

## Detection of Peanut in Foods by the Nima Peanut Sensor

### Abstract:

*To address the growing public health concerns regarding food allergies, Nima is developing handheld devices that integrate food processing, assay chemistry and data interpretation, enabling consumers to readily test their foods for allergens. The first device, now on the market, is the Nima™ gluten sensor, providing a food testing solution for millions of patients with celiac disease or other gluten sensitivities. Here, we describe product verification testing to evaluate performance of the newly developed Nima™ peanut sensor in a variety of foods. A total of 64 foods were tested, most with and without spiking with peanut. The results demonstrated successful device performance across a wide range of food matrices. In particular, foods with  $\geq 10$  ppm peanut were identified with 97.6% accuracy. Another collection of 30 peanut free flours and spices were analyzed to assess cross-reactivity. Importantly, results with this handheld consumer device were comparable to those obtained with a standard immunoassay designed only for laboratory use. The study also identified several limitations of the device, particularly certain foods causing errors or false positives, now undergoing further investigation. In the meantime, such limitations will be described in the product literature for the Nima™ peanut sensor.*

### I. Introduction

Food allergies are a growing food safety and public health concern in the US (Gupta et al., 2013; Liu et al., 2010). It is estimated that at least 3% of the US population has a food allergy (NIAID Expert Panel, 2010; Rona et al., 2007). A National Institute of Allergy and Infectious Diseases (NIAID) report estimated a prevalence of 5% in children under 5 years old and 4% in teens and adults, numbers that are appearing to increase (NIAID Expert Panel, 2010). Peanut allergy is the most common cause of fatalities related to food allergies in the US (Bock et al., 2007). Among children, it alone accounts for 25%, the highest prevalence, of food allergies. A strict and life-long peanut-free diet is the only current treatment for these patients, but it can be difficult to adhere to because of cross-contamination during food manufacturing or preparation and inadequate labeling and testing (Remington et al., 2013). One potential solution to this public health challenge would be for consumers, including parents of allergic children, to test their food for peanut prior to consumption. However, traditional peanut tests are: complicated, with processing steps not suitable for regular consumers and results that are difficult to interpret; expensive, costing at least \$14 per test; and time consuming, taking longer than 18 min per test. Fundamentally, there

does not exist a consumer-oriented product that peanut-sensitive individuals can use to ensure safety of their food.

To address this need, Nima developed a handheld device, Nima™, integrating food processing, allergen detection, results interpretation and data transmission, enabling consumers to readily test for dietary allergens. Recently, the company launched the Nima™ sensor for dietary gluten detection ([nimasensor.com/gluten/](http://nimasensor.com/gluten/)). The newly developed peanut sensor is modeled on the gluten sensor, but was designed to address distinct technical requirements, including not only specificity for peanut allergen but also greater sensitivity. As with the gluten sensor, Nima developed novel proprietary antibodies for use in the peanut sensor. The antibodies were raised against the Ara h3 protein, as Ara h3 is reported to be more stable in processed foods than other peanut proteins (Comstock et al., 2016). The technology platform will eventually be extended, with necessary modifications, to detect other common food allergens or irritants, including tree nuts, dairy products, eggs, soy and many others. Results by users and official testers are reported via the Nima mobile application ([nimasensor.com/nima-app-review-contribute-discover/](http://nimasensor.com/nima-app-review-contribute-discover/)), providing a cloud-based database of food testing results to the community concerned about food allergies.

This report describes results of product testing to evaluate performance of the Nima™ peanut sensor to analyze a wide array of foods, many containing either no peanut or a 10 ppm spiked peanut concentration, by mass, along with some containing higher peanut levels.

## **II. Methods**

### **A. Summary:**

The Nima™ device is composed of a disposable capsule — where sample grinding, extraction and lateral flow immunoassay (LFIA) are performed — inserted into a reusable sensor component that performs the LFIA reading, interpretation and reporting to the user (**Figure 1**). Unless otherwise indicated, foods were tested in the device at 0.300 g, representing small to medium sample sizes. Two different lots of capsules were tested, containing two different batches of the LFIA strip and extraction buffer. The testing plan involved evaluation of parameters including limit of detection (LoD) and stability. Where indicated, foods were also analyzed using a standard peanut enzyme-linked immunosorbent assay (ELISA).



