



Validation Report for Reveal for Pork (Neogen item #9532)

Revision 1, May 2017

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Reveal for Pork

SUMMARY

Reveal for Pork is an immunochromatographic lateral flow assay used for the rapid, qualitative detection of pork meat in raw meats and processed raw meat products, such as sausages or burgers. Reveal for Pork is a qualitative test and should only be used as a preliminary screen for the presence of raw pork. The test is not suitable for cooked meat products. The validation report details the findings of the experimental evaluation to establish product claims for the Reveal for Pork test.

Limit of Detection: The limit of detection in raw meat samples was found to be 0.5% pork meat in a non-target meat matrix.

The limit of detection for environmental swabbing was found to be 15 µg/100 cm² pork protein on plastic and stainless steel surfaces.

The limit of detection for rinse waters was found to be 5 µg/mL pork protein.

Cross-reactivity: Reveal for Pork has been tested on a panel of raw meat cross-reactants. Cross-reactivity is expected in raw meat from the Suidae family, comprising of pigs, hogs and boars. No cross-reactivity against meat from non-target species such as beef, horse, poultry or sheep was observed.

Spike and Recovery: The Reveal for Pork test kit demonstrated excellent recovery in a variety of raw meat matrices.

Robustness: Variations in the extraction and test procedure were investigated over multiple days with no significant effect on the functionality of the test kit.

MATERIALS AND METHODS

All tests were conducted on standard quality control (QC) approved lots of Reveal for Pork test kits. All assays were performed in accordance with the test kit insert. Scoring of the lines: Throughout the data presented in this report, line intensity of the control and test line was scored by comparing the device to a reference card. The scale was measured between 0 (no line intensity) – 5 (highest line intensity).

LIMIT OF DETECTION

The sensitivity of Reveal for Pork was determined using spikes into a non-target meat matrix across three lots (L1, L2 and L3). The data is summarised in the table and graphs below.

Sample	Lot	Test Line (TL) scores			Average TL Score	Control Line (CL) scores			Average CL Score
Buffer	L1	0	0	0	0.00	3.5	3.5	3	3.33
	L2	0	0	0	0.00	3	3	2.75	2.92
	L3	0	0	0	0.00	2.5	3	3	2.83
100% Beef	L1	0	0	0	0.00	3.5	3	3	3.17
	L2	0	0	0	0.00	3	3	3	3.00
	L3	0	0	0	0.00	3	3	3	3.00
100% Horse	L1	0	0	0	0.00	3	3	3	3.00
	L2	0	0	0	0.00	2.75	2.75	3	2.83
	L3	0	0	0	0.00	2.5	2.5	2.5	2.50
100% Lamb	L1	0	0	0	0.00	3.5	3	3	3.17
	L2	0	0	0	0.00	3	3	3	3.00
	L3	0	0	0	0.00	2.5	3	3	2.83
100% Chicken	L1	0	0	0	0.00	3	3	3.25	3.08
	L2	0	0	0	0.00	2.5	3	2.75	2.75
	L3	0	0	0	0.00	2.5	2.5	2.5	2.50
0.1% Pork spiked into beef mince	L1	0.5	0.5	0.5	0.50	3	3	3	3.00
	L2	0.25	0.25	0.5	0.33	3	2.5	3	2.83
	L3	0.75	0.5	0.5	0.58	3	3	3	3.00
0.25% Pork spiked into beef mince	L1	1	0.75	0.75	0.83	3.5	3.25	3.5	3.42
	L2	1	0.5	1	0.83	3	3	3	3.00
	L3	1	1	1	1.00	3	3	3	3.00
0.5% Pork spiked into beef mince	L1	1.5	1.5	1.5	1.50	3.5	3.5	3.5	3.50
	L2	1.5	1.5	1.5	1.50	3	3	3	3.00
	L3	1.5	1.5	1.5	1.50	3	3	3	3.00

