

## Customer Validation Report for the BioKits RAPID 3-D™ Peanut Test

### 1. Summary/Abstract

In order to compile the data required for the Validation of the *BioKits* RAPID 3-D™ Peanut Test (RPT) Kit, the following experiments were performed and are reported within:

- Sensitivity & specificity
- Cross reactivity & interference
- Robustness study.
- *BioKits* Peanut Assay (ELISA) Kit (PAK) Comparison
- Swab Testing

This report details the findings of the testing for sensitivity and specificity (selectivity) of the kit for six sample matrices (milk chocolate, plain chocolate, breakfast cereal, ice cream, biscuit and milled almond samples). The robustness of the method (broadly as described by Youden & Steiner, Statistical Manual of the AOAC, 1975). Cross reactivity & interference of a group of food commodities identified by FDA. Method comparison between RPT and PAK (Peanut Assay Kit) for retail processed food samples. Swab testing using the same sample extraction solution as the BASK. The samples tested in the RPT were the same as those tested for the PAK AOAC-RI Performance Tested Method 030402 Validation.

The results identified that recovery of peanut spiked into food commodities proved variable both for the sensitivity/specificity and cross reactivity testing and was considered to be matrix dependent. However, good spike recovery was observed for foods prepared ready for consumption.

The RPT is routinely quality control tested using a 10PPM control made from raw, defatted peanut. Using commodities, a range of which are identified in this report, detection levels are shown to be matrix dependent, with levels of less than 5PPM being detected in some instances.

Overall, the sensitivity of the test is set at below 5-50PPM for a negative result and above 5-50PPM for a positive result, dependent on the matrix tested within this trial.

**The RPT is designed to screen for the presence or absence of Peanut.  
It is not intended for quantitative use.**



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Where peanut is known to be present and the RPT is used to monitor levels, it is recommended that customers prepare their own spiking protocol as necessary. If several matrices are to be tested, a spiked “standard/control” will be required for each. This will give a visual comparative for each matrix. The “control” should be tested in accordance with the RPT Product Insert.

The functionality of the RPT is not compromised when performed within the conditions as applied for the robustness testing.

Method comparison testing for all commodities assayed were compliant with the Acceptance Criteria and it is concluded that the RPT offers a reliable qualitative testing method for preliminary screening for the presence of peanut.

## 2. Introduction

In order to obtain the data required for internal Validation of the *BioKits* RAPID 3-D™ Peanut Test (RPT) kit five separate experiments were performed and are reported within.

Note: This report includes an additional test evaluation (Swab Testing) and identifies any method amendments to those described in the Protocol (CD03001D02P).

### ➤ Sample matrices testing:

Sensitivity and specificity of the kit for six sample matrices (milk chocolate, plain chocolate, breakfast cereal, ice cream, biscuit and milled almond samples) were tested by the analysis of multiple replicates of these matrices with and without added peanut extract. Chocolate samples were spiked to contain 50PPM peanut butter. All other samples were spiked to contain 25PPM peanut butter.

### ➤ Cross reactivity & interference:

The possible cross reactivity (false positivity) of a group of food commodities (identified by FDA – as listed in Appendix 1) was evaluated. The possible interference of the same food commodities was also tested by the analysis of multiple replicates of these matrices with added peanut extract.

### ➤ Robustness study:

Robustness of the method, when key variables are changed, were carried out broadly as described by [Youden & Steiner](#)® .

### ➤ *BioKits* RPT versus *BioKits* PAK method comparison (retail, processed foods)

In order to test the efficacy of the new RPT method in detecting peanut content in retail, processed foods, a panel of higher risk products e.g. with “may contain peanuts/nuts” labelling and/or products known from previous testing to contain peanut were compared.



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➤ Swab Testing

Swab testing was carried out in order to test the efficacy of the new RPT for environmental testing. NIST peanut butter extract was used to spike 4 mL of Swabbing solution at various concentrations.

### 3. Method

In order to maintain the “blinded” identities of the samples used in these experiments, they were overseen by Quality . Analytical samples were prepared and coded by one person and analysed by another.

