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Multi-laboratory evaluation of staphylococcal enterotoxin detection by the RIDASCREEN SE A, B, C, D, E ELISA kit

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Abstract

The RIDASCREEN® SE A, B, C, D, E kit was evaluated for use as a secondary test for the detection of staphylococcal enterotoxins in meat matrices. Seven state agriculture and public health laboratories participated in the study. The study demonstrated that the kit is able to detect each of the five enterotoxin serotypes in hot dogs, breaded chicken nuggets, bologna, and ready-to-eat barbeque meat. Staphylococcal enterotoxins were detected at a minimum of 1.0 ng/g in all matrices and at levels as low as 0.375 ng/g in some matrices. While cross-reaction between some serotypes is seen, false negative and false positive results were minimally observed. When used along with the BioMerieux VIDAS® SET2 automated immunoassay test, this kit provides a secondary assay to use as confirmation for the presence of staphylococcal enterotoxins in a meat sample.

Keywords: RIDASCREEN, ELISA, Staphylococcal enterotoxins

1. Introduction

A variety of foods have been associated with staphylococcal enterotoxin (SET) illness, such as dairy products, pastries, sandwiches and meats [10, 7, 5]. SET related illness is caused by as many as 20 or more different protein toxins causing emetic and/or diarrheal symptoms [7, 6, 2]. Diagnosis of staphylococcal enterotoxin illness commonly relies on isolating coagulase-positive staphylococcal organisms from the patient and/or identification of the presence of staphylococcal enterotoxins in the associated food [7]. The detection of staphylococcal enterotoxins in foods has been accomplished by several methods, both automated and or manual, in regulatory food testing laboratories. While staphylococcal enterotoxin A is the most commonly identified food-related toxin, commercially available immunoassays also detect toxins B, C, D, and E [2, 3, 6, 9]. Testing laboratories often utilize two separate assay kits for analysis of samples to first detect the presence of the toxins as a group and then to identify the individual toxin serotype(s) present. Recently, a set of commonly used and commercially available SET detection kits, 3M Tecra SET VIA and SET VIA ID, were discontinued (discontinuation date of September 2016); these test kits detected the presence of toxins as a group or by individual serotype, respectively. It is vital that laboratories have an appropriate and sensitive testing kit to replace the discontinued Tecra tests. A workgroup consisting of state food testing laboratories performed a verification study of the RIDASCREEN® SE A, B, C, D, E ELISA kit to be used along with the VIDAS® SET2 assay for detection and confirmation of staphylococcal enterotoxins (SET) in meat matrices. The goal of this workgroup was to provide useful data on the performance of the RIDASCREEN® SE A, B, C, D, E kit as a confirmatory test for regulated commodities that initially test positive by the VIDAS® SET2 kit.

2. Validation Study Overview

2.1. Food Matricies

The RIDASCREEN® SE A, B, C, D, E kit was evaluated on four different meat matrices: bologna, breaded chicken nuggets, pork-based hot dogs, and ready-to-eat pork barbeque. The participating laboratories were assigned specific meat matrices in which to perform the verification (Table 1). For each assigned matrix, the laboratories acquired three different product types from local area retail markets. Laboratories were instructed to choose different product types based on criteria such as variety, class, formulation, or manufacturer. For instance, hot dogs produced by different manufacturers or hot dogs that were “jumbo” vs “smoked” were considered different product types.

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	Bologna	**Breaded Chicken Nugget, cooked**	**Pork-based Hot Dogs**	**Barbeque Meat (RTE)**
Laboratory 1	Toxins A/C [a]		Toxins A/C	
Laboratory 2		Toxins A/C		Toxins A/C
Laboratory 3	Toxins B/D		Toxins B/D	
Laboratory 4	Toxin E		Toxin E	
Laboratory 5		Toxins B/D		Toxins B/D
Laboratory 6		Toxin E	Toxin E	Toxin E
Laboratory 7		Toxins A/D	Toxins A/D	

Table 1: Toxin and food assignments by laboratory.

[a]Each participating laboratory was assigned toxin(s) and food matrices for this validation according to the table above, allowing for each toxin/matrix combination to be evaluated.

