

AllerFlow Gluten Validation & Comparison

Background:

AllerFlow Gluten is an environmental gluten residue test kit used for the detection of gluten residue on food processing surfaces. The test consists of an environmental sample collection swab and individually packaged lateral flow device.

This goal of this report is three-fold:

- Validation of limit of detection using dry and wet samples at varying gluten concentration
- Performance comparison of AllerFlow Gluten with other gluten surface residue test kits with gluten food samples
- Validation of non-reactivity with non-gluten and gluten-free labeled foods

Testing Plan:

1. Side-by-Side Comparison:

Compare Hygiena's AllerFlow Gluten, Neogen's Reveal® 3-D Gluten and r-biopharm's RIDA® QUICK Gliadin test using wheat flour and gluten (Sigma) as wet and dry samples at various dilutions.

2. Blind Food testing:

Test gluten, gluten-free labeled, and non-gluten food samples with AllerFlow Gluten.

Testing Procedure:

Sample preparation

1. Make 10% solution of samples to be tested using sterile water.
2. Make serial dilutions 10%, 1%, 0.1%, 0.01% and 0.001% of the above samples.

Sample collection and testing

1A. Dry sample: Dry 10 µl of sample on petri-dish. Swab surface according to kit insert sample collection instructions.

1B. Wet sample: Pipette 10 µl of sample on to swab. Continue according to kit insert instructions.

Part 1: Side by Side Comparison
Wheat Flour: Wet Samples

Table 1: AllerFlow Gluten, Wheat Flour, Wet Sample

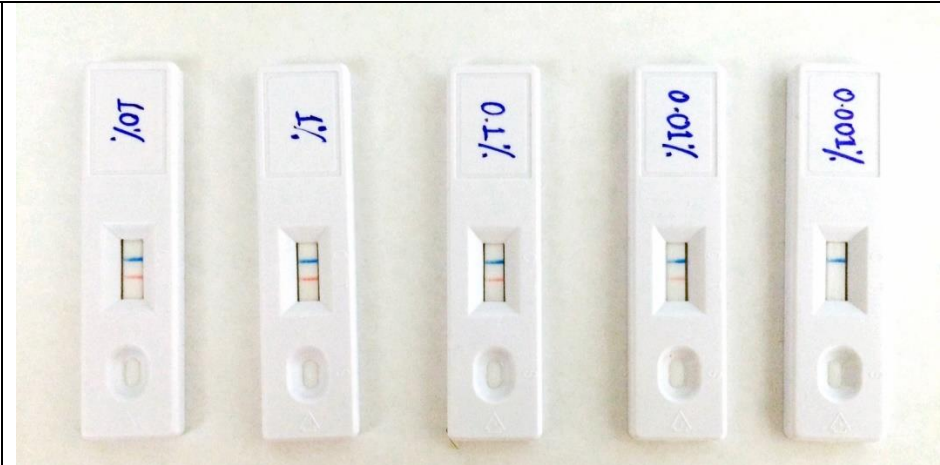
Results					
Dilution	10%	1%	0.1%	0.01%	0.001%
Interpretation	Fail	Fail	Fail	Fail	Pass

Table 2: r-biopharm RIDA QUICK Gliadin, Wheat Flour, Wet Sample


Results					
Dilution	10%	1%	0.1%	0.01%	0.001%
Interpretation	Fail	Fail	Fail	Fail	Pass

Table 3: Neogen Reveal 3-D Gluten, Wheat Flour, Wet Sample

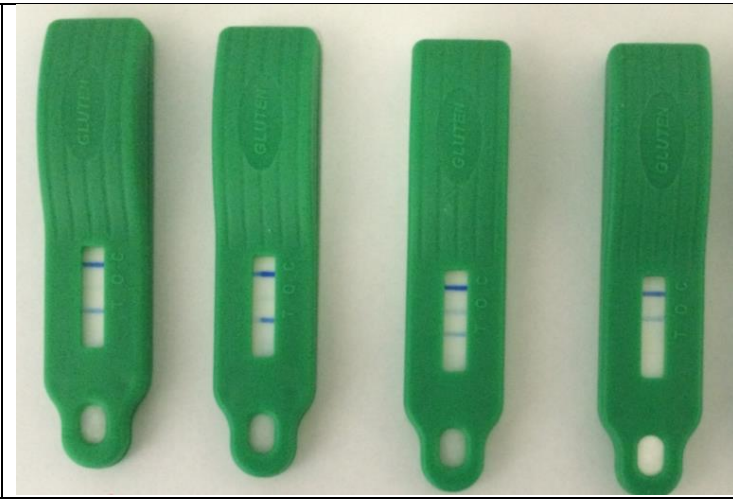
Results				
	Dilution	10%	1%	0.1%
Interpretation	Fail	Fail	Fail	Pass