The RPTs were performed as indicated in the *BioKits* RAPID 3-D™ Peanut kit insert (9J985.v01), and analysed in accordance with TBS QC Specification.

The PAK assays were performed as indicated in the *BioKits* RAPID 3-D™ Peanut kit insert (9J578.v06 ) and analysed in accordance with TBS QC Specification.

### 4. Results

#### A. Sensitivity & Specificity

Six food matrices were studied:

Breakfast Cereal  
Ice Cream  
Biscuit  
Plain Chocolate  
Milk Chocolate  
Milled Almond “flour”

The operator extracted and tested each of the prepared sample matrices using one RPT device (Note: chocolate samples were melted at 60°C prior to extraction).

#### Milk Chocolate

The RPT correctly identified all spiked and blank samples

#### Plain chocolate

All three spiked samples were correctly identified  
Two of the three blank samples were correctly identified



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### **Cereal**

All three spiked samples were correctly identified  
Two of the three blank samples were correctly identified

### **Biscuit**

The RPT correctly identified all spiked and blank samples

### **Ice cream**

The RPT correctly identified all spiked and blank samples

### **Milled Almonds**

Two of the three spiked samples were correctly identified  
All three blank samples were correctly identified

## **B. Robustness**

The following extraction conditions were varied (as identified below), for two replicates each of three pre-prepared 0.25g samples:

Soya protein (known high cross reactant)

PAK Negative biscuit crumb

25PPM spiked PAK biscuit crumb

### **Extraction Variables**

#### **Sample weight**

The positive samples were all positive.  
The negative samples were all negative.  
The Soya samples were all negative.

#### **Extraction volume**

The positive samples were all positive.  
The negative samples were all negative.  
The Soya samples were all negative.

#### **Extraction buffer components**

The positive samples were all positive.  
The negative samples were all negative.  
The Soya samples were all negative.



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**Extraction buffer temp**

The positive samples were all positive.  
The negative samples were all negative.  
The Soya samples were all negative.

**Extraction time**

The positive samples were all positive.  
The negative samples were all negative.  
The Soya samples were all negative.

**Extraction motion**

The positive samples were all positive.  
The negative samples were all negative.  
The Soya samples were all negative.

**Reading time**

The positive samples were all positive.  
The negative samples were all negative.  
The Soya samples were all negative.

**Control Set**

The positive control was positive.  
The negative control was negative.  
The Soya sample was negative

**C. Cross reactivity/interference**

A sample of each of the 32 commodities (Appendix 1) was prepared.

**Summary of Results (for detailed results see Appendix 2)**

Spiked samples correctly identified when spiked at 25ppm = >65%

Blank samples correctly identified = >75%



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#### **D. Method Comparison**

A method comparison was carried out in order to test the efficacy of the new RPT method in detecting peanut content. A panel of 39 retail, processed foods (at least 15 at a level of 5PPM peanut or above) was assembled and tested using both the *BioKits* PAK (ELISA) and new RPT test.

In order to improve the likelihood of encountering peanut-containing foods, higher risk products e.g. with “may contain peanuts/nuts” labelling and/or products known from previous testing to contain peanut were compared.

#### **For detailed results see Appendix 3**

When compared to the PAK, the RPT correctly identified all products that had a peanut content above 5PPM. The RPT positively identified three products (Jaffa cakes, jelly lolly & muesli cookies) that were less than 1PPM as quantified in the PAK. All other samples identified as negative in the RPT were reported to be less than 1.2PPM in the PAK.

Given that Dosoy flour was consistently observed to give a positive result in the cross reactivity study (section 5C), products containing Soya, ranging from 100% down to “contains” but not listed as a percentage, were included in this section.

Of the twelve samples tested only one positive sample (Moroccan Style Wrap Kit fajita wrap) was observed in the RPT which was quantified as 0.16PPM in the PAK. Similarly, one of the other samples in the non-soya specific commodities to show as positive in the RPT below 5PPM, was a fajita sauce mix. All other products were negative in the RPT and less than 0.5PPM in the PAK

#### **E Swab Testing**

Testing was carried out in order to test the efficacy of the new RPT for environmental testing of peanut allergen in the *BioKits* Allergen Swabbing kit Swabbing Solution. NIST peanut butter extract was used to spike 4 ml of Swabbing Solution at a levels of 50, 6.25, 3.125, 1.25, 0.5, 0.25, 0.125 & 0 PPM.