**Figure 1. Nima™ peanut sensor, showing the reusable sensor (left) and the disposable capsule (right).**

### **B. Equipment and Materials:**

Foods tested. Samples of a variety of foods (Appendix, **Table A1**) regarded as being peanut-free were tested, with and without added peanut (see **Procedures**). Of these, 13 foods were spiked prior to cooking and another 42 were cooked first and then spiked. All food preparation was done at Nima. Of these 55 foods, 10 samples each, at each peanut level, were tested. In addition, 7 "as is" foods were tested, without spiking, at 10 samples each. Of these 7 "as is" foods, 2 (peanut butter and Snickers bar) were known to contain peanut and the remaining 5 were confirmed as peanut-free by R-Biopharm RIDASCREEN®FAST Peanut ELISA testing performed by Bia Diagnostics (Colchester, VT, USA). In addition, 30 spices and flours considered to be peanut-free were tested, again at 10 samples each, to assess cross-reactivity. Unless otherwise indicated, testing was performed on 0.300 g samples. Depending on the food, this would be considered a small or medium sample size for the Nima™ device.

Lots tested. Two different capsule lots were tested, each containing different sublots of LFIA strips and buffer. These were LOT1 (LFIA strip lot #1, buffer lot #1) and LOT2 (LFIA strip lot #2, buffer lot #2). The LFIA strip and buffer lots were prepared with the same manufacturing processes, but using different sets of raw materials, including two different lots of proprietary Nima antibodies.

Nima peanut sensors tested. The analyses were performed using a total of 10 different Nima peanut sensors, all with firmware (FW) version 2.4. Each food type was tested on 5 different sensors, with each test replicated twice, for a total of 10 analyses per food sample.

### **C. Procedures:**

Food testing. Across regulatory agencies there is currently no recommended threshold for peanut ppm levels in foods. However, findings presented by the FDA (Threshold Working Group (FDA), 2008) supported a target detection sensitivity for the device of 10 ppm peanut in foods. The designated 55 foods were, therefore, either left at their original 0 ppm or spiked to a level of 10 ppm peanut. For each ppm level, 10 replicates were tested, each at a target weight of 300 mg. As described under **Equipment and Materials**, samples were either prepared in-house and spiked or purchased in a prepared form and spiked. Additional samples (7 "as is" foods, some, such as peanut butter, containing high levels of peanut) were tested without spiking. Results reported by each Nima™ peanut detector were recorded.

Calculations. The Nima™ device reports a binary output, either "Peanut Found", indicating that peanut was detected, or "Smile" indicating that peanut was not detected. Test result outcomes were quantitated in accordance with the following definitions: True Positive (TP), "Peanut Found" reading for a sample containing peanut, as prepared; False Negative (FN), "Smile" reading for a sample containing peanut, as prepared; True Negative (TN), "Smile" for a sample with 0 ppm peanut; False Positive (FP), "Peanut Found" for a sample with 0 ppm peanut. Sensitivity and specificity of the technology were assessed based on the following parameters: True Positive Rate (TPR) =  $TP/(TP + FN)$ ; True Negative Rate (TNR) =  $TN/(TN + FP)$ ; False Negative Rate (FNR) =  $1 - TPR = FN/(TP + FN)$ ; False Positive Rate (FPR) =  $1 - TNR = FP/(TN + FP)$ ; Accuracy =  $(TP + TN)/(TP + FN + TN + FP)$ ; Precision =  $TP/(TP + FP)$ ; Test Error Rate (TER) = Error or No Result / Total Tests. In most cases, these values were converted to percentages (e.g.  $TPR (\%) = [TP/(TP + FN)] * 100\%$ .)

