APPLICATION NOTE

Comparison of KPL BacTrace® Anti-*Salmonella* CSA-Plus Antibody to Two Other Anti-*Salmonella* species Antibodies in an Indirect ELISA.

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Food poisoning due to *Salmonella* contamination of undercooked meat, usually chicken and eggs, is a major concern for human health and agribusiness. Infection with *Salmonella* causes Salmonellosis characterized by diarrhea, fever and abdominal cramps 12-72 hours after ingestion. According to the US Centers for Disease Control (CDC) the annual incidence of Salmonellosis in the US is approximately 42,000 cases; however, many infections are not reported and the actual rate is thought to be ~1.2 million cases.

There are over 2500+ *Salmonella* serovars and all can cause Salmonellosis. Most (99.5%) of the *Salmonella* serovars are from one subspecies, *Salmonella enterica* subsp. *enterica.* To classify the *Salmonella* serovars further, the serovars are grouped by the O-antigen(s) they express. Historically, O-antigens were designated by letters; however, with the discovery of over 20 O-antigens, the nomenclature has changed to designate O-antigens by number (Grimont). O-antigen expression is important because the O-antigen dictates the host antibody response. Thus, for *Salmonella* detection purposes, researchers need multiple antibodies to detect the various serovars.

In order to eliminate the blending of multiple antibodies specific to every *Salmonella* O-antigen, SeraCare has developed a polyclonal antibody that recognizes a majority of the *Salmonella* O-antigens: BacTrace® Anti-*Salmonella* CSA-Plus (Cat. No. 01-91-90). The antibody was developed in a two pronged approach. First, goats were immunized using multiple serovars of *Salmonella* that represent the various O-antigens. The broad coverage of O-antigens in the immunogen ensures that no one antigen becomes immuno-dominant. Second, the antibody is purified using a series of affinity purifications utilizing proprietary Encapsulated Column Affinity Purification (ECAP) technology. Utilization of ECAP reduces cross-reactivity while maintaining potency. Combined, these techniques provide Anti-*Salmonella* CSA-Plus with several advantages over other anti-*Salmonella* antibodies: 1) Reactivity to most *Salmonella* O-antigens; and 2) High specificity to *Salmonella* with low cross-reactivity to non-*Salmonella* bacteria due to SeraCare's affinity purification methods.

To help researchers visualize the advantages of using BacTrace antibodies in their applications, we have compared Anti-*Salmonella* CSA-Plus to two commercially available Anti-*Salmonella* species antibodies in an indirect ELISA format. One of the commercial antibodies is a rabbit polyclonal IgG while the other is a mouse monoclonal IgG2a with specificity to the following O-antigens: A, B, C, D, E, F & G Groups. Assays were performed to compare both the sensitivity and specificity of the antibodies.

MATERIALS AND METHODS

1. ELISA

   a. Nunc-Immuno Maxisorp high binding microwell plates (8 well strip format) were coated with 100 µL of various heat-killed bacteria in Phosphate Buffered Saline (PBS, pH 7.4) normalized to OD 7.0 at 650 nm and blocked with 1% Bovine Serum Albumin (BSA) (1:10 dilution of KPL's 10% BSA Diluent/Blocking Solution (Cat# 50-61-00)). Each ELISA plate contained an eight well strip used as a normalization control that was coated with 100 µL of KPL BacTrace *Salmonella* Typhimurium Positive Control (Cat# 50-74-01) and blocked with 1% BSA. Specific bacterial strains used in plate coating are listed in Table 1.

   b. Primary antibodies: KPL BacTrace (Goat) Anti-*Salmonella* CSA-Plus (Cat# 01-91-90), USBiological (Rabbit) Anti-*Salmonella* species (Cat# S0060-29) and Abnova (Mouse) *Salmonella* species (A, B, C, D, E, F & G Groups) monoclonal antibody, clone B343M (Cat# MAB4900). The primary antibodies were diluted to 2 µg/mL in 1% BSA.