All positive samples tested were positive down to level of ~1PPM NIST peanut butter in Swabbing Solution. The negative control was negative.



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## 5. Conclusions / Recommendations

- ◆ The results identified that spike recovery proved variable both for the sensitivity / specificity and cross reactivity testing according to the matrices tested. In particular, for raw ingredients with absorbent properties and high surface area (e.g. flour, rice), low pH etc. However, good spike recovery was observed for foods prepared ready for consumption.
- ◆ Given that the above samples tested in the RPT were the same as those tested for the PAK AOAC-RI Performance Tested Method 030402 Validation, this report concludes the following:

Using commodities, a range of which are identified in this report, detection levels are shown to be matrix dependent, with levels of less than 5PPM being detected in some instances.

Overall, the sensitivity of the test is set at below 5-50PPM for a negative result and above 5-50PPM for a positive result, dependent on the matrix tested within this trial.

**The RPT is designed to screen for the presence of absence of Peanut. It is not intended for quantitative use.**

Where peanut is known to be present and the RPT is used to monitor levels, it is recommended that customers prepare their own spiking protocol as necessary. If several matrices are to be tested, a spiked "standard/ control" will be required for each. This will give a visual comparative for each matrix. The "control" should be tested in accordance with the RPT Product Insert. For future reference the "control" device, once run, may be stored -20°C to maintain the line intensity at point of reading. (Note: The line intensity of the control device may increase as the test dries when left at room temperature). It is advisable to prepare "controls" in duplicate.

- ◆ The RPT is functional within the following conditions:

|                                |                             |         |
|--------------------------------|-----------------------------|---------|
| Sample weight:                 | 0.25g                       | +/- 10% |
| Extraction volume:             | 4 mL                        | +/- 10% |
| Extraction buffer:             | pH7.2                       | - 0.02  |
| Extraction buffer temperature: | 4 - 25°C                    |         |
| Extraction time:               | 1 minute                    | +1      |
| Extraction motion:             | Hand shaken or vortex mixed |         |
| Reading time:                  | 5 minutes                   | +/-2    |



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- ◆ Method comparison testing for all commodities assayed were compliant with TBS Acceptance Criteria and concludes that the RPT offers a reliable qualitative testing method for the preliminary screening for the presence of peanut.

## 6. References/Bibliography

W. J. Youden & E.H. Steiner; Statistical Manual of the AOAC, Pub:AOAC, 1975



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## Appendix 1- Cross reactivity/Interference samples

### Potential Cross-Reactive Proteins

#### Grains (8)

Barley  
Buckwheat  
Wheat  
Wheat gluten  
Rice  
Rye  
Oat  
Corn (maize)

#### Legumes & Vegetables (4)

Soy bean (as "Dosoy" soya flour)  
Pea  
Lima (butter bean)  
Chick pea

#### Seeds (4)

Sunflower  
Pumpkin  
Sesame

Poppy

#### Nuts (10 + Almond)

Almond (6 positive & 6 negative in Section A)  
Brazil  
Cashew  
Chestnut  
Coconut  
Hazelnut  
Macademia  
Pistachio  
Pecan  
Pine kernel  
Walnut

#### Miscellaneous (5)

Milk protein as skim milk powder  
Cocoa  
Lecithin  
Bovine gelatin  
Porcine gelatin