## **III. Results**

### **A. Accuracy and precision in test results:**

**Table 1** shows summary results obtained from 62 foods (Appendix, **Table A1**) for the two lots, as well as data for both lots combined. The results indicated that LOT2 had a higher sensitivity, based on a higher TPR and lower FNR, than did LOT1. However, LOT2 also had a higher FPR than did LOT1. Future production efforts will be aimed at maintaining sensitivity (TPR) and FNR values within the performance parameters observed in this analysis. It should be noted that these performance characteristics are based on a spiking level of 10 ppm. Foods with higher peanut concentrations ( $\geq 21$  ppm), included in the "as is" foods, had a 100% detection rate when tested with both lots.

Test Results: 62 Foods									
	LOT1			LOT2			COMBINED		
	Counts	%	CL (+/-)	Counts	%	CL (+/-)	Counts	%	CL (+/-)
True Positive	552	98.2%	1.09%	557	99.8%	0.35%	1109	99.0%	0.58%
False Negative	10	1.8%	1.09%	1	0.2%	0.35%	11	1.0%	0.58%
True Negative	526	95.5%	1.74%	527	92.8%	2.13%	1053	94.1%	1.38%
False Positive	25	4.5%	1.74%	41	7.2%	2.13%	66	5.9%	1.38%
Accuracy	1078	96.9%	1.03%	1084	96.3%	1.11%	2162	96.6%	0.75%
Precision	552	95.7%	1.66%	557	93.1%	2.03%	1109	94.4%	1.32%

Table 1. Results of food testing with 62 foods.

**B. Accuracy and precision with data from nightshade foods excluded:**

Foods in the nightshade family (e.g. tomato and eggplant) consistently gave FP results with at least one of the two lots tested. This issue is currently under further investigation. In the meantime, the analysis was repeated with those foods excluded (**Table 2**). Interestingly, samples of potato, also in the nightshade family, did not give FP results so were not excluded from this analysis. Potential differences between potatoes and the other nightshade family members will also be investigated. The foods excluded from this analysis are marked in the list of foods tested (see Appendix **Table A1**). Removing data from nightshade foods increased accuracy for both lots to 97.6%.

Test Results: Nightshade Foods Excluded									
	LOT1			LOT2			COMBINED		
	Counts	%	CL (+/-)	Counts	%	CL (+/-)	Counts	%	CL (+/-)
True Positive	532	98.2%	1.13%	541	99.8%	0.36%	1073	99.0%	0.60%
False Negative	10	1.8%	1.13%	1	0.2%	0.36%	11	1.0%	0.60%
True Negative	515	97.0%	1.45%	527	95.5%	1.73%	1042	96.2%	1.14%
False Positive	16	3.0%	1.45%	25	4.5%	1.73%	41	3.8%	1.14%
Accuracy	1047	97.6%	0.92%	1068	97.6%	0.90%	2115	97.6%	0.64%
Precision	532	97.1%	1.41%	541	95.6%	1.69%	1073	96.3%	1.11%

Table 2. Results of food testing with nightshade foods (except for potato) excluded.

Furthermore, certain foods caused more FN responses. These included meatballs and potato pancakes, which were insufficiently ground in the capsule, and Cheerios, which gave relatively low signals on the LFIA strips. Visual inspection confirmed that, in many of the FN cases, the sample was insufficiently ground and/or stuck inside the cap of the capsule, resulting in incomplete peanut protein extraction. This is a known limitation of the Nima™ device, and is most apparent at peanut concentrations near the limit of detection. As the concentration of peanut proteins in the sample is further increased, sensitivity is also increased and the potential for FN results is significantly decreased.

### **C. Cross-reactivity:**

To assess cross-reactivity (**Table 3**), the Nima™ device was used to test a selection of peanut-free foods, consisting of 30 flours and spices (Appendix, **Table A1**). This analysis again identified nightshade foods (e.g. paprika) as well as certain others that appeared to interfere with or be otherwise incompatible with the device, resulting in FP results (see **Table 4**). For most of these, sample preparation was subsequently modified as described in **Table 4**. Instructions for such alternative sample preparation, once finalized, will be provided in product literature. Sesame seeds were not included in the final analysis because they cross-reacted with the Nima™ antibody in both lots, yet were negative by the standard ELISA. This limitation will be included in the product literature. Paprika and cayenne, both nightshade foods, were also removed from this analysis. (However, research to improve assay of such foods is underway (**Table 4**.) The results (**Table 3**) showed cross-reactivity of 9.0% and 7.3%, respectively, for LOT1 and LOT2.

