

# ***BIOKITS***

## ***RAPID 3-D GLUTEN TEST***

*Cat. No. 901031P*

*CUSTOMER VALIDATION REPORT*

The *BIOKITS* RAPID 3-D™ Gluten Test (RGT, Cat no. 901031P) is designed to detect the presence or absence of gluten in a wide range of cooked and uncooked foods, i.e. a screening test. It is not intended to be used quantitatively or for 100% raw ingredients, whereby the matrix may interfere with the functionality of the test.

The results section of this validation report is divided into two sections.

**Section 4.** (page 8) provides details of the experimental evaluation undertaken to determine the test parameters and establish product claims for the previous version of the RGT which utilised a one step extraction method (Method 1). This information is still included as the majority of the information is still valid and can be applied or compared to the improved product.

**Section 5.** (page 24) provides a validation update detailing investigations to support the NEW Improved Gluten RAPID 3-D test which utilises a new two step extraction methodology (Method 2) for a demonstrated enhancement in test sensitivity.

## **Summary / Abstract**

The *BIOKITS RAPID 3-D™* Gluten Test (RGT, Cat no. 901031P) is designed to detect the presence or absence of gluten in a wide range of cooked and uncooked foods, i.e. a screening test. It is not intended to be used quantitatively or for 100% raw ingredients, whereby the matrix may interfere with the functionality of the test. This validation report details the findings of the experimental evaluation undertaken to determine the test parameters and establish product claims of the RGT.

This report also details the investigations of a **NEW** two step extraction procedure (method 2) which demonstrates an enhanced sensitivity to Gluten when testing food samples. The detection of less than 20 ppm Gluten is demonstrated to be routinely possible even in previously problematic matrices\*.

\*It is important that validation work is performed on each sample matrix to be routinely tested in order to establish the detection level. Note: possible improvement only applies for the testing of food samples and not environmental swabs.

### **1.1 Inter & Intra-assay variability**

No significant variation in test result was evident between operators or between RGT batches.

### **1.2 Sensitivity**

1 ppm (0.0001%) Vital Wheat Gluten protein

### **1.3 Specificity, cross reactivity & interference**

## **Method 1**

**Gluten Free Samples:** Results were as expected with the exception of the following samples: Caramel Powder, Cookie – Apricot & Coconut, Rice Pollard and Ground paprika, unexpected results for which may have been due to possible matrix effects. The Cake mix-chocolate was subsequently found to contain Gluten by ELISA (>50 ppm). Overall >90% detection accuracy.

**Samples of known gluten content:** The RGT successfully detected gluten in samples at the levels of 15.6 and 89.9 ppm. Levels of Gluten <10.9 ppm were not detected in these particular sample types / matrices.

**Cross Reactants:** A panel of potential cross reactants was assessed. No cross reactants were detected except Brown Linseed, although gluten contamination cannot be ruled out. One sample of oats was found to be contaminated.

**Beer / Stout / Lager:** The test is capable of detecting gluten in various beers. Validation of sensitivity of detection is recommended.

**Other Grains:** The RGT can detect both Rye and Barley derived gluten. Validation is recommended for each individual matrix to determine the sensitivity of the test.

## **Method 2**

All samples extracted with the new 2 two step method gave results that were at least the same, in most cases better, than previous testing with method 1. Comparative ELISA results indicate the RGT detected all the samples with a known gluten content down to at least 4ppm.

### **1.4 Spike recovery in representative problematic matrices**

#### **Method 1**

Retail Food Commodities (Spiked 20 ppm): Observed 70% Spike recovery for selected panel. False negatives observed for problematic matrices.

The test detected 10ppm VWG extract spiked into GF Sausage, and GF Biscuit

The test detected 20ppm VWG extract spiked into GF Baby Formula, and GF Yogurt (0.5g sample only)

The test did not detect 50 ppm VWG extract spiked into GF Chocolate Cake

#### **Method 2**

Samples selected are known to be problematic matrices; flours & powders (high surface area), high absorbency and 100% raw ingredient. The results highlight the matrix effect, and demonstrate the ability to detect low levels of gluten. The 2-step extraction has improved the detection of low levels of gluten in the majority of these problematic sample matrices.

### **1.5 Environmental swab testing**

In this series of tests, the RGT is capable of detecting down to the level of 20µg/25cm<sup>2</sup> on certain surfaces.