State	**Toxin/Lot**	**Toxin/Lot**	**RIDASCREEN Lot**	**VIDAS SET2 Lot**
Laboratory 1	SEA 012412A	SEC 32602C1	13426	1005014110
Laboratory 2	SEA 012412A	SEC 32602C1	13426	Not applicable [a]
Laboratory 3	SEB 50916B	SED 12516D	13426	1005172170
Laboratory 4	SEE 100714E	Not applicable [a]	13426	1005344730
Laboratory 5	SEB 121712B	SED 32415D	13426	1005344730& 1005172170
Laboratory 6	SEE 100714E	Not applicable [a]	13426	1005172170& 1004931710
Laboratory 7	SEA 012412A	SED 32415D	13426	1005182170

Table 2: Toxin and test kit lot numbers used by each laboratory.

[a]Lab did not have a VIDAS instrument available during the study.

2.2. Staphylococcal enterotoxins

Each participating laboratory was assigned a specific serotype(s) of staphylococcal enterotoxin used for matrix spiking in the study. Staphylococcal enterotoxin standards were obtained by each laboratory from Toxin Technology (https://www.toxintechnology.com) and included toxins from more than one production lot for serotypes B and D (Table 2). The detection of each serotype was performed at three levels: 0 ng/g, 0.375 ng/g and 1 ng/g. The manufacturer's instructions for the RIDASCREEN® kit includes a statement stating the presence of cross-reaction between particular serotypes (cross-reactions are known to occur between antibody/serotype: A/E, E/A, B/C and C/B). Laboratories spiking samples with two toxins utilized toxins that are not known to cross-react based on the manufacturer's kit instructions.

2.3. Test Kits

Each laboratory acquired RIDASCREEN® SE A, B, C, D, E kits from r-bio-pharm AG (https://food.r-biopharm.com/). All kits tested in this study were from the same manufacturer's lot (Table 2). Samples analyzed by the RIDASCREEN® SE A, B, C, D, E kit were extracted and analyzed according to the manufacturer's protocol for solid foods. Laboratories analyzed samples in parallel utilizing the BioMerieux (http://www.biomerieux-industry.com/) VIDAS® SET2 kit. Two laboratories utilized two different manufacturing lots of VIDAS® SET2 kits in this study (Table 2).

2.4. Study Protocol

The meat matrices and individual serotypes of SET were assigned to the participating laboratories so that each enterotoxin was tested in each meat matrix (Table 1). Individual lab-

Matrix	Laboratory [a]	Avg OD Well F	Avg OD Well G
Hot Dogs	1	0.041	0.042
	3	0.003	0.002
	6	0.054	0.058
	7	0.040	0.040
Bologna	1	0.043	0.042
	3	0.006	0.003
	4	0.039	0.042
BBQ Meat	2	0.032	0.032
	5	0.006	0.005
	6	0.0049	0.059 w/ one outlier at 1.035
Chicken Nuggets	2	0.045	0.048
	5	0.012	0.009
	6	0.060	0.065
	7	0.060	0.057

Table 3: Average background levels by commodity (measurement of matrix interference).

[a]Shakers/Mixers used (by Lab): Laboratory 1 - GenoGrinder; Laboratories 3, 4, 7 - Orbital Shaker; Laboratories 2, 6 - Wrist Action Shaker; Laboratory 5 - Plate Shaker

oratories were responsible for preparing and analyzing spiked samples following a written protocol to ensure consistency between participating laboratories. For samples analyzed using the RIDASCREEN® SE A, B, C, D, E kit, a 10-g sample portion was weighed. For samples analyzed in parallel using VIDAS® SET2, a 25-g sample portion was weighed. Samples were spiked with SET diluted in phosphate buffer saline (PBS) from the 100-μg/mL stock and allowed to sit for 30 min before the analysis. For each of the three matrix brands, two samples were spiked at either 0.375 ng/g or 1 ng/g of the assigned SET serotype(s) and one sample had no toxin added. Samples analyzed using the RIDASCREEN® SE A, B, C, D, E kit were extracted following the manufacturer's protocol for solid food. Samples analyzed using the VIDAS® SET2 kit were extracted following the AOAC OMA 2007.06 extraction method for raw meat, seafood and delicatessen meat [1]. Testing for all samples spiked at 0.375 ng/g and 1 ng/g were performed in triplicate using the RIDASCREEN® SE A, B, C, D, E kit and VIDAS® SET2 kit. The non-spiked samples were only subjected to one analysis. This protocol allowed for the laboratories to limit the amount of staphylococcal enterotoxin they needed to purchase and store in the laboratory. It also allowed a comparison of matrix interference with the kit, as demonstrated or reported by individual laboratories.