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## Appendix 2 Cross Reactivity Testing; Spike Recovery

| commodity                              | status | Pos / Neg | Repeat<br>Test Pos /<br>Neg | commodity       | status | Pos / Neg | Repeat<br>Test Pos /<br>Neg |
|--|--------|-----------|-----------------------------|-----------------|--------|-----------|-----------------------------|
| <b>Grains</b>                          |        |           |                             |                 |        |           |                             |
| Barley                                 | Blank  | Neg       |                             | Buck wheat      | Blank  | Neg       |                             |
|  | Spike  | Pos       | Pos                         |                 | Spike  | Neg       | Pos                         |
|  | Spike  | Neg       |                             |                 | Spike  | Pos       |                             |
| Wheat                                  | Blank  | Neg       |                             | Wheat<br>gluten | Blank  | Pos       |                             |
|  | Spike  | Pos       |                             |                 | Spike  | Pos       |                             |
|  | Spike  | Pos       |                             |                 | Spike  | Neg       |                             |
| Rice                                   | Blank  | Neg       |                             | Rye             | Blank  | Neg       |                             |
|  | Spike  | Pos       |                             |                 | Spike  | Pos       |                             |
|  | Spike  | Neg       |                             |                 | Spike  | Pos       |                             |
| Oat                                    | Blank  | Neg       |                             | Corn<br>(maize) | Blank  | Neg       |                             |
|  | Spike  | Pos       |                             |                 | Spike  | Pos       |                             |
|  | Spike  | Pos       |                             |                 | Spike  | Pos       |                             |
| <b>Legumes &amp; Vegetables</b>        |        |           |                             | <b>Seeds</b>    |        |           |                             |
| Soy bean<br>(as 'Dosoy'<br>soya flour) | Blank  | Neg       |                             | Sunflower       | Blank  | Pos       |                             |
|  | Spike  | Neg       |                             |                 | Spike  | Neg       |                             |
|  | Spike  | Neg       |                             |                 | Spike  | Pos       |                             |
| Pea                                    | Blank  | Neg       |                             | Pumpkin         | Blank  | Pos       |                             |
|  | Spike  | Neg       | Neg                         |                 | Spike  | Pos       |                             |
|  | Spike  | Pos       |                             |                 | Spike  | Pos       |                             |
| Lima (butter<br>bean)                  | Blank  | Neg       |                             | Poppy           | Blank  | Neg       |                             |
|  | Spike  | Pos       | Neg                         |                 | Spike  | Pos       | Neg                         |
|  | Spike  | Neg       |                             |                 | Spike  | Pos       |                             |
| Chick pea                              | Blank  | Neg       |                             | Sesame          | Blank  | Neg       |                             |
|  | Spike  | Neg       |                             |                 | Spike  | Pos       |                             |
|  | Spike  | Neg       |                             |                 | Spike  | Pos       |                             |
| <b>Nuts</b>                            |        |           |                             |                 |        |           |                             |
| Brazil                                 | Blank  | Neg       |                             | Hazelnut        | Blank  | Pos       |                             |
|  | Spike  | Pos       |                             |                 | Spike  | Pos       |                             |
|  | Spike  | Pos       |                             |                 | Spike  | Pos       |                             |



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|                                  |       |     |     |                 |       |     |     |
|----------------------------------|-------|-----|-----|-----------------|-------|-----|-----|
| Cashew                           | Blank | Neg |     | Macadamia       | Blank | Neg |     |
|                                  | Spike | Pos |     |                 | Spike | Pos |     |
|                                  | Spike | Pos |     |                 | Spike | Pos |     |
| Chestnut                         | Blank | Neg |     | Pistachio       | Blank | Neg |     |
|                                  | Spike | Neg | Neg |                 | Spike | Pos |     |
|                                  | Spike | Neg |     |                 | Spike | Neg | Pos |
| Coconut                          | Blank | Neg |     | Pecan           | Blank | Neg |     |
|                                  | Spike | Neg |     |                 | Spike | Neg | Pos |
|                                  | Spike | Pos |     |                 | Spike | Neg |     |
| Walnut                           | Blank | Neg |     | Pine kernel     | Blank | Pos |     |
|                                  | Spike | Neg | Pos |                 | Spike | Pos |     |
|                                  | Spike | Neg |     |                 | Spike | Pos |     |
| <b>Miscellaneous</b>             |       |     |     |                 |       |     |     |
| Milk protein as skim milk powder | Blank | Neg |     | Bovine gelatin  | Blank | Pos |     |
|                                  | Spike | Neg |     |                 | Spike | Neg |     |
|                                  | Spike | Neg |     |                 | Spike | Pos |     |
| Cocoa                            | Blank | Neg |     | Porcine gelatin | Blank | Neg |     |
|                                  | Spike | Neg |     |                 | Spike | Neg |     |
|                                  | Spike | Neg |     |                 | Spike | Pos |     |
| Lecithin                         | Blank | Neg |     |                 |       |     |     |
|                                  | Spike | Pos |     |                 |       |     |     |
|                                  | Spike | Pos |     |                 |       |     |     |



