

BIOKITS

RAPID 3-D EGG TEST

Cat. No. 902082Q

CUSTOMER VALIDATION REPORT

1. Summary / Abstract

The RAPID 3-D EGG Test (RET) (Cat.No.902082Q) is designed to detect the presence or absence of **egg white protein** (EWP). Based on highly specific polyclonal antibodies to hen’s egg Ovomuroid (Gal d 1), a relatively heat-stable, allergenic protein purified from egg white, this test employs the principles of lateral flow chromatographic EIA and is a highly sensitive and specific test designed to screen for very low (parts per million; PPM) levels of Egg White Protein (EWP) powder content in raw and cooked food products. This Validation Report details the findings of the experimental evaluation undertaken to determine the test parameters and establish the Performance Characteristics for the suitability for testing a variety of food matrices in RAPID 3-D Egg Test.

1.1 Inter- and intra-assay variability

Observed line intensities are comparable between device Batches -03 & -04 and between operators.

1.2 Sensitivity

Whole Egg Extract: Detects 0.5 ppm powdered whole egg protein extract (<0.00005%) .
The results (line intensity Grades) represent the SENSITIVITY parameters of the RET.

1.3 Specificity / Cross reactivity /Interference

Fresh Whole Hen Egg: Detects 10 ppm (0.001%).

Fresh Hen Egg Yolk: Detects 1000 ppm (0.1%).

Fresh Hen Egg White: Detects <100 ppm (<0.01%).

Commercial Egg Powder: Detects 1 ppm (<0.0001%).

Purified ovomucoid (Trypsin inhibitor type III- 0): Detects 1 ppm (<0.0001%).

The results highlight how the sensitivity of the test is reduced due to the liquid content of the fresh versus powdered egg and therefore the test is more sensitive for the detection of powdered egg.

100% Heterologous egg ingredients.

Duck yolk negative	Duck white positive @10%
Quail yolk positive @ 0.1%	Quail white negative
Goose yolk negative	Goose white negative
Ostrich white negative	

In most instances 100% raw ingredients saturated and/or interfered with the test (wiped out the Control line). In addition, the functionality of the test was compromised at very high raw ingredient concentrations (100% ≡ 10⁶ppm). In most cases, when such samples

were further diluted 1/10, the retest results were as expected. This “matrix effect” is a recognised phenomenon and has been observed with other RAPID 3-D allergen tests. Sample types that fit into this category include 100% high protein raw ingredients, high fat content, flours, finely ground products and products with high absorbance characteristics. In many cases a “real” matrix evaluation can be achieved by **testing the risk matrix at a similar level (%) as would be normally be present in the final product**. Alternately, a spike recovery protocol may be implemented for each particular risk matrix.

Egg Containing Commodities

Of the 25 egg containing commodities tested the RET successfully detected the presence of egg in 22 (88% of samples). Of these 4 samples required additional dilutions in order to avoid the associated matrix effects (Meringue nests, Chicken egg albumin, liquid pasteurised egg albumin and Madeira cake). The matrices where egg was not detected include Baby Food (EWP content found to be 7.95 ppm by ELISA), Egg Noodles and Quiche (although further dilutions were not investigated with this matrix).

NOTE: Egg Noodles should be highlighted as a particular sample type requiring careful validation for use with the RET.

This study also emphasizes the test is not designed to test 100% homologous egg derived ingredients. **The RET is most appropriate for the detection of powdered egg as an ingredient in food commodities. All other samples should be validated on a matrix specific basis.**

1.4 Spike recovery in representative matrices

The RET detected 1ppm EWP from Whole Egg extract spiked into margarine, biscuit crumb and tomato ketchup, and 5ppm EWP from Whole Egg extract spiked into Thai fish style cakes, vanilla flavoured ice cream and plain chocolate.

NOTE: Ice Cream and Plain Chocolate should be highlighted as a particular sample type requiring careful validation for use with the RET.

