

Validation REPORT

AgraStrip® Gluten G12 (COKAL0200AS)

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Summary

Validated AgraStrip® Gluten:

Limit of Detection:	2 ppm Gluten (In extraction solution)
Specificity:	Cross reactivity with Rye, Spelt, Barley and some oat varieties. Good correlation with AgraQuant G12 ELISA. Under recovery observed with some processed foods such as Baby Food.
Spiked commodities:	Detection of 5ppm protein with the majority of spiked commodities tested, 10ppm with Chocolate
Rinse Water:	Variation in pH (5-9) does not affect result
Extraction:	Molten chocolate and some powders may block filter tip
Swabbing:	Recovery of 4µg from Plastic and Stainless Steel Surfaces
Test time:	Around 10 minutes

All samples were extracted and assayed in accordance with the kit insert.

Materials

Romer Labs AgraStrip G12 Gluten Kit

Greek Style 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

Internal Quality Control Gluten Extract

Method

Calibration:

The calibrant used in the validation of Romer Lab's G12 Gluten AgraStrip was created using Vital Wheat Gluten (Roquette, UK) which was extracted using 60% ethanol. A set of standard solutions with 5 ng/mL, 25 ng/mL, 100 ng/mL and 250 ng/mL gliadin using the PWG gliadin (1) was prepared. Assuming that gliadin concentration multiplied by two is giving the concentration for gluten the PWG set of standards was used to calibrate the Vital Wheat Gluten Extract. This was achieved by making a serial dilution of the Vital Wheat Gluten extract in dilution buffer and running the AgraQuant G12 ELISA using the PWG gliadin as the standards. The Vital Wheat Gluten Extract could then be diluted using the dilution buffer to a final concentration of 10 ng/mL, 50 ng/mL, 200 ng/mL and 500 ng/mL gluten. The dilution buffer was used as blank. Both set of standards were run in six replicates on the G12 sandwich assay.

Limit of Detection:

Gluten 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 G12 Gluten test.

Specificity:

A range of commodities, finished good and Gluten Free foods were tested with the results being compared with the AgraQuant G12 Gluten ELISA.

Rinse Waters:

Negative and Positive (100ng/ml Gluten) rinse waters were tested over a range of pH values (5-9) and their effect on the AgraStrip G12 Gluten test, if any, was noted.

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 Gluten protein extract (Romer IQC). 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 protein spike was also added direct to the extraction solution to act as a control.

Table 1

Prepare 0.5mg/ml (500 µg/ml) (Romer IQC)

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
1	0.02	0.04	0.96	10ul
2	0.04	0.08	0.92	10ul
5	0.1	0.2	0.8	10ul
10	0.2	0.4	0.6	10ul

Swabbing

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

Each surface was sectioned into 8 x 25 cm² 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 Gluten G12 AgraStrip Kit Insert.

Table 2

Prepare 0.5mg/ml (500 µg/ml) (Romer IQC)

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
0.2	0.02	0.04	0.96	10µl
0.4	0.04	0.08	0.92	10µl
1	0.1	0.2	0.8	10µl
2	0.2	0.4	0.6	10µl
4	0.04	0.08	0.92	100µl
10	0.1	0.2	0.8	100µl
20	0.2	0.4	0.6	100µl

Results

Limit of Detection

	LOD	Material Tested
Gluten	2 ppm	Vital Wheat Gluten

Specificity

	Food Sample	AgraStrip G12 Gluten	AgraQuant G12 ELISA (ppm Gluten)	
Nuts	Pecan	-	<4	
	Walnut	-	<4	
	Almond	-	<4	
	Cashew	-	<4	
	Macadamia	-	<4	
	Peanut	-	<4	
	Hazelnut	-	<4	
	Pine Nuts	-	<4	
	Pistachio	-	<4	
Oils	Hazelnut Oil	-	<4	
	Walnut Oil	-	<4	
	Vegetable Oil	-	<4	
	Sunflower Oil	-	<4	
Seeds	Golden Linseed	-	<4	
	Brown Linseed	-	<4	
	Poppy	-	<4	
	Sesame	-	<4	
	Sunflower	-	N/A	
	Pumpkin	-	N/A	
	Mustard	-	<4	
	Soya Mince	-	<4	
Naturally gluten free foods	Soy Bean	-	<4	
	Soya Flour*	+	37	
	Millet	-	<4	
	Buckwheat	-	<4	
	Rice Flour	-	<4	
	Quinoa	-	<4	
	Lupin	-	N/A	
	Corn	-	<4	
	Gluten containing grains	Wheat Flour	+	>200
		Rye	+	>200
Spelt		+	N/A	
Barley		+	>200	
Firth Oat		+	7	
Avena Oat		+	153	
Gluten Free Oat		-	<4	

