Characterization of an affinity-purified antibody specific for *Listeria spp.*

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ABSTRACT

Listeria species are ubiquitous in the environment, in the soil and on plants and animals. Among them, *Listeria monocytogenes* is major foodborne pathogen which causes human listeriosis. Listeriosis is associated with consumption of contaminated food products. A high mortality rate of infection prompted government to establish standards for detection of *Listeria spp.* in ready-to-eat foods. Immunoassay systems are efficient methods for early detection of *Listeria spp* in order to fulfill these regulatory needs. However, rapid detection of *Listeria* using immunoassays is highly dependent on antibodies with a high degree of sensitivity and specificity for the organism. We have developed a highly specific polyclonal antibody specific for *Listeria spp.* for this purpose. The *Listeria spp.* antibody was compared to other commercially available antibodies and demonstrated high reactivity to all six *Listeria* species, as well as low cross-reactivity against closely related Gram positive organisms including *Enterococcus spp*., *streptococcus spp.*, *Lactococcus spp.*, and *Staphylococcus spp*. The antibody is capable of detection of greater than or equal to 10^4 cfu of *Listeria* cells. The results indicate that this antibody has excellent sensitivity and specificity towards detection of *Listeria spp* following primary enrichment of suspect samples.

INTRODUCTION

The genus *Listeria consists of six different species of* small rod shaped gram-positive bacteria: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. ivanovii*, *L. seelingeri*, and *L. grayi*. Only two of these species are pathogenic: *L. monocytogenes* causes severe illness both in humans and animals whereas *L. ivanovii* is primarily an animal pathogen. Human listeriosis is overwhelmingly a foodborne disease-- ~99% of all human listeriosis cases are caused by consumption of contaminated food. In 2000, the CDC reported that of all the foodborne pathogens tracked by the organization, *L. monocytogenes* had the second highest case fatality rate (21%) and the highest hospitalization rate (90.5%). The ubiquity of *Listeria* bacteria enables them to enter the food-processing environments and the food chain. Moreover, the ability of the bacterium to grow at refrigerating temperatures increases the risk of food contamination.

Rapid detection methods have been developed for *Listeria spp.* using either molecular based systems or antibody-based immunoassays. In either case, antibodies may also be used for immunocapture of *Listeria* from food. For immunoassay-based methods, the antibody is the key determinant of the sensitivity and specificity of the test. Broad-spectrum antibodies to *Listeria spp*. with good sensitivity and specificity are needed for rapid screening methods for Listeria in food. In this poster, we describe the characterization of these parameters for a new antibody directed against *Listeria spp.*

MATERIALS AND METHODS

Antibody production: Goats were immunized with heat-killed *Listeria* cells according to standard protocols. Antisera were tested to monitor the titer of antibody. Qualified antisera were collected and pooled for purification. The antibody was purified according to proprietary methods.

Cell treatments: Bacterial cells were grown overnight in BHI media at 30ºC. Cells were collected by centrifugation. Cell number were determined by viable plate count. Cells for making heat killed materials were resuspended in 0.01M PBS, followed by heat killing at 95ºC for 15 min in a boiling water bath. Cells for formalin fixed materials were resuspended in 2% of formalin solution and placed in 4 ºC overnight, followed by washing several times with PBS to remove the trace of formalin.

Antibody testing: The antibody was characterized for specificity, sensitivity and avidity according to the following methods. The specificity assay was performed using a direct ELISA method. Bacterial cells (10^6 cfu/mL of heat-killed bacterial culture) were coated on the plate. The plate was incubated with anti-*Listeria* antibody (2 µg/mL) followed by HRP-labeled mouse anti-goat antibody. Detection was performed using the chemiluminescent substrate LumiGLO® (KPL). The bacterial strains used included 10 *Listeria* serotypes, 10 closely related Gram-positive and 4 Gram-negative organisms. Sensitivity testing was performed using a sandwich (capture) ELISA method. 5 µg/mL of anti-*Listeria* antibody was coated on the plate. Live, heat-killed or formalin fixed species of *Listeria* (100 µL) were added in a dilution series. The goat anti-*Listeria spp* was labeled with biotin (KPL's SureLINK™ Chromophoric Biotin Labeling Kit) and used at 2.5 µg/mL for the detection step. Detection was performed using subsequent incubations of the plate with streptavidin-HRP and LumiGLO® chemiluminescent substrate. The avidity test was performed using the direct ELISA method described above, except that after the antibody binding step was completed, the plate was treated with a concentration series of ammonium thiocyanate in order to disrupt binding. Avidity was determined by measuring the thiocyanate concentration at which 50% inhibition occurs (IC50)

RESULTS

Specificity of the anti-*Listeria* antibody: The specificity assay was performed in a direct ELISA format described in Materials and Methods. The antibody showed broad spectrum activity against different serotypes of *L. monocytogenes* and other *Listeria spp.* with minimal cross- reactivity to *Enterococcus faecelis* and other *non-Listeria* organisms (Figure 1):

Figure 1: Specificity Testing of Goat anti-*Listeria* antibody

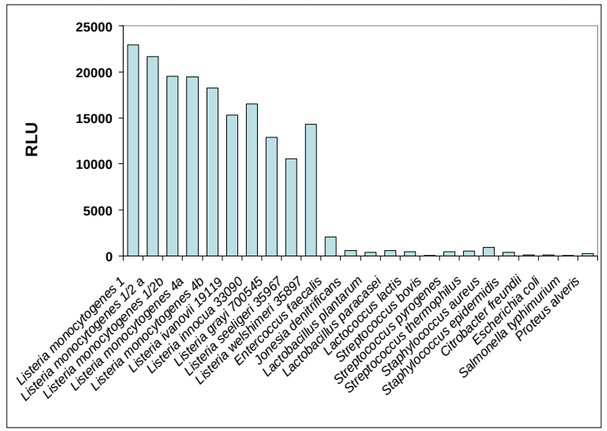

Figure 1: The specificity assay was performed in direct ELISA format. RLU represents Relative Light Units when the assay was read by adding the chemiluminscent substrate LumiGlo (KPL).