**2. Contents**

<b>Section</b>	<b>Page</b>
1. Summary.....	3
2. Contents.....	5
3. Materials / Methods .....	6
4. Results – Method 1	
4.1. Inter & Intra assay variability.....	8
4.2. Sensitivity.....	8
4.3. Specificity, cross reactivity & interference.....	9
4.4. Spike recovery in representative problematic matrices.....	19
4.5. Environmental swab testing.....	22
5. Results – Method 2	
5.1. Sensitivity .....	24
5.2. Specificity, cross reactivity & interference .....	25
5.3. Spike recovery in representative problematic matrices .....	26
6. Conclusions and recommendations.....	28

**3. Materials / method**

**3.1 Materials**

Production manufactured batches of RGT Reagents:

Device

Rapid Extraction Buffer Type 3 (4 mL)

Rapid Extraction Buffer Type 4 (1 mL)

Rapid Extraction Buffer Type 5 (3 mL)

Vital Wheat Gluten Extract

The positive (analyte under test) allergen source was extracted Vital Wheat Gluten with known protein content determined by Kjeldahl analysis.

## **3.2 Methods**

### **3.2.1 Method 1 –single step extraction**

RGT's were performed as described below. All variations are reported as applicable in the respective experimental section

Sample volume (ml) / weight (g) = 0.25 & 0.5

Volume (ml) of extraction buffer type 4 = 4.0

Hand Shaken 1 minute

Dip into REB cap or 100µl pipette Sample application

Device Graded after 5 minute incubation at ambient temperature

Test, Overload and Control (TOC) are graded as follows:

High line intensity 5 to low line intensity 1, with no line being 0

### **3.2.2 Method 2 – NEW two step extraction**

RGT's were performed as described below. All variations are reported as applicable in the respective experimental section

Sample volume (ml) / weight (g) = 0.25

Volume (ml) of REB Type 4 = 1.0

Hand shaken 1 minute

Volume (ml) of REB Type 5 = 3.0

Hand shaken 1 minute

Dip into REB cap or 100µl pipette Sample application

Device Graded after 5 minute incubation at ambient temperature

Test, Overload and Control (TOC) are graded as follows:

High line intensity 5 to low line intensity 1, with no line being 0

**4. Results - Method 1, single step extraction**

**4.1 Inter & Intra-assay variability**

Intra-assay variability was evaluated by testing a dilution series of the allergen using two (2) different operators with devices from the same Production batch of RGT. No significant variation in test result was evident between operators or RGT batches.

**4.2 Sensitivity**

Sensitivity (Limit of Detection) of the test, determined by serially diluting the allergen, expressed as parts per million (ppm), is reported as the lowest concentration of the allergen at which the RGT was observed to be POSITIVE.

<b>Test Parameters:</b> • Inter & intra-assay variability • Sensitivity <b>Sample:</b> • Gluten extract	ppm	operator #1	operator #2	Comparison test Device –08
		TOC Sign:DN	TOC Sign:DS	
1000		325	<1<15	1<15
		325	<1<15	
100		535	435	435
		535	425	
10		335	445	235
		445	335	
1		245	<145	<135
		245	<145	
0.1		<145	<145	035
		<145	<135	
0.01		045	035	035
		045	045	
0		045	035	045
		045	045	

**Conclusion:**

Observed line intensities are comparable between device Batches #1 & #2 and between operators. Detects 1 ppm (0.0001%) Gluten protein.

These results (line intensities) represent the SENSITIVITY test parameters of the RGT.



**4.3 Specificity, cross reactivity & interference**

The following experimental conditions evaluated the efficacy of the RGT method in detecting the allergen content in a number of retail commodity sample matrices (raw & processed foods, & raw ingredients) with and without added Gluten extract.