Table 4: Limit of Detection Summary, Wheat Flour (wet)

Dilution	Hygiena	r-biopharm*	Neogen
10%	+	+	+
1%	+	+	+
0.1%	+	+	+
0.01%	+	+	-
0.001%	-	-	NA

Summary:

Kits are equivalently sensitive and can detect a wide range of results without error. When a very high gluten concentration sample is used with Neogen Reveal 3-D test kit, the device shows 1 single line. With many lateral flow kits on the market, a single line indicates a passing (no gluten) result. Thus, design of Reveal 3-D confuses user interpretation as well as side-by-side comparison of kit performance.

*Note: r-biopharm RIDA QUICK Gliadin detects gliadin, which makes up approximately 50% of gluten. (1 µg gliadin ≈ 2 µg gluten)

Wheat Flour: Dry Samples

Table 5: AllerFlow Gluten, Wheat Flour, Dry Sample


Results					
	Dilution	10%	1%	0.1%	0.01%
Interpretation	Fail	Fail	Fail	Pass	Pass

Table 6: r-biopharm RIDA QUICK Gliadin, Wheat Flour, Dry Sample


Results				
	Dilution	10%	1%	0.1%
Interpretation	Fail	Fail	Fail	Pass

Table 7: Neogen Reveal 3-D Gluten, Wheat Flour, Dry Sample


Results				
	Dilution	10%	1%	0.1%
Interpretation	Fail	Pass	Pass	Pass

Table 8: Limit of Detection Summary, Wheat Flour (dry)

Dilution	Hygiena	r-biopharm	Neogen
10%	+	+	+
1%	+	+	-
0.1%	+	+	-
0.01%	-	-	-
0.001%	-	-	NA

Summary:

Hygiena and r-biopharm kits are the most sensitive and can detect a wide range of results without error. Neogen Reveal 3-D kit was not able to successfully detect wheat flour from a dried surface sample, unlike other kits which were successful. See table below for user experience comparison.

Test Kit User Experience Comparison:

	Ease of Sample Collection	Ease of Interpretation	Value for Market Price
Hygiena AllerFlow Gluten	Easy	Clear	Superior
r-biopharm RIDA Quick Gliadin	Cumbersome	Clear	Fair
Neogen Reveal 3-D Gluten	Cumbersome	Confusing	Poor

Part 2: Blind Food Testing
Gluten Food (dry and wet samples)

Table 9: Gluten-containing Grains


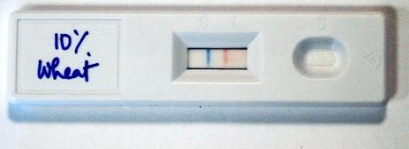
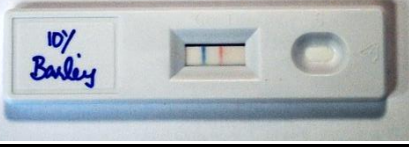
	10% Dilution Sample	Results
	Rye	Fail
	Wheat	Fail
	Barley	Fail

Table 10: Bread with Gluten




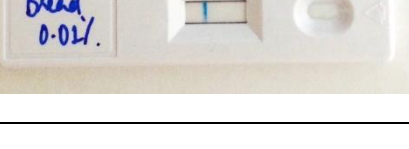
	Dilution	Results (wet)	Results (Dry)
	10%	Fail	Fail
	1%	Fail	Fail
	0.1%	Fail	Fail
	0.01%	Pass	Pass

Table 11: Donut


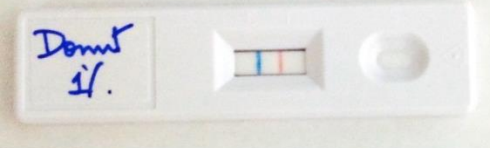

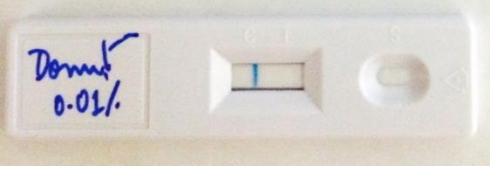
	Dilution	Results (wet)	Results (Dry)
	10%	Fail	Fail
	1%	Fail	Fail
	0.1%	Fail	Fail
	0.01%	Pass	Pass