Sample	Lot	Test Line (TL) scores			Average TL Score	Control Line (CL) scores			Average CL Score
10% Pork spiked into beef mince	L1	2.75	2.75	2.5	2.67	3.25	3.25	3.5	3.33
	L2	2.5	3	3	2.83	3	3	3	3.00
	L3	3	3	3	3.00	3	3	3	3.00
50% Pork spiked into beef mince	L1	2.5	2.5	2.25	2.42	2.75	2.75	3	2.83
	L2	2.5	2.5	2.5	2.50	2.5	2.5	2.5	2.50
	L3	2.5	2.5	2.5	2.50	2.5	2.5	2.5	2.50
100% Pork	L1	2.5	2.25	2.25	2.33	3	3	2.75	2.92
	L2	2.25	2.5	2.25	2.33	2.5	2.5	2.5	2.50
	L3	2.25	2.25	2.5	2.33	2.25	2	2.5	2.25

The limit of detection for Reveal for Pork is 0.5 % raw pork meat in a non-target raw meat matrix.

CROSS-REACTIVITY

A panel of 34 samples including raw meats, processed raw meats and offal were tested for cross-reactivity and results are summarised in the table below:

Sample	Result
Beef burger	Negative
Beef fat	Negative
Beef liver	Negative
Beef, diced meat	Negative
Beef steak mince	Negative
Beef, peppered grill steak	Negative
Bresaola	Negative
Buffalo burger	Negative
Chicken breast	Negative
Chicken breast mince	Negative
Chicken liver	Negative
Chicken sausage	Negative

Sample	Result
Chicken skin	Negative
Chicken, Chinese style	Negative
Chicken, hickory BBQ	Negative
Chicken, Piri Piri	Negative
Duck meat	Negative
Goat meat	Negative
Goat mince	Negative
Horse steak mince	Negative
Lamb fat	Negative
Lamb kidney	Negative
Lamb liver	Negative
Lamb meatball	Negative

Sample	Result
Lamb mince	Negative
Lamb, leg steak	Negative
Ox kidney	Negative
Rabbit meat	Negative
Turkey breast mince	Negative
Turkey meat	Negative
Turkey thigh mince	Negative
Turkey, sweet chilli burger	Negative
Venison meat	Negative
Venison mince	Negative

Reveal for Pork

SPECIFICITY TESTING

A panel of 26 samples containing pork were tested including processed raw meats, cured meats, fermented meats and offal. Of these, black pudding, Peperami –style sausage and smoked German frankfurters returned negative results. It is likely that these products were heat-treated and not suitable for testing using the Reveal for Pork.

Sample	Pork content of Sample	Result
Pork loin	100% Pork loin	Positive
Pork mince	100% Pork mince	Positive
Wild boar meat	100% Wild boar meat	Positive
Wild boar mince	100% Wild boar mince	Positive
Pork in Chinese style sauce	90% Chinese style pork leg steak	Positive
Beef sausage with a natural pork casing	Pork casing	Positive
Pork casing from beef sausage	100% Pork casing	Positive
Lorne Sausage	19.5% Pork	Positive
Pork sausage	40% Pork	Positive
Beef and Pork meatball	34% Pork	Positive
Pork & Apple Burger	72% Pork	Positive
Smoked bacon and maple syrup pork sausage	65% Pork, 15% bacon	Positive
Sausage roll	11% Pork	Positive

Sample	Pork content of Sample	Result
Smoked diced bacon	100% Smoked Bacon Lardons	Positive
Chestnut smoked sweet cured gammon steak	91% Pork	Positive
Parma ham	130g pork per 100g finished product	Positive
Spanish Chorizo	133g Pork per 100g finished product	Positive
German Salami	135g Pork per 100g finished product	Positive
Peperami – style sausage*	147% Pork	Negative
German Smoked Frankfurters*	80% Pork	Negative
Pork liver	100% Pork liver	Positive
Pig kidney	100% Pig kidney	Positive
Pig heart	100% Pig heart	Positive
Pork fat	100% Pork fat	Positive
Black Pudding*	Pork content was unspecified	Negative
Pig skin	100% Pig skin	Positive