<b>Test Parameter: Selectivity, Cross reactivity &amp; Interference</b>					
<b>Nominal Sample dilution. Further diluted 1/10 where applicable</b>					
<b>Samples listed as GLUTEN FREE. Expected result NEG / Pass</b>					
<b>Sample</b>	<b>Sample size 0.25 or 0.5</b>	<b>TOC @ nominal</b>	<b>STATUS: POS / NEG Pass / Fail</b>	<b>TOC @ 1/10</b>	<b>STATUS: POS / NEG Pass / Fail</b>
custard mix (gluten free)	0.25	045	NEG/Pass		
	0.5	045	NEG/Pass		
bread mix - plain (gluten free)	0.25	04<1	NEG/Pass	032	NEG/Pass
	0.5	000		032	NEG/Pass
bread mix - wholemeal (gluten free)	0.25	050		044	NEG/Pass
	0.5	000			
cake mix - chocolate (gluten free)	0.25	031	NEG/Pass		
	0.5	030			
muesli - fruit and almond (gluten free)	0.25	033	NEG/Pass		
	0.5	032	NEG/Pass		
fruit bar (gluten free)	0.25	031	NEG/Pass		
	0.5	031	NEG/Pass		
buckwheat pancake mix	0.25	033	NEG/Pass		
	0.5	031	NEG/Pass		
fine maize starch	0.25	0<12	NEG/Pass	045	NEG/Pass
	0.5	000			
maize starch	0.25	035	NEG/Pass		
	0.5	035	NEG/Pass		
potato starch	0.25	045	NEG/Pass		
	0.5	045	NEG/Pass		
tapioca starch	0.25	045	NEG/Pass		
	0.5	045	NEG/Pass		
caramel powder	0.25			<143	POS/Fail
	0.5			<153	POS/Fail
biscotti - chocolate chip	0.25	032	NEG/Pass		
	0.5	031	NEG/Pass		
cookie - apricot and coconut	0.25	<141	POS/Fail		

	0.5	15<1	POS/Fail		
crispbread corn	0.25	045	NEG/Pass		
	0.5				
pasta - italian style shells	0.25	031	NEG/Pass		
	0.5	031	NEG/Pass		
pasta - rice and corn spirals	0.25	041	NEG/Pass		
	0.5	031	NEG/Pass		
BBQ flavoured powder	0.25	035	NEG/Pass		
	0.5	035	NEG/Pass		
soy milk powder	0.25	02<1	NEG/Pass	042	NEG/Pass
	0.5	03<1	NEG/Pass	041	NEG/Pass
soy milk powder	0.25	02<1	NEG/Pass	041	NEG/Pass
	0.5	02<1	NEG/Pass	032	NEG/Pass
parboiled rice (type P)	0.25	03<1	NEG/Pass	045	NEG/Pass
	0.5	031	NEG/Pass		
parboiled rice (type R)	0.25	033	NEG/Pass		
	0.5	03<1	NEG/Pass		
rice cakes - flavoured	0.25	042	NEG/Pass		
	0.5	041	NEG/Pass		
rice cakes - plain	0.25	041	NEG/Pass		
	0.5				
whole rice - basmati	0.25	042	NEG/Pass		
	0.5	031	NEG/Pass		
whole rice - brown	0.25	031	NEG/Pass		
	0.5	03<1	NEG/Pass		
whole rice - jasmine	0.25	032	NEG/Pass		
	0.5	031	NEG/Pass		
whole rice - white	0.25	032	NEG/Pass		
	0.5	041	NEG/Pass		
rice flour - fine	0.25	041	NEG/Pass		
	0.5	041	NEG/Pass		
rice flour - medium	0.25	031	NEG/Pass		
	0.5	041	NEG/Pass		
rice grits	0.25	042	NEG/Pass		
	0.5	041	NEG/Pass		
rice bran	0.25	04<1	NEG/Pass	031	NEG/Pass

	0.5	040			
rice pollard	0.25	040		041	NEG/Pass
	0.5	<130	POS/Fail	041	NEG/Pass
cooked white rice - heat & eat (gluten free)	0.25	045	NEG/Pass		
	0.5	045	NEG/Pass		
cooked white rice with meat, vegetables and flavouring - heat & eat (with gluten)	0.25	045	NEG/Fail		
	0.5	045	NEG/Fail		
cake mix - chocolate	0.25	241	POS/Fail		
	0.5	341	POS/Fail		
corn flour (gluten free)	0.25	045	NEG/Pass		
	0.5	035	NEG/Pass		
energy food sticks - chocolate	0.25	031	NEG/Pass		
	0.5	02<1	NEG/Pass		
cake mix - butter (gluten free)	0.25	044	NEG/Pass		
	0.5	043	NEG/Pass		
soup mix - creamy chicken and vegetable (gluten free)	0.25	033	NEG/Pass		
	0.5	033	NEG/Pass		
gravy mix – beef stroganoff (gluten free)	0.25	034	NEG/Pass		
	0.5	033	NEG/Pass		
cake mix – lemon (gluten free)	0.25	034	NEG/Pass		
	0.5	034	NEG/Pass		
biscuits - hi fibre (gluten free)	0.25	043	NEG/Pass		
	0.5	041	NEG/Pass		
biscuits - chocolate coated chocolate (gluten free)	0.25	021	NEG/Pass		
	0.5	02<1	NEG/Pass		
corn and rice snack - cheese supreme (gluten free)	0.25	022	NEG/Pass		
	0.5	011	NEG/Pass		
muesli (gluten free)	0.25	041	NEG/Pass		
	0.5	031	NEG/Pass		
ground paprika 1	0.25	15<1	POS/Fail	042	NEG/Pass
	0.5			051	NEG/Pass
groud paprika 2	0.25	130		042	NEG/Pass
	0.5				
psyllium husk powder (gluten free)	0.25			044	NEG/Pass
	0.5				
vitamin tablets (B group & C)	0.25	044	NEG/Pass		