	Cross-reactivity results								
	LOT1			LOT2			COMBINED		
	Counts	%	CL (+/-)	Counts	%	CL (+/-)	Counts	%	CL (+/-)
<b>True Positive</b>	-	-	-	-	-	-	-	-	-
<b>False Negative</b>	-	-	-	-	-	-	-	-	-
<b>True Negative</b>	242	<b>91.0%</b>	3.44%	241	<b>92.7%</b>	3.16%	483	<b>91.8%</b>	2.34%
<b>False Positive</b>	24	<b>9.0%</b>	3.44%	19	<b>7.3%</b>	3.16%	43	<b>8.2%</b>	2.34%

**Table 3. Cross-reactivity results.** The analysis included data from selected peanut-free (non-spiked) foods only, with sesame seeds, paprika and cayenne excluded, as described in the text and shown in Appendix, **Table A1**.

### **D. Test error rate (TER):**

The Nima™ device is designed to report test errors whenever a system error is detected. Normally, inside the disposable capsule, the food sample is ground and mixed with extraction buffer and then the extract is injected onto the LFIA strip to enable performance of the immunoassay for peanut. Thus, conditions such as incomplete grinding or inadequate dilution of a food sample may cause clogging within the capsule or inadequate mixing, resulting in test error messages. In the cross-reactivity analysis (**Table 3**), there were 1.9% errors out of 904 tests. Of these, 70.6% were system errors in which mixing inside the capsule was not completed successfully. **Table 4** summarizes the major foods we've identified as causing such issues. (The remaining errors were caused by buffer ejection and mixing errors, not necessarily dependent on the foods.) Based on observations with these foods, we will recommend to users that flours and spices be tested at a lower weight (150 mg) and diluted 1:1 with water. The limitations with

nightshade foods, including cayenne and paprika, remain under investigation. However, when nightshade foods were included (e.g. **Table 1**), we processed them by dilution in milk, as indicated in **Table 4**.

Sample name	Issue	Recommendation
Cayenne (nightshade)	FP	Dilution 1:1 with milk, still under development
Cinnamon	System error, due to sample clogging	Decreased sample weight
Nutmeg	System error, due to sample clogging	Decreased sample weight, dilution 1:1 with water
Paprika (nightshade)	FP	Dilution 1:1 with milk, still under development
Pumpkin seed	System error, due to sample clogging	Decreased sample weight, dilution 1:1 with water
Red lentil flour	System error, due to sample clogging	Decreased sample weight, dilution 1:1 with water
Sesame seed	FP, cross-reactivity	Cannot be accurately tested
Tamarind	FP, cross-reactivity	Cannot be accurately tested
Turmeric	FP, due to coloring	Decreased sample weight, dilution 1:1 with water

**Table 4. Foods causing system errors or false positive readings in the Nima™ peanut sensor.**

To follow up on the errors observed in the cross-reactivity analysis, the test error rate (TER) was evaluated in the larger collection of food samples, with results summarized in **Table 5**. The results indicated a TER of 3.9% in 4200 tests. More than 75% of these errors were caused by insufficient mixing of the food within the capsule, while 14% were caused by incomplete development of the LFIA strip. Nearly 9% of the errors were caused by capsule assembly issues, as detected by internal system controls. Such system errors result from internal process control tests incorporated into the system design to prevent false but believable results and improve overall test accuracy.