*Samples are likely to have undergone heat process

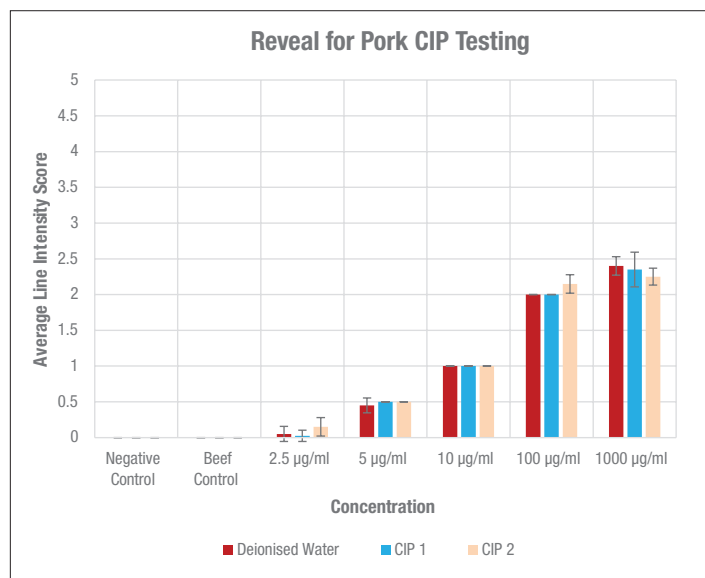
SPIKE AND RECOVERY

A panel of 26 negative target matrices were spiked with 0.5% pork meat spike. Following spiking all samples returned positive results.

Sample Type	Product Description	Pork Spike	Result
Beef meat	Scotch Diced Beef	0.5% Pork spike	Positive
Horse steak	Horse Portioned Steak	0.5% Pork spike	Positive
Lamb leg steak	New Zealand Lamb Leg Steak	0.5% Pork spike	Positive
Chicken breast	Chicken Breast Portions	0.5% Pork spike	Positive
Turkey meat	British Turkey Breast	0.5% Pork spike	Positive
Goat meat	Frozen Goat Goulash (Bone In)	0.5% Pork spike	Positive
Venison meat	N/A	0.5% Pork spike	Positive
Rabbit meat	Rabbit Fillet. Rabbit 100%.	0.5% Pork spike	Positive
Duck meat	Duck breast meat 100%.	0.5% Pork spike	Positive
Beef steak mince	British Lean Minced Beef Steak. 5% fat.	0.5% Pork spike	Positive
Horse steak mince	Made from horse steak	0.5% Pork spike	Positive
Lamb mince	British Minced Lamb	0.5% Pork spike	Positive
Chicken breast mince	Made from Chicken Breast	0.5% Pork spike	Positive
Turkey breast mince	British Turkey Breast Mince	0.5% Pork spike	Positive
Turkey thigh mince	British Turkey Thigh Mince	0.5% Pork spike	Positive
Goat mince	Made from goat meat	0.5% Pork spike	Positive
Venison mince	Made from venison meat	0.5% Pork spike	Positive
Rabbit mince	Made from rabbit fillet	0.5% Pork spike	Positive
Duck mince	Made from duck meat	0.5% Pork spike	Positive

Sample Type	Product Description	Pork Spike	Result
Piri Piri Marinated Chicken	Hot Piri Piri Marinated Chicken Stir Fry. Chicken 95%.	0.5% Pork spike	Positive
Hickory BBQ Chicken	Hickory BBQ Chicken Steak. Chicken 95%.	0.5% Pork spike	Positive
Chicken with Chinese style sauce	British Chicken Chinese Style Drumsticks & Thighs - Fresh class A skin-on chicken drumsticks and thighs with a Chinese style marinade. Chicken 93%.	0.5% Pork spike	Positive
Sweet chilli turkey burger	Sweet Chilli British Turkey Burgers - Gluten free turkey burgers with sweet chilli seasoning. Turkey 86%.	0.5% Pork spike	Positive
Peppered beef grillsteak	British Beef Peppered Grillsteaks - Chopped and shaped beef coated in pepper. Beef 81%.	0.5% Pork spike	Positive
Beef burger	British Beef Burgers - Burgers made with chopped and shaped, lightly seasoned, formed beef, with onion. Beef 63%.	0.5% Pork spike	Positive
Lamb meatball	Lamb meatballs with salt and black pepper. Lamb 94%.	0.5% Pork spike	Positive
Chicken sausage	85% Chicken Chipolatas with Mozzarella and Tomatoes	0.5% Pork spike	Positive