1.5 Robustness

The robustness parameters tested concludes the following conditions do not compromise the functionality of the RET:

Sample weight or volume	0.25 g or ml	± 10%
Extraction Buffer volume	4 ml	± 5%
Extraction Buffer temperature	Ambient	or 2-8°C
Sample extraction time	1 minute	± 30 seconds
Sample extraction motion	Hand shaken or Whirly-mix	
Incubation time before Grading	5 minutes	± 2 minutes

1.6 Environmental Swab Testing

The RET detected residue swabbed from plastic, Teflon and stainless steel surfaces contaminated with 5µg Whole egg extract. It is anticipated that not all the extract residue would be transferred to the swab, so the test detected ≤5ppm EWP.

1.7 Standard Reference Material

The RET is able to detect low levels of egg white protein in reference materials, as identified by the results obtained from FAPAS sample analysis (7.6 ppm EWP).

1.8 Method comparison Egg ELISA kit parallel testing (Cat.No.902072T)

The observed (corrected) data confirms the RET extraction procedure is at least comparable to the ELISA extraction procedure. The RET was able to detect levels of EWP from certain matrices down to the level of 4.6 ppm EWP. This qualifies the use of the RET as an initial screening test.

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3. Materials / method

3.1 Materials

Production manufactured batches of RET Reagents:

Device 502404Z-03/-04

Rapid Extraction Buffer 502345P-18

The positive (analyte under test) allergen source is whole egg powder extracted in Tris (hydroxymethyl) methylamine 0.05M / NaCl 0.2M pH8.5 and protein content determined using the Kjeldahl method.

Whole Egg Extract 403608K

Egg ELISA kit 902072T

Equipment Identified as required

3.2 Method

RET'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 extraction buffer = 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

4. Results

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 RET.

Inter-assay variability was evaluated by testing a dilution series of the allergen in duplicate with devices from two (2) separate Production batches of RET.

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 RET was observed to be POSITIVE.

Test Parameters:		Operator #1	Operator #2	STATUS:	
• Intra-assay variability	ppm	TOC	TOC	POS / NEG	Comments
• Sensitivity					
Sample:					
KA Egg extract 1mg/ml (1000 ppm)	1000	205	205	POS	
serially diluted	100	315	215	POS	
	10	245	235	POS	
	1	145	235	POS	
	0.5	T45	135	POS	
	0.1	T45	045	NEG	
	0	045	045	NEG	

Conclusion:

Observed line intensities of Batch 502404Z-03 devices tested by 2 different operators are comparable. Detects 0.5 ppm (<0.00005%) Egg protein.

The results (line intensities) represent the SENSITIVITY test parameters of the RET.

The RET was found to detect powdered whole egg protein extract down to the level of 0.5 ppm

Status: Complies with Acceptance criteria

Test Parameters: • Inter assay variability • Sensitivity	ppm	502404Z-03		502404Z-04		STATUS: POS / NEG	Observed score range between operators & batches, run on different days
		Dev	QC	Dev	QC		
Sample: KA Egg extract 1mg/ml (1000 ppm) serially diluted	1000	205	215	315	2<15	POS	T 2,3 O 0,1,4 C 4,5
		205		244			
	100	315	235	225	325	POS	T 2,3 O 1,2,3 C 5
		215		235			
	10	245	235	245	345	POS	T 2,3 O 3,4 C 5
		235		345			
	1	145	145	<155	135	POS	T 1,2 O 3,4,5 C 5
		235		<145			
	0.5	<145	<145	<155	<135	POS	T 1 O 3,4,5 C 5
		135		<155			
	0.1	<145		055			
	0	045	045	045	035	NEG	T 0 O 3,4,5 C 5
		045		055			

Conclusion:

Observed line intensities of Batches 502404Z-03 & -04 devices tested are comparable. Detects 0.5 ppm (<0.00005%) Egg protein.

The results (line intensities) represent the SENSITIVITY test parameters of the RET.

A serial dilution of the analyte of a known protein concentration is the recommended format against which other RET results may be referenced.

Status: Complies with Acceptance criteria

4.3 Specificity, cross reactivity & interference

The efficacy of the RET method in detecting the allergen content in a number of retail commodity sample matrices (raw & processed foods, & raw ingredients) with and without added Egg extract was evaluated. Products include cholesterol-free and fat-free egg products, pre-cooked frozen and refrigerated entrees, pre-cooked frozen scrambled and diced eggs, refrigerated and frozen liquid pasteurised eggs and scrambled egg mixes, and peeled hard-cooked eggs.