Table 12: Cake


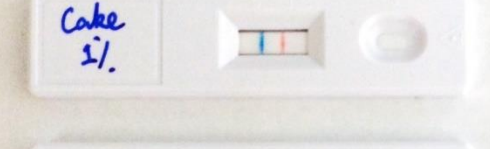
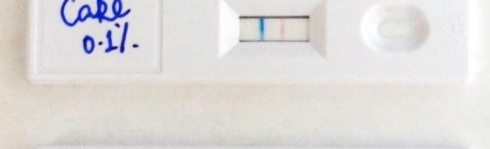

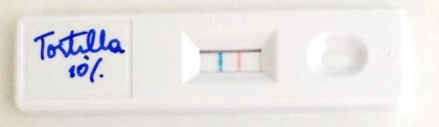

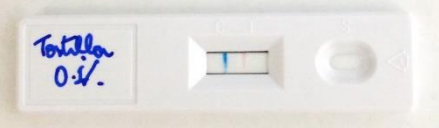
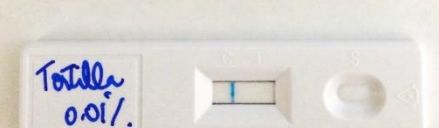
	Dilution	Results (wet)	Results (Dry)
	10%	Fail	Fail
	1%	Fail	Fail
	0.1%	Fail	Fail
	0.01%	Pass	Pass

Table 13: Tortilla

	Dilution	Results (wet)	Results (Dry)
	10%	Fail	Fail
	1%	Fail	Fail
	0.1%	Fail	Fail
	0.01%	Pass	Pass

Summary:

AllerFlow Gluten successfully detects a wide range of gluten products from wet and dried samples, including rye, wheat, and barley.

Non-Gluten and Gluten-Free Foods (dry and wet samples)

Table 14: Non-gluten foods (Testing for cross-reactivity)

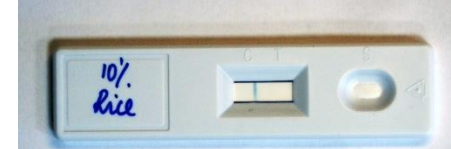


	10% Dilution Sample	Results
	Rice	Pass
	Corn	Pass
	Soy	Pass

Table 15: Quinoa

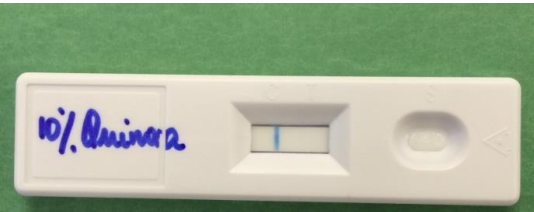

	Dilution	Results
	10%	Pass
	Control	-

Table 16: Butter

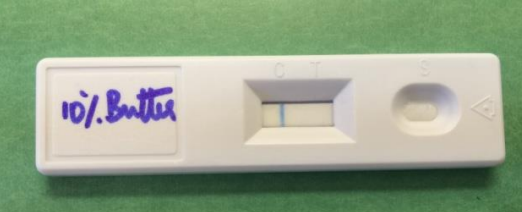
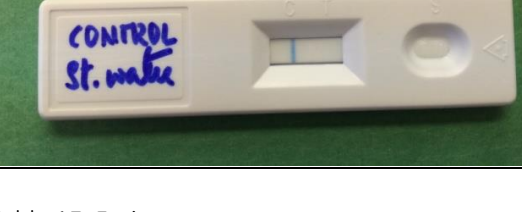
	Dilution	Results
	10%	Pass
	Control	-

Table 17: Fruit

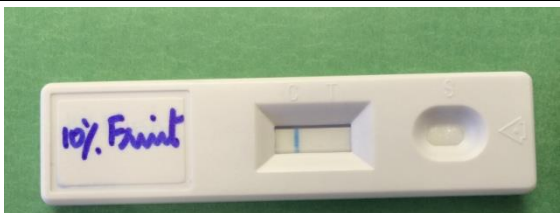

	Dilution	Results
	10%	Pass
	Control	-

Table 18: Nut





	Dilution	Results
	10%	Pass
	Control	-

Table 19: Vegetables

	Dilution	Results
	10%	Pass
	Control	-

Summary:

AllerFlow Gluten has no cross reactivity with rice, corn, soy, quinoa, or many other non-gluten foods.