

BioKits

RAPID 3-D ShellfishTest

Cat. No. 902081S

CUSTOMER VALIDATION REPORT



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1. Summary / Abstract

The RAPID 3-D Shellfish Test (RST) (Cat.No.902081S) is designed to detect the presence or absence of Shellfish. This Validation Report details the findings of the experimental evaluation undertaken to determine the test parameters and establish Product claims for the RAPID 3-D Shellfish Test

1.1 Inter- and intra-assay variability:

Observed line intensities are comparable between device Batches and between operators.

1.2 Sensitivity: Detects 0.5 ppm cooked prawn extract

1.3 Specificity / Cross reactivity /Interference:

Shellfish: Tested thirteen (13) species of the same genus. All were positive, except for Clam.

Miscellaneous Commodities: Tested eleven (11). Ten (10) gave NEGATIVE results. One (1) could not be tested.

Spike recovery in representative matrices:

Vegetable Oil (Fish Frying Oil) - Detected 5ppm cooked prawn extract spike.

Fish stock cube and Mayonnaise – Detected 10ppm cooked prawn extract spike.

Minced White Fish. – Detected 40ppm cooked prawn extract spike.

Surimi Sticks and Seafood Sauce - Detected 50ppm cooked prawn extract spike.

Retail Food Commodities: Unspiked:

26 products from various ranges, manufacturers and suppliers were tested. With the exception to eight (8), all gave the expected result. (Overall 69% accuracy).

Retail Food Commodities: Spiked:

15 Samples were spiked with 10ppm cooked prawn extract, 12 samples gave positive results. The remaining 3 samples gave positive results with a 20ppm spike level.

1.4 Robustness:

The robustness parameters tested do not compromise the functionality of the RST

1.5 Environmental Swab Testing:

The RAPID 3-D test detected residue swabbed from plastic and stainless steel surfaces contaminated with 20 µg / 25cm² cooked prawn extract. And from a Teflon surface contaminated with 10 µg / 25 cm² cooked prawn extract.

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3. **Materials and Methods**

3.1 **Materials**

3.1.1 Production manufactured batches of RST Reagents:

Device	502392E-01 /-02
Rapid Extraction Buffer	502345P-17

3.1.2 The positive (analyte under test) allergen source is cooked prawn extract with known protein content.

3.2 **Method**

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

Sample volume (ml) / weight (g) =	0.5
Volume (ml) of extraction buffer =	4.0
Total extraction dilution =	1/8 \equiv 0.125g/ml \equiv 125,000 ppm \equiv 12.5%

Hand Shaken 1 minute

Dip into REB cap or 100 μ l pipette Sample application

Device Graded after 5 minute incubation at ambient temperature

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

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

4. Results

4.1 Inter- and intra-assay variability & Stability

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 RST.

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

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 RST was observed to be POSITIVE.

Test Parameters: • Inter & intra-assay variability	ppm	Operator #1 TOC	Operator #2 TOC	STATUS: POS / NEG Pass / Fail
Sample: Cooked Prawn extract	1000	534	534	POS / Pass
		534	523	
	100	535	535	POS / Pass
		545	535	
	10	435	435	POS / Pass
		445	445	
	1	235	235	POS / Pass
		245	245	
	0.1	035	035	NEG / Pass
		045	045	
	0.01	035	035	NEG / Pass
		045	045	
	0	-	-	NEG / Pass
		045	045	

Conclusion:

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

Test Parameter: • Sensitivity	ppm	TOC	STATUS: POS / NEG Pass / Fail	Comments
Sample: Cooked Prawn extract	1000	534	POS / Pass	
	100	545	POS / Pass	
	10	445	POS / Pass	
	1	245	POS / Pass	
	0.5	145	POS / Pass	
	0.25	045	NEG / Pass	Line appeared at 0.25ppm after 15 minutes.
	0.1	045	NEG / Pass	
	0.01	045	NEG / Pass	
	0	045	NEG / Pass	

Conclusion: Cooked Prawn Extract can be detected at 0.5 ppm.

4.3 Specificity, Cross reactivity & Interference

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

Test Parameter: Selectivity, Cross reactivity & Interference

SHELLFISH	TOC @ 1/ 8	STATUS: POS / NEG Pass / Fail
Crab	435	POS/Pass
Lobster	545	POS/Pass
Brown Shrimp	545	POS/Pass
Tiger Prawn	535	POS/Pass
Langoustine (Scampi)	545	POS/Pass
Crayfish	545	POS/Pass
Scallop	445	POS/Pass
Oyster	445	POS/Pass
Mussel	335	POS/Pass
Cockle	245	POS/Pass
Clam*	045	NEG/Fail
Squid	334	POS/Pass
Blank	045	NEG/Pass

*This is a tinned sample, which would have been subjected to extremely high temperatures during the tinning process, therefore destroying the Tropomyosin content.