CIP RINSE WATERS: Clean-in-place (CIP) rinse waters and deionised water were spiked with pork protein to determine sensitivity. Deionised water, CIP1 and CIP2 all produced negative results at 0 ppm and positive results at 5 µg/mL pork protein.



ENVIRONMENTAL SWABBING: Plastic and stainless steel surfaces were spiked with known concentrations of pork protein extract. Spiked surfaces were allowed to dry for 2 hours prior to testing. Positive results were obtained on both stainless steel and plastic surfaces at 15 µg/100 cm².

Note: The environmental swabs that have been validated with this test are available from Neogen, Reveal for Meat Speciation Swabbing Kit #9541.

Surface Type	Sensitivity Summary (µg/100 cm ²)	Result
Plastic	15	Positive
Stainless Steel	15	Positive

ROBUSTNESS

Samples of mince were prepared for the randomised blind robustness experiment. Mince samples were 100% beef, 100% horse, 100% lamb, 100% chicken, 0.5% pork (limit of detection) and 10% pork. Each operator extracted 3 samples of each sample type and ran samples against three different lots of Reveal for Pork test strips.

The robustness experiments were carried out with three operators across two days.

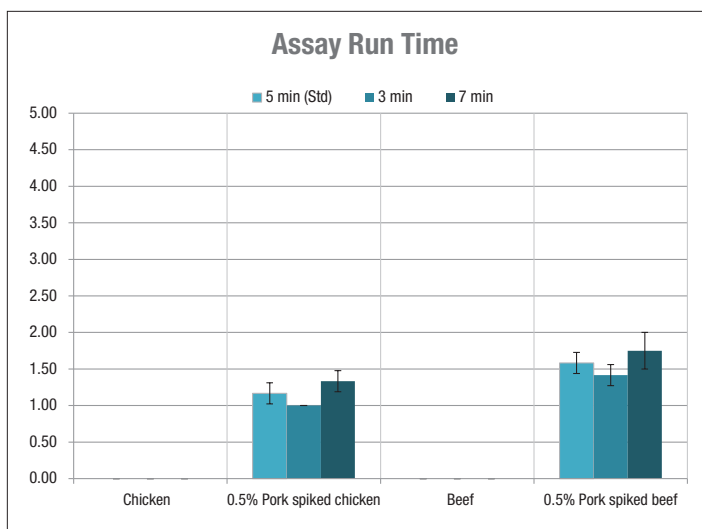
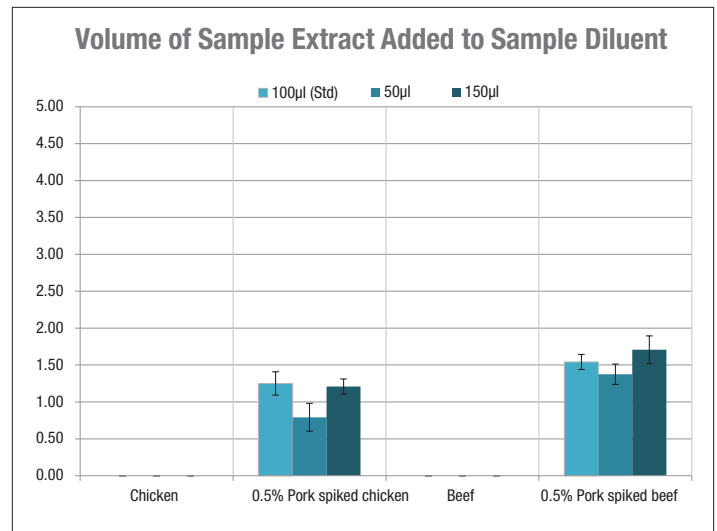
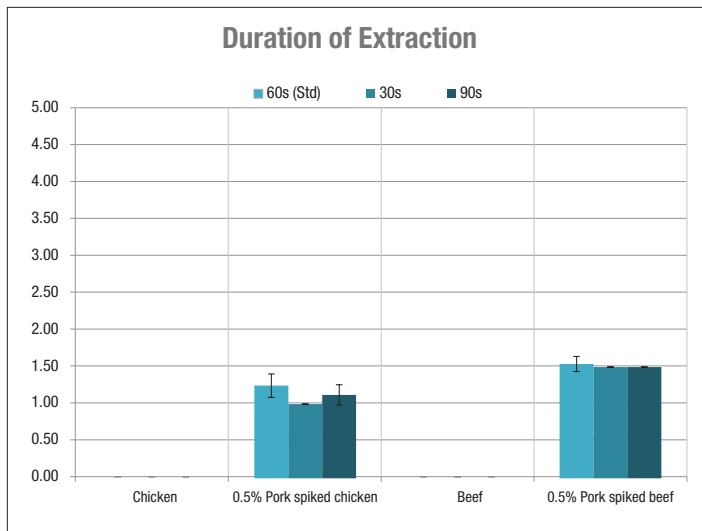
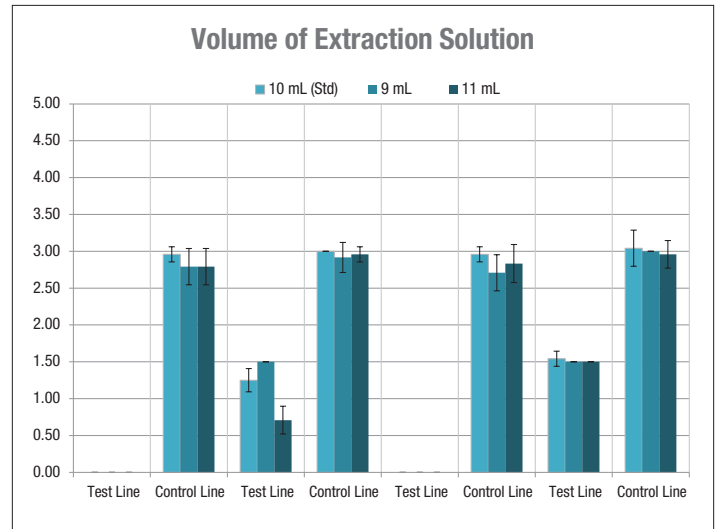
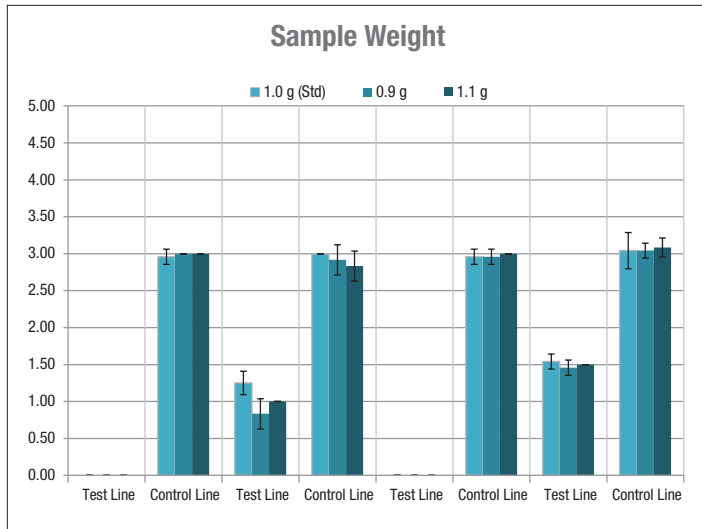
A total of 216 test devices were evaluated. All 144 test devices that gave negative results were expected to be negative. All 72 test devices that gave positive results were expected to be positive.