   c. Either 100 µL of primary antibody was added to wells (n=5) or 100 µL of 1% BSA was added to wells (n=3) as a negative background control. Wells were incubated for 1 hour at room temperature.

   d. Wells were washed using a 1X washing solution (1:20 dilution of KPL's Wash Solution (Cat# 50-63-00)) in a BioTek 405 TS plate washer. The wash cycle includes 3 automatic washes followed by a 9 minute incubation in washing solution ending with 3 more automatic washes.

   e. 100 µL of the appropriate secondary antibody (KPL's Anti-Goat IgG (H+L) Antibody, Peroxidase Labeled (Cat# 14-13-06), KPL's Anti-Rabbit IgG (H+L) Antibody, Peroxidase Labeled or KPL's Anti-Mouse IgG (H+L) Antibody, Human Serum Adsorbed and

Peroxidase Labeled (Cat# 074-1806) was added to wells in 1% BSA and incubated for 30 minutes.

f. Wells were washed as in step d.

g. 100 µL of KPL's ABTS ELISA HRP substrate (Cat# 50-66-18) was added to each well. Development time varied depending on the primary and secondary antibody combination. Development was stopped with 100 µL of 1X ABTS Stop Solution (1:5 dilution of KPL's ABTS Peroxidase Stop Solution (Cat# 50-85-01)).

h. Plates were read using a Molecular Devices Versamax Tunable Plate Reader set at 405 nm.

i. Individual absorbance readings were background subtracted before averaging. Error bars represent the standard deviation. Unless noted otherwise, each ELISA plate was normalized so that the absorbance value of the *S*. Typhimurium control strip was 2.

RESULTS AND DICUSSION

Sensitivity Comparison

1. We developed a broad-spectrum anti-*Salmonella* antibody which recognizes the numerous O-antigens expressed by *Salmonella*. The sensitivity of this antibody was measured in an indirect ELISA and compared with two commercially available antibodies. Figure 1 shows the comparison of the normalized absorbance values obtained using various heat-killed *Salmonella* serovars as antigens. The results show that KPL Anti-*Salmonella* CSA-Plus antibody has greater sensitivity to most serovars compared to a rabbit polyclonal. In particular, the KPL Anti-*Salmonella* CSA-Plus showed more sensitivity to O-Groups 8 (C^2-C^3); 9 (D^1); 3, 10 (E^1); 1, 3, 19 ($E^{4)}$ and 13 (G). While the rabbit polyclonal was generally less sensitive than KPL Anti-Salmonella CSA-Plus, the mouse monoclonal was very sensitive to a particular strain or not sensitive at all. The mouse monoclonal recognized O-Groups 9 (D^1); 3, 10 (E^1) and 1, 3, 19 (E^4) well. However, even within the same O-Group, 4 (B) and 7 (C^1), the mouse monoclonal had mixed sensitivity results. Polyclonal antibodies are a better choice for researchers needing broad spectrum bacterial detection because they recognize more epitopes than monoclonal antibodies.

Specificity Comparison

Researchers also value low antibody cross-reactivity. Polyclonal antibodies generally have more cross-reactivity than monoclonal antibodies, and the cross-reactivity can be detrimental when it leads to false positives. However, the cross-reactivity of a polyclonal can be significantly reduced when antigen-specific affinity purification methods are used (i.e., ECAP technology). In contrast to the KPL BacTrace antibody line, most antibacterial polyclonal antibodies are purified using Protein G or equivalent. While the resulting antibody is "affinity purified", the polyclonal antibody still contains non-specific antibody which can cause cross-reactivity in an immuno-assay.

