9. PERFORMANCE EVALUATION DATA

9.1. Analytical Sensitivity

9.1.1. Limit of Detection

This is defined as the lowest concentration of human IgG detectable by the Food Detective™.

Method: Serial dilutions of human IgG from 1mg/ml to 0.1μg/ml were bound to the wells on the Reaction Tray (10μl/well). The tray was then blocked using a commercially available biomolecular stabiliser and dried. Antibody Detector Solution was then added to the Reaction Tray and incubated for 10 minutes as per the Instructions for Use. After washing with Wash Buffer, Developer Solution was added for 2 minutes. The Reaction Tray was then washed with Wash Buffer and dried. The lowest concentration of IgG giving a definite blue spot was then identified.

Results: Clear blue spots were detected in wells coated from 1 mg/ml to 1.6μg/ml IgG.

Conclusions:

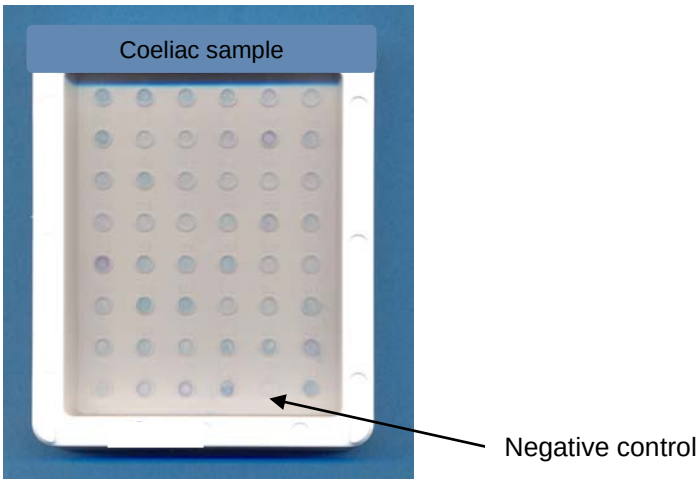
The limit of IgG detection was estimated to be 1.6μg/ml or the equivalent of 16ng protein.

9.1.2 Detection of antibodies to 59 Foods

Method: Samples from coeliac patients that gave positive IgG reactions to all 101 foods in a Mediterranean Food IgG microplate-based ELISA manufactured by Genesis Diagnostics Ltd, UK were used to confirm the detection by the Food Detective of IgG antibodies to 59 foods common to both assays. Assays were conducted according to the Instructions for Use.

Results: Figure 5 shows the binding of IgG in the coeliac patient sample. It can be seen that all wells except the negative control (well 47) contain a blue spot. The intensities of the blue spots vary depending on the food in the wells.

Figure 5.



Conclusion:

The results from this study demonstrate that The Food Detective™ is able to detect IgG antibodies to all foods arrayed on the Food Detective Reaction Tray where these are present in concentrations that give positive results in the microplate-based ELISA.

9.2. Sensitivity, specificity and accuracy relative to conventional microplate-based ELISA

Method: Relative sensitivity and specificity of the Food Detective was assessed in relation to the detection of positive IgG reactions (>12 U/ml) and negative reactions (<12 U/ml) by the microplate-based 93 Food IgG and Mediterranean Food IgG ELISAs both manufactured by Genesis Diagnostics Ltd. Since the Food Detective is a qualitative assay, borderline results obtained in the semi-quantitative microplate-based assays are considered negative in this study.

Results and Conclusions: Table 2 shows the % sensitivity, specificity and accuracy for the detection of IgGs to each food/mixture included in the Food Detective in relation to the target response derived from the microplate-based assay. These data confirm that the Food Detective is suitable for its intended use.

Table 2.

