Romer Labs Allergen Solutions

February 2015
**Commercial analytical methods**

detecting Proteins – Peptides - DNA

**Proteins**

ELISA or LFD

*AgraQuant® Allergen*

*AgraQuant® + Allergen*

*AgraStrip® Allergen*

Antibodies recognizing Epitopes (3D structures) allergens (antigen-antibody interaction)

**Peptides**

LC/MSMS

Tryptic digestion into peptides, which are recognized by LC/MSMS

**DNA**

PCR or RT-PCR

Amplification of nucleic acid sequence
Romer Labs® – Experts in food allergen testing

**AgraStrip® lateral flow devices**
19 different allergens

**AgraQuant® ELISA**
20 different allergens

**AgraQuant® Plus ELISA**
8 different allergens

**ISO 17025 accredited service laboratory for allergen testing**
RomerLabs solution for detecting allergens

AgraQuant Test kits

- ELISA Method
- Quantitative Results
- Lab equipment needed (Reader, centrifuge, water bath)
- 48 (some 96) microwells

AgraStrip Test kits

- Lateral Flow Strip Method
- Qualitative Results
- On site testing
- No Lab equipment needed (all components included)
- 10 test strips & swabs
Allergen detection with the AgraStrip®
Why using a strip test for allergens?

- Real time testing – implement decisions quickly
- Trouble shooting abilities
- **Simplicity of use – less training**
- Cost effective - single sample
- No additional equipment for AgraStrip® Allergen Tests
- **On-site testing**
- Confidence through testing
Why using AgraStrip® Allergen Kits?

Testkit details:

☑ Everything included in kit
☑ On-site method
☑ Same procedure (ex. G12, soy)
☑ Sensitive - Low LOD
☑ Certificate of Performance
☑ Validation reports
AgraStrip® Allergen On-site Test Kit

Components in the kit:

- 1 Tube containing 10 AgraStrip® Strips
- 1 Dropper Bottle (green label) containing 35mL Extraction Buffer
- 10 incubation vials
- 10 Extraction Tubes
- 10 Tube Caps
- 10 Extraction Tube Dropper Tips
- 10 Breakpoint Swabs
- 1 Tube Holder

→ Store at Room Temperature
→ Time-to-result: **11min**
On-site Testing for Trace Amounts of Allergens

3 extraction procedures

Risk Management
- samples collected according to HACCP or other Quality Management scheme

Samples for Allergen Testing
- Products: raw materials, in-process, finished products
- Liquids: liquid products, liquid raw materials, rinse waters
- Swabs: production line, personnel, packaging material, environmental

AgraStrip® Allergen test strips
- Results within 11 minutes, no equipment or lab space needed

ALLERGENS DETECTED
- Intervention required

NO ALLERGENS DETECTED
- OK to continue
**Finished Products & Rinse Water Testing - Extraction**

**Homogenize** the sample (i.e. blend, crush, grind)

Weigh **0.2g** of sample (with Balance or estimate by filling up one of the extraction tube caps) and add to an extraction tube or just add 0.2 mL rinse water

Fill Extraction Tube with *Extraction Buffer* to level shown below (blue arrow)

Close tube with tube cap, and shake vigorously by hand for **1 minute**.

Remove cap from the Extraction Tube and replace with dropper tip. And transfer **12 drops** (400 µL) to an incubation vial.
**Swabbing - Extraction**

Fill extraction tube with **Extraction Buffer**, take a swab and wet the end by dipping into the buffer.

Wipe an area of **5cmx5cm** using side to side movements, rotating the swab tip as you go (we recommend the “cross-hatch” swabbing technique indicated below).

Place the swab into the extraction tube. Carefully break off the end at the pre-scored point.

Close the tube with a cap and shake vigorously for **1 minute**.

Remove cap from the Extraction Tube and replace with dropper tip. And transfer **12 drops (400µl)** to a new tube (incubation Vial).
Running the Assay for all sample types

Shake Incubation Vial vigorously by hand for **15 seconds** (ensuring liquid comes in contact with the lid of the vial) then leave to stand at room temperature for **5 minutes**.

Open the container of AgraStrips®, remove the necessary number of strips and close the tube. Open the Incubation Vial and dip the bottom end of test strip into the vial.