Fresh and powdered eggs

	ppm mg/ml %	1,000,000 1000 100	500,000 500 50	100,000 100 10	10,000 10 1	1000 1 0.1	100 0.1 0.01	10 0.01 0.001	1 0.001 0.0001	0.5 0.0005 0.00005	0.1 0.0001 0.00001	STATUS
		TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	
Purified Ovomucoid (Trypsin Inhibitor type III – O)		035^		025^	135	235	335	135	<145	045		POS
Conalbumin 1mg/ml						245	<145					POS
Lysozyme 10mg/ml					145	<145						POS
Egg powder						135	235	125	<145	045	045	POS
Fresh hen whole egg				1<15	225	345	145	<145	045		045	POS
Fresh hen yolk		10<1		204	315	<135	035					POS
Fresh hen egg white		045^		145	245	245	145					POS
Fresh Casein free hen egg yolk		10<1		203	215	<125						POS
Fresh Casein free hen egg white		045^		145	235	334	<145					POS
Fresh Casein free hen egg shell		135										POS
Fresh Casein free hen egg shell washed		235										POS
Whole Hen Egg Shell unwashed		035^		255	355	345	<155					POS

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Whole Hen Egg Shell washed	255									POS
Fresh duck yolk	001^		005^	015^	035	035				NEG
Fresh duck egg white	<135		<135	035	035					POS
Fresh duck egg shell	235									POS
Fresh duck egg shell washed	<135									POS
Fresh quail yolk	00<1		<104	1<15	<115	025				POS
Fresh quail egg white	045									NEG
Fresh quail egg shell	055									NEG
Fresh quail egg shell washed	<145									POS
Fresh goose yolk	001^		0<13^	025	035	045				NEG
Fresh goose egg white	035									NEG
Fresh goose egg shell	045									NEG
Fresh goose egg shell washed	035									NEG
Fresh ostrich egg white	055									NEG
Egg albumin powder	005^		025^	135	235	345	235	155		POS
Egg yolk powder	000^		103	215	135	<135				POS
Powdered egg	001^		105	215	235	135	<135	035		POS
Commercial dried egg (13g + 70ml liquor equivalent to one whole egg)	000^		003^	115	335	245	<145	<145		POS

^ Matrix effect

The results highlight how the sensitivity of the test is reduced due to the liquid content of the fresh versus powdered egg and therefore the test is more sensitive for the detection of powdered egg. This study also emphasizes the test is not designed to test 100% homologous egg derived ingredients. The RET is most appropriate for the detection of powdered egg as an ingredient in food commodities. All other samples should be validated on a matrix specific basis.

Egg Containing Commodities

ppm mg/ml %	1,000,000 1000 100	500,000 500 50	100,000 100 10	10,000 10 1	1000 1 0.1	100 0.1 0.01	10 0.01 0.001	1 0.001 0.0001	0.5 0.0005 0.00005	0.1 0.0001 0.00001	STATUS
	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	
Malted cocoa drinks (Ovaltine)	1<12		125								POS
Meringue nests	025^	<135	245	345	345	<145					POS
Baby food a) Heinz cauliflower cheese,pasta & potatoes –contains Egg	035	035	035	045	045	Found to contain 7.95 PPM EWP by ELISA					FALSE NEG
Puff pastry steak & Kidney pie	145										POS
Salad dressing (creamy)-caesar	205	105	<125	035	045						POS
Egg papadel past	4<15										POS
Egg noodles-Asda medium noodles	024	024	035	045		Found to contain >20 PPM EWP by ELISA Problematic Matrix with RET					FALSE NEG
Eggnog Oooymans advocaat	201	302	314	345							POS
Hollandaise sauce	105										POS
Lemon curd	215	3<15	135	045							POS
Tartar sauce	205										POS