	Food Detective		
Food	% Sensitivity	% Specificity	% Accuracy
Oat	100	100	100
Wheat	88	100	92
Rice	100	100	100
Corn	100	100	100
Rye	88	100	92
Durum Wheat	100	100	100
Gluten	88	100	92
Almond	100	100	100
Brazil	100	100	100
Cashew	100	100	100
Tea	100	100	100
Walnut	75	100	92
Cow Milk	100	100	100
Whole Egg	100	100	100
Chicken	88	100	92
Lamb	100	100	100
Beef	100	100	100
Pork	100	100	100
White Fish	100	100	100
Freshwater Fish	100	100	100
Tuna	100	100	100
Shellfish	100	100	100
Broccoli	100	100	100
Cabbage	100	100	100
Carrot	100	100	100
Leek	100	100	100
Potato	100	100	100
Celery	100	100	100
Cucumber	100	100	100
Peppers	100	100	100
Legume Mix	83	100	92
Grapefruit	100	100	100
Melon Mix	100	100	100
Peanut	100	100	100
Soya Bean	100	100	100
Cocoa Bean	100	100	100
Apple	100	100	100
Blackcurrant	100	100	100
Olive	100	100	100
Orange/Lemon	100	100	100
Strawberry	100	100	100
Tomato	100	100	100
Ginger	100	100	100
Garlic	100	100	100
Mushroom	100	100	100
Yeast	86	100	92

9.3 Antigen absorption studies

Specificity has been confirmed using antigen absorption studies.

Method: Food IgG positive sera were pre-incubated with the reactive food antigen prior to assay. This allows any IgG antibodies to the foods to complex to the food antigens rendering the antibodies unavailable for binding to the food in the subsequent immunoassay. The results for absorbed serum are compared with those for unabsorbed serum.

In the example in Table 3, samples positive for IgGs to tea, lentil, oat, almond, egg, beef, shellfish, cabbage, tomato, mustard, yeast and potato were absorbed with the corresponding antigen at room temperature for 1 hour. Unabsorbed sera were treated with the buffer in which the extracts are dissolved. Absorbed and unabsorbed sera were then diluted in Sample Diluent and the assay was performed as per the Instructions for Use. The intensity of blue spots was scored with 6+ indicating a very intense spot and 1 + a weak positive spot; (-) indicates a negative result.

Where no effect of absorption was observed following a one hour incubation at room temperature, absorptions were carried out at 37^{o}C for 2 hours (Table 4).

Cross-over absorptions were also performed to confirm food IgG specificity (Table 5 and Table 6).

Results: It can be seen from Table 3 that absorption of food IgG positive sera with the cognate antigen significantly decreased antibody binding to the corresponding food on the Reaction Tray for all foods except potato.

Table 3.

Comparison of unabsorbed sera and sera absorbed with various antigens for 1 hour at room temperature.

	Tea	Lentil	Oat	Almond
Unabsorbed	2+	3+	6+	1+
Absorbed	+/-	1+	3+	+/-
	Egg	**Beef**	**Shellfish**	**Cabbage**
Unabsorbed	5+	2+	3+	5+
Absorbed	1+	1+	1+	+/-
	Tomato	**Mustard**	**Yeast**	**Potato**
Unabsorbed	4+	4+	5+	1+
Absorbed	2+	1+	+/-	1+

Longer absorptions at 37^{o}C reduced binding further as shown in Table 4.

Table 4.

Comparison of unabsorbed sera and sera absorbed with various antigens for 2 hours at 37^{o}C.

Plate 1	**Tea**	**Lentil**	**Oat**	**Almond**
Unabsorbed	**2+**	**3+**	**6+**	**1+**
Absorbed	**-**	**-**	**1+**	**-**
	Egg	**Beef**	**Shellfish**	**Cabbage**
	5+	**2+**	**3+**	**5+**
	-	**-**	**-**	**-**
Plate 3	**Tomato**	**Mustard**	**Yeast**	**Potato**
Unabsorbed	**4+**	**4+**	**6+**	**1+**
Absorbed	**1+**	**2+**	**1+**	**-**

Table 5 shows the effect of absorption with yeast extract on the binding of IgG to non-yeast extracts and to yeast. It can be seen that only IgG binding to yeast is affected by absorbing serum with yeast.

