# Comparison of KPL BacTrace® Anti-Salmonella CSA-Plus Antibody to Two Other Anti-Salmonella Species Antibodies in an Indirect ELISA

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## INTRODUCTION

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 approximate 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 as, 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 developed a polyclonal antibody which recognizes a majority of the *Salmonella* O-antigens: BacTrace® Anti-*Salmonella* CSA-Plus (Material Number 5310-0321). 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.

## HIGHLIGHTS

Antigen affinity purified polyclonal antibodies maximize the need for high sensitivity and unparalleled specificity in immunoassays

Antibodies to food-borne pathogens:
*E. Coli*
*Salmonella*
*Listeria*
*campylobacter*
*Vibrio*
*Shigella*

## 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 10% BSA Diluent/Blocking Solution (Material Number 5140-0006). Each ELISA plate contained an eight well strip used for a normalization control that was coated with 100 µL of KPL BacTrace Salmonella Typhimurium Positive Control (Material Number 5370-0002) 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 (Material Number 5310-0319), USBiological (Rabbit) Anti-Salmonella species and Abnova (Mouse) Salmonella species (A, B, C, D, E, F, & G Groups) monoclonal antibody, clone B343M. 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 Wash Solution (Material Number 5150-0008) 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 Anti-Goat IgG (H+L) Antibody, Peroxidase Labeled (Material Number 5220-0362), KPL Anti-Rabbit IgG (H+L) Antibody, Peroxidase Labeled or KPL Anti-Mouse IgG (H+L) Antibody, Human Serum Adsorbed and Peroxidase Labeled (Material Number 5220-0336 or 5220-0341) was added to wells and incubated for 30 minutes.

f. Wells were washed as in step d.

g. 100 µL of KPL ABTS ELISA HRP substrate (Material Number 5120-0046) was added to each well. Development time varied depending on the primary and secondary antibody combination. Development was stopped with 100 µL of KPL 1X ABTS Stop Solution (1:5 dilution of KPL ABTS Peroxidase Stop Solution (Material Number 5150-0017).

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 DISCUSSION

**Sensitivity Comparison**

We developed a broad-spectrum anti-Salmonella antibody which recognizes the numerous O-antigens expressed by Salmonella. The sensitivity of this strain 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 BacTrace 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 (C2-C3); 9 (D1); 3, 10 (E1); 1, 3, 19 (E4), 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 (D1), 3, 10 (E1), and 1, 3, 19 (E4) well. However, even within the same O-Group, 4 (B) and 7 (C1), 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 to detection assays. 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 anti-bacterial 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 immunoassay.

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, produce 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 (l) [IV, Salmonella enterica subsp. houtenae] |
| S. Paratyphi A | 9150 | 2 (A) |
| S. Chester | 11997 | 4 (B) |
| S. Sloterdijk | 15791 | 4 (B) |
| S. Typhimurium | 14028 | 4 (B) |
| S. Choleraesuis | 10708 | 7 (C1) |
| S. Infantis | 51741 | 7 (C1) |
| S. Tennessee | 10722 | 7 (C1) |
| S. Hadar | 51956 | 8 (C2-C3) |
| S. Kentucky | 9263 | 8 (C2-C3) |
| S. Muenchen | 8388 | 8 (C2-C3) |
| S. Tallahassee | 12002 | 8 (C2-C3) |
| S. Newport | 6962 | 8 (C2-C3) |
| S. Berta | 8392 | 9 (D1) |
| S. Enteritidis | 49214 | 9 (D1) |
| S. Gallinarum | 9184 | 9 (D1) |
| S. Typhi | 9992v | 9 (D1) |
| S. Pullorum | 9120 | 9 (D1) |

| Strain/Serovar | ATCC | O-Antigen# |
|---|---|---|
| S. Give | 9268 | 3, 10 (E1) |
| S. Newington | 29628 | 3, 10 (E1) |
| 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*.

