New Rapid Allergen Test Kits With Sensitivity From 0.5 to 2.0ppm:
Soy, Milk, Almond, Egg and Coconut Proteins with Sensitivity From 0.5 to 2.0ppm Incurred Samples In Less Than Five Minutes.


Presented by Bia Diagnostics, LLC,
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Validation of New Rapid Allergen Test Kits for: Soy, Milk, Almond, Egg and Coconut Proteins: With Sensitivity From 0.5 to 2.0ppm In Incurred Samples In Less Than Five Minutes.


Abstract:
The incidence of food related allergen hospitalization is continuously increasing in industrialized nations worldwide. In a recent study in the journal of PEDIATRICS, Gupat states that 8% of the US population under the age of 18 suffers from the effects of food allergies and another study reported that at least 4% of the overall population suffers the effects of food allergies.

Under the Food Allergen Labeling and Consumer Protection Act of 2004 food manufacturers are required to have in place a process which will eliminate or reduce the likelihood of cross-contamination between non-allergenic and allergenic foods. This often requires time consuming, extensive cleaning and verification of processes. With economies of scale such that short downtimes between production runs are critical to a company’s productivity, quick reliable methods are needed to certify not only that the equipment is clean but that the final product is free of trace amounts of the food allergens.

Here we look at five new rapid LFD (Lateral Flow Device) test kits to determine their efficacy in detecting specific allergens in various finished product samples down to 1ppm in less than five minutes. All methods were able to detect processed food allergens (pasteurized: soy, bovine milk, almond, egg and coconut proteins) in a wide variety of food matrices. CIP and swab contaminated samples had detection limits up to ten times lower than finished products. There were no false positive or false negative results with all matrices tested including highly processed incurred spiked samples.

Total Food Allergens and Sensitivities of Children in US 2007
National Health Interview Survey and Center for Celiac Research University of Maryland School of Medicine

- Gluten Sensitivity (37.5%)
- Milk (15.6%)
- Egg (14.8%)
- Peanut (7.5%)
- Tree Nut (7.5%)
- Other, Soy, Sesame, Celery, etc. (17.5%)

Statistics are from America Academy of Allergy Asthma and Immunology and University of Maryland School of Medicine Center for Celiac Research
Method:
All five methods (egg, soy, coconut, almond and bovine milk) employ polyclonal antibodies with colloidal gold conjugate imbedded in the sample dispense area and capture the antibody imbedded on the strip. Each assay includes both a hook line (to check for the prozone effect) and a control line (to check for matrix interferences) to assure accurate results. The hook line contains bound purified protein specific for the antibody employed in the method. All methods were tested for sensitivity, reproducibility and recovery in naturally incurred (FAPAS®, ‘Food Analysis Performance Scheme’, in house samples), artificially incurred (baked cookies) and spiked samples. Negative controls were assessed in all but the naturally incurred samples, where there was no negative matrix available.

All (pasteurized) spiking material was provided by a leading international food manufacturer and verified to contain labeled proteins as indicated using the Kjeldahl method for protein determination. All samples were spiked from 1:49 to 1:9 but not less than 1:9 in order to maintain consistency of the matrices. Levels of contamination of incurred samples were determined by ELISA and some samples were found to be at the threshold of detection with this method (whole milk ELISA). Artificially incurred cookies were made with predetermined levels of protein (5ppm pasteurized allergen protein) mixed and then baked in a convection oven at 350F for 20min. All assays were performed according to instructions outlined in the kit insert.

Discussion and Results:
All assays performed as expected with excellent reproducibility (+/- 10%) and within the norm of what one would expect from an ELISA method (see Figure 4), n=10. It should be noted that all values were graded on a relative scale of 0-10 based on the control line after 10min. Throughout the study only one false positive result was noted and that was with the egg method and corrected by centrifugation per kit instructions (see Figure 3). There were no false negative samples detected, with the exception of the total milk analysis on the FAPAS® casein sample (assigned value between 1.4 - 2.2ppm casein by ELISA) which did not detect any milk in the 10min time allotted for the analysis.

With all of the 27 complex matrices tested, no method had difficulty in detecting allergen protein contamination in the very low ppm levels (1-5ppm). Of the more than 50 analyses made there was only one false positive result as noted above and this was ameliorated by centrifuging the sample at 3K for 2min. (see Figure 3, Vanilla Smoothie) and confirmed by ELISA. One value was discarded because there was an anomaly with the strip and this was flagged by the presence of a smeared hook line and most likely caused by a defect in the strip itself. Samples were also tested at the very high level of contamination (500-1000ppm or greater) to check for the prozone effect (false negative reporting) and each assay method was able to flag the user on overloaded test samples with a disappearing “hook” line as the allergen protein contamination increased. On all LFD methods evaluated the hook line is the line just below the control line and both lines are visible with negative test samples. Three visible lines indicate a positive result, (test line, hook line and control line). An over loaded test has only one line visible and that is the control line. With all but one method (Milk LFD) the upper limit of saturation was not reached, even at the 1mg/ml protein level, so that neither the test line nor hook line disappeared completely. When testing allergen rich protein samples (1-10mg/ml), (Almond Milk, Eggnog, Coconut Milk, etc.) these methods showed strong positive result as well without complete diminution of the hook line (with the exception of the coconut method -data not shown).