	0.5	03<1	NEG/Pass	
electrolyte drink powder	0.25	035	NEG/Pass	
	0.5	035	NEG/Pass	
supplementary sports food powder	0.25	033	NEG/Pass	
	0.5	033	NEG/Pass	
supplementary sports food bar	0.25	022	NEG/Pass	
	0.5	022	NEG/Pass	
instant formula powder	0.25	022	NEG/Pass	
	0.5	022	NEG/Pass	
instant baby powder	0.25	021	NEG/Pass	
	0.5	02<1	NEG/Pass	
soya flour (gluten free)	0.25	020		045 NEG/Pass
	0.5	020		034 NEG/Pass
soya flour (gluten free)	0.25	040		052 NEG/Pass
	0.5	050		051 NEG/Pass
textured soy protein (gluten free)	0.25	042	NEG/Pass	
	0.5	041	NEG/Pass	

Results were as expected with the exception of the following samples: Caramel Powder, Cookie – Apricot & Coconut, Rice Pollard, cooked white rice with meat, vegetables and flavouring and Ground paprika, unexpected results for which may have been due to possible matrix effects. The Cake mix-chocolate was subsequently found to contain Gluten by ELISA (>50 ppm)

**Test Parameter: Selectivity, Cross reactivity & Interference**  
**Nominal Sample dilution. Further diluted 1/10 where applicable**  
**Samples of UNKNOWN GLUTEN content.**

Sample	Sample size	TOC @ nominal	STATUS: POS / NEG Pass / Fail	TOC @ 1/10	STATUS: POS / NEG Pass / Fail
Infant formula	0.25	032	NEG / Pass		
Farmhouse Vegetable simmer soup	0.25	223	POS/ Pass		
Peppered Beef Casserole	0.25	243	POS/ Pass		
Jacob's Digestive biscuits for cheese with added wheat grain	0.25	544	POS/ Pass		
Clusters almonds, hazelnuts & whole grain wheat flakes	0.25	444	POS/ Pass		
FAPAS Dry Cake mix test material (2708 A )	0.25	414	POS/ Pass		
FAPAS Dry Cake mix test material (2708 B )	0.25	414	POS/ Pass		
FAPAS biscuit powder test material (2703 A)	0.25	543	POS/ Pass		
FAPAS biscuit powder test material (2703 B )	0.25	534	POS/ Pass		

**Test Parameter: Selectivity, Cross reactivity & Interference**  
**Nominal Sample dilution. Further diluted 1/10 where applicable**  
**Samples of KNOWN GLUTEN content.**

Sample	Sample size	TOC @ nominal	STATUS: POS / NEG Pass / Fail	BIOKITS Gluten ELISA
FAPAS dry bread mix test material (2712 A )	0.25	042	NEG	10.9 ppm
FAPAS dry bread mix test material (2712 B )	0.25	<154	POS	15.6 ppm
FAPAS cake mix test material (2705 A )	0.25	040	NEG	9.5 ppm
FAPAS cake mix test material (2705 B )	0.25	15<1	POS	87.9 ppm

The RGT successfully detected gluten in samples at the levels of 15.6 and 89.9 ppm. Levels of Gluten <10.9 ppm were not detected in these particular sample types / matrices.

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**Test Parameter: Selectivity, Cross reactivity & Interference**  
**Nominal Sample dilution. Further diluted 1/10 where applicable**  
**POTENTIAL CROSS REACTANTS.**

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<b>Sample</b>	<b>Sample size</b>	<b>TOC @ nominal</b>	<b>STATUS: POS / NEG Pass / Fail</b>	<b>Comments</b>
Black eyed bean	0.25	044	NEG / Pass	
Coconut	0.25	043	NEG / Pass	
Kidney bean	0.25	044	NEG / Pass	
Chick pea	0.25	044	NEG / Pass	
Lima bean	0.25	044	NEG / Pass	
Haricot	0.25	044	NEG / Pass	
Puy lentil	0.25	044	NEG / Pass	
Corn (maize)	0.25	043	NEG / Pass	
Pinto	0.25	043	NEG / Pass	
Red split lentil	0.25	044	NEG / Pass	
Soya bean	0.25	044	NEG / Pass	
Green pea	0.25	044	NEG / Pass	
Brazil	0.25	043	NEG / Pass	

