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Validation *REPORT*

AgraStrip® Walnut

(COKAL0910AS)

KEYdiagnostics

T: 02 8212 4074 F: 02 9423 6992
info@keydiagnostics.com.au
www.keydiagnostics.com.au
PO Box 1038, Gymea, NSW, 2227

Summary

Validated AgraStrip® Walnut:

Limit of Detection:	**10 ppm Walnut**
Specificity:	**Cross reactivity observed with pecan and rye.**
Spiked commodities:	**Detection of 20ppm Walnut in Biscuit, Curry Sauce and Sausage. In Dairy Free Spread, Chocolate and Rice Flour. 40ppm Walnut could be detected. In Yogurt a Faint Line was observed with the 40ppm Walnut spike.** **Increasing the incubation time of the reaction vial to 10 minutes will allow the detection of 20ppm Walnut in chocolate.**
Rinse Water:	**All results as expected.**
Extraction:	**Molten chocolate and some powders may block filter tip.**
Swabbing:	**Recovery of 20µg Walnut from a Plastic surface and 50µg Walnut from a stainless steel surface.**
Test time:	**Around 10 minutes. (15 minutes for chocolate samples)**

The following protocol was performed in collaboration with The Institut fuer Produktqualitaet, Berlin, Germany using the Romer Walnut AgraStrip Kit. All samples were extracted and assayed in accordance with the kit insert.

Materials

Romer Labs AgraStrip Walnut Kit (WA5001-40610 Exp 29/02/2012)

Plain Yoghurt
Digestive Biscuit
Dairy Free Soya Spread
Casein and Nut Free Chocolate
Balti Curry Cooking Sauce
Pork Sausage (Wheat listed as an ingredient)
Rice Flour

Plastic Chopping Board
Stainless Steel Surface

Fresh Walnut Extract, 0.2g of Walnut was extracted in accordance with the kit insert. This extract was given an assumed value of 1,000,000ppm (1000mg/ml Walnut)

Method

Limit of Detection: Walnut was spiked into extraction solution, as detailed in Table 1 below. The lowest spike level to give a positive result was recorded as the Limit of Detection for the AgraStrip Walnut test.

Specificity: Specificity of the strip was evaluated in a total of 34 matrices, including grains, nuts, seeds and other miscellaneous samples. Samples were prepared in accordance with the current kit insert.

Real Samples: A range of processed products were obtained from local retailers and tested according to the kit instructions.

Rinse Waters: The impact of rinse water was evaluated with blank and positive control (water spiked with 2 ppm and 10 ppm allergen) samples to test for the appearance of false positive and false negative results.

Spiked Commodities: Representative sample matrices Spiked & Unspiked

Unspiked samples: Sensitivity and specificity of the kits were evaluated for seven (7) representative sample matrices: Dairy free margarine; sausage; biscuit crumb; dark chocolate; rice flour; yoghurt and curry sauce. Samples were prepared in accordance with the current kit insert.

The representative samples were further analysed with the addition of various levels of Walnut extract. Spiked samples were prepared as per Table 1 below, mixed well and set aside for at least 1 hour. Spiked samples were then extracted in accordance with the current kit insert.

The allergen spike was also added direct to the extraction solution to act as a control.

Table 1

Prepare 5mg/ml (5000 µg/ml)

Req'd spike (ppm)	Prepare Spike (mg/ml)	Volume of 0.5mg/ml extract (ml)	Vol. Diluent (ml)	Vol. of spike added to 0.2g Sample
0	0	0	1000	10ul
5	0.1	0.02	0.98	10ul
10	0.2	0.04	0.96	10ul
20	0.4	0.08	0.92	10ul
40	0.8	0.16	0.84	10ul

Swabbing

The transfer of protein from two surfaces onto a swab and detection by the AgraStrip Walnut Kit was investigated. The allergen source is an extracted sample of assumed Walnut concentration. Two surfaces were assessed: Plastic chopping board and Stainless Steel.

Each surface was sectioned into 8 x 25 cm^2 squares.

Protein solutions, prepared as per Table 2, were applied using a micropipette to each of the two surfaces.

The solution in each square was left to dry for 2 hours at room temperature.

Each area was swabbed, following the guidelines in the Romer Walnut AgraStrip Kit Insert.