Threshold levels were determined using processed proteins diluted into DiH₂O and showed that all test methods could detect down to 1ppm protein or better within 10min and many could detect the 0.5ppm protein within 5min (milk, egg and almond). Upper limit of detection was set at 1mg/ml protein for this study and all methods with the exception of the milk assay were able to reliably detect the protein at this high level without overt quenching. The milk assay showed some quenching (both test line and hook line) at 100ppm with complete quenching at 1mg/ml protein level. This was ameliorated by employing the standard dilution used for incurred samples of 1:4 (instead of 1:1 used for CIP), without a significant effect on the LLOD of the method (~1ppm).

All samples for the reproducibility analysis were spiked in DiH₂O at 5ppm allergen protein. Reproducibility showed that there was less than 10% variation in the test lines and hook lines with all test methods (n=10). No false negative samples were detected.
Scoring of test line intensities was subjective, based on a scale of 0-10 and relative to the control line which in some cases with highly contaminated samples was slightly less intense. Repeated strip was voided because of muted test line, hook line and control line.

**KEY**
- **0 = NO RESPONSE**
- **10 = MAXIMUM RESPONSE**
- All samples were run in **replicates (ex. 0,0)**

**Eggs DILUTION 1:9**
- **5 MIN**
- **10 MIN**

**Almond DILUTION 1:9**
- **5 MIN**
- **10 MIN**

**Soy**
- **5 MIN**
- **10 MIN**

**Coconut**
- **5 MIN**
- **10 MIN**

**Milk**
- **5 MIN**
- **10 MIN**

Dilution 1:1

**Figure 2**

Scoring of test line intensities was subjective, based on a scale of 0-10 and relative to the control line which in some cases with highly contaminated samples was slightly less intense. Repeated strip was voided because of muted test line, hook line and control line.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Dilution</th>
<th>5 MIN</th>
<th>10 MIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>1:9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Almond</td>
<td>1:9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coconut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>1:1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LFD Min/Max Data
### Dynamic Range

![Dynamic Range chart]

### Relative value

- **Egg**
  - Sample: 0.0 > > > 0.0
  - Spiked: 6.0 > > > 1.0
- **Soy**
  - Sample: 0.0 > > > 0.0 0.0 0.0 > > > 0.0
  - Spiked: 2.0 > > > 2.0 2.0 2.0 > > > 2.0
- **Almond**
  - Sample: 0.0 > > > 0.0 0.0 0.0 0.0 0.0 0.0
  - Spiked: 4.0 > > > 4.0 4.0 4.0 4.0 4.0 4.0
- **Coconut**
  - Sample: 0.0 > > > 0.0 0.0 0.0 0.0 0.0 0.0
  - Spiked: 2.0 > > > 2.0 2.0 2.0 2.0 2.0 2.0

### LLOD Validation of Matrices and Incurred Samples

#### Figure 3

All samples were analyzed per instructions outlined in kit inserts. Spiked samples were spiked in accordance with the exception of Apple & Eve cranberry juice which was spiked at 4ppm for milk (2ppm was not detected). The spike was made using processed protein and verified via the Kjeldahal method.

- **Egg**
  - LLOD: Not Applicable
  - Max: 10.0
  - ULLOD: 100ppm
  - LOQ: >1mg/mg

- **Soy**
  - LLOD: 0.5ppm
  - Max: 10.0
  - ULLOD: 100ppm
  - LOQ: >1mg/mg

- **Almond**
  - LLOD: 1.0
  - Max: 4.0
  - ULLOD: 100ppm
  - LOQ: >1mg/mg

- **Coconut**
  - LLOD: 1.0
  - Max: 2.0
  - ULLOD: 100ppm
  - LOQ: >1mg/mg

#### NOTE: All Relative values are based on the control line intensity (0-10)

- **Egg**
  - Control: 0.0
  - Spiked: 6.0

- **Soy**
  - Control: 0.0
  - Spiked: 2.0

- **Almond**
  - Control: 0.0
  - Spiked: 4.0

- **Coconut**
  - Control: 0.0
  - Spiked: 2.0

### Example Table

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Concentration</th>
<th>Spike</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td>0.0 ppm</td>
<td></td>
</tr>
<tr>
<td>Soy</td>
<td>2ppm</td>
<td></td>
</tr>
<tr>
<td>Soy</td>
<td>4ppm</td>
<td></td>
</tr>
</tbody>
</table>

### Annex

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**LFD Validation of Matrices and Incurred Samples**
Samples were run in parallel un-spiked diH2O and spiked H2O (5ppm protein). All methods were run n=10 and showed ≤10% variation in scoring positive samples at the 5ppm protein level. All negative samples reported a score of 0 on a scale of 0-10 compared to the control line (10). There was no discernible effect on the hook line with the addition of these low level concentrations of allergen proteins.

Figure 4

Average Reproductivity of LFD testing on 10 strips
Conclusion:
These methods were shown to be robust and reliable for QA/QC screening in both finished product and CIP samples.

References:
3. AOAC Official Methods 2000- Method 950.10