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Almond	0.25	043	NEG / Pass	
Pecan	0.25	042	NEG / Pass	
Hazelnut	0.25	042	NEG / Pass	
Macadamia	0.25	042	NEG / Pass	
Cashew	0.25	043	NEG / Pass	
Pistachio	0.25	041	NEG / Pass	
Buckwheat	0.25	044	NEG / Pass	
Pine nut	0.25	041	NEG / Pass	
Rice	0.25	041	NEG / Pass	
Chestnut	0.25	045	NEG / Pass	
Oats Source 1	0.25	044	NEG / Pass	
Oats Source 2	0.25	T43	Pos / Fail	Contamination - Positive in ELISA
Sunflower seeds	0.25	042	NEG / Pass	
Semolina	0.25	544	Pos. / Pass	
Brown linseed	0.25	243	Poss / Fail	Possible contamination
Poppy seed	0.25	045	NEG / Pass	
Sesame seed	0.25	042	NEG / Pass	
Pumpkin seed	0.25	042	NEG / Pass	

Millet seed	<b>0.25</b>	053	NEG / Pass
Milk powder	<b>0.25</b>	044	NEG / Pass
Hazelnut spread	<b>0.25</b>	035	NEG / Pass
Hazelnut oil	<b>0.25</b>	045	NEG / Pass
Almond oil	<b>0.25</b>	045	NEG / Pass
Walnut oil	<b>0.25</b>	045	NEG / Pass

No cross reactants were detected except Brown Linseed, although gluten contamination cannot be ruled out. One sample of oats was found to be contaminated.

**Test Parameter: Selectivity, Cross reactivity & Interference**  
**Nominal Sample dilution. Further diluted 1/10 where applicable**  
**BEERS**

<b>Sample</b>	<b>TOC @ nominal</b>	<b>STATUS: POS / NEG Pass / Fail</b>	<b>Comments</b>
Guinness 1/16 dilution	435	Pos / Pass	
Guinness 1/160 dilution	245	Pos / Pass	
Guinness 1/1600 dilution	T45	Pos / Pass	
Guinness neat	T15	Pos / Pass	
Mackeson stout 1/16 dilution	445	Pos / Pass	
Mackeson stout 1/160 dilution	145	Pos / Pass	
Mackeson stout 1/1600 dilution	045	NEG / Fail*	



Mackeson stout Neat	125	Pos / Pass
Carlsberg 1/16dilution	435	Pos / Pass
Carlsberg 1/160 dilution	145	Pos / Pass
Carlsberg 1/1600dilution	045	NEG / Fail*
Carlsberg Neat	T15	Pos / Pass
Marstons Pedegree 1/16 dilution	445	Pos / Pass
Marstons Pedegree 1/160 dilution	145	Pos / Pass
Marstons Pedegree 1/1600 dilution	045	Neg / Fail*
Marstons Pedegree Neat	125	Pos / Pass
Gluten Free Beer 1/16 dilution	045	NEG / Pass

The test is capable of detecting gluten in various beers.

Fail\* indicates dilution at which gluten is no longer detected in the particular matrix

**Test Parameter: Selectivity, Cross reactivity & Interference**  
**Nominal Sample dilution. Further diluted 1/10 where applicable**

**OTHER GRAINS**

	<b>Sample</b>	<b>Sample size</b>	<b>TOC @ nominal</b>	<b>STATUS: POS / NEG Pass / Fail</b>	<b>Comments</b>
Rye					
1/16	6.25%	0.25	544	POS / Pass	
1/160	0.625%	0.25	445	POS / Pass	
1/1600	0.0625%	0.25	145	POS / Pass	
1/16,000	0.006%	0.25	045	NEG / Fail	
1/160,000	0.0006%	0.25	045	NEG / Fail	
1/1,600,000	0.00006%	0.25	045	NEG / Fail	
Barley					
1/16	6.25%	0.25	531	POS / Pass	
1/160	0.625%	0.25	345	POS / Pass	
1/1600	0.0625%	0.25	145	POS / Pass	
1/16,000	0.006%	0.25	045	NEG / Fail	
1/160,000	0.0006%	0.25	045	NEG / Fail	
1/1,600,000	0.00006%	0.25	045	NEG / Fail	

The RGT can detect both Rye and Barley derived gluten. Validation is recommended for each individual matrix to determine the sensitivity of the test.