Remove the test strip from the Incubation Vial **after 5 minutes** and read off the result immediately.
**Reading Results**

One single purple line in the central part of the test = negative result

Two purple lines in the result zone = positive result

No control line appears = invalid result

- After 5 min strip development cut off filter pad for proper read out
Video AgraStrip® Allergen Procedure

http://www.youtube.com/watch?v=AWmB3jk8Q1I
# AgraStrip® Allergens (19)

<table>
<thead>
<tr>
<th>AgraStrip® Peanut (COKAL0110AS)</th>
<th>AgraStrip® Hazelnut (COKAL0310AS)</th>
<th>AgraStrip® Almond (COKAL0710AS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgraStrip® Egg (COKAL1810AS)</td>
<td>AgraStrip® Gluten G12 (COKAL0200AS)</td>
<td>AgraStrip® Casein (COKAL1210AS)</td>
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<tr>
<td>AgraStrip® Walnut (COKAL0910AS)</td>
<td>AgraStrip® Lupin (COKAL1510AS)</td>
<td>AgraStrip® Mustard (COKAL2110AS)</td>
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<tr>
<td>AgraStrip® Sesame (COKAL1910AS)</td>
<td>AgraStrip® Macadamia (COKAL1610AS)</td>
<td>AgraStrip® Brazil Nut (COKAL1710AS)</td>
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<td>AgraStrip® Soy (COKAL0410AS)</td>
<td>AgraStrip® Milk (COKAL2610AS)</td>
<td>AgraStrip® Coconut (COKAL2610AS)</td>
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<tr>
<td>AgraStrip® Cashew/Pistachio (COKAL1310AS)</td>
<td>AgraStrip® BLG (COKAL1010AS)</td>
<td>AgraStrip® Gluten (COKAL0210AS)</td>
</tr>
<tr>
<td></td>
<td>AgraStrip® Crustacea (COKAL1010AS)</td>
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</tbody>
</table>
Allergen detection with the AgraQuant®
<table>
<thead>
<tr>
<th>AgraQuant® Peanut (COKAL0148)</th>
<th>AgraQuant® Hazelnut (COKAL0348)</th>
<th>AgraQuant® Almond (COKAL0748)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgraQuant® Egg white (COKAL0848)</td>
<td>AgraQuant® Gluten G12 (COKAL0200)</td>
<td>AgraQuant® Casein (COKAL1200)</td>
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<tr>
<td>AgraQuant® Walnut (COKAL0948)</td>
<td>AgraQuant® Lupin (COKAL1548)</td>
<td>AgraQuant® Mustard (COKAL2148)</td>
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<tr>
<td>AgraQuant® Sesame (COKAL1948)</td>
<td>AgraQuant® Pistachio (COKAL2748)</td>
<td>AgraQuant® Crustacea (COKAL2248)</td>
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<tr>
<td>AgraQuant® Soy (COKAL0448)</td>
<td>AgraQuant® Milk (COKAL2448)</td>
<td>AgraQuant® Fish (COKAL2548)</td>
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<tr>
<td>AgraQuant® Cashew (COKAL3148)</td>
<td>AgraQuant® BLG (COKAL1048)</td>
<td>AgraQuant® Lysozyme (COKAL2848)</td>
</tr>
<tr>
<td>AgraQuant® Ovalbumin (COKAL2948)</td>
<td>AgraQuant® Gluten (COKAL0248)</td>
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</tr>
</tbody>
</table>
**ELISA assays**

- Microplates coated with specific antibody
- Antibody-enzyme conjugates
- Substrate changing color in presence of enzyme
Why AgraQuant Kits?