To demonstrate the effect of ECAP on reducing cross-reactivity, the KPL Anti-*Salmonella* CSA-Plus antibody was compared to a mouse monoclonal Anti-*Salmonella* species antibody and a rabbit polyclonal Anti-*Salmonella* species antibody, both of which were presumably purified by Protein G. Figure 2 compares the normalized absorbance values obtained in an indirect ELISA with possible cross-reacting bacterial antigens (heat-killed bacterial strains). It is evident in Figure 2 that the rabbit polyclonal shows higher levels of cross-reactivity than either the polyclonal Anti-*Salmonella* CSA-Plus or the mouse monoclonal antibody. Indeed the level of background exhibited by the rabbit polyclonal antibody would obscure many of the positive signals shown in Figure 1. In contrast, the KPL BacTrace Anti-*Salmonella* CSA-Plus shows cross-reactivity which is almost equal to that of a monoclonal antibody.

The results demonstrate that careful antibody development, from immunogen selection through antigen specific affinity purification, produces higher quality antibodies. KPL Anti-*Salmonella* CSA-Plus reacts with higher sensitivity to *Salmonella* species than either a rabbit polyclonal or a mouse monoclonal antibody to a broad range of *Salmonella* serovars. In addition, Anti-*Salmonella* CSA-Plus has the highly desirable trait of low cross-reactivity bordering on the levels typically observed for monoclonal antibodies.

Table 1

Strain/Serovar	***ATCC***	***O-Antigen#***
S. Arizonae	13314	51 [IIIa, *Salmonella enterica* subsp. *arizonae*]
S. Maartensdijk	15790	40 (R) [IIIa, *Salmonella enterica* subsp. *arizonae*]
S. Diarizonae	29934	[IIIb, *Salmonella enterica* subsp. *diarizonae*]
S. Harmelen	15783	51 [IV, *Salmonella enterica* subsp. *houtenae*]
S. Ochsenzoll	29932	16 (I) [IV, *Salmonella enterica* subsp. *houtenae*]
S. Paratyphi A	9150	2 (A)
S. Newington	29628	4 (B)
S. Sloterdijk	15791	4 (B)
S. Typhimurium	14028	4 (B)
S. Choleraesuis	10708	7 (C_1)
S. Infantis	51741	7 (C_1)
S. Tennessee	10722	7 (C_1)
S. Hadar	51956	8 (C_2-C_3)
S. Kentucky	9263	8 (C_2-C_3)
S. Muenchen	8388	8 (C_2-C_3)
S. Tallahassee	12002	8 (C_2-C_3)
S. Newport	6962	8 (C_2-C_3)
S. Berta	8392	9 (D_1)
S. Enteritidis	49214	9 (D_1)
S. Gallinarum	9184	9 (D_1)
S. Typhi	9992v	9 (D_1)
S. Pullorum	9120	9 (D_1)
S. Give	9268	3, 10 (E_1)
S. Newington	29628	3, 10 (E_1)
S. Senftenberg	8400	1, 3, 19 (E4)
S. Simsbury	12004	1, 3, 19 (E4)
S. Rubislaw	10717	11 (F)
S. Cubana	12007	13 (G)
S. Putten	15787	13 (G)
S. Havana	NCTC 6086*	13 (G)
S. Mississippi	NCTC 6487*	13 (G)
S. Florida	10727	6, 14 (H)
S. Minnesota	9700	21 (L)
Citrobacter braakii	6750	N/A
Citrobacter freundii	6879	N/A
Enterobacter aerogenes	13048	N/A
Enterobacter cloacae	13047	N/A
Escherichia coli	25922	N/A
E. coli O157	35150	N/A
Hafnia alvei	29926	N/A
Klebsiella pneumoniae	27736	N/A
Kluyvera ascorbata	33433	N/A
Proteus hauseri	13315	N/A
Serratia marcescens	14756	N/A
Shigella boydii	8702	N/A
Shigella flexneri	12025	N/A
Shigella sonnei	11060	N/A
Staphylococcus aureus	6538	N/A
Staphylococcus epidermidis	12228	N/A
Yersinia ruckeri	29473	N/A

*National Collection of Type Cultures, Public Health England #Based on WHO reference, format: WHO numeric O-group (historic letter O-group). Unless otherwise noted *Salmonella* serovars are *Salmonella enterica* subsp. *enterica.*