Table 5.

The effect of absorption with yeast on IgG binding to various antigens.

Plate 1	**Tea**	**Lentil**	**Oat**	**Almond**
Unabsorbed	**3+**	**4+**	**4+**	**2+**
Absorbed with yeast	**3+**	**4+**	**4+**	**2+**
Plate 2	**Egg**	**Beef**	**Shellfish**	**Cabbage**
Unabsorbed	**3+**	**2+**	**2+**	**5+**
Absorbed with yeast	**3+**	**3+**	**2+**	**5+**
Plate 3	**Tomato**	**Mustard**	**Yeast**	**Potato**
Unabsorbed	**4+**	**4+**	**5+**	**3+**
Absorbed with yeast	**3+**	**3+**	**1+**	**3+**

Table 6.

The effect of absorption with mustard, oat and cabbage on IgG binding to various antigens.

	Tea	Lentil	Oat	Almond
Unabsorbed	**2+**	**4+**	**4+**	**2+**
Absorbed with mustard	**2+**	**4+**	**4+**	**2+**
	Egg	**Beef**	**Shellfish**	**Cabbage**
Unabsorbed	**3+**	**2+**	**2+**	**5+**
Absorbed with oat	**3+**	**3+**	**2+**	**5+**
	Tomato	**Mustard**	**Yeast**	**Potato**
Unabsorbed	**4+**	**4+**	**5+**	**3+**
Absorbed with cabbage	**Neg**	**3+**	**5+**	**1+**

It can be seen from Table 6 that absorption of serum with mustard had no effect on IgG binding to tea, lentil, oat and almond. Absorption with oat had no effect on IgG binding to egg, beef, shellfish and cabbage. Absorption of serum with cabbage significantly reduced binding of IgG to tomato and to a lesser extent to mustard and potato, but had no effect on IgG binding to yeast.

Conclusions:

Absorption studies, examples of which are shown in Tables 3-6 have confirmed the specificity of food antibody binding for almost all foods. The finding that absorption with cabbage reduced IgG binding to mustard probably reflects antibody cross-reactivity, since cabbage and mustard are both members of the Mustard family. Antibody cross-reactivity may also explain the effect of cabbage absorption on IgG reactivity with tomato and potato. The latter are both members of the Nightshade family. Food antibody cross-reactivity is well documented; further examples are given in Table 7.

Table 7. Food antibody cross reactivity

Reactive foods	Cross-reactive foods
Cows' milk	Other animal milk
Hens' eggs	Eggs of other birds
Peanuts	Various tree nuts; rarely other legumes
Various tree nuts	Cross react with one another and with peanut
Soya bean	Seldom cross-reacts significantly with other legumes
Fish	Other fish
Wheat	Other cereals

9.4 Intralot reproducibility

Method: 5 randomly selected plates were assayed using corresponding kit components and sera with characterised food IgG immunoreactivity. Patterns of immunoreactivity shown by the kits are compared by visual inspection and recorded to confirm reproducibility.

