

# ***Affinity Purified Antibodies for the Detection of Food Pathogens***

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*ISO9001:2000 Registered*

# KPL's BacTrace<sup>®</sup> Antibody Products

- Goat anti-*E. coli* O157:H7
- Goat anti-*Salmonella*, CSA-1
- Goat and mouse anti-*Listeria*: in development
- Goat anti-*Camphylobacter*: Research-scale only

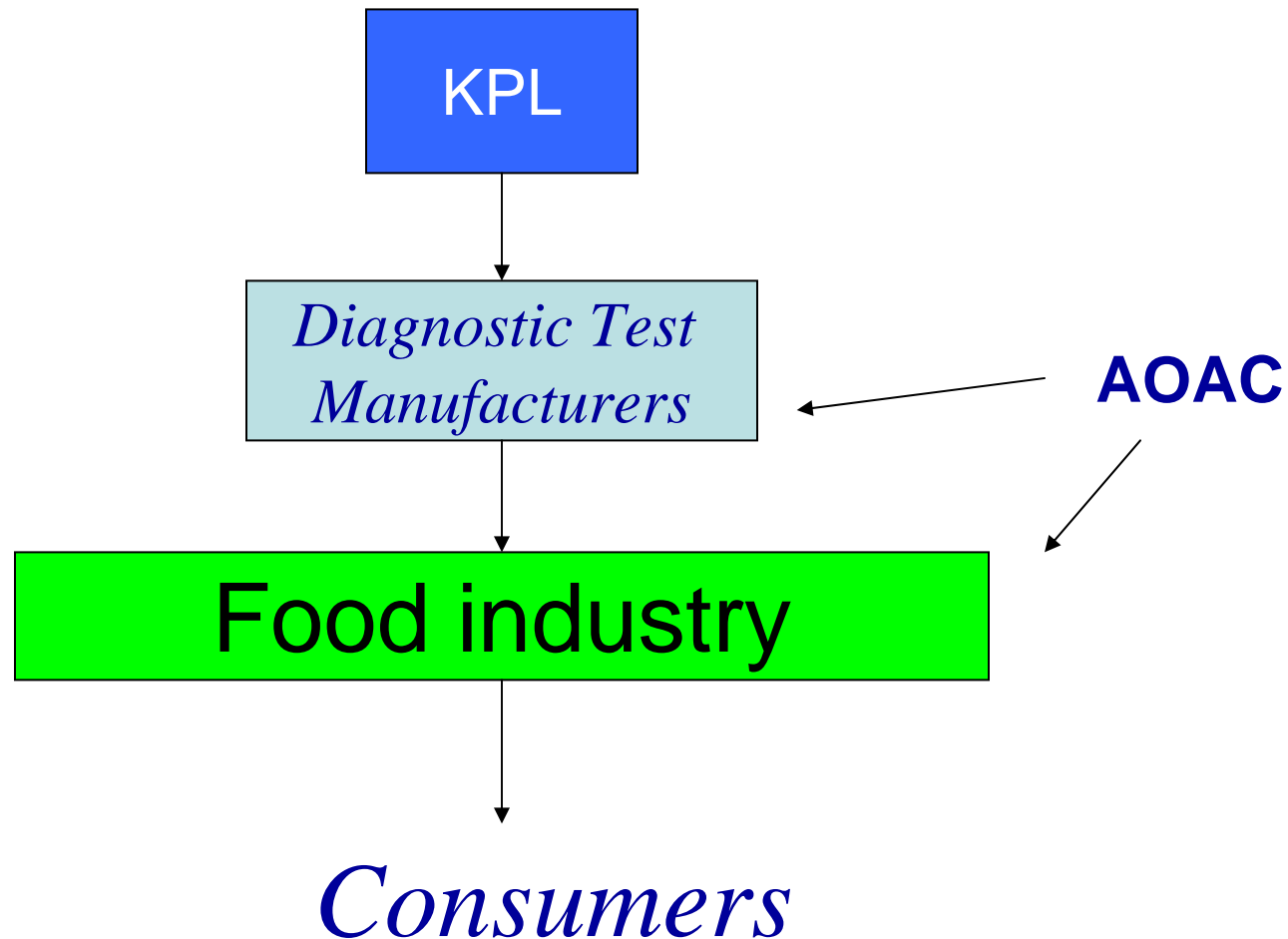


# *The importance of antibodies in food testing*

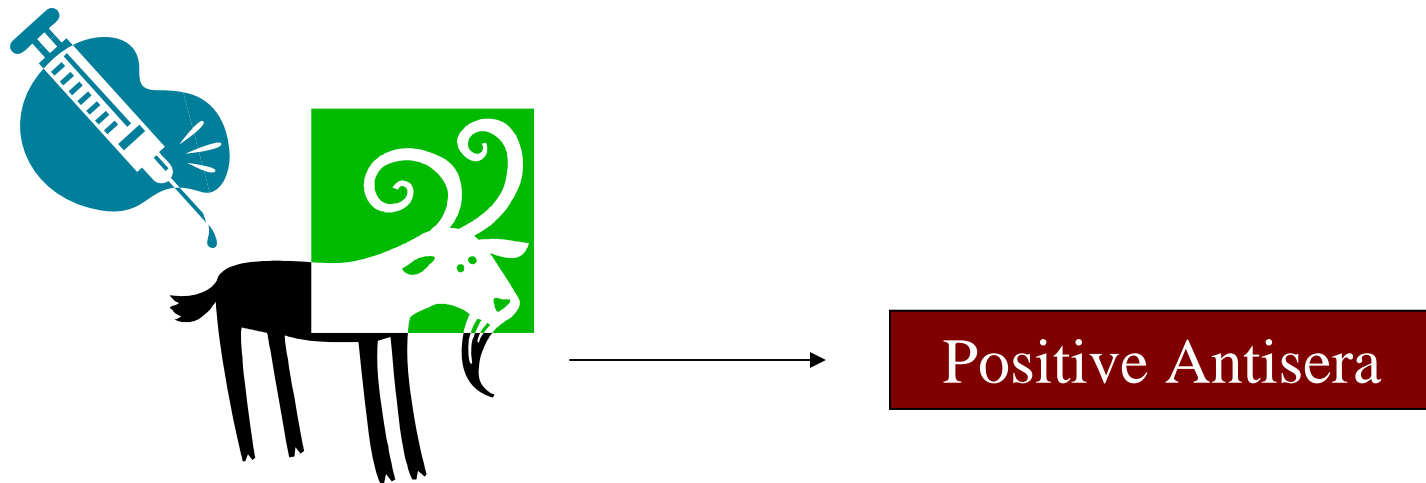
- For detection: as the capture or detection antibody in diagnostic assays
- Immuno-concentration after culturing from food
- May be conjugated to beads, enzymes, fluorophores, etc.
- ***The antibody is the key determinant of specificity and sensitivity of the test.***