Bernard Matthews Southern Fried Crispy Chicken	<124					Found to contain 18.75 PPM EWP by ELISA				POS
Scotch egg – sausage meat only	1<15									POS
Scotch egg sausage meat and breadcrumbs	244					Found to contain >20 PPM EWP by ELISA				POS
Scotch egg-whole(egg and meat)	<101									POS
Scotch egg-egg only	<101		115							POS
Quiche Lorraine	015	Further dilution required – possible overload				Found to contain >20 PPM EWP by ELISA				FALSE NEG
Cottage Pie	035					Found to contain <1ppm EWP by ELISA				NEG
Chicken Egg Albumin					045^	155	255	155	055	POS
Liquid pasteurised egg albumin (cholesterol & fat free)	035^		145	445	345	145	035			POS
Fox's Viennese biscuits	205	115	<145	045						POS
Egg custard filling	102									POS
Madeira cake	0<15^	0<15	315	035		Found to contain 12.28 PPM EWP by ELISA				POS
Turkish Delight (May Contain Egg)	0T4^	014	014	035						PASS

^ Matrix effect

Of the 25 egg containing commodities tested the RET successfully detected the presence of egg in 22 (88% of samples). Of these 4 samples required additional dilutions in order to avoid the associated matrix effects (Meringue nests, Chicken egg albumin, liquid pasteurised egg albumin and Madeira cake). The matrices where egg was not detected include Baby Food (EWP content found to be 7.95 ppm by ELISA), Egg Noodles and Quiche (although further dilutions were not investigated with this matrix). **NOTE:** Egg Noodles should be highlighted as a particular sample type requiring careful validation for use with the RET.

Egg Free Commodities / Cross reactivity

	ppm mg/ml %	1,000,000 1000 100	500,000 500 50	100,000 100 10	10,000 10 1	1000 1 0.1	100 0.1 0.01	10 0.01 0.001	1 0.001 0.0001	0.5 0.0005 0.00005	0.1 0.0001 0.00001	STATUS
		TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	
Chicken serum albumin						055	055	055				NEG
Turkey Egg Albumin						055						NEG
Turkey serum						055						NEG
Lecethin		025										NEG
Legumes / vegetables												
Soy bean		015^		035	035	045	045	045				NEG
Pea		014^		035	045	035						NEG
Chick pea		115^		035	045	045						NEG
Haricot bean		<1<15^	0<11	044								NEG
Adzuki beans		025										NEG
Kidney bean		<1<15^	023	045	044							NEG
Lima bean		<1<15^	014	045								NEG
Black-eyed bean		<1<14^	0<12	044								NEG
Milks												
Cow milk raw		024										NEG
Cow milk pasteurised		024										NEG
Sheep milk		033										NEG
Goat milk pasteurised		014^										NEG
Goat milk raw		024										NEG
Whey protein in infant formula (15g in 100ml water)		045										NEG
Baby rice (made up as per instructions)		045										NEG

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Coconut milk	021^										NEG
Barley	0<13^	034	034								NEG
Buckwheat	0<14^	0<1<1	044								NEG
Wheatgerm	100^	1<12	014	035	035						NEG
Vital Wheat gluten	015^		045								NEG
Oatmeal	044										NEG
Corn	023^										
Nuts											
Brazil nut	022^										
Cashew	000^	000^	023^	045							NEG
Desiccated Coconut	031^	024	035	035							NEG
Almond	<1<15^	0<14^	013^	035							NEG
Peanut	00<1^	0<15^	025	035							NEG
Pecan	<1<11^	015^	035	045	045						NEG
Pine kernel	<1<14^		024^	045							NEG
Pistachio	0<11^	0<11^	023^	035							NEG
Walnut	002^		024^	045							NEG
Macadamia	015^										
Hazelnut	<1<14^		033^	045							NEG
Seeds											
Sunflower	0<14^		024^	045							NEG
Pumpkin	0<14^		034	045							NEG
Sesame	025										NEG
Poppy	035										NEG
Millet	023^		035	045							NEG
Linseed	<125^		034	035							NEG
Pork	025										NEG

^ Matrix effect

Again the functionality of the test was compromised at very high raw ingredient concentrations. Samples of this type should be tested at the final concentration they are found in the final product.