Results: See Table 8 and Table 9

Table 8. Positive sample

C10271	Sample 18029	Plate				
Well	**Food**	**1**	**2**	**3**	**4**	**5**
1	Oat	+	+	+	+	+
2	Wheat	+	+	+	+	+
3	Rice	+	+	+	+	+
4	Corn	+	+	+	+	+
5	Rye	+	+	+	+	+
6	Durum Wheat	+	+	+	+	+
7	Gluten	+	+	+	+	+
8	Almond	+	+	+	+	+
9	Brazil	+	+	+	+	+
10	Cashew	+	+	+	+	+
11	Tea	+	+	+	+	+
12	Walnut	+	+	+	+	+
13	Cow Milk	+	+	+	+	+
14	Whole Egg	+	+	+	+	+
15	Chicken	+	+	+	+	+
16	Lamb	+	+	+	+	+
17	Beef	+	+	+	+	+
18	Pork	+	+	+	+	+
19	White Fish	+	+	+	+	+
20	Freshwater Fish	+	+	+	+	+
21	Tuna	+	+	+	+	+
22	Shellfish	+	+	+	+	+
23	Broccoli	+	+	+	+	+
24	Cabbage	+	+	+	+	+
25	Carrot	+	+	+	+	+
26	Leek	+	+	+	+	+
27	Potato	+	+	+	+	+
28	Celery	+	+	+	+	+
29	Cucumber	+	+	+	+	+
30	Peppers	+	+	+	+	+
31	Legume Mix	+	+	+	+	+
32	Grapefruit	+	+	+	+	+
33	Melon Mix	+	+	+	+	+
34	Peanut	+	+	+	+	+
35	Soya Bean	+	+	+	+	+
36	Cocoa Bean	+	+	+	+	+
37	Apple	+	+	+	+	+
38	Blackcurrant	+	+	+	+	+
39	Olive	+	+	+	+	+
40	Orange/Lemon	+	+	+	+	+
41	Strawberry	+	+	+	+	+
42	Tomato	+	+	+	+	+
43	Ginger	+	+	+	+	+
44	Garlic	+	+	+	+	+
45	Mushroom	+	+	+	+	+
46	Yeast	+	+	+	+	+
47	Negative					
48	Positive	+	+	+	+	+

Table 9. Negative sample

C10271	Sample MIR	Plate				
Well	**Food**	**1**	**2**	**3**	**4**	**5**
1	Oat	-	-	-	-	-
2	Wheat	-	-	-	-	-
3	Rice	-	-	-	-	-
4	Corn	-	-	-	-	-
5	Rye	-	-	-	-	-
6	Durum Wheat	-	-	-	-	-
7	Gluten	-	-	-	-	-
8	Almond	-	-	-	-	-
9	Brazil	-	-	-	-	-
10	Cashew	-	-	-	-	-
11	Tea	-	-	-	-	-
12	Walnut	-	-	-	-	-
13	Cow Milk	-	-	-	-	-
14	Whole Egg	-	-	-	-	-
15	Chicken	-	-	-	-	-
16	Lamb	-	-	-	-	-
17	Beef	-	-	-	-	-
18	Pork	-	-	-	-	-
19	White Fish	-	-	-	-	-
20	Freshwater Fish	-	-	-	-	-
21	Tuna	-	-	-	-	-
22	Shellfish	-	-	-	-	-
23	Broccoli	-	-	-	-	-
24	Cabbage	-	-	-	-	-
25	Carrot	-	-	-	-	-
26	Leek	-	-	-	-	-
27	Potato	-	-	-	-	-
28	Celery	-	-	-	-	-
29	Cucumber	-	-	-	-	-
30	Peppers	-	-	-	-	-
31	Legume Mix	-	-	-	-	-
32	Grapefruit	-	-	-	-	-
33	Melon Mix	-	-	-	-	-
34	Peanut	-	-	-	-	-
35	Soya Bean	-	-	-	-	-
36	Cocoa Bean	-	-	-	-	-
37	Apple	-	-	-	-	-
38	Blackcurrant	-	-	-	-	-
39	Olive	-	-	-	-	-
40	Orange/Lemon	-	-	-	-	-
41	Strawberry	-	-	-	-	-
42	Tomato	-	-	-	-	-
43	Ginger	-	-	-	-	-
44	Garlic	-	-	-	-	-
45	Mushroom	-	-	-	-	-
46	Yeast	-	-	-	-	-
47	Negative	-	-	-	-	-
48	Positive	**+**	**+**	**+**	**+**	**+**

Conclusions: Manufacturing procedures give rise to highly reproducible intralot assay performance.

9.5 Interlot reproducibility

Method: Each kit lot is assayed at QC using sera with characterised food IgG immunoreactivity and the results are compared with those obtained with a previously passed kit lot to confirm reproducibility.

Results: See Table 10

Table 10.

