory declaration of gluten-containing cereals on product labels (allergen labeling)
• The Enzyme Linked Immunosorbent Assay
1. Add sample/standards
2. Incubation
3. Washing
4. Add conjugate
5. Incubation
6. Washing
7. Add substrate
8. Add stop solution
9. Read

Pre-coated antibody
Analyte (allergen)
Ab - conjugate (HRP-labelled)
• Lateral Flow Devices
• Incubation – AgraStrip® Allergens

**Low** addition of allergen

5 min incubation

sandwich complex

**No** addition of allergen

no sandwich complex
• Lateral Flow Technology

AgraStrip® Allergens

Absorption Pad
Control Line
Test Line
Nitrocellulose Membrane
Gold Conjugate Pad
Sample Pad

Negative

Positive

Gold AB conjugate
Anti-DIG AB
Streptavidin
Sandwich complex
• Analytical Challenges

- Every food is different and presents its own unique challenges with analytical analysis

- If there is any doubt about how a food will perform, validate

- Knowledge is power, know your food
Why Consumers Want to Test

Brazil meat-packing giants 'exported rotten beef'

The authorities in Brazil suspended 53 government officials amid allegations that some of the country's biggest meat processors have been selling rotten beef and poultry for years.

Three meat processing plants have also been closed and another 23 are under scrutiny.
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Fipronil egg scandal: What we know

Millions of eggs have been pulled from supermarket shelves in more than a dozen European countries - including the UK - after it was discovered that some had been contaminated with the potentially harmful insecticide fipronil.

Fipronil is commonly used to get rid of flies, lice and ticks but is banned by the European Union for use on animals destined for human consumption, such as chickens.

The insecticide got into the food chain in the Netherlands, which is one of Europe's biggest egg producers, but contaminated eggs have now been found as far away as Hong Kong. There is no evidence yet that it has harmed anyone.
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EUR 230 MILLION WORTH OF FAKE FOOD AND BEVERAGES SEIZED IN GLOBAL OPSON OPERATION TARGETING FOOD FRAUD

39 April 2017

Press Release

Operation “OPSON II”, the joint European (INTERPOL), operation targeting counterfeit and substandard food and drinks, as well as fake cosmetics and pharmaceuticals, has resulted in the seizure of EUR 230 million worth of counterfeit food and beverages ranging from baby products such as baby food, beverages and交汇点 comp pills, to alcohol and EUR 4.0 to 5.0 million euros in counterfeit cigarettes, as well as a total of 220,000 counterfeit and substandard goods.

The new OPSON II operation (also known as MISPW10), which was carried out for the second time in a row and saw an increase in participating countries (32 countries in 2015), saw authorities across 32 countries in a criminal operation to be carried out on 19 November 2016 to 28 March 2017, involving police, customs and health regulatory bodies and partners from the private sector. More than 10,000 authorities were involved in the operation, targeting.operator partners.

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Why Consumers Want to Test

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The authorities in Brazil suspended a prominent meat processor and allegations that some of the country’s biggest meat companies have been selling rotten beef and poultry for years.

Three meat processing plants have also been closed and another 24 are under scrutiny.

FOOD ALLERGENS

Bert Popping

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Fipronil egg scandal: What we know

© 2018 FOCUS

E 2.23 MILLION WORTH OF FAKE FOOD AND BEVERAGES SEIZED IN GLOBAL OPSON OPERATION TARGETING FOOD FRAUD

Press Release:

The Dutch Food Safety Authority (NVWA), in cooperation with national authorities in 10 countries and the EU industry, conducted a large operation to seize fake food and beverages in the Netherlands and in the EU. The operation was conducted in parallel with similar operations in 23 other countries.

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Why Consumers Want to Test
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Consumer’s distrust of food industry grows

By Mike Huggett | MARCH 16, 2016 – 12:30PM

The majority of Americans surveyed by a Wall Street food industry analyst say they are becoming more distrustful of the food system.

“We seem to be at a tipping point in the food industry as an increasing number of people are becoming more distrustful of the food system,” wrote Alexis Howard, a stock analyst at Sanford Bernstein, which this week released a proprietary survey of 888 U.S. consumers.

Fifty-five percent of consumers agreed that they are becoming more distrustful of the food system, up from 48 percent in 2013, the last time Sanford Bernstein did such a survey.

The finding parallels a 10 percentage point increase — to 69 percent — in consumers concerned about food safety issues such as salmonella and listeria, according to the Bernstein survey.