4.4 Spike recovery in representative problematic matrices

Test Parameter: • Spike recovery in representative matrices	Gluten extract Spike (ppm)	0.5g TOC	STATUS: POS / NEG Pass / Fail	0.25g TOC	STATUS: POS / NEG Pass / Fail
<b>Sample:</b> GF baby formula	1	021	NEG/Fail	023	NEG/Fail
	5	031	NEG/Fail	033	NEG/Fail
	10	031	NEG/Fail	034	NEG/Fail
	20	<132	POS/Pass	<133	POS/Pass
<b>Sample:</b> GF Chocolate cake	1	041	NEG/Fail	042	NEG/Fail
	5	041	NEG/Fail	042	NEG/Fail
	10	041	NEG/Fail	041	NEG/Fail
	20	041	NEG/Fail	042	NEG/Fail
	35	051	NEG/Fail	054	NEG/Fail
	50	051	NEG/Fail	054	NEG/Fail
<b>Sample:</b> GF Biscuit	1	041	NEG/Fail	042	NEG/Fail
	5	051	NEG/Fail	041	NEG/Fail
	10	<151	POS/Pass	<151	POS/Pass
	20	<151	POS/Pass	<141	POS/Pass

<b>Sample:</b> GF yoghurt	1	023	NEG/Fail	023	NEG/Fail
	5	023	NEG/Fail	023	NEG/Fail
	10	023	NEG/Fail	023	NEG/Fail
	20	<122	POS/Pass	023	NEG/Fail

<b>Sample:</b> GF sausage	0	031	NEG/Fail	042	NEG/Fail
	5	031	NEG/Fail	042	NEG/Fail
	10	<132	POS/Pass	<142	POS/Pass
	20	132	POS/Pass	<132	POS/Pass

Expected result: NEGATIVE for unspiked samples and POSITIVE for spiked samples.  
 The test detected 10ppm VWG extract spiked into GF Sausage, and GF Biscuit  
 The test detected 20ppm VWG extract spiked into GF Baby Formula, and GF Yogurt  
 (0.5g sample only)  
 The test did not detect 50 ppm VWG extract spiked into GF Chocolate Cake

This highlights the matrix effect and the need for specific determination of sensitivity of the test with the matrix in question.

<b>Test parameter:</b> • <b>Problematic Food Commodities, matrices spiked with VWG extract @ 20 ppm</b>	<b>0.5g TOC</b>	<b>STATUS: POS / NEG Pass / Fail</b>	<b>0.25g TOC</b>	<b>STATUS: POS / NEG Pass / Fail</b>
Muesli-fruit and almond (GF)	141	POS/Pass	<143	POS/Pass
Parboiled rice (type P)	<b>035</b>	<b>NEG/Fail</b>	<b>045</b>	<b>NEG/Fail</b>
Maize starch	145	POS/Pass	<145	POS/Pass
<b>Rice bran</b>	<b>031</b>	<b>NEG/Fail</b>	<b>031</b>	<b>NEG/Fail</b>
Cake Mix-Butter (GF)	<143	POS/Pass	<145	POS/Pass
<b>Soya flour (GF)</b>	<b>034</b>	<b>NEG/Fail</b>	<b>034</b>	<b>NEG/Fail</b>
Fruit bar (GF)	<141	POS/Pass	<b>04&lt;1</b>	<b>NEG/Fail</b>
Tapioca Starch	145	POS/Pass	<145	POS/Pass
Rice cakes-flavoured	35<1	POS/Pass	141	POS/Pass
Corn flour (GF)	<145	POS/Pass	<145	POS/Pass

Observed 70% Spike recovery for selected panel. 20 ppm spike was not detected in some problematic matrices. In such instances extra validation guidance should be sought from Tepnel. It may be possible to improve the sensitivity of the test by undertaking further validation work (e.g. increasing incubation times, or extracting from 0.5g of sample). However this should only be performed under guidance.

**4.5 Environmental swab testing**

The transfer of Gluten extract from a range of surfaces onto a swab and detection by RGT was investigated as follows. Three surfaces were assessed: Teflon, Plastic and Stainless Steel. Each of the three surfaces were sectioned into 6 x squares, each with an area of 25 cm<sup>2</sup> using masking tape and labelled A-F. Following mixing, six volumes of the allergen solution was applied (using a micropipette) to the six squares on each of the three surfaces to give final allergen amount of 100, 50, 20, 10, 5 and 0 µg. REB was used for the controls (0 µg/mL). (See table below for volumes). The solution in each square was left to dry before swabbing. This was repeated for each allergen on each surface. Once contaminated and dried the squares were swabbed and tested according to the method.