3. Results and Discussion

Overall, the RIDASCREEN® SE A, B, C, D, E kit showed favorable results for the identification of specific enterotoxins in the selected meat matrices. Based on achieving greater than 50% detection, the limit of detection for all matrices except breaded chicken was 0.375 ng/g of food tested (Table 4). Breaded chicken spiked at low level (0.375 ng/g) with serotype A showed no positive results. Additional breaded chicken samples spiked at low levels with toxin serotype C showed one positive result from nine samples. Breaded chicken spiked with toxin serotype E at 0.375 ng/g level tested positive for all nine samples. Previous studies performed on breaded chicken for the detection of SET have shown significant matrix interference (data not shown). The overall evaluation results showed that breaded chicken nuggets limit of detection (LOD) was 1 ng/g of food for toxins A, B and C and 0.375 ng/g of food for toxin D and E (Table 4).

There are known cross-reactivity issues with this test kit as described by the manufacturer in the test kit insert. Cross-reactions are known to occur between serotypes A/E, E/A, B/C and C/B. The cross-reactivity was confirmed (Table 5) as those laboratories spiking with serotype A and serotype C saw cross-reactivity with serotype E and serotype B, respectively. Cross-reactivity was more pronounced in samples that received the higher spiking level of 1 ng/g. According to the manufacturer, cross-reactivity issues may be resolved by diluting a positive sample 1:10 and re-analyzing.

Within the cumulative dataset, only breaded chicken nuggets displayed potential matrix interference with the detection of SET. When reanalyzed, breaded chicken nuggets continued to show high OD (signal) values in the negative control wells (data not shown) which is indicative of potential matrix interference. The analyzing laboratory substituted a second brand of chicken nuggets and analyzed it. The results from this different brand showed low interference as indicated by low OD values for the negative controls; this second brand of breaded

Matrix	Toxin	Level (ng/g)	Total Tested	Total Positive	Detection Rate, % [a]	LOD/g [b]	Specificity [c]	False Positive, %
Bologna	A	0.375	9	9	100	0.375	100	0
		1	9	9	100			
		0	3	0	0			
	B	0.375	9	8	89	0.375	100	0
		1	9	9	100			
		0	3	0	0			
	C	0.375	9	9	100	0.375	100	0
		1	9	9	100			
		0	3	0	0			
	D	0.375	9	8	89	0.375	100	0
		1	9	9	100			
		0	3	0	0			
	E	0.375	9	7	78	0.375	33	67
		1	9	9	100			
		0	3	2	67			
Breaded Chicken Nuggets	A	0.375	18	9	50	1	100	0
		1	18	18	100			
		0	6	0	0			
	B	0.375	9	0	0	1	100	0
		1	9	9	100			
		0	3	0	0			
	C	0.375	9	1	11	1	100	0
		1	9	9	100			
		0	3	0	0			
	D	0.375	18	13	72	0.375	100	0
		1	18	18	100			
		0	6	0	0			
	E	0.375	9	9	100	0.375	100	0
		1	9	9	100			
		0	3	0	0			

Table 4: RIDASCREEN method performance results.