“Rarely a week goes by without some form of recall being announced by the (U.S. Food and Drug Administration),” Howard wrote. “Already this year we have had more than 60 different food-related recalls in the U.S. alone.”
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Why Consumers Want to Test
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Bert Popping

Consumers' distrust of food industry grows

By Mike Hughelett

The public’s mistrust of the food industry is growing, according to a new study.

The latest in a series of consumer surveys by the Food Allergy Research and Education (FARE) Institute for Food Allergy Research, the study found that 70% of adults are concerned about food allergies and 60% have avoided a product due to potential allergens.

The findings also highlight the significant impact of food allergies on consumers.

“Millennials are more likely to distrust major food companies: report

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Why Consumers Want to Test

Trends for 2017 show wellness and foods link to grow

Chooag Ming | @chooagming
Published 2:04 PM ET Jan 3, 2017 | Updated 11:01 AM ET Feb 7, 2017

Getting serious about physical fitness is a common New Year’s resolution, but the coming year may help some stay on track, as emerging food and beverage trends point to healthy living.

Consumers Want Healthy Foods--And Will Pay More For Them

Nancy Gagliardi, CONTRIBUTOR

Health is front and center for those who choose out the food products that will line supermarket shelves in the next year. At Campbell’s, organic and all natural are the buzzwords as the company prepares to launch a variety of soups using USDA-certified organic ingredients and shelf-stable fruit and vegetable juices with no added sugar or artificial ingredients. At the 2015
Why Consumers Want to Test

Trends for 2017 show wellness and foods link to grow

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Why Consumers Want to Test
• Personal Allergen Detection Devices
• Personal Allergen Detection Devices
The Pro’s

- Can help consumer identify risks early
- Can support healthy lifestyle / support dietary needs
- Can help manufacturer reduce food safety risks
- Are Affordable
The Challenges
Challenge: Sampling

FOOD ALLERGENS

Bert Popping

© 2018 FOCOS
• Sampling
• Sampling
• Sampling

Judith Morrison
@JLMo
## Challenge: Misinterpretation

<table>
<thead>
<tr>
<th>What the British say</th>
<th>What the British mean</th>
<th>What others understand</th>
</tr>
</thead>
<tbody>
<tr>
<td>I hear what you say</td>
<td>I disagree and do not want to discuss it further</td>
<td>He accepts my point of view</td>
</tr>
<tr>
<td>With the greatest respect...</td>
<td>I think you are an idiot</td>
<td>He is listening to me</td>
</tr>
<tr>
<td>That’s not bad</td>
<td>That’s good</td>
<td>That’s poor</td>
</tr>
</tbody>
</table>
Challenges: Specificity / Selectivity / Imprecision
Challenge: Data Quality

Possible description by consumers:
- Fruits
- Apples
- Granny Smith or Braeburn
- Cooking apple

Specific details are important if only one variety has a problem
Challenge: Technology Abuse
Consequence: Food Manufacturers
What is needed

Guidelines acceptable by all stakeholders:
- Device manufacturers
- Food industry
- Consumer representatives
- Laboratories
- Lab Assay Developers
- Regulators
What has been done so far

Guidelines acceptable by all stakeholders:

✓ Device manufacturers
✓ Food industry
✓ Consumer representatives
✓ Laboratories
✓ Lab Assay Developers
✓ Regulators
• What’s Being Done

• Stakeholders’ Guidance Document for Consumer Analytical Devices with Focus on Gluten and Food Allergens

- Customer support or help line
- Test performance and limitations
- Sampling
• What’s Being Done

- Interpreting results
- Reagent safety and disposal
- Independent validations/certifications

Special Allergen addition of JAOAC published Jan 2018
• Other opinions

• https://www.glutenfreewatchdog.org/

• @GFWatchdog

• “At GFWD we've been testing a variety of products w/ Nima. It's difficult to put results into context due to lack of published validation report. In our opinion Nima was released into marketplace prematurely. We can't support its use by gluten-free community at this time”
• Other opinions

• [https://www.glutenfreewatchdog.org/](https://www.glutenfreewatchdog.org/)

• **Charlie**  
  I have celiac and I am incredibly discerning in what I eat. I do my due diligence and when eating out I use the Nima. When I’ve gotten positives I’ve chosen not to eat that dish. At best, I’ve saved myself from being glutened. At worst … well I don’t see any downside. I’m grateful to have this tool.