- Ready to use reagents
- Extraction solution included
- Same procedure all kits
- Sensitive - Low LOD/LOQ
- Certificate of Performance
- Spreadsheet provided
  - Validation service
- Full product pipeline
- Customer support
Kit components:

1 bottle extraction solution (10x)
1 bottle wash buffer (10x)

5 vials of standards

1 vial conjugate
1 vial substrate
1 vial stop solution

Antibody coated wells
## Sample Preparation - Example

<table>
<thead>
<tr>
<th>Sample size</th>
<th>1 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction solution</td>
<td>20 mL extraction buffer (provided)</td>
</tr>
<tr>
<td>Extraction time</td>
<td>15 min at 60°C water bath, shake vigorously every 2 minutes</td>
</tr>
<tr>
<td>Centrifugation/Filtration</td>
<td>10 min at 2000g at room temperature or filter</td>
</tr>
<tr>
<td>Dilution</td>
<td>Supernatant is ready for testing</td>
</tr>
</tbody>
</table>
1. Add sample/standards
2. Incubation
3. Washing
4. Add conjugate
5. Incubation
6. Washing
7. Add substrate
8. Add stopp solution
9. Read

Sandwich ELISA

1. Ab coated well
2. Analyte (Allergen)
3. Ab coated well
4. Pre-coated Ab
5. Ab-Conjugate
Procedure - AgraStrip Allergen

Add samples & Standards
Incubate **20 minutes**
Wash

Add Conjugate
Incubate **20 minutes**
Wash

Add Substrate
Incubate **20 minutes**
Add Stop & Read results
Spreadsheet

### Section I: Calibration Curve

<table>
<thead>
<tr>
<th>Std. Level [ppm]</th>
<th>Abs.</th>
<th>B/Bo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.83</td>
</tr>
<tr>
<td>1</td>
<td>0.40</td>
<td>0.27</td>
</tr>
<tr>
<td>4</td>
<td>0.70</td>
<td>0.47</td>
</tr>
<tr>
<td>10</td>
<td>1.20</td>
<td>0.80</td>
</tr>
<tr>
<td>40</td>
<td>1.50</td>
<td>1.00</td>
</tr>
</tbody>
</table>

![Calibration Curve Graph]

### Section II: Sample Calculations

<table>
<thead>
<tr>
<th>No.</th>
<th>ABS</th>
<th>SAMPLE</th>
<th>ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0 ppm</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.40</td>
<td>1 ppm</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0.70</td>
<td>4 ppm</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>1.20</td>
<td>10 ppm</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>1.50</td>
<td>40 ppm</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>0.50</td>
<td>0.5 ppm</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>0.30</td>
<td>0.3 ppm</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>0.60</td>
<td>0.6 ppm</td>
<td>0.6</td>
</tr>
<tr>
<td>9</td>
<td>1.10</td>
<td>1.1 ppm</td>
<td>1.1</td>
</tr>
<tr>
<td>10</td>
<td>1.40</td>
<td>1.4 ppm</td>
<td>1.4</td>
</tr>
<tr>
<td>11</td>
<td>1.20</td>
<td>1.2 ppm</td>
<td>1.2</td>
</tr>
</tbody>
</table>

![Sample Calculation Table]

Sandwich ELISA

- direct signal to concentration curve
- more analyte, higher OD value
AgraQuant® Swabbing Kit

- 20 swabbs
- 20 temper proof vials
- Swabbing buffer

Validations for:
- Plastic, stainless steel, teflon
Allergen detection with the AgraQuant® PLUS
AgraQuant® Plus Allergens

- AgraQuant® Plus Egg (COKAL1848F)
- AgraQuant® Plus Peanut (COKAL0148F)
- AgraQuant® Plus Hazelnut (COKAL0348F)
- AgraQuant® Plus Cashew (COKAL3148F)
- AgraQuant® Plus Almond (COKAL0748F)
- AgraQuant® Plus Macadamia nut (COKAL1648F)
- AgraQuant® Plus Casein (COKAL1248F)
- AgraQuant Plus Mustard (COKAL2148F)

...more to come soon (BLG, Pistachio, Lupin, Sesame, Soy)
AgraQuant® PLUS Allergens

Fastest Allergen ELISA on the market
- Extraction: 1min
- ELISA Assay: 30min
- Time-To-Result: 31min

Accurate
- low cross-reactivity
- good recovery from challenging matrices
- good inter- and intra-assay precision

Sensitive
- low LODs

Technology
- time saving extraction technology
- capsules used for extraction
Kit components:

- Stop solution
- Substrate
- Conjugate
- Washbuffer
- 5 standards
- Antibody coated wells
- Transfer wells
- Extraction additive 1 & 2
Extraction Procedures:

1. Food

2. Environmental samples (swab and sponge)

3. Rinse water
Extraction procedure:

1. Accurately weigh 1g (mL) of sample into a 50mL centrifuge tube and add one capsule each of extraction additive 1 & 2.

2. Bring destilled water to 80-100°C and Add 20mL hot, but not boiling water to the tube.

3. Shake tube vigorously for 15 seconds (until sample and capsules are dissolved).

4. Transfer an aliquot to a 1.5 or 2 mL reaction vial and centrifuge OR alternatively, filtrate through a pleated filter and use the filtrate in the test.
ELISA Assay:

1. Dispense 150 µL sample extract or standards into transfer wells (blue).

2. Transfer 100 µL sample extract or standards into the antibody coated wells (colourless) using a multichannel pipette and incubate for 10 min.

3. Wash 5 times with wash buffer.

4. Tap dry washed wells.

5. Pipette 100 µL conjugate solution into the antibody coated wells and incubate for 10 min, wash the wells afterwards as described in step 3 & 4.

6. Pipette 100 µL substrate solution into the antibody coated wells and incubate for 10 min in the dark.

7. Add 100 µL stop solution into the antibody coated wells.

8. Read Results at 450 nm with an ELISA reader.

Running the assay: 30 min
The big advantages:

- fast extraction of **up to 24 samples** at the same time
- no need for preparing extraction buffer
- **one extract** can be used to measure several allergens (exception egg)

10 vs 70 samples
Online Video Instructions on YouTube
but also the extracts of the normal AQ kits can be used in different assays...

<table>
<thead>
<tr>
<th>Extraction</th>
<th>Sample Dilution</th>
<th>Standard Dilution</th>
<th>Add Sample/Standards</th>
<th>1st Incubation</th>
<th>Washing</th>
<th>Add Conjugate</th>
<th>2nd Incubation</th>
<th>Washing</th>
<th>Add Substrate</th>
<th>3rd Incubation</th>
<th>Add Stop Solution</th>
<th>Read with ELISA Reader</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 g in 20 mL Extraction Buffer at 60 °C</td>
<td>No</td>
<td>No</td>
<td>100 μL Sample/Standard into Well - incubate 20 min in the dark</td>
<td>Wash 5x with Wash Buffer</td>
<td>100 μL Conjugate into Well - incubate 20 min in the dark</td>
<td>Wash 5x with Wash Buffer</td>
<td>100 μL Substrate into Well - incubate 20 min in the dark</td>
<td>100 μL Stop Solution into Well</td>
<td>Read at 450 nm (with 630 nm differential filter recommended)</td>
<td>1:100</td>
<td>1:100</td>
<td>1:100</td>
</tr>
</tbody>
</table>

- **Crustacea**: 1 g in 20 mL Extraction Buffer (40 °C)
- **Casein**: 0.5 g in 10 mL Extraction Buffer (60 °C)
- **Gluten**: 1 g in 10 mL 40 % EtOH
- **Gluten G12**: 0.25 g in 2.5 mL Extraction Solution (50 °C, 40 min) + 7.5 mL, 80 % EtOH
- **Milk**: 0.5 g in 10 mL Extraction Buffer (60 °C)

> taken from the AQ Allergen Quick Guide
The next generation Gluten Antibody
Gluten G12

AgraQuant® Gluten G12

AgraStrip® Gluten G12

AOAC Official Method 2014.03

AACC INTERNATIONAL

PERFORMANCE TESTED
AOAC RESEARCH INSTITUTE
LICENSE NUMBER 010902
Romer offers solutions for the whole process!

Ingredients

In-Process

Analytical Service

Cleaning Validation

Certification

Finished Products

Covering all Stages
Analytical Service

ISO 17025:2005 accredited through the American Association for Laboratory Accreditation
  • for allergens
  • for mycotoxins

→ For customized validation
→ For confirmation of positives

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Union, MO 63084
Laboratory Director: Christy Brewe
Phone: 636-583-8600

ISO 17025:2005
ISO 9001:2008
ISO 17025:2005
Romer Labs supports you with:

✓ Broad Range of Allergen Solutions
✓ Custom based validations
✓ Confirmation of positives
✓ Customer service
✓ Technical service/support
✓ Trainings and Certification
✓ Check Sample Surveys
Thanks for your attention!

"Bit of a bummer really, you've got a nut allergy"

Questions?