Test Error Rate		
	Counts	%
<b>Insufficient mixing</b>	125	<b>77.16%</b>
<b>Capsule assembly issues</b>	14	<b>8.64%</b>
<b>Incomplete LFIA development</b>	23	<b>14.20%</b>
<b>Total Test Error</b>	162	<b>100.00%</b>
<b>Total Test</b>	4200	<b>3.9%</b>

**Table 5. Test error rate**

### **E. Stability testing:**

The stabilities of LFIA strips, buffers and complete capsules were assessed, by analyzing foods spiked with 10 ppm peanut. This long-term stability testing (9 months) is ongoing. Stability of the reusable sensor component (**Figure 1**) was considered to be equivalent to that in the gluten device (unpublished data) and was not tested further. To test shipping stability, capsules were exposed to either 1, 2, 3 or 6 cycles of temperature extremes. Cycles ranged from 45°C to -5°C and testing was conducted over 7 days with 6 time points. At each time point, capsules were removed from the environmental chamber, food samples added and results analyzed. Evaluated this way, the LFIA system showed no signs of degradation, as compared with its performance on Day 0 (data not shown).

## **IV. Conclusions**

The results of this food testing study indicated successful performance of the Nima™ peanut sensor across a wide variety of food matrices. In particular, the device identified foods with  $\geq 10$  ppm peanut with 97.6% accuracy in both capsule lots tested. Thus, the LoD was at the appropriate target level for assessing food safety for peanut allergic subjects. The study also identified several limitations of the device, primarily certain foods that gave inconsistent or otherwise erroneous results, or test errors. These problematic foods included sesame seeds, tamarind, certain nightshade family members (eggplant, tomato paste, cayenne pepper and paprika), pure turmeric and brightly dyed candies. Some of these, especially flours and spices, caused clogging of the device or similar effects leading to system errors. For these, we propose measures such as smaller sample size and dilution into water. For the nightshade foods, we are investigating potential measures, including dilution into milk, to decrease FP results. Foods that brightly stain the LFIA membrane, such as turmeric, might not be possible to analyze reliably. Limitations on foods that can be analyzed, as well as special considerations for sample preparation, will be provided in the product literature for the Nima™ peanut sensor.

## **V. Acknowledgements**

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## VI. References

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## Appendix

Table A1. List of foods tested with the Nima™ peanut sensor.

<b>Foods tested with and without spiking to 10 ppm</b> (results in <b>Table 1</b> ; foods marked with * were removed for the analysis shown in <b>Table 2</b> ; foods marked with ** were removed for analyses shown in <b>Tables 1 and 2</b> .)		
Almond butter	Donut	Pea soup
Banana bread	* Eggplant	Pesto
Berry jam	French fries	Potato pancake
Bread	Fried chicken	Pumpkin seed
Brownie	Frosting	Sandwich macaroons
Canned tuna	Fudge	**Scrambled egg
Caramel	Garlic french fries	Soy sauce
Cheerios	Garlic powder	Taffy candy
Chili	Granola bar	Tea
Chocolate cookie	Gummy candy	Tikka masala
Chocolate cupcake	Hoisin sauce	Tofu
Chocolate ice cream	Hot sauce	* Tomato paste
Chocolate muffin	Marinade	Tortilla
Chocolate pudding	Meatball	Vanilla cupcake
Cinnamon bun	Milk chocolate	Vanilla ice cream
Coconut milk	Muffin	**Walnut and cashew butter
Cookie	Mushroom	White chocolate
Cream cheese	Oatmeal	White rice
Dark chocolate	Pasta	Yogurt

<b>Foods tested in cross-reactivity analysis, with no spiking</b> (results in <b>Table 3</b> ; foods marked * were removed from the analysis shown in <b>Table 3</b> )		
Almond flour	Fava bean flour	Pecan flour
Black bean flour	Fenugreek	Pistachio flour
Brazil nut	Green pea flour	Pumpkin seed flour
Brown lentil flour	Hazelnut flour	Red lentil flour
Cashew flour	Lima bean flour	* Sesame seed, ground
* Cayenne pepper	Lupin flour	Soy bean flour
Celery seed, ground	Mustard	Turmeric
Chickpea flour	Mustard seed	Walnut flour
Cinnamon	Nutmeg	White bean flour
Cumin	* Paprika	Yellow split pea flour

<b>Foods tested as is, with no spiking</b> (results in <b>Tables 1 and 2</b> )
NOS Energy Drink
Peanut butter
Peanut oil, refined
Peanut oil, unrefined
Snickers Bar
Tai Pei- Pork Egg Rolls
Tai Pei- Pork Egg Rolls Sauce