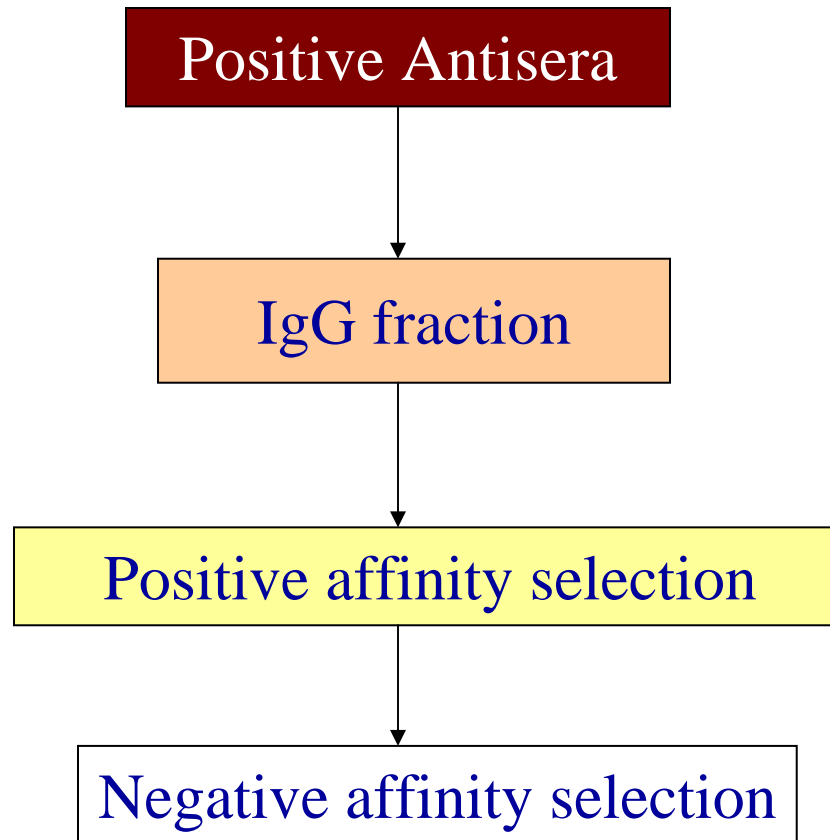
# *The food testing “food chain”*



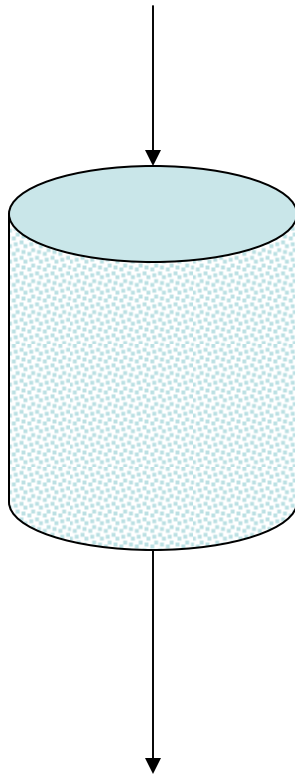
# *Immunization of animals*



# *General Scheme for Antibody Purification*



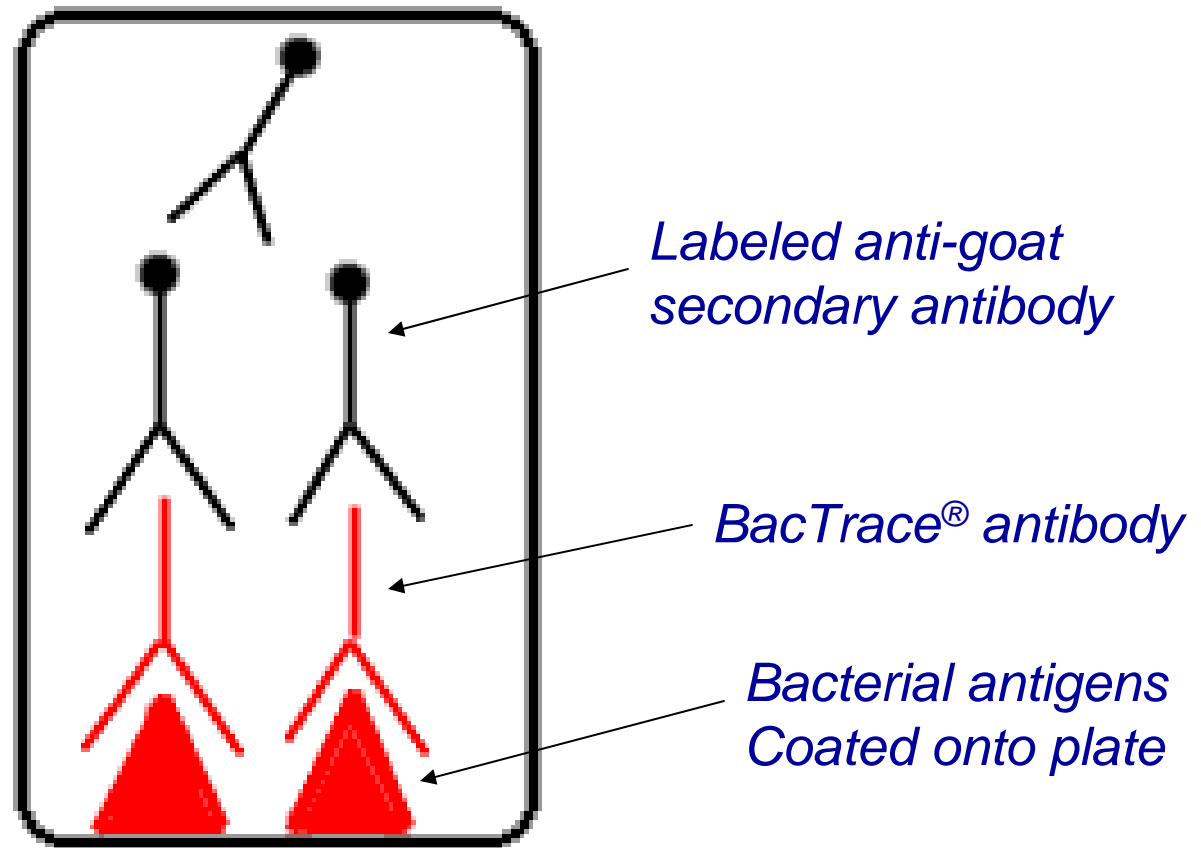
# Affinity Purification



← Affinity  
Column matrix

Unbound antibody is washed through  
Bound antibody is eluted with higher-strength  
buffer