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Test Parameter: Spike recovery in representative matrices

Test Parameter: • Spike recovery in representative matrices	Cooked Prawn extract Spike (ppm)	TOC	STATUS: POS / NEG Pass / Fail	Comments
Sample:	0	045	NEG / Pass	
Fish Stock Cube (dissolved ready to use)	5	<145	POS / Pass	
1/8	10	145	POS / Pass	
	15	245	POS / Pass	
	20	245	POS / Pass	

Comments: Test Line observed after five minutes for all spike levels tested.

Sample:	Cooked Prawn extract Spike (ppm)	TOC	STATUS: POS / NEG Pass / Fail	Comments
Sample:	0	045	NEG / Pass	
Vegetable Oil (fish frying oil)	5	145	POS / Pass	
1/8	10	145	POS / Pass	
	15	245	POS / Pass	
	20	245	POS / Pass	

Comments: Test Line observed after five minutes for all spike levels tested.

Sample:	Cooked Prawn extract Spike (ppm)	TOC	STATUS: POS / NEG Pass / Fail	Comments
Sample:	0	045	NEG / Pass	
Mayonnaise	5	<145	NEG / Fail	} 'T' line grade too low
1/8	10	145	POS / Pass	
	15	245	POS / Pass	
	20	245	POS / Pass	

Comments: Test Line observed after five minutes for all spike levels except 5ppm, which was too faint to grade.

Sample:	Cooked Prawn extract Spike (ppm)	TOC	STATUS: POS / NEG Pass / Fail	Comments
Sample:	0	035	NEG / Pass	
Minced White Fish	5	035	NEG / Fail	} 'T' line grade too low
1/8	10	045	NEG / Fail	
	15	045	NEG / Fail	
	20	<135	NEG / Fail	
	40	135	POS / Pass	
	50	235	POS / Pass	

Comments: Test Line observed after five minutes for 40 and 50ppm spike levels, 20ppm was too faint to grade. Poor spike recover may be due to high levels of protein in the sample.

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Sample:				Comments
Surimi Sticks 1/8	0	035	NEG / Pass	
	5	035	NEG / Fail	
	10	035	NEG / Fail	
	15	045	NEG / Fail	
	20	<135	NEG / Fail	} 'T' line grade too low
	50	135	POS / Pass	
	100	235	POS / Pass	

Comments: Test Line observed after five minutes for 50 and 100ppm spike levels, 20ppm was too faint to grade. Poor spike recover may be due to high levels of protein in the sample.

Sample:				Comments
Seafood Sauce 1/8	0	035	NEG / Pass	
	5	034	NEG / Fail	
	10	034	NEG / Fail	
	15	034	NEG / Fail	
	20	<134	NEG / Fail	} 'T' line grade too low
	50	244	POS / Pass	
	100	235	POS / Pass	

Comments: Test Line observed after five minutes for 50 and 100ppm spike levels, 20ppm was too faint to grade.

Test Parameter: Spike recovery in a group of food matrices

Test Parameter: • Spike recovery in a group of food commodities	Cooked Prawn extract Spike (ppm)	TOC	STATUS: POS / NEG Pass / Fail	Comments
Sample:	0	044	NEG / Pass	
Wheatflour	5	045	NEG / Fail	
1/8	10	<145	NEG / Fail	} 'T' line grade too low
	20	145	POS / Pass	
	50	245	POS / Pass	
Comments: Test Line observed after five minutes for 20 and 50ppm spike levels.				
Sample:	0	022	NEG / Pass	
Soya Flour	5	022	NEG / Fail	
1/8	10	022	NEG / Fail	
	20	<122	NEG / Fail	
	50	121	POS / Pass	
Comments: Test Line observed after five minutes for 50ppm spike level. Poor recovery due to high protein sample.				
Sample:	0	013	NEG / Pass	
Milk Powder	5	023	NEG / Fail	
1/8	10	<123	NEG / Fail	} 'T' line grade too low
	20	123	POS / Pass	
	50	123	POS / Pass	
Comments: Test Line observed after five minutes for 20 and 50ppm spike levels.				
Sample:	0	011	NEG / Pass	
Ground Peanut	5	011	NEG / Fail	
1/8	10	011	NEG / Fail	
	20	011	NEG / Fail	
	50	112	POS / Pass	
Comments: Test Line observed after five minutes for 20 and 50ppm spike levels. Poor recovery due to high protein sample.				
Sample:	0	041	NEG / Pass	
Egg	5	031	NEG / Fail	
1/8	10	131	POS / Pass	
	20	232	POS / Pass	
	50	332	POS / Pass	
Comments: Test Line observed after five minutes for all spike levels except 5ppm.				