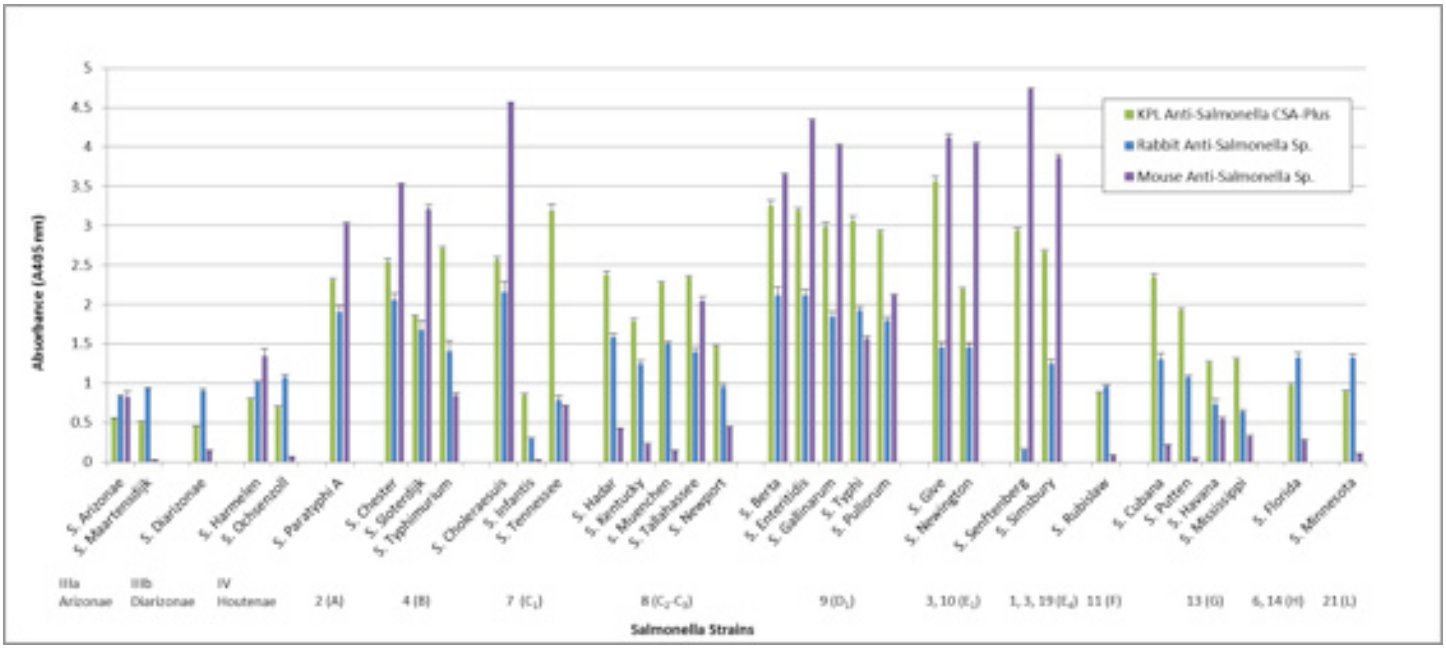

Figure 1: Normalized absorbance data for an indirect ELISA using KPL Anti-*Salmonella* CSA-Plus (green), a rabbit polyclonal Anti-*Salmonella* species (blue) and a mouse monoclonal Anti-*Salmonella* species (purple) against various *Salmonella* serovars. Roman numbers represent different *Salmonella enterica* subspecies. Numbers and letters in parenthesis represent different conventions of *Salmonella enterica* subsp. *enterica* O-antigens. Data and error bars represent the mean ± 1 SD (n=5).