Sample	Total Number of Samples Tested	Total Number of LFDs Tested with Samples	Expected Result	% Required Results Obtained
100% Beef	18	36	Negative	100%
100% Lamb	18	36	Negative	100%
100% Horse	18	36	Negative	100%
100% Chicken	18	36	Negative	100%
0.5% Pork	18	36	Positive	100%
10% Pork	18	36	Positive	100%

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RUGGEDNESS

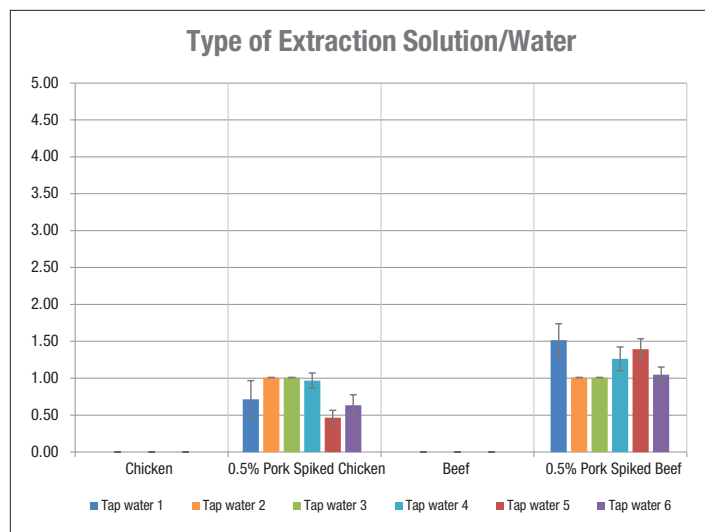
During development the Reveal for Pork assay was assessed for ruggedness by varying a number of conditions from the standard operating procedure. Conditions that were varied and represented in the graphs below included sample weight, volume of extraction solution, duration of extraction, volume of sample extract added to sample diluent and assay run time.



No significant variation was found by altering the conditions of the test. Sensitivity of the device was met irrespective of varying the conditions. This demonstrates that the Reveal for Pork assay is rugged when compared against normal operating conditions.

Extraction Evaluation – Water Type

During development mains water from a number of sources were evaluated against deionised water to determine if mains tap water would be a suitable for sample extraction.



In the test kit insert, we recommend that distilled or deionized water is used for sample extraction. However, from the data summarised in the graph below, it may be possible for mains tap water to be used for sample extraction. For customers wishing to use mains tap water, we would recommend that a validation is carried out prior to routine testing to determine suitability.

BETA SITE RESULTS

The beta site evaluation included six independent testing locations. Each site was asked to evaluate the method and performance of the test kit by evaluating blind samples provided by Neogen.

Six samples extracts were tested in duplicate using a new Reveal for Pork device and results can be seen below:

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Horse mince	Negative	Negative	Negative	Negative	Negative	Negative
0.5% pork spiked beef mince	Negative*	Positive	Positive	Positive	Positive	Positive
Lamb mince	Negative	Negative	Negative	Negative	Negative	Negative
Chicken mince	Negative	Negative	Negative	Negative	Negative	Negative
10% pork spiked beef mince	Positive	Positive	Positive	Positive	Positive	Positive
Beef mince	Negative	Negative	Negative	Negative	Positive/Negative **	Negative

* Site reported negative, test lines could be seen on returned photographs

** Site reported negative on one strip and faint positive on replicate strip

CONCLUSION

Throughout a wide range of tests, the Reveal for Pork test kit demonstrated excellent spike and recovery and superior test robustness. This coupled with its reproducibility make Reveal for Pork a good choice for the quick accurate detection of pork contamination in raw meat matrices, surface swabbing and rinse waters.



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