Table 2

Prepare 5mg/ml (5000 µg/ml)

Req'd spike (µg)	Prepare Spike (mg/ml)	Volume of 0.5mg/ml extract (ml)	Vol. Diluent (ml)	Vol. of spike added to Surface
0	0	0	1000	10µl
5	0.5	0.1	0.9	10µl
10	1	0.2	0.8	10µl
20	2	0.4	0.6	10µl
50	0.5	0.1	0.9	100µl
100	1	0.2	0.8	100µl

Results

NOTE: Where the AgraStrip Walnut Kit produces a Faint Line for a known positive matrix, the result was treated as a negative. As the test is interpreted by eye a Faint Line may not be seen by all individuals.

Limit of Detection

	LOD	Material Tested
Walnut	10 ppm	Crushed Walnut

Specificity

	Sample No.	Walnut AgraStrip
Sweet low fat milk powder	105	-
Low fat milk powder	35	-
Sweet whey powder	104	-
Whey concentrate (35%)	95	-
Whey concentrate (85%)	99	-
Cream powder (42%)	96	-
Cashew	9	-
Pecan nut	107/133	+
Macadamia	5	-
Sesame	79	-
Hazelnut	134	-
Sunflower seed	135	-
Walnut	119	+
Lineseed	44	-
Almond	113	-
Pistachio	111	-
Brazil nut	108	-
Peanut	7	-
Pine kernel	25	-
Whole egg powder	121	-
Red lentil	19	-
Green peas	17	-
Wheat flour	15	-
Dark chocolate with 20 ppm almond	3	-
Lupin	20	-
White beans	16	-
Chick peas	18	-
Oat	77	-
Rice flour	30	-
Apricot	10	-
Soybean	21	-
Barley	73	-
Rye	74	+
Corn	55	-

During testing undertaken at IFP cross reactivity was observed with Brazil Nut, Lentil, Pecan and Rye. Testing at Romer Labs confirmed the cross reactivity with Pecan. No such cross reactivity was

observed with Brazil Nut and Lentil when testing was repeated at Romer Labs. The testing of Rye was not repeated due to availability of material at the time of validation.

Real Samples

Sample No.	Test Material	Walnut AgraStrip	PCR
11/6973	chocolate product	-	-
11/6974	Joghurt	-	-
11/6975	wheat product	-	-
11/6976	wheat product	-	-
11/6977	wheat product	-	-
11/6978	wheat product	-	-
11/10890	Salami	-	Not Tested
11/10891	Salami	+	+
11/10892	Salami	-	Not Tested

Rinse Waters

	Walnut AgraStrip Result
10ppm: 400 µL water + 5 drops Buffer	+
2ppm: 400 µL water + 5 drops Buffer	+
Blank: 400 µL water + 5drops Buffer	-

Tap water was spiked with 10ppm and 2ppm reference material

400 µl water + 5 drops of Extraction Buffer was used

All results were as expected

Specificity & Interference

Walnut Protein (PPM)	Walnut (PPM)	Yogurt	Biscuit	Dairy Free Spread	Chocolate*
0	0	-	-	-	-
0,5	1,5	-	-	-	-
1	3	-	Faint Line	Faint Line	-
2	6	Faint Line	+	+	-
5	15	+	+	+	-

Walnut Protein (PPM)	Walnut (PPM)	Curry Sauce	Sausage	Rice Flour	No Matrix
0	0	-	-	-	-
0,5	1,5	-	-	-	Faint Line
1	3	Faint Line	Faint Line	Faint Line	+
2	6	+	+	+	+
5	15	+	+	+	+

The AgraStrip Walnut Kit was able to detect 20ppm Walnut in Biscuit, Curry Sauce and Sausage. In Dairy Free Spread, Chocolate and Rice Flour. 40ppm Walnut could be detected. In Yogurt a Faint Line was observed with the 40ppm Walnut spike. The chocolate samples were heated to 60°C in a water bath prior to extraction. When extracted the chocolate has the potential to block the filter

tip. This can be avoided by transferring the extract directly from the extraction tube to the reaction vial using a pipette or by hand to a level just under the 0.5ml graduation of the reaction vial. The same process can be used for some flours which may also block the filter tip.
As the level of recovery of Walnut spiked into chocolate was low, the experiment was repeated but leaving the reaction vial to incubate for 10 minutes instead of 5 minutes as stated in the kit insert.

Walnut (PPM)	Chocolate
0	-
5	-
10	Faint Line
20	+
40	+

When the reaction vial is left to incubate for 10 minutes instead of 5 minutes then 20ppm Walnut can be detected in chocolate.

Swabbing

Walnut on surface (µg)	Stainless Steel	Plastic
0	-	-
5	-	-
10	-	-
20	+	-
50	+	+
100	+	+

The AgraStrip Walnut Kit was able to recover 20µg Walnut when swabbing a stainless steel surface and 50µg Walnut when swabbing a plastic surface.