Sample:	0	044	NEG / Pass	
Coconut	5	043	NEG / Fail	
1/8	10	144	POS / Pass	
	20	244	POS / Pass	
	50	344	POS / Pass	

Comments: Test Line observed after five minutes for all spike levels except 5ppm.

Sample:	0	045	NEG / Pass	
Starch	5	<145	NEG / Fail	} 'T' line grade too low
1/8	10	145	POS / Pass	
	20	245	POS / Pass	
	50	345	POS / Pass	

Comments: Test Line observed after five minutes for all spike levels except 5ppm.

Sample:	0	035	NEG / Pass	
Oily Fish (Anchovy)	5	035	NEG / Fail	
1/8	10	035	NEG / Fail	
	20	035	NEG / Fail	} 'T' line grade too low
	50	<135	NEG / Fail	

Comments: Poor recovery due to high protein content of the sample.

Sample:	0	035	NEG / Pass	
Raw Meat (Beef)	5	035	NEG / Fail	
1/8	10	035	POS / Pass	
	20	035	POS / Pass	'T' line grade too low
	50	<125	POS / Pass	

Comments: Poor recovery due to high protein content of the sample.

Sample:	0	031	NEG / Pass	
Chocolate Mousse	5	031	NEG / Fail	} 'T' line grade too low
1/8	10	131	POS / Pass	
	20	231	POS / Pass	
	50	331	POS / Pass	

Comments: Test Line observed after five minutes for all spike levels except 5ppm.

The test was unable to detect the Cooked Prawn spikes in Oily Fish and Beef. There were very faint lines visible at 50ppm, but these were graded as <1

This poor spike recovery is due to the “matrix effect” and is a recognised phenomenon, which has been observed with other RAPID 3-D allergen tests. Sample types that fit into this category include 100% high protein raw ingredients, 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.

Test Parameter: Commodity Testing

Test parameter: • Commodity testing 1/8	TOC	STATUS: POS / NEG Pass / Fail
Egg & Cress Sandwiches	044	NEG / Pass
Breaded Wholetail Scampi (45% Shellfish)	542	POS / Pass
King Prawn Flavour Tails (Crab Extract) *	035	NEG / Fail
Chinese Egg Fried Rice	045	NEG / Pass
Prawn Crackers (14% Shellfish) *	045	NEG / Fail
Fisherman's Pie	034	NEG / Pass
Breaded Cod Fishcakes	034	NEG / Pass
Mariner's Pie	034	NEG / Pass
Chow Mein (6.2% shellfish)	545	POS / Pass
Prawn Spring Rolls	545	POS / Pass
Tuna Napolitana Mix (Shrimp Powder)	245	POS / Pass
Taramasalata	044	NEG / Pass
Prawn Cocktail Flavour Rice Snack	044	NEG / Pass
Chicken & Vegetable Instant Soup	035	NEG / Pass
Prawn Toast (35% Shellfish)	445	POS / Pass
Medium Curry Paste	034	NEG / Pass
Smart Price Salmon Paste	035	NEG / Pass
Crab Paste (10% Shellfish) *	035	NEG / Fail
Chicken in Black Pepper Sauce (Oyster Sauce) *	045	NEG / Fail
Thai Green Curry Sauce	045	NEG / Pass
Tuna & Sweetcorn Sandwich Filler	035	NEG / Pass
Shrimps in Brine *	035	NEG / Fail
Edible Roasted Seaweed Sheets	035	NEG / Pass
Tom Yum Paste (Shrimp Extract) *	035	NEG / Fail
Oyster Sauce (0.2% Oyster Powder, Oyster Extract) *	045	NEG / Fail
Oyster Extract *	045	NEG / Fail

***Possible cause of FALSE results are related to processing conditions:**

King Prawn Flavour Tails (Crab Extract) Flour - 'Extract' probably highly rendered

Prawn Crackers (14% Shellfish) - Deep-fried at high temperatures so destroying protein

Crab Paste (10% Shellfish) - Very highly processed sample, likely to have been heat-treated.

Chicken in Black Pepper Sauce (Oyster Sauce) - Very small amount of Oyster sauce, which contains even smaller amount of Oyster extract which is highly processed.