Miscellaneous

	ppm mg/ml %	1,000,000 1000 100	500,000 500 50	100,000 100 10	10,000 10 1	1000 1 0.1	100 0.1 0.01	10 0.01 0.001	1 0.001 0.0001	0.5 0.0005 0.00005	0.1 0.0001 0.00001	STATUS
		TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	
HiSoy												
DoSoy		000^		015^	035							NEG
Cocoa (as powder)		000^	000^	0<10^	035							NEG
Cocoa (made up into drink as per instructions)		024										NEG
Lecithin		025										NEG
Porcine gelatin		045										NEG
Pasta		045										NEG
Noodles –Amoy straight to wok noodles		045										NEG
Yoghurt		044										NEG
Dark Chocolate		023										NEG
Milk Chocolate-dairy milk		035										NEG
Chicken Flavour Instant Noodles		045										NEG
Pancakes		015										MATRIX EFFECT
Biscuit		034										NEG
Breakfast Cereal		023										NEG
Cauldron Mushroom Burger (Vegetarian product)		<134					Possible Egg Contamination					FALSE POS
Sandwiches- cheese & tomato –no mayo		045										NEG

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pasta Bolognese –no egg visible on label	035										NEG
Chocolate cake mix	034										NEG
Batters (Pancake batter mix)	015^		045	045							NEG
Peppercorn sauce (pre-prepared cooking sauce)	035										NEG
Bouillon (in restaurants to clear it)											
Fairy cakes	<104^	005^	0<15^	035	045						NEG
Meatballs	055										NEG
thick sausages	025										NEG
Sherbets (sherbert fountain)	055										NEG
Tomato soup (asda smart price)	015^	025	035	045	045						NEG
Cream of Chicken soup	045										NEG
Chocolate mousse	0<13^		025								NEG
Cocoa (made up into drink as per instructions)	024										NEG
Soya Grits	002^	002^	013^	045							NEG
Custard (Egg Free)	024	035									NEG
Salad cream	4<14										POS
Truffles	024										NEG
Marshmallows	045										NEG
Coconut mushrooms	124					Possible Egg Contamination				POS	
Cheese	045										NEG
White Fish	035										NEG

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Mini duck and Orange Pate	044										NEG
Trifle sponge cake	015^	035	035								NEG
Pork, Ham and Apricot sausages	025										NEG
Turkey Fillet	015^		035								NEG
Duck Filet	015^		035								NEG
Horse Meat	025		035								NEG
Lamb	025										NEG
Haggis	044										NEG
Egg Free Omelette Mix	<134					Possible Egg Contamination					POS
Organic chocolate Spread	0<1<1^		035								NEG
Egg Free Houmous	001^		025								NEG
Spicy Pinto Bean Dip	0<1<1^		035								NEG
Fish Free Fish Style steaks-Egg Free	0<11^		035								NEG
Falafel-Egg Free	034										NEG
Thai Fish Style cakes-Egg Free	045										NEG
Tempeh Rashers	013^		013^	045							NEG
Cocoa drink (made up as per instructions)	024										NEG
Tiger king prawn	<134^ 144^		035								NEG
Fish stock	035			045							NEG
Whey protein	055										NEG
Soya drink	<134										
Soya PP500F	000^		023^	045							NEG
Soya	000^		013^	045							NEG
Roller dried sodium caseinate	022^										
Rennet casein	045										NEG

Whey protein	132 [^]										
Sodium caseinate	Void too viscous		033								NEG
Roller dried calcium caseinate	000 [^]		044								NEG
Spray dried sodium caseinate	000 [^]		034								NEG
Calcium caseinate	000 [^]		033								NEG
Spray dried casein	000 [^] 000 [^]		044								NEG
BLG	035										NEG