Gluten Extract(500 µg/mL)	Amount (µg)
100	200 µL
50	100 µL
20	40 µL
10	20 µL
5	10 µL
0	200 µL REB

Test Parameter: Environmental swab testing	Gluten Extract µg / 25 cm <sup>2</sup>	TOC@ 5min	STATUS: POS / NEG Pass / Fail
<b>Surface:</b> Plastic	100	<135	POS/Pass
Sample: Gluten extract	50	<135	POS/Pass
	20	035	NEG/Fail
	10	035	NEG/Fail
	5	035	NEG/Fail
	0	035	NEG/Pass
<b>Surface:</b> Teflon	100	145	POS/Pass

Sample: Gluten extract	50	145	POS/Pass
	20	<145	POS/Pass
	10	045	NEG/Fail
	5	045	NEG/Fail
	0	035	NEG/Pass
<b>Surface: Stainless Steel</b>			
Sample: Gluten extract	100	045	NEG/Fail
	50	045	NEG/Fail
	20	045	NEG/Fail
	10	045	NEG/Fail
	5	045	NEG/Fail
	0	045	NEG/Pass

The following levels were detectable on Plastic, Teflon and stainless steel respectively:  
20, 50 and >100 µg/25 cm<sup>2</sup>.

**5. Results - Method 2, NEW two step extraction**

**5.1 Sensitivity**

Sensitivity (Limit of Detection) of the test, determined by serially diluting the allergen, expressed as parts per million (ppm), is reported as the lowest concentration of the allergen at which the RGT was observed to be POSITIVE.

Direct comparison of one step and two step extraction methods.

<b>Test Parameters:</b>	<b>ppm</b>	<b>Method 1</b>	<b>Method 2</b>
• Sensitivity		<b>TOC</b>	<b>TOC</b>
<b>Sample:</b>			
• VWG extract 1mg/ml (1000 ppm) serially diluted	1000	215	1<15
	100	535	525
	10	245	335
	1	145	145
	0.1	055	045
	0.01	055	045
	0	055	045

**Repeat testing**

<b>Test Parameters:</b>	<b>ppm</b>	<b>Method 1</b>	<b>Method 2</b>
• Sensitivity		<b>TOC</b>	<b>TOC</b>
<b>Sample:</b>			
• VWG extract 1mg/ml (1000 ppm) serially diluted	1000	315	115
	100	535	525
	10	245	345
	1	155	155
	0.1	055	055
	0	055	055

**Comments:** All samples extracted with Method 2 gave results that were at least the same, in most cases better, than samples extracted with Method 1. The LOD is 1 ppm VWG protein

See section 4.1 for information on Inter and Intra assay variability.



**5.2 Specificity, Cross reactivity and Reference Materials**

The following experimental conditions evaluated the efficacy of the new RGT extraction method (method 2) in comparison with the old extraction method (method1) at detecting the allergen content in a number of proficiency samples as well as retail commodity sample matrices (raw & processed foods, & raw ingredients) with (POSITIVE) and without (NEGATIVE) added Gluten extract.

Sample		Method 1	Method 2	ELISA
		TOC	TOC	(ppm)
Baby food; pasta in tomato sauce			433	-
Infant formula (2718 )		032	044	-
Peppered Beef Casserole mix		243	521	-
Digestive biscuits for cheese with added wheat grain		544	313	-
Cereal : almonds, hazelnuts & whole grain wheat flakes		444	314	-
Chocolate cake mix		241	52<1	-
FAPAS biscuit powder test material (2703 A)		543	412	-
FAPAS biscuit powder test material (2703 B )		534	413	-
FAPAS Dry Cake mix test material (2705 A)		051	<151	9.5
FAPAS Dry Cake mix test material (2705 B )		151	351	87.9
FAPAS Dry Cake mix test material (2708 A )		414	312	-
FAPAS Dry Cake mix test material (2708 B )		414	312	-
FAPAS bread mix test material (2712 A )		052	<153	10.9
FAPAS bread mix test material (2712 B )		<153	153	15.6
FAPAS infant cereal test material (2719 B )	Slow to run	<131	142	7.9
FAPAS Dry Cake mix test material (2722 A )		231	431	7830
FAPAS Dry Cake mix test material (2722 B )	Slow to run	<151	150	6.8
FAPAS Dry Cake mix test material (2732A)			521	-
FAPAS Dry Cake mix test material (2732B)			531	-
cheese & chive potato snack			155	10.4
Sample 1			134	27.6
Sample 2			<141	3.7
Sample 3			142	11.9
Sample 4		<141	<142	16.4
Sample 5		041	<142	11.0

**Comments:** All samples extracted with method 2 gave results that were at least the same, in most cases better, than previous testing with method 1. The comparative ELISA results for the CA samples indicate the RGT detected all the samples with a known gluten content down to at least 4ppm.