Shrimps in Brine - This is a canned sample and has been subjected to temperatures of 116-121°C during the canning process, therefore destroying the Tropomyosin content.

Tom Yum Paste (Shrimp Extract) - Shrimp extract most likely to be processed in the same way as Oyster extract (below).

Oyster Sauce (0.2% Oyster Powder, Oyster Extract) - Oyster extract has a high salt content and is heat-treated to concentrate the flavour.

Test Parameter: Spiked Food Commodity Testing

Test parameter:		STATUS:
• Food Commodities matrices 1/8 spiked with Cooked Prawn extract @ 10ppm	TOC	POS / NEG Pass / Fail
Egg & Cress Sandwiches	142	POS / Pass
Chinese Egg Fried Rice	145	POS / Pass
Prawn Crackers (14% Shellfish)	145	POS / Pass
Fisherman's Pie*	034	NEG / Fail
Breaded Cod Fishcakes*	024	NEG / Fail
Mariner's Pie	134	POS / Pass
Taramasalata	132	POS / Pass
Prawn Cocktail Flavour Rice Snack	243	POS / Pass
Chicken & Vegetable Instant Soup	135	POS / Pass
Medium Curry Paste	134	POS / Pass
Crab Paste (10% Shellfish)*	<125	NEG / Fail
Chicken in Black Pepper Sauce (Oyster Sauce)	135	POS / Pass
Thai Green Curry Sauce	144	POS / Pass
Tuna & Sweetcorn Sandwich Filler	135	POS / Pass
Edible Roasted Seaweed Sheets	145	POS / Pass

*Possible cause of failed spike recovery (Fisherman's Pie, Breaded Cod Fishcakes and Asda Crab Paste (10% Shellfish)) is the high content of white fish in each sample.

Test parameter:		STATUS:
• Food Commodities matrices 1/8 spiked with Cooked Prawn extract @ 20ppm	TOC	POS / NEG Pass / Fail
Smart Price Fisherman's Pie	134	POS / Pass
Breaded Cod Fishcakes	124	POS / Pass
Crab Paste (10% Shellfish)	125	POS / Pass

4.4 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. Samples were prepared as described below for each variable under test.

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

Sample weight or volume	0.5 g or ml	± 4%
	0.5g or ml	±10%
Extraction Buffer volume	4 ml	± 5%
	4 ml	± 10%
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

4.5 Environmental Swab Testing

The transfer of Cooked Prawn extract from a range of surfaces onto a swab and detection by RST 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 cm² 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. Rapid Extraction Buffer was used for the controls (0 µg/mL). The solution in each square was left to dry for 1 hour 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.

Test Parameter: Environmental swab testing	Cooked Prawn Extract (µg)	Cooked Prawn Extract (µg/cm²)	TOC	STATUS: POS / NEG Pass / Fail
Surface: Plastic	100	4	345	POSS / Pass
Sample: Cooked Prawn extract	50	2	245	POSS / Pass
	20	0.8	145	POSS / Pass
	10	0.4	<145	NEG / Fail
	5	0.2	045	NEG / Fail
	0	0	045	NEG / Pass
Surface: Teflon	100	4	445	POSS / Pass
Sample: Cooked Prawn extract	50	2	445	POSS / Pass
	20	0.8	345	POSS / Pass
	10	0.4	145	POSS / Pass
	5	0.2	<145	NEG / Fail
	0	0	045	NEG / Pass
Surface: Stainless Steel	100	4	345	POSS / Pass
Sample: Cooked Prawn extract	50	2	345	POSS / Pass
	20	0.8	245	POSS / Pass
	10	0.4	045	NEG / Fail
	5	0.2	045	NEG / Fail
	0	0	045	NEG / Pass

The RST detected residue swabbed from plastic and stainless steel surfaces contaminated with 20µg (20 µg/ 25cm²) Cooked Prawn extract. It also detected residue swabbed from Teflon surfaces contaminated with 10µg (10 µg/ 25cm²) Cooked Prawn extract. It is anticipated that not all the extract residue would be transferred to the swab.

5. Conclusions

The RAPID 3-D Shellfish Test is suitable for the detection of shellfish 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.

Users should perform 'in house' matrix specific spike recovery validation work in conjunction with a validated laboratory assay e.g. the *BioKits* Shellfish Assay kit to help confirm RAPID 3-D Shellfish Test results.

Highly processed shellfish 'extracts' may not be suitable for testing, see Section 4.3.

6. References/Bibliography

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

BioKits RAPID 3-D Shellfish Insert

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