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### Appendix 3 Panel of 39 retail processed foods

| Status of Peanut Content | Commodity                                | Pos/Neg | ELISA / PPM |
|--------------------------|--|---------|-------------|
| Yes                      | Satay sauce                              | Pos     | >>>         |
| Yes                      | Organic leek cheese & mushroom nut roast | Pos     | 19.5        |
| Yes                      | Roasted nut chewy bars                   | Pos     | >>>         |
| Yes                      | Fresh chicken satay take-away            | Pos     | >>>         |
| Yes                      | Salad bar nut and celery salad           | Pos     | >>>         |
| Yes                      | Nougat                                   | Pos     | >>>         |
| May Contain              | Fresh cornish pasty                      | Neg     | 0.19        |
| May Contain              | Fajita sauce mix                         | Pos     | 0.56        |
| May Contain              | Milk chocolate spread                    | Neg     | 0.29        |
| May Contain              | Milk chocolate break bar                 | Neg     | 0.11        |
| May Contain              | Rock choc chip cookies                   | Neg     | 0.27        |
| May Contain              | Chicken and turkey satay sticks          | Neg     | 1.14        |
| May Contain              | Fresh chicken tikka massala              | Neg     | 0.04        |
| Not Identified           | Jaffa cakes mini roll                    | Pos     | 0.38        |
| Not Identified           | Steak cornish pasty                      | Neg     | 0.16        |
| Not Identified           | Sweet soy and sesame sauce               | Neg     | 0.44        |
| Not Identified           | Fajita sauce mix                         | Neg     | 0.44        |
| Not Identified           | Quiche lorraine                          | Neg     | <<<         |
| Not Identified           | Mushroom & garlic pate                   | Neg     | 0.52        |
| Not Identified           | Kids tangy BBQ cheesy pizza fingers      | Neg     | 0.05        |
| Not Identified           | Stir fry cracked black pepper sauce      | Neg     | <<<         |



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|                |                                       |     |       |
|----------------|---------------------------------------|-----|-------|
| Not Identified | Raisin loaf                           | Neg | <<<   |
| Not Identified | Xmas milk chocolate lollies           | Neg | <<<   |
| Not Identified | Milk chocolate                        | Neg | 0.06  |
| Not Identified | White chocolate                       | Neg | <<<   |
| Not Identified | Groovy chic milk chocolates           | Neg | <<<   |
| Not Identified | Hulk jelly lolly                      | Pos | 0.175 |
| Yes            | Choc Chip Tracker                     | Pos | >>>   |
| Yes            | Crunchy nut clusters                  | Pos | >>>   |
| Yes            | Indonesian Satay sauce                | Pos | >>>   |
| Yes            | Peanut chocolate bar                  | Pos | >>>   |
| Yes            | Peanut mighty chocolate bar           | Pos | >>>   |
| Yes            | Crispy nut cornflakes                 | Pos | >>>   |
| Yes            | Peanut Chocolate ice cream bar        | Pos | >>>   |
| May Contain    | Chocolate coated praline crispy bites | Pos | 1.996 |
| May Contain    | Caramel fruit & Nut slices            | Neg | 0.035 |
| May Contain    | Swiss style muesli fruit and nut      | Neg | 0.502 |
| May Contain    | Mini nut muesli cookies               | Pos | 0.352 |
| Refined From   | Pure ground nut oil                   | Neg | 0.412 |

<<< Less than 1ppm

>>> More than 20ppm



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