	Food Sample	AgraStrip G12 Gluten	AgraQuant G12 ELISA (ppm Gluten)	
Starches	Tapioca Starch	-	<4	
	Wheat Starch	-	<4	
	Potato Starch	-	<4	
Miscellaneous	Fennel	-	<4	
	Paprika	-	<4	
	Chickpea	-	<4	
	Soy Sauce	-	<4	
	Coriander	-	<4	
	Black Eyed Beans	-	<4	
	Puy Lentils	-	<4	
	Golden Syrup	-	N/A	
	Maple Syrup	-	N/A	
	Prof Khoehler Bread	+	139,00	
	Prof Khoehler Maize Flour	+	6,67	
	Prof Khoehler Snack	+	90,70	
	Bisto Best (Gravy Granules)	-	<4	
	Corn Flakes	+	>200	
	Multi Grain Pringles	+	>200	
	Cardamom	-	<4	
Food labelled as "Gluten free"	Gluten Free Cookie Mix	-	<4	
	Gluten Free Chocolate Bar	-	<4	
	Gluten Free Cake Mix	-	<4	
	Gluten Free Breadcrumbs	-	<4	
	Gluten Free Pancake Mix	-	<4	
	Gluten Free Bread Mix	-	<4	
	Pitta Bread	-	<4	
	English Muffins	-	<4	
	Cherry Bakewell	-	<4	
	Lemon Shortbread	-	<4	
	Spaghetti	-	<4	
	Bread	-	<4	
	Shortbread	-	<4	
	Chocolate Brownie	-	<4	
	Honey and Oat Cookie	+	26,3	
	Mini Muffin	-	<4	
	Custard Cream	-	<4	
	Gluten Free Cake Mix	-	<4	
	Beers	Hoegaarden (Wheat Beer)	+	>200
		Pedigree (Ale)	-	<4
Budweiser (Lager)		+	N/A	
Budvaar (Lager)		N/A	<4	
Baby Food	Apple and Banana Breakfast	-	4,70	
	Fruity Muesli	-	50,73	
	Pasta Bolognese	+	>200	
	Mini Beef Casserole	-	<4	
	Creamed porridge	-	7,30	

The AgraStrip G12 Gluten test correlates well with the results given by the AgraQuant G12 Gluten ELISA. Some highly processed foods such as Baby Food may give a negative result when compared to the ELISA method. The AgraStrip G12 Gluten test may not be suitable for highly processed samples.

Rinse Waters

	Blank	Positive Control*
pH 5: 500 µL water + 5 drops Buffer	-	+
pH 6: 500 µL water + 5 drops Buffer	-	+
pH 7: 500 µL water + 5 drops Buffer	-	+
pH 8: 500 µL water + 5 drops Buffer	-	+
pH 9: 500 µL water + 5 drops Buffer	-	+

*100ng/ml Gluten

Testing Rinse waters within a pH range of 5 to 9 has no observable effect on the results obtained with the Gluten G12 AgraStrip

Specificity & Interference

Gluten Protein (PPM)	Yogurt	Biscuit	Dairy Free Spread	Chocolate
0	-	+	-	-
1	-	+	-	-
2	-	+	-	-
5	+	+	+	-
10	+	+	+	+

Gluten Protein (PPM)	Curry Sauce	Sausage**	Rice Flour	No Matrix
0	-	+	-	-
1	-	+	-	-
2	-	+	-	+
5	+	+	+	+
10	+	+	+	+

** Contains Wheat

The AgraStrip G12 Gluten Kit was able to detect 5ppm Gluten with all matrices tested. 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.

The biscuit and sausage samples used in these experiments had wheat listed as an ingredient.

Swabbing

Gluten Protein on surface (µg)	Stainless Steel	Plastic
0	-ve	-ve
0,2	-ve	-ve
0,4	-ve	-ve
1	-ve	-ve
2	-ve	-ve
4	+ve	+ve
10	+ve	+ve
20	+ve	+ve

The AgraStrip Gluten Kit was able to recover 4µg Gluten when swabbing both a stainless steel and a plastic surface.