• **Terry**  
  Reply  
  Trisha, I also use my Nima sensor when I must eat out or go to family potlucks, etc. Most gluten eating non-Celiacs do not understand gluten or contamination. Prior to my Nima, my mantra was, “if in doubt, do without”. With my Nima, I have some comfort in knowing the food is okay or not. I honestly have not been poisoned since I have used my Nima sensor.  
  The original instructions was to stir soups/sauces, take sample from several parts and/or rub the sample around the plate. Until there are better tools available to the Celiac/gluten sensitive lay persons, this is all we have for now.
• Other Opinions

- [Link](https://www.anaphylaxis.org.uk/)
- [@Anaphylaxiscoms](https://twitter.com/Anaphylaxiscoms)

• Expert Panel at Corporate Conference
• “Don’t touch with a bargepole”
• Gluten Detective: Consumer Test Kits for Detecting Gluten in Stool and Urine


• Lateral Flow Device: Analytically OK

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<table>
<thead>
<tr>
<th></th>
<th>urine</th>
<th>stool</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIP Ab Specificity</td>
<td>&gt;96%</td>
<td>&gt;96%</td>
</tr>
<tr>
<td>Full Window</td>
<td>1- to 24-hours</td>
<td>1- to 7-days</td>
</tr>
<tr>
<td>Optimal Window</td>
<td>6-16 hours</td>
<td>2-4 days</td>
</tr>
<tr>
<td>Minimum Intake</td>
<td>500mg+ (2 bites of bread)</td>
<td>50mg+ (a crumb of bread)</td>
</tr>
<tr>
<td>Notes</td>
<td>1st morning urine ideal</td>
<td>diarrhea can shorten time windows</td>
</tr>
<tr>
<td>Application</td>
<td>identifying major transgressions</td>
<td>monitoring total weekly gluten consumption</td>
</tr>
</tbody>
</table>
Gluten Detective: Consumer Test Kits for Detecting Gluten in Stool and Urine

What I would like to see

In the US, we tend to use 10 mg of gluten per day as the “safe” threshold for folks with celiac disease. If the optimal window for the stool test is 2 to 4 days, it would be useful if the lower limit of detection for this test could be reduced from 50 mg to 20 to 40 milligrams of gluten.

Bottom line

If you are concerned that you have been regularly exposed to cross contact from gluten over a period of days then you may find the stool test useful (assuming the test works as the manufacturer claims). However, if you are concerned that a single food or meal may have contained a level of gluten above 20 ppm, this test will likely not provide you with an answer (unless the food/meal is highly contaminated).
• Conclusions

  ▪ Testing food is difficult
  ▪ Big data good/bad
  ▪ The technology is wonderful (I wish we had done it)
  ▪ In my opinion should not be used by an allergic individual
Improved reference materials for gluten analysis

Katharina Scherf, Eszter Schall, Lívia Hajas, Zsuzsanna Bugyi, Kitti Török, Stefano D'Amico, Regine Schönlechner, Peter Köhler, Roland Poms, Sandór Tömösközi

2nd MoniQA International Symposium on Food Fraud Prevention and Effective Food Allergen Management, Vienna, June 8, 2018
Consumer-Operated Analytical Devices
Pros & Cons

Bert Popping & Carmen Diaz-Amigo
FOCOS - Food Consulting Strategically
THE PRODUCT IS NOT A MEDICAL DEVICE; IS NOT REGULATED OR APPROVED BY THE U.S. FOOD AND DRUG ADMINISTRATION; AND IS NOT DESIGNED TO DETERMINE WHETHER YOU WILL HAVE AN ALLERGIC REACTION OR SENSITIVITY TO THE FOODS THAT YOU TEST. **YOU SHOULD NOT RELY ON NIMA ALONE TO IDENTIFY THE PRESENCE OF ALLERGENS.** YOU WILL STILL NEED TO ASK QUESTIONS OF THOSE WHO PREPARE FOOD, READ PACKAGING LABELS AND MENUS, AND CONSIDER ALL SOURCES OF INFORMATION IN ADDITION TO TESTS CONDUCTED WITH A NIMA. IF A PRODUCT IS LABELED AS CONTAINING AN ALLERGEN, YOU SHOULD RELY ON THE LABEL. THE PRODUCT IS INTENDED TO BE USED WITH THE NIMA ONE-TIME-USE CAPSULES AND THE NIMA MOBILE APPLICATION TO DETERMINE WHETHER A THRESHOLD LEVEL OF GLUTEN IS IN THE FOOD SAMPLE THAT YOU TEST, AND IS LIMITED TO THAT SAMPLE, WHICH MAY NOT BE REPRESENTATIVE OF THE FOOD SERVING AS A WHOLE. THE PRODUCT IS NOT A SUBSTITUTE FOR MEDICAL CARE OR ADULT SUPERVISION. **THE PRODUCT IS NOT INTENDED FOR USE IN WHICH ERRORS OR FAILURE COULD LEAD TO PERSONAL INJURY OR DEATH**
Thank you!

www.romerlabs.com