	Sample IW	**Kit lot**		
Well	**Food**	**10276**	**10289**	**10303**
1	Oat			
2	Wheat	+	+	+
3	Rice			
4	Corn			
5	Rye	+	+	+
6	Durum Wheat			
7	Gluten	+	+	+
8	Almond			
9	Brazil			
10	Cashew	+	+	+
11	Tea	+	+	+
12	Walnut			
13	Cow Milk	+	+	+
14	Whole Egg	+	+	+
15	Chicken			
16	Lamb			
17	Beef			
18	Pork			
19	White Fish			
20	Freshwater Fish			
21	Tuna			
22	Shellfish	+	+	+
23	Broccoli			
24	Cabbage			
25	Carrot			
26	Leek			
27	Potato			
28	Celery			
29	Cucumber			
30	Peppers			
31	Legume Mix			
32	Grapefruit			
33	Melon Mix			
34	Peanut			
35	Soya Bean	+	+	+
36	Cocoa Bean	+	+	+
37	Apple			
38	Blackcurrant			
39	Olive			
40	Orange/Lemon			
41	Strawberry			
42	Tomato			
43	Ginger			
44	Garlic			
45	Mushroom			
46	Yeast			
47	Negative			
48	Positive	+	+	+

Conclusions: Manufacturing procedures assure interlot reproducibility

9.6 Matrix Effects

Method: The effects of common, potentially interfering substances were assessed by spiking samples with triglycerides to 3g/dL, haemoglobin to 100mg/dl and bilirubin to 40mg/dL. Blood samples with added triglycerides, haemoglobin and bilirubin were then tested in parallel with controls in Food Detective.

Results: See Figure 6 for representative results.
Figure 6.