[^] Matrix effect

4.4 Spike recovery in representative problematic matrices

Test parameter: Spike recovery in representative negative (egg free) matrices	Results of matrix testing to determine negative (baseline) response					Spike recovery results					
	Ref 100% 0.25g sample + 4 ml REB	Ref 50%	Ref 10%	Ref 1%	Ref 0.1%	mg/ml ppm %	0.05 50 0.005	0.02 20 0.002	0.01 10 0.001	0.005 5 0.0005	0.001 1 0.0001
	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC	TOC
Margarine melted	035					235	245	145	145	135	
Margarine ambient	035										
Comments:	Reference testing meets Acceptance Criteria. Whole egg protein spike detected at 1ppm										
Biscuit crumb	013	045				434	444	234	134	<124	
Comments:	Reference testing indicates slight matrix effect. Meets Acceptance Criteria when diluted 1/2. Whole egg protein spike detected at 1ppm										
Tomato sauce	034					324	334	223	123	<123	
Comments:	Reference testing meets Acceptance Criteria. Whole egg protein spike detected at 1ppm										
Thai fish style cakes	034					134	144	<134	<134	034	
Comments:	Reference testing meets Acceptance Criteria. Whole egg protein spike detected at 5 ppm										
Ice cream vanilla flavoured	003		0<15	035	045	203	103	<1<14	<1<14	003	
Ice cream vanilla flavoured @1/10						025	025	025	025	025	
Comments:	Reference testing indicates matrix effect. Meets Acceptance Criteria when diluted to 1%. Spike recovery detected at 5ppm but matrix affects the functionality of the overload line. When spike sample diluted 1/10 the overload line returns. This indicates that the Whole egg protein spike can be observed in the matrix, but the matrix should be identified as problematic.										
Recommendation:	When testing ice cream products users should evaluate matrix effect										
Plain chocolate grated not melted	0<1<1	011	045								
Plain chocolate melted and warmed to aid mixing with REB	<1<1<1	0<11	023	055		1<11	<11<1	<1<12	>112	<11<1	
Plain chocolate grated not melted @1/10						<133	034	023	035	035	
Comments:	Reference testing indicates matrix effect. Meets Acceptance Criteria when grated and diluted to 10%, and at 1% when melted and warmed to aid mixing. This suggests that the spike material was adsorbed by the un-melted chocolate and absorbed by the melted chocolate. Unless dealing with surface contamination, in the case of a real time sample if egg was present it would be absorbed within the matrix. Spike recovery at all levels was undetected for 100% samples, and only slightly at 50ppm when further diluted 1/10. However, this is effectively equivalent to 5ppm. The matrix affected the overload line. When spiked sample was diluted 1/10 the overload line returned. This indicates that the Whole egg protein spike can be observed in the matrix, but the matrix should be identified as problematic.										
Recommendation:	When testing plain chocolate products users should evaluate matrix effect										

4.5 Robustness

The robustness of the method (adapted from Youden & Steiner, Statistical Manual of the AOAC, 1975), was tested. The variations are in some instances, designed to exacerbate the assumed effect of the variation e.g. a low sample weight for a Positive sample, but high weight for a potential false Positive sample. Prepare and test samples as described below for each variable under test.

Test Parameter: • Test Robustness	Reference parameter	Test parameter	Sample volume / weight	Volume of extraction buffer	TOC	STATUS: POS / NEG Pass / Fail
Positive sample (ref)			0.25g	4 ml	<104	POS/Pass
Negative sample (ref)			0.25g	4 ml	035	NEG/Pass
Sample weight (A)						
Positive sample A (PA)	PA = 0.25g					
Positive sample a +		= PA+10%	0.275g	4 ml	<104	POS/Pass
Positive sample a -		=PA-10%	0.225g	4 ml	<105	POS/Pass
Negative sample A (NA)	NA = 0.25g					
Negative sample a +		= NA+10%	0.275g	4 ml	034	NEG/Pass
Negative sample a -		= NA-10%	0.225g	4 ml	045	NEG/Pass
Extraction Buffer volume (B)						
Positive sample B	PB = 4mL					
Positive sample b +		= PB+5%	0.25g	4.2ml	<104	POS/Pass
Positive sample b -		= PB-5%	0.25g	3.8ml	<104	POS/Pass
Negative sample B	PB = 4mL					
Negative sample b +		= NB+5%	0.25g	4.2ml	045	NEG/Pass
Negative sample b -		= NB-5%	0.25g	3.8ml	045	NEG/Pass
Extraction buffer temp (C) Sample further diluted 1/10 as previous samples observed as strong overload						
Positive sample C	Ambient		0.25g	4ml	(Ref) 215	POS/Pass
Positive sample c		c = 2-8°C	0.25g	4ml	215	POS/Pass
Negative sample C	Ambient		0.25g	4ml	(ref)	
Negative sample c		c = 2-8°C	0.25g	4ml	035	NEG/Pass
Extraction time (D)						
Positive sample D	1 minute					
Positive sample d +		=PD+30sec	0.25g	4ml	215	POS/Pass
Positive sample d -		=PD-30sec	0.25g	4ml	215	POS/Pass
Negative sample D	1 minute					
Negative sample d +		=ND+30sec	0.25g	4ml	045	NEG/Pass
Negative sample d -		=ND-30sec	0.25g	4ml	045	NEG/Pass
Extraction motion (E)						
Positive sample E	Hand shaken		0.25g	4ml		
Positive sample e		Whiry-mix	0.25g	4ml	2<15	POS/Pass
Negative sample E	Hand shaken		0.25g	4ml		
Negative sample e		Whiry-mix	0.25g	4ml	034	NEG/Pass
Reading time (F)						