[a]Detection rate = total number of positive test samples/total number of test samples spiked x 100
[b]LOD/g = limit of detection of the assay per gram/mL
[c]Specificty = total number of true negative samples/(total number of true negative test samples + false positie samples) x 100

Matrix	Toxin	Level (ng/g)	Total Tested	Total Positive	Detection Rate, %	LOD/g	Specificity	False Positive, %
Pork Hot Dogs	A	0.375	18	12	67	0.375	100	0
		1	18	18	100			
		0	6	0	0			
	B	0.375	9	6	67	0.375	100	0
		1	9	9	100			
		0	3	0	0			
	C	0.375	9	9	100	0.375	100	0
		1	9	9	100			
		0	3	0	0			
	D	0.375	18	15	83	0.375	100	0
		1	18	18	100			
		0	6	0	0			
	E	0.375	9	9	100	0.375	100	0
		1	9	9	100			
		0	3	0	0			
BBQ (RTE)	A	0.375	9	9	100	0.375	100	0
		1	9	9	100			
		0	3	0	0			
	B	0.375	9	8	89	0.375	100	0
		1	9	9	100			
		0	3	0	0			
	C	0.375	9	9	100	0.375	100	0
		1	9	9	100			
		0	3	0	0			
	D	0.375	9	9	100	0.375	100	0
		1	9	9	100			
		0	3	9	0			
	E	0.375	9	9	100	0.375	100	0
		1	9	9	100			
		0	3	0	0			

Table 4: Continued.

chicken nuggets was used in the study summary and conclusions. The data from the breaded chicken nugget samples also showed poor detection of serotypes A, B and C at the 0.375 ng level. It is possible that the toxin was bound to proteins within the sample matrix as the extraction method used for the RIDASCREEN® SE A, B, C, D, E kit samples is a phosphate buffer extraction with no pH lowering step to aid in release of the toxin from the meat precipitate. Overall, the OD readings for toxin-specific and the positive control wells were consistent between all laboratories (data not shown). The negative control wells (wells F and G) also showed consistent OD readings between all participating laboratories (Table 3).

A false positive was determined in two different ways due to the known cross-reactivity of the toxin serotypes (A/E, B/C). Only one instance of a false positive result in a blank well occurred during this study. When included as part of the whole study, these results give a false positive rate of only 2.7%. Positive results due to the cross-reactions identified by the manufacturer where not included in false positive calculations. For example, samples spiked with serotype A that also showed a positive signal in serotype E were not counted as false positives. False positive rates are calculated only from samples with no toxin added. The sample is positive for SET; however, it was ambiguous for the serotype. The same would be true for samples spiked with serotype E that are positive in serotype A wells and for the samples containing serotypes B and C. However, samples spiked with only serotype A that were also positive for serotypes B, C, or D would have been considered false positives as the known cross-reactivity does not cover these situations. The false positive rate for these occurrences is shown in Table 5. One laboratory recorded false positive results for serotype D in samples spiked with toxin serotypes A and C in two of nine samples, leading to a false positive rate of 1.1% for this particular serotype. No other false positive results for serotype D were identified with other matrices or SET spiking patterns. The results are shown in Table 5.

The ability to demonstrate a specific serotype(s) of staphylococcal enterotoxin in a food sample can increase a laboratory's confidence in positive results. When used in combination with the VIDAS® SET2 test, the RIDASCREEN® SE A, B, C, D, E kit can be relied upon to analyze a variety of meat-based matrices for the presence of SET. While cross-reactions between serotypes A/E, E/A, B/C and C/B are known to occur, there are ways to distinguish between cross-reactivity and a true positive. According to the test kit manufacturer, the OD of the true positive sample should be approximately 40% greater than the OD of the serotype suspected as cross-reacting. To distinguish between a true positive reading and cross-reactivity, the sample may be diluted in PBS and re-analyzed. As the number of identified serotypes increases, identification of specific serotypes may not always be possible. Laboratories may need to adopt more robust assays such as mass spectrometry [4, 8] or polymerase chain reaction assays [7].

4. Declaration of Conflicting Interest

The authors declare no conflicts of interest.

5. Disclaimer

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view(s) of the U. S. Department of Agriculture or the Food Safety and Inspection Service.

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7. Article Information

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8. References

[1] AOAC International. Official Methods of Analysis. Retrieved from http://www.eoma.aoac.org/methods/info.asp?ID=49057

[2] Argudín, M. A., Mendoza, M. C., & Rodicio, M. R. (2010). Food poisoning and Staphyloococcus aureus enterotoxins. *Toxins, 2(7),* 1751-1773.