Sensitivity of the anti-*Listeria* antibody: Sensitivity of the *anti-Listeria spp*. antibody was performed by a sandwich ELISA as described. Figure 2 shows the results from such an assay where heat-killed *Listeria monocytogenes1/2a* were prepared at dilutions of 10^2 to 10^6 cfu. The results show that the limit of detection was ~10^3-10^4 cfu.

Figure 2: Detection of *L. monocytogenes* 1/2a with a sandwich ELISA.

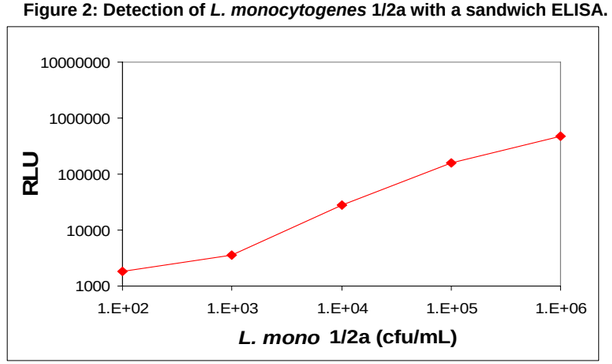

Figure 2: The assay was performed in sandwich (capture) ELISA format as described. RLU represents Relative Light Units as described above. Background is ~2000 RLU.

We also evaluated the detection of antibody against bacterial cells with different treatments. About 10^4 and 10^3 cfu of live cells, heat killed cells, or chemically (formalin) fixed *Listeria* cells were assayed in the sandwich ELISA (Table 1). The results indicated that the antibody can detect live cells at 10^4 cfu in ELISA. Among three cell forms, the highest signal was seen for detection of heat killed bacteria, while the lowest signal was for chemically fixed cells.

Table 1: Sensitivity of the anti-*Listeria spp.* antibody to different cell treatments

	Live cells	Heat-killed cells	Chemically fixed cells
Listeria monocytogenes (serotype 1)	75813	197100	18762
Listeria monocytogenes (serotype 1/2a)	53468	189592	28770
Listeria monocytogenes (serotype 4b)	75559	227127	16797
Listeria innocua	71400	164235	12424
Listeria ivanovii	54501	345759	26423
Listeria grayi	113837	173728	37036
Listeria seelingeri	37999	90305	19465
Listeria welshmeri	77354	206086	44394

Table 1: The assay was performed in sandwich (capture) ELISA format as described. The values listed are Relative Light Units (RLU) as described above using 10^4 cfu cells. Background is ~2000 RLU.

Avidity of anti-*Listeria* antibody: Antibody avidity is an important parameter of the antibody because it measures the strength of the antibody-antigen interaction and how difficult it is to disrupt this interaction. This parameter is particularly important when using the antibody for immunocapture of bacterial cells from complex food samples. Avidity of antibody was evaluated based on a standard published method using ammonium thiocyanate. The avidity of the KPL antibody was compared to the avidity of other commercially available anti-*Listeria* antibodies. The KPL antibody had the highest overall avidity for a range of *Listeria spp.* and was similar or higher than other antibodies for Listeria monocytogenes serotypes (Table 2):

Table 2: Avidity (IC50 values) of anti-*Listeria spp.* antibodies

	KPL Goat anti-*Listeria*	Antibody B	Antibody C
Listeria monocytogenes (serotype 1)	1.4	1.4	1.0
Listeria monocytogenes (serotype 1/2a)	1.5	1.4	0.5
Listeria monocytogenes (serotype 4b)	1.25	1.25	0.75
Listeria innocua	1.3	0.8	0.4
Listeria ivanovii	1.3	1.4	0.75
Listeria grayi	1.0	0.8	0.5
Listeria seelingeri	1.0	1.3	0.8
Listeria welshmeri	1.2	0.75	0.4

Figure 3: Characterization of the avidity of the anti-*Listeria spp*. antibody and other commercially available anti-*Listeria* antibodies. Avidity testing was described in Materials and Methods using the thiocyanate method for inhibiting antibody-antigen interactions. IC50 values are shown for each antibody with each *Listeria spp*.strain. Note that a higher IC50 corresponds to a higher avidity.

SUMMARY

KPL's Goat anti-*Listeria spp*. antibody provides:

- High specificity: a broad spectrum of reactivity against all *Listeria spp* and serotypes
- Low cross-reactivity against non-*Listeria* organisms
- Improved sensitivity: a detection limit of <10^4 cfu for both heat killed cells and live cells
- High avidity: overall high avidity against *Listeria spp*. as compared with other commercial antibodies.

KPL plans to commercialize this antibody as part of our BacTrace® line of antibodies to food pathogens.