Positive sample	F	5 minutes					
Positive sample	f +		=PF+2min	0.25g	4ml	215	POS/Pass
Positive sample	f -		=PF- 2min	0.25g	4ml	2<15	POS/Pass
Negative sample	F	5 minutes					
Negative sample	f +		=PF+2min	0.25g	4ml	034	NEG/Pass
Negative sample	f -		=PF- 2min	0.25g	4ml		
Comments: Complies with Acceptance Criteria							

The robustness parameters tested concludes the following conditions do not compromise the functionality of the RET:

Sample weight or volume	0.25 g or ml	± 10%
Extraction Buffer volume	4 ml	± 5%
Extraction Buffer temperature	Ambient	or 2-8°C
Sample extraction time	1 minute	± 30 seconds
Sample extraction motion	Hand shaken or Whirly-mix	
Incubation time before Grading	5 minutes	± 2 minutes

4.6 Environmental swab testing

The transfer of Egg extract from a range of surfaces onto a swab and detection by RET was investigated. 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 cm². 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. 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	Egg Extract µg /25cm²	TOC	STATUS: POS / NEG Pass / Fail
Surface: Plastic Sample: Whole Egg extract	100	345	POSS/Pass
	50	245	POSS/Pass
	20	155	POSS/Pass
	10	155	POSS/Pass
	5	155	POSS/Pass
	0	055	NEG/Pass
Surface: Teflon Sample: Whole Egg extract	100	345	POSS/Pass
	50	245	POSS/Pass
	20	155	POSS/Pass
	10	155	POSS/Pass
	5	155	POSS/Pass
	0	055	NEG/Pass
Surface: Stainless Steel Sample: Whole Egg extract	100	245	POSS/Pass
	50	155	POSS/Pass
	20	155	POSS/Pass
	10	155	POSS/Pass
	5	<155	POSS/Pass
	0	055	NEG/Pass

Comments: Whole Egg Extract residue detected on all surfaces at all levels tested.

4.7 Reference Material

A recent FAPAS Proficiency sample (2732 Egg in Cake Mix) and the National Institute of Standards and Technology reference material – Whole Egg Powder 8415 were assessed by the RET.

Test Parameter: Standard Reference Material	Egg Extract Sample %	TOC	STATUS: POS / NEG Pass / Fail
100% Sample: NIST Whole Egg Powder 8415 TBS Part No.401270H	100	000	OVERLOAD
	10	104	POSS/Pass
	1	225	POSS/Pass
	0.1	135	POSS/Pass
	0.01	<145	POSS/Pass
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100% Sample FAPAS Proficiency Test 2732A Cake Mix Assigned value for the <i>BIOKITS</i> Egg Assay Kit = 7.6 ppm egg white protein	100	<102	POSS/Pass
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100% Sample FAPAS Proficiency Test 2732B Cake Mix Assigned value for the <i>BIOKITS</i> Egg Assay Kit = not detected	100	0<13	NEG/Pass
	10	023	
	1	024	
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The RET is able to detect low levels of egg white protein in reference materials, as identified by the results obtained from FAPAS sample analysis (7.6 ppm EWP).

4.8 Method Comparison

A panel of non-egg and egg containing sample matrices was tested in parallel in the RET and the *BIOKITS* Egg ELISA kit to evaluate:

Sensitivity and Extraction efficiency

Extract sample and test in RET as per Insert (DRAFT)

Dilute extracted sample in Egg ELISA assay Diluent. Assay as per current Insert

Assay: *BIOKITS* Egg Assay Kit Lab temp: 22.5°C

STANDARD	CONC. (ng/ml)	LOT NO.
1	1	502346M
2	2	502347K
3	5	502348H
4	10	502349F
5	20	502333W

REAGENT	TYPE	LOT NO.
PLATE	Anti-ovomuroid	502334U
BIOTIN	Anti-ovomuroid Bio	502336Q
ENZYME	Avidin HRP	502087K70
DILUENT	Type 8	502269D26
SUBSTRATE	TMB	502266K51
STOP	Acid	502205G50A
Spike Control	Egg	502335S