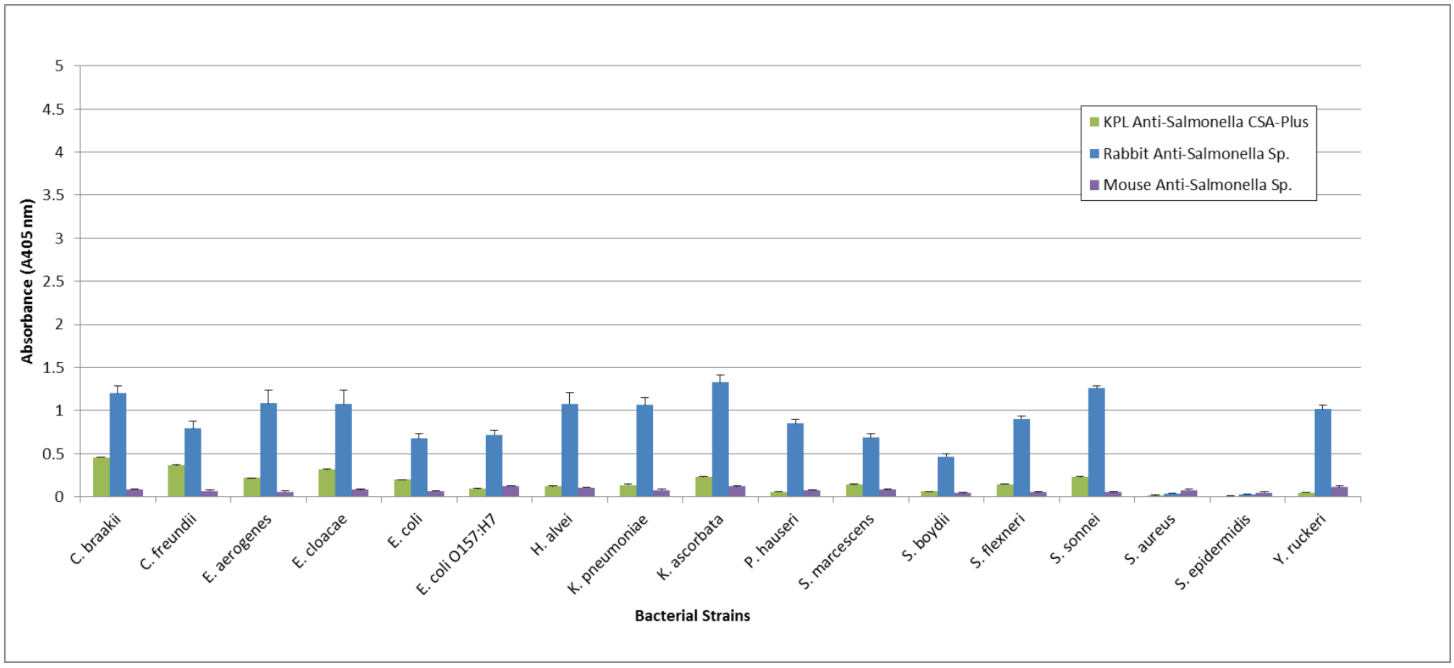

Figure 2: Cross-reactivity data showing normalized absorbance data for an indirect ELISA using KPL Anti-*Salmonella* CSA-Plus (green), a rabbit polyclonal Anti-*Salmonella* species (blue) and a mouse monoclonal Anti-*Salmonella* species (purple) against various bacterial strains. Data and error bars represent the mean ± 1 SD (n=5).

REFERENCES

Grimont, PAD and Weill, FX. "Antigenic Formulae of the Salmonella Serovars, 9th edition" 2007. WHO Collaborating Centre for Reference and Research on *Salmonella*, World Health Organization, Institut Pasteur, Paris, France.

RELATED PRODUCTS

Product	***Cat. No.***
Anti-Salmonella CSA+ Plus Antibody	01-91-90
Anti-Salmonella CSA-1 Antibody	01-91-99
Anti-Salmonella CSA-1 Magnetic Beads	082-01-91-99
Anti-Salmonella CSA-1 Latex Beads	082-02-91-99
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