[3] Bennett, R. W. (2005). Staphylococcal enterotoxin and its rapid identification in foods by enzyme-linked immunosorbent assay-based methodology. *Journal of Food Protection, 68(6),* 1264-1270.

[4] Dupuis, S., Hennekinne, J-A., Garin, J., & Brun, V. (2008). Protein Standard Absolute Quantification (PSAQ) for improved investigation of staphylococcal food poisoning outbreaks. *Proteomics, 8(22),* 4633-4636.

[5] Genigeorgis, C. A. (1989). Present state of knowledge on staphylococcal intoxication. *International Journal of Food Microbiology, 9(4),* 327-360.

[6] Hait, J. M., Tallent, S. M., & Bennett R. W. (2014). Screening, detection, and serotyping methods for toxin genes and enterotoxins in Staphylococcus strains. *Journal of AOAC International, 97,* 1078-1083.

[7] Hennekinne, J-A., De Buyser, M-L., & Dragacci, S. (2012). Staphylococcus aureus and its food poinsoning toxins: characterization and outbreak investigation. *FEMS Microbiology Review, 36,* 815-836.

[8] Mamone, G., Picariello, G., Caira, S., Addeo, F., & Ferranti, P. (2009). Analysis of food proteins and peptides by mass spectrometry-based techniques. *Journal of Chromatography A, 1216(43),* 7130-7142.

[9] Ostyn, A., Guillier, F., Prufer, A-L., Papinaud, I., Messio, S., Krys, S., Lombard, B., & Hennekinne, J-A. (2011). Intra-laboratory validation of the Ridascreen® SET Total kit for detecting staphylococcal enterotoxins SEA to SEE in cheese. *Letters of Applied Microbiology, 52(5),* 468-474.

[10] Schelin, J., Susilo, Y. B., & Johler, S. (2017). Expression of staphylococcal enterotoxins under stress encountered during food production and preservation. *Toxins, 9,* 401-410.

[11] Jechorek, R. P. & Johnson, R. L. (2008). Evaluation of the VIDAS staph enterotoxin II (SET 2) immunoassay method for the detection of staphylococcal enterotoxins in selected foods: collaborative study. *Journal of AOAC International, 91(1),* 164-73.

Lab/Matrix/ Spike Level	Toxin Assigned	SEA Result	SEB Result	SEC Result	SED Result	SEE Result	VIDAS SET2 Result
1 -Bologna-L1	A & C	9/9 Positive	9/9 Negative	9/9 Positive	9/9 Negative	9/9 Negative	9/9 Positive
2 -Bologna-L2	A & C	9/9 Positive	9/9 Positive [a]	9/9 Positive	9/9 Negative	9/9 Positive [a]	9/9 Positive
1 -Bologna-MB	A & C	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative
3 -Bologna-L1	B & D	9/9 Negative	8/9 Positive	9/9 Negative	8/9 Positive	9/9 Negative	9/9 Positive
3- Bologna-L2	B & D	9/9 Negative	9/9 Positive	5/9 Positive [a]	9/9 Positive	9/9 Negative	9/9 Positive
3 -Bologna-MB	B & D	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative
4 -Bologna-L1	E	9/9 Negative	1/9 Positive [b]	9/9 Negative	1/9 Positive [b]	7/9 Positive	9/9 Positive
4 -Bologna-L2	E	8/9 Positive [a]	9/9 Negative	9/9 Negative	1/9 Positive [b]	9/9 Positive	9/9 Positive
4 -Bologna-MB	E	3/3 Negative	1/3 Positive [c]	1/3 Positive [c]	3/3 Negative	3/3 Negative	3/3 Negative
1 -Hot Dog-L1	A & C	3/9 Positive	9/9 Negative	9/9 Positive	9/9 Negative	9/9 Negative	9/9 Positive
1 -Hot Dog-L2	A & C	9/9 Positive	9/9 Positive [a]	9/9 Positive	2/9 Positive [b]	9/9 Positive [a]	9/9 Positive
1 -Hot Dog-MB	A & C	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative
3 -Hot Dog-L1	B & D	9/9 Negative	6/9 Positive	9/9 Negative	6/9 Positive	9/9 Negative	9/9 Positive
3 -Hot Dog-L2	B & D	9/9 Negative	9/9 Positive	7/9 Positive [a]	9/9 Positive	9/9 Negative	9/9 Positive
3 -Hot Dog-MB	B & D	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative
6 -Hot Dog-L1	E	3/9 Positive [a]	9/9 Negative	9/9 Negative	9/9 Negative	9/9 Positive	9/9 Positive
6 -Hot Dog-L2	E	8/9 Positive [a]	9/9 Negative	9/9 Negative	9/9 Negative	9/9 Positive	9/9 Positive
6 -Hot Dog-MB	E	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative
7 -Hot Dog-L1	A & D	9/9 Positive	9/9 Negative	9/9 Negative	9/9 Positive	9/9 Negative	9/9 Positive
7 -Hot Dog-L2	A & D	9/9 Positive	9/9 Negative	9/9 Negative	9/9 Positive	9/9 Positive [a]	9/9 Positive
7 -Hot Dog-MB	A & D	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative
2 -BBQ-L1	A & C	9/9 Positive	9/9 Negative	9/9 Positive	9/9 Negative	9/9 Negative	Not performed [d]
2 -BBQ-L2	A & C	9/9 Positive	3/9 Positive [a]	9/9 Positive	9/9 Negative	9/9 Positive [a]	Not performed [d]
2 -BBQ-MB	A & C	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	Not performed [d]
5 -BBQ-L1	B & D	9/9 Negative	8/9 Positive	9/9 Negative	9/9 Positive	9/9 Negative	9/9 Positive
5 -BBQ-L2	B & D	9/9 Negative	9/9 Positive	4/9 Positive [a]	9/9 Positive	9/9 Negative	9/9 Positive
5 -BBQ-MB	B & D	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3 Negative	3/3 Negative