REAGENT	TIME (min)	VOLUME (µl)	SHAKEN
SAMPLE	30	100	Yes
ENZYME	15	50	Yes
BIOTIN	15	50	Yes
TMB	15	100	No
STOP	N/a	50	N/a

CONTROLS: assayed in duplicate

Zero (working diluent 8)
Standards 1-5
Spike Control
NSB

ELISA Sample prep:

Extracted & tested as per ELISA Insert
(Total dilution 1/100)

Sample prep:

Extracted as per RAPID Insert (1/16) then dilute in assay diluent (1/10) and tested as per ELISA Insert
(Total dilution 1/160, 1.6 standard curve factor adjustment required)

Assay :	Egg					
Purpose:	Rapid Egg Test validation					
Stds ppm	Rep 1	Rep 2	Mean	stdev	% cv	ppm
0	0.113	0.096	0.105	0.0120	11.5	
0.5	0.313	0.319	0.316	0.0042	1.3	
1	0.571	0.567	0.569	0.0028	0.5	
2	0.995	0.977	0.986	0.0127	1.3	
5	2.221	2.220	2.221	0.0007	0.0	
10	3.146	3.298	3.222	0.1075	3.3	
control	0.885	0.885	0.885	0.0000	0.0	1.70
SB	0.076	0.075	0.076	0.0007	0.9	0.00

Extracted as per ELISA (Total dilution 1/100)						
Quiche	3.248	3.358	3.303	0.0778	2.4	>10
Southern Fried Crispy Chicken	3.124	3.182	3.153	0.0410	1.3	9.47
Scotch egg	3.606	3.421	3.514	0.1308	3.7	>10
Baby food	1.849	1.680	1.765	0.1195	6.8	3.72
Cottage pie	0.165	0.158	0.162	0.0049	3.1	<0.5
Cooked egg noodles	0.174	0.184	0.179	0.0071	4.0	<0.5
Madeira cake	2.464	2.518	2.491	0.0382	1.5	6.06
Uncooked egg noodles	3.384	3.537	3.461	0.1082	3.1	>10

Extracted as per RET (Total dilution 1/160)							Corrected ppm (x1.6)
Quiche	3.164	3.158	3.161	0.0042	0.1	9.52	15.23
Southern Fried Crispy Chicken	3.045	3.182	3.114	0.0969	3.1	9.22	14.75
Scotch egg	3.305	3.421	3.363	0.0820	2.4	>10	>16
Baby food	1.498	1.496	1.497	0.0014	0.1	3.04	4.86
Cottage pie	0.140	0.137	0.139	0.0021	1.5	<0.5	<0.8
Cooked egg noodles	0.150	0.149	0.150	0.0007	0.5	<0.5	<0.8
Madeira cake	1.509	1.361	1.435	0.1047	7.3	2.89	4.62
Uncooked egg noodles	3.233	3.382	3.308	0.1054	3.2	>10	>16

ELISA and RET Extraction Comparison

Sample	ELISA (ppm EWP)	RET (ppm EWP)	% Efficiency of RET extraction relative to ELISA	T O C
Quiche	>10	15.23	-	0 1 5 NEG
Southern Fried Crispy Chicken	9.47	14.75	156	<1 2 4 POS
Scotch egg	>10	>16	-	2 4 4 POS
Baby food	3.72	4.86	134	0 3 5 NEG
Cottage pie	<0.5	<0.8	-	0 3 5 NEG
Cooked egg noodles	<0.5	<0.8	-	- -
Madeira cake	6.06	4.62	83.2	3 1 5 POS*
Uncooked egg noodles	>10	>16	-	0 2 4 NEG

EWP = egg white protein

*1/10 Dilution required before testing due to matrix effect

The observed (corrected) data confirms the RET extraction procedure is at least comparable to the ELISA extraction procedure. The RET was able to detect levels of EWP from certain matrices down to the level of 4.6 ppm EWP. This qualifies the use of the RET as an initial screening test.

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5. References

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

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