See section 4.3 for information on cross reactivity.

**5.3 Spike recovery in representative problematic matrices**

Test Parameter: • Representative GLUTEN FREE samples Unspiked & Spiked @ 5 & 10 ppm	VWG extract Spike (ppm)	Method 1	Method 2	Comments
Muesli-fruit and almond (GF)	0		051	
	5		<152	
	10		<142	
Parboiled rice (type P)	0	053	043	
	5	041	<143	
	10	051	<142	
Maize starch	0		045	
	5		045	
	10		145	
Rice bran	0	040	030	Slow to run; sample clogged reservoir
	5	050	<140	
	10	050	14<1	
Cake Mix-Butter (GF)	0		041	
	5		<142	
	10		<142	
Soya flour (GF)	0		041	
	5		04<1	
	10		040	
Fruit bar (GF)	0	050	040	Slow to run
	5	050	<141	
	10	050	131	
Tapioca Starch	0		045	
	5		<145	
	10		145	
Rice cakes-flavoured	0		045	
	5		044	
	10		143	
Corn flour (GF)	0		055	
	5		<145	
	10		<145	

**Test Parameter:**

• Representative GLUTEN FREE samples Unspiked & Spiked @ 5 ,10 and 20 ppm	VWG extract Spike (ppm)	Method 1	Method 2	Comments
GF Baby Formula	5	033	<144	
	10	033	<144	
	20	<133	<144	
GF Plain Chocolate	10	041	<141	
	20	041	141	
	50	041	241	
GF Biscuit	1	043	<143	
	5	043	<143	
	10	042	<143	
GF Hazelnut Yoghurt	5	<133	143	
	10	<133	144	
	20	134	>144	
GF Sausage	1	033	<143	
	5	<132	143	
	10	132	143	
Tomato Ketchup	1	042	043	
	5	<142	143	
	10	<142	243	

**SUMMARY**

Gluten Free Sample matrix	<u>Method 1</u>	<u>Method 2</u>
	Detection level (ppm)	Detection level (ppm)
Parboiled rice (type P)	<b>Not detected (&gt;10)</b>	<b>5</b>
Rice bran	<b>Not detected (&gt;10)</b>	<b>5</b>
Fruit bar (GF)	<b>Not detected (&gt;10)</b>	<b>5</b>
<b>Baby Formula</b>	<b>20</b>	<b>5</b>
<b>Plain Chocolate</b>	<b>Not detected (&gt;50)</b>	<b>10</b>
<b>Biscuit</b>	<b>Not detected (&gt;10)</b>	<b>1</b>
<b>Hazelnut Yogurt</b>	<b>5</b>	<b>5</b>
<b>Sausage</b>	<b>5</b>	<b>1</b>
<b>Tomato Ketchup</b>	<b>5</b>	<b>5</b>

**Comments;** Samples selected are known to be problematic matrices; flours & powders (high surface area), high absorbency and 100% raw ingredient. The results highlight the matrix effect, and demonstrate the ability to detect low levels of gluten. The 2-step extraction has improved the detection of low levels of gluten in the majority of these problematic sample matrices.

See section 4.5 for information on the environmental swabbing validation which still applies for the updated method.

## **6. Conclusions & recommendations**

The RAPID 3-D Gluten Test is suitable to detect gluten in a wide range of cooked and uncooked foods. While every effort has been made to validate as many food types as possible, there may be some foods that are not suitable for testing.

The results indicate that with the addition of the **NEW** two step extraction procedure the RAPID 3-D Gluten test demonstrates an enhanced sensitivity to Gluten when testing food samples. The detection of less than 20 ppm Gluten is demonstrated to be routinely possible even in previously problematic matrices.

**It is important that validation work is performed on each sample matrix to be routinely tested in order to establish the detection level. Users should perform ‘in house’ matrix specific spike recovery validation work in conjunction with a validated laboratory assay e.g. *BIOKITS* Gluten Assay kit to help confirm RAPID 3-D Gluten Test results.**

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