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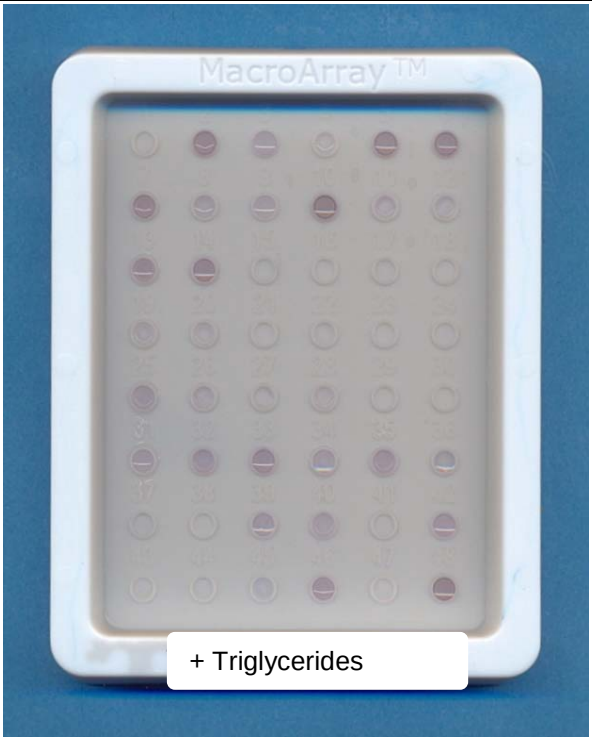
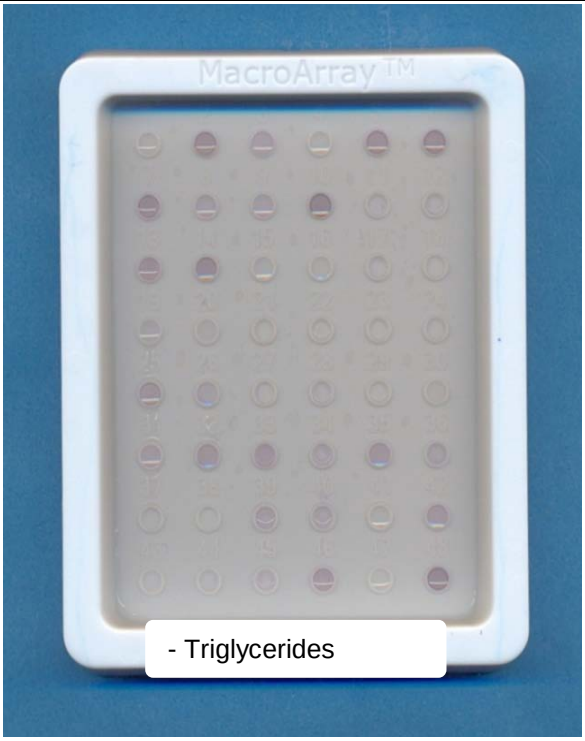
<tr><td> 
</td><td> 
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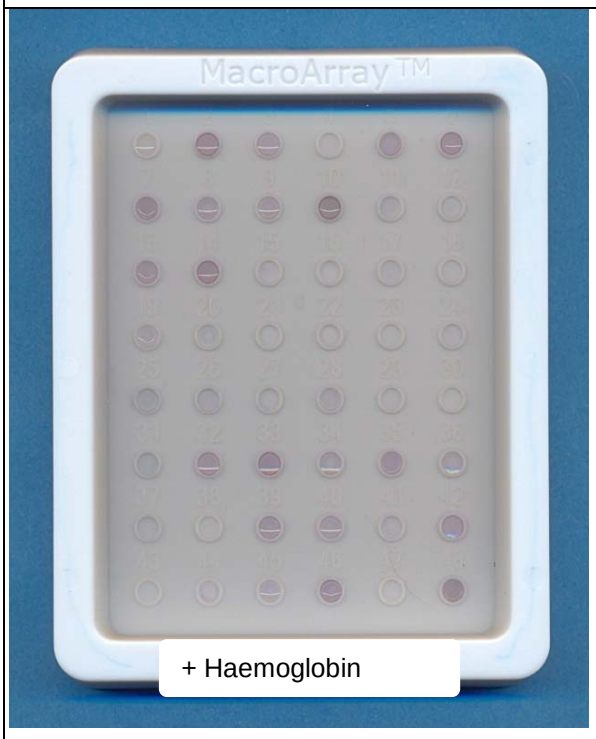
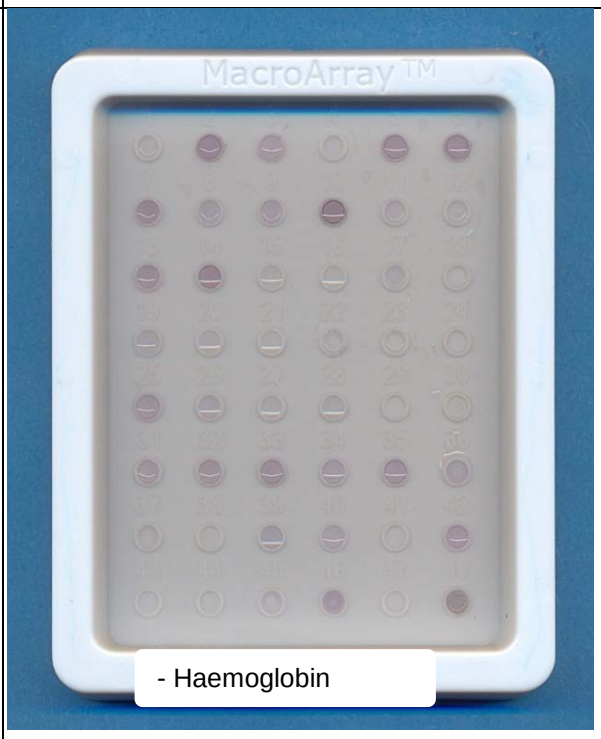
<tr><td> 
</td><td> 
</td></tr>
<tr><td>GD blood sample with 1000mg/dlL of haemoglobin</td><td>GD blood sample without haemoglobin</td></tr>
</table>

Figure 6 contd

	
GD blood sample with added 40mg/dl of bilirubin	GD blood sample without bilirubin

Conclusions: No interference was observed with hemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing blood samples.