# Antibacterial Antibody ELISA

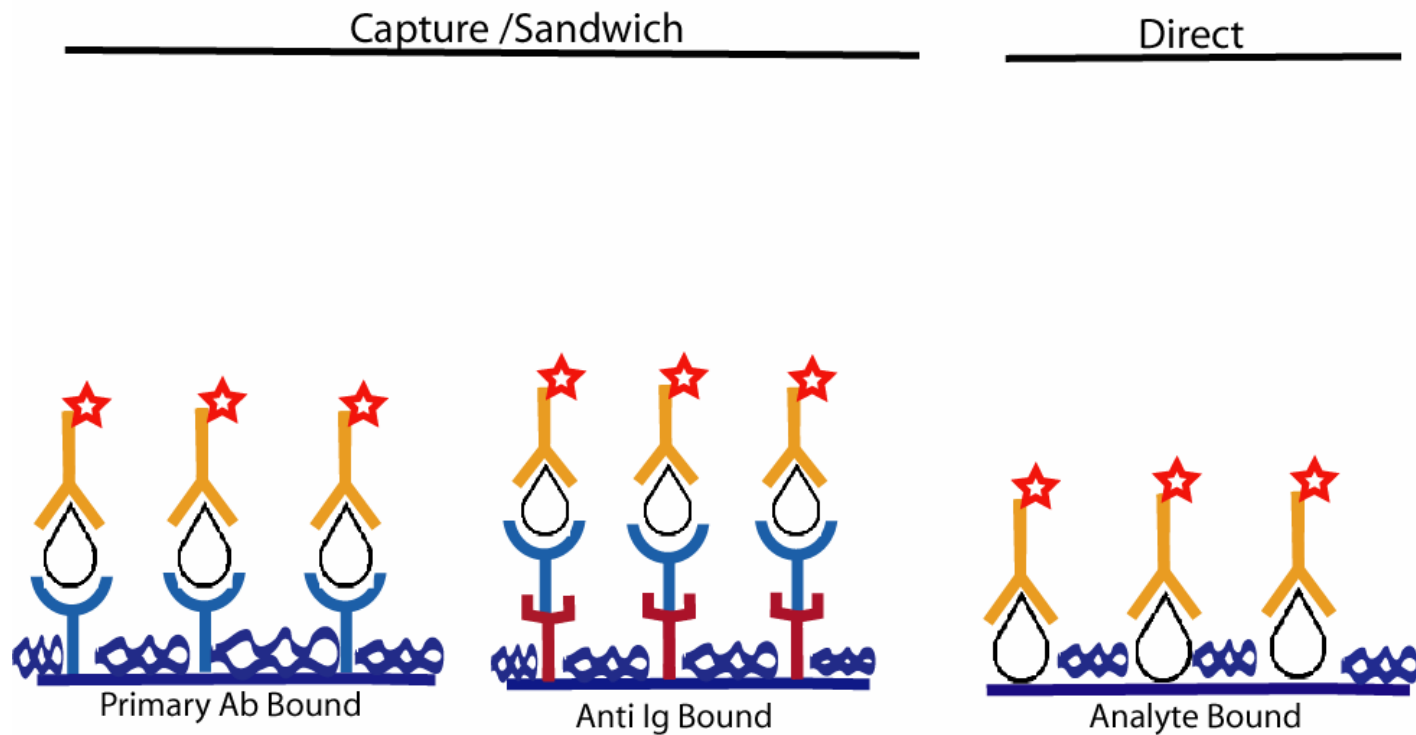




# ELISA Assays for Specificity and Sensitivity Testing

Figure 1

Assay Formats



# *Escherichia coli* O157:H7

- Enterohemorrhagic strain of *E. coli*– causes acute bloody diarrhea
- Produces Shiga-like toxin which attacks the lining of the intestine
- 5-8 cases reported per 100,000 people per year in developed countries
- Mortality rate of 1-2% (up to 50% in elderly)
- Recent outbreak: Bagged spinach from California

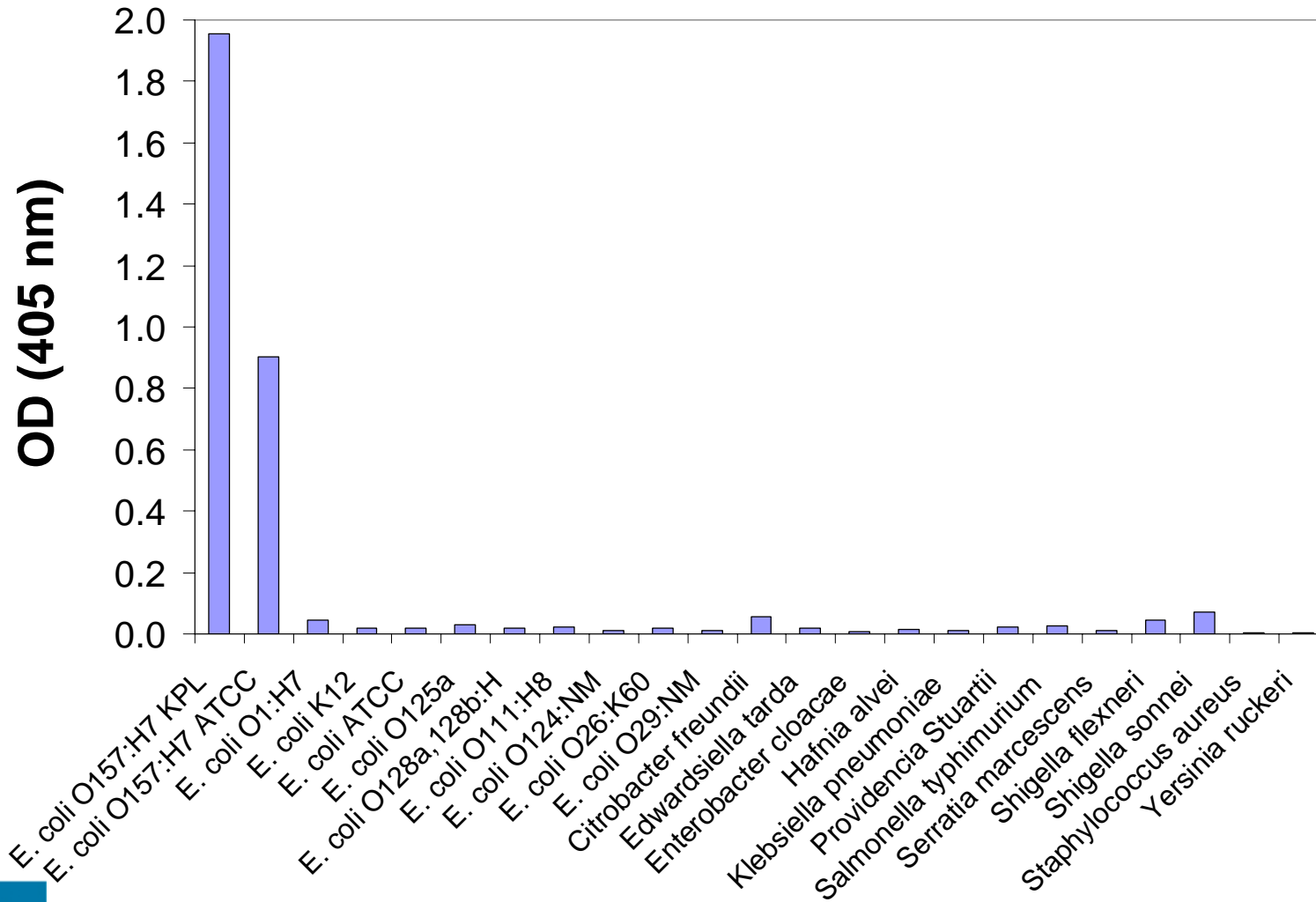


# *Antibody to Escherichia coli O157:H7*

- Key requirements:
  - Specific for the O157:H7 serotype
  - Low crossreactivity against other organisms (esp. *Salmonella*)



# Specificity of BacTrace<sup>®</sup> Antibody to *Escherichia coli* O157:H7



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# *Salmonella spp.*

- Motile, gram-negative bacterium (non motile strains include *S. pullorum* and *S. gallinarum*)
- *S. typhi* and *S. paratyphi* cause typhoid fever- other strains cause nausea, vomiting, cramps, diarrhea, and headache.
- 2-4 million cases annually in the U.S.
- Mortality rate is 1%, except for typhoid producing strains, which have a mortality rate of 10%
- Recent outbreaks include contaminated peanut butter, melons, milk, and poultry

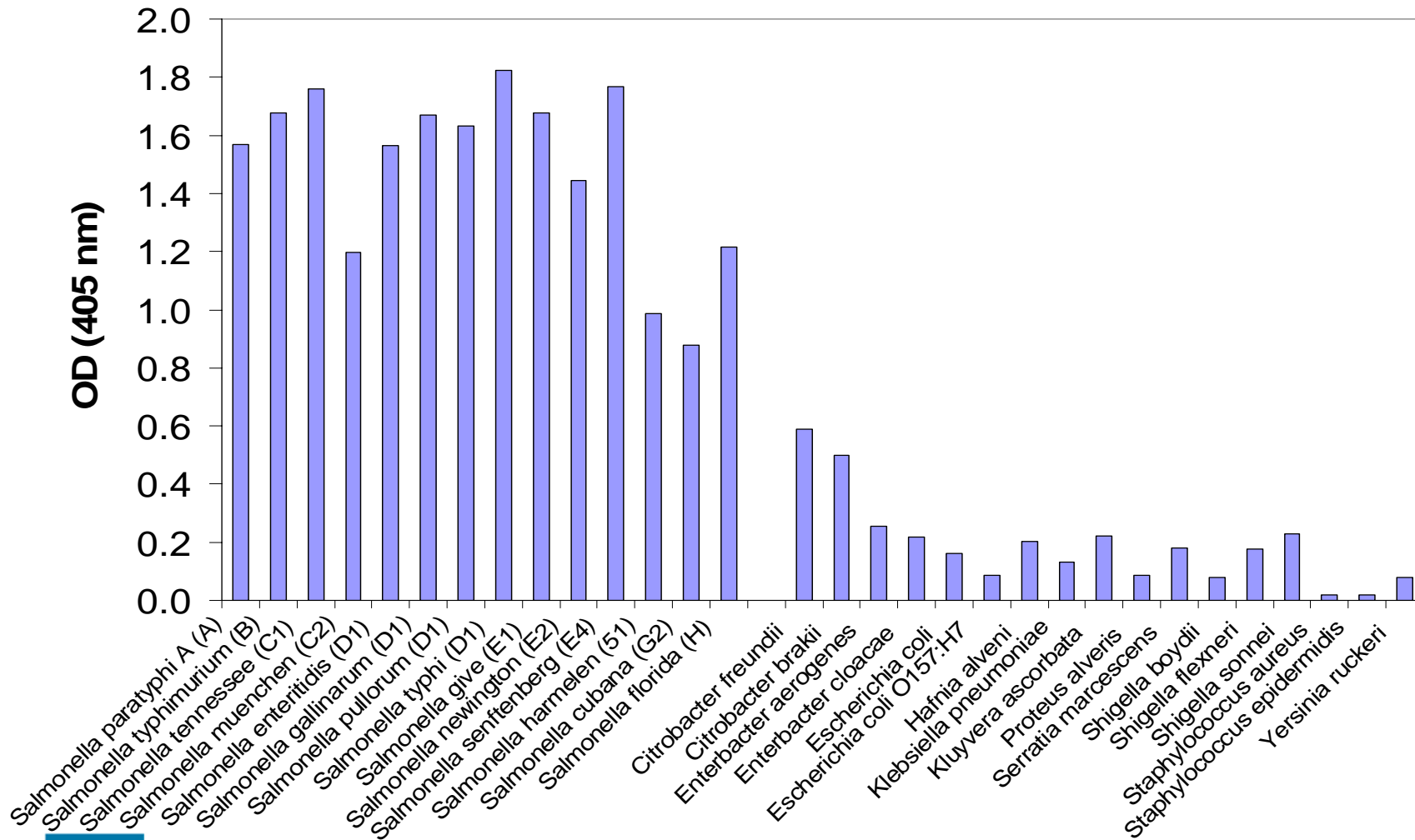


# *Antibody to Salmonella spp.*

- Key requirements:
  - Broadly reactive against multiple *Salmonella* types
    - CSA-1– “Common Structural Antigens”
  - Low cross-reactivity against other organisms (e.g. *E. coli*)



# Specificity of BacTrace<sup>®</sup> Antibody to Salmonella, CSA-1



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# *Listeria monocytogenes*

- Gram-positive, motile bacterium
- Causes listeriosis– flu-like symptoms, gastrointestinal symptoms, meningitis or infections– miscarriage or stillbirth in pregnant women
- At least 1600 cases per year in the U.S. with a mortality rate of ~25%
- Listeria genus includes *L. monocytogenes* and five species with no pathogenicity in humans (*L. ivanovii* is pathogenic in other mammals)
- Recent outbreak in chicken tenders



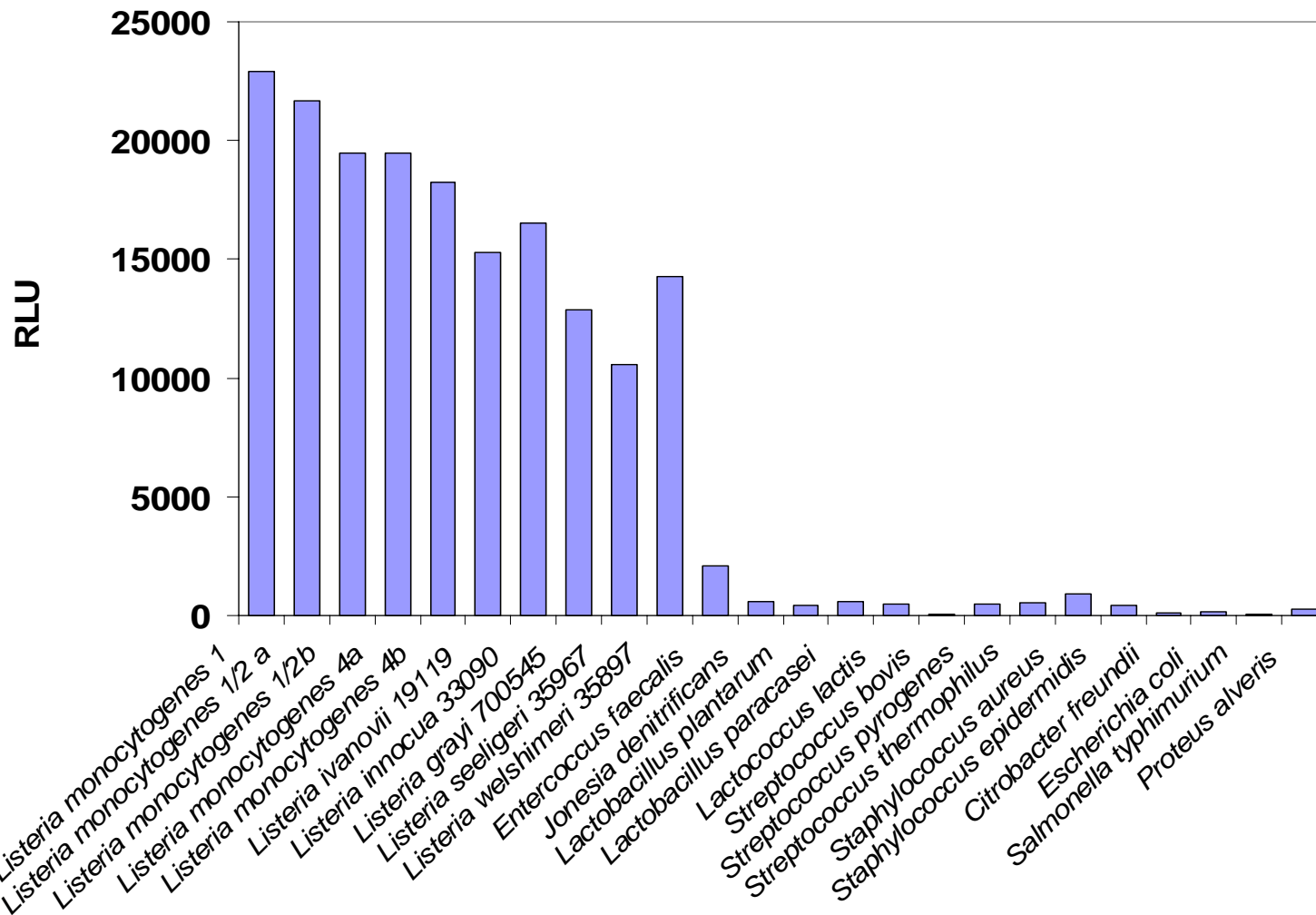


# *Antibodies for Listeria spp.*

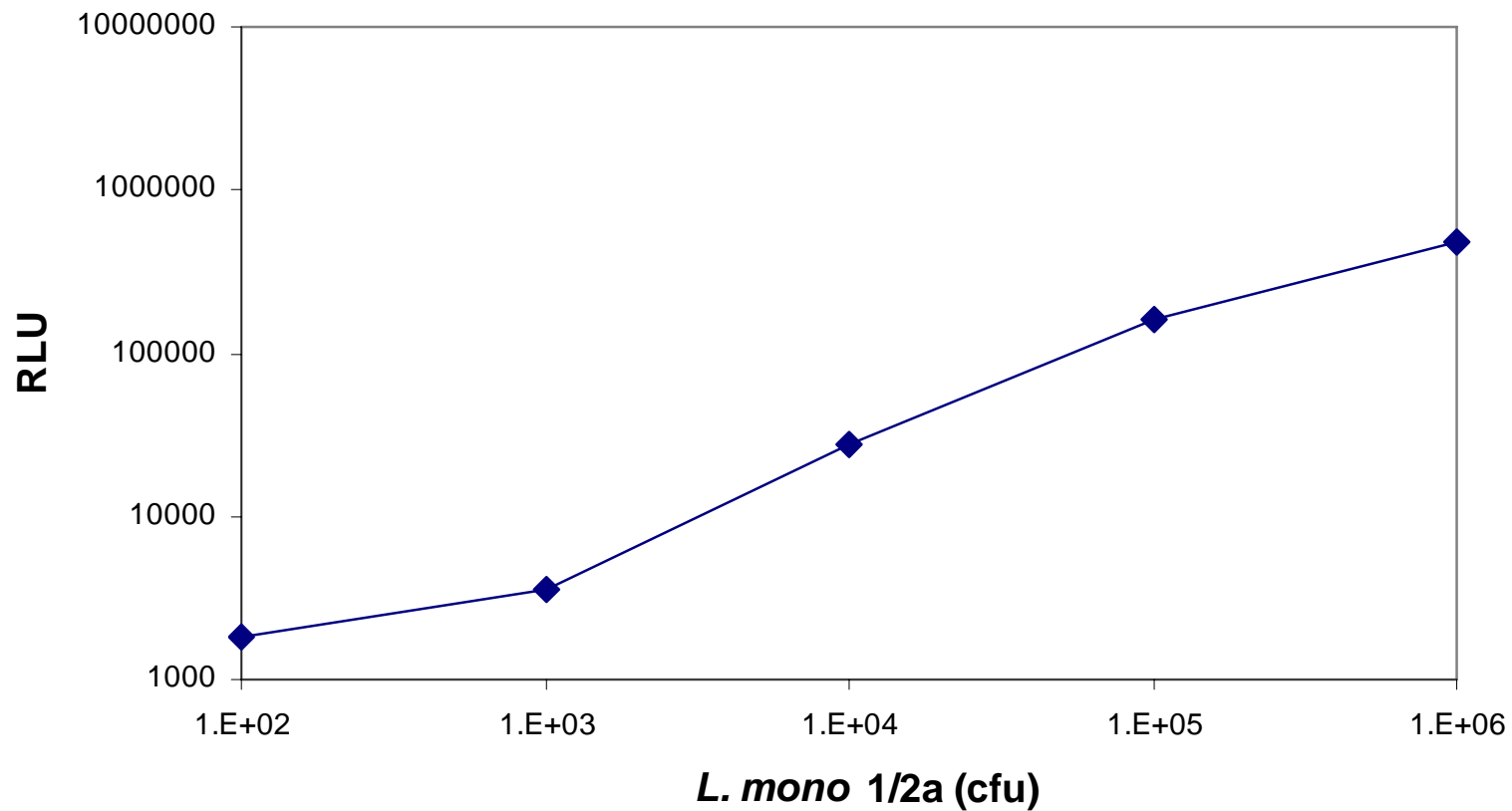
- Requirements for an Antibody to *Listeria spp.*:
  - Broadly reactive against all *Listeria* species and serotypes
  - Low cross-reactivity against other organisms (both gram positive and gram negative)
  - Very sensitive detection and high avidity



# Specificity of BacTrace<sup>®</sup> Goat Antibody to *Listeria* spp. (prototype)

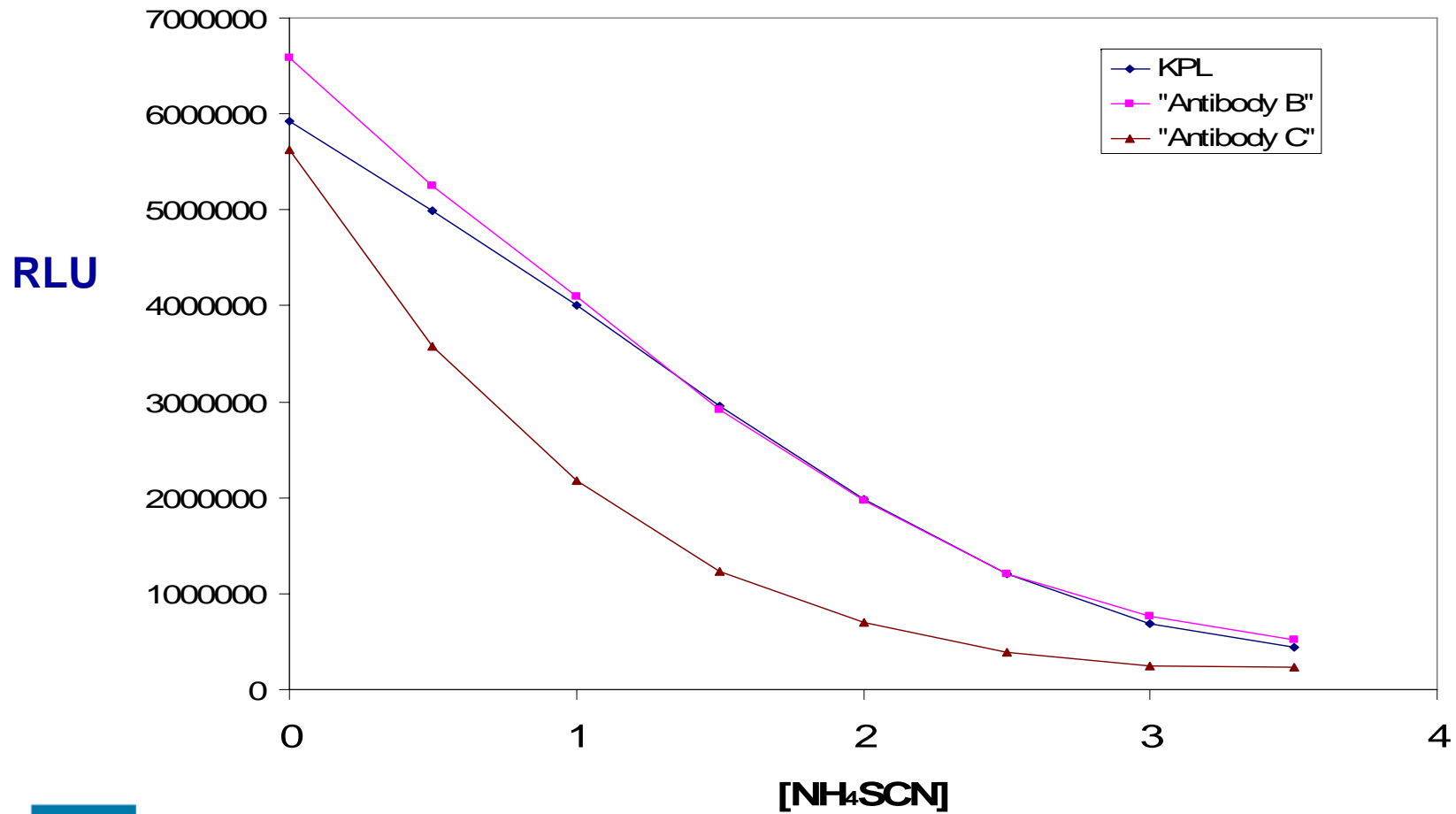


# *Sensitivity of BacTrace<sup>®</sup> Goat Antibody to Listeria spp. (prototype)*



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# Measurement of Antibody Avidity



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