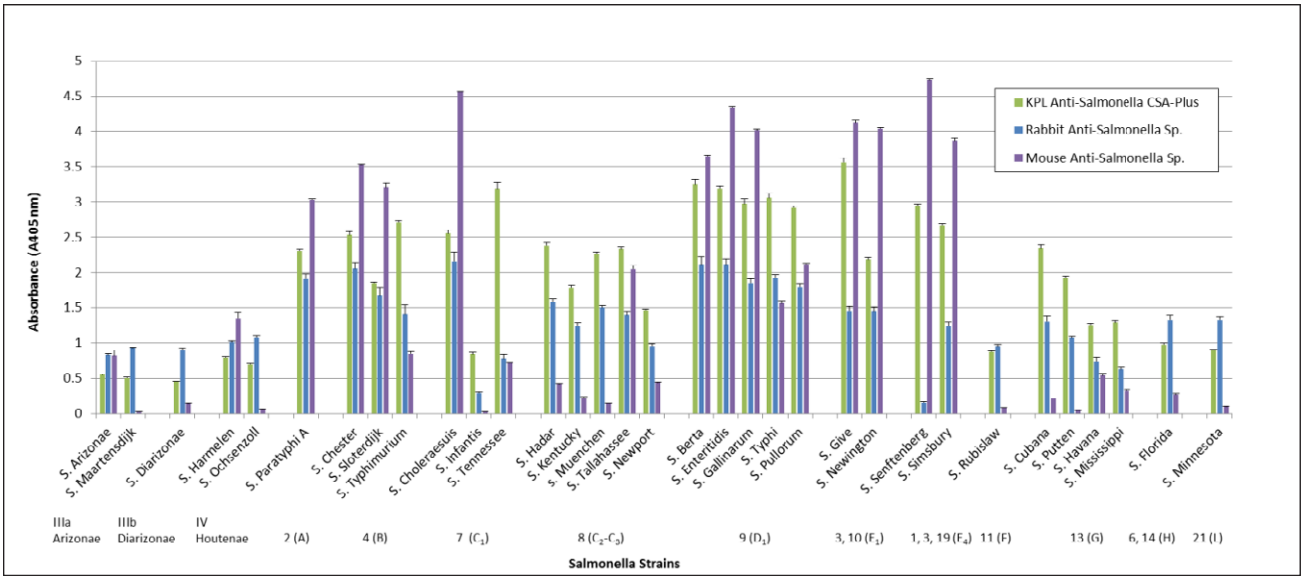


**Figure 1:** Normalized absorbance data for an indirect ELISA using KPL Anti-Salmonella CSA-Plus (green), a rabbit polyclonal Anti-Salmonella species (blue), and a mouse monoclonal Anti-Salmonella species (purple) against various Salmonella serovars. Roman numbers represent different Salmonella enterica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

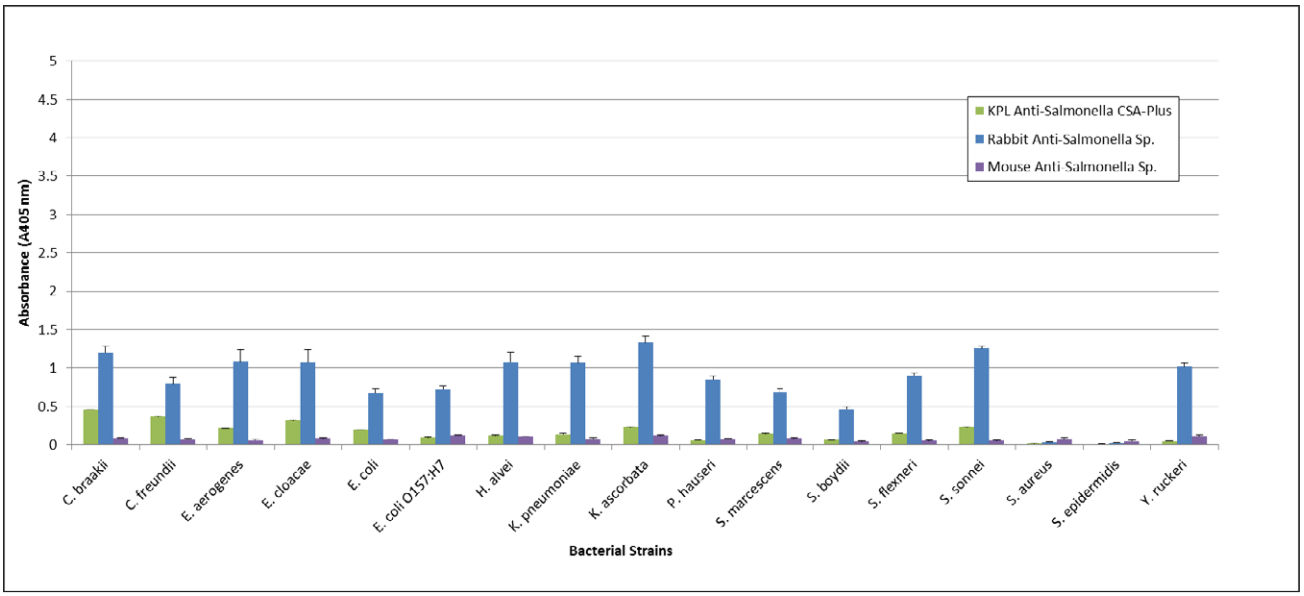


**Figure 2:** Cross-reactivity data showing normalized absorbance data for an indirect ELISA using KPL Anti-Salmonella CSA-Plus (green), a rabbit polyclonal Anti-Salmonella species (blue), and a mouse monoclonal Anti-Salmonella species (purple) against various bacterial strains. Data and error bars represent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

## REFERENCES

1. Grimont, PAD and Weill, FX. “Antigenic Formulae of the Salmonella Serovars, 9th edition” 2007. WHO Collaborating Centre



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