Table 5: Summary of the individual samples and reported results.

[a]Cross-reactivity observed - OD was just above the calculated positive threshold.
[b]False positive in a sample previously spiked with SET that does not follow the known cross-reactivity as described by the manufacturer.
[c]False positive in a matrix negative sample not containing SET.
[d]Labratory 2 did not have a VIDAS instrument for use during this study.

Lab/Matrix/ Spike Level	Toxin Assigned	SEA Result	SEB Result	SEC Result	SED Result	SEE Result	VIDAS SET2 Result
6 -BBQ-L1	E	1/9 Positive [a]	9/9 Negative	9/9 Negative	9/9 Negative	9/9 Positive	9/9 Positive
6 -BBQ-L2	E	4/9 Positive [a]	9/9 Negative	9/9 Negative	9/9 Negative	9/9 Positive	9/9 Positive
6 -BBQ-MB	E	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative
2 -Chicken-L1	A & C	0/9 Positive	9/9 Negative	1/9 Positive	9/9 Negative	9/9 Negative	Not performed [d]
2-Chicken-L2	A & C	9/9 Positive	9/9 Negative	9/9 Positive	9/9 Negative	9/9 Positive [a]	Not performed [d]
2 -Chicken-MB	A & C	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	Not performed [d]
5 -Chicken-L1	B & D	9/9 Negative	0/9 Positive	9/9 Negative	4/9 Positive	9/9 Negative	9/9 Positive
5 -Chicken-L2	B & D	9/9 Negative	9/9 Positive	9/9 Negative	9/9 Positive	9/9 Negative	9/9 Positive
5 -Chicken-MB	B & D	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative
6 -Chicken-L1	E	6/9 Positive [a]	9/9 Negative	9/9 Negative	9/9 Negative	9/9 Positive	9/9 Positive
6 -Chicken-L2	E	9/9 Positive [a]	9/9 Negative	9/9 Negative	9/9 Negative	9/9 Positive	9/9 Positive
6 -Chicken-MB	E	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative
7 -Chicken-L1	A & D	9/9 Positive	9/9 Negative	9/9 Negative	9/9 Positive	9/9 Negative	9/9 Positive
7 -Chicken-L2	A & D	9/9 Positive	9/9 Negative	9/9 Negative	9/9 Positive	5/9 Positive [a]	9/9 Positive
7 -Chicken-MB	A& D	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative	3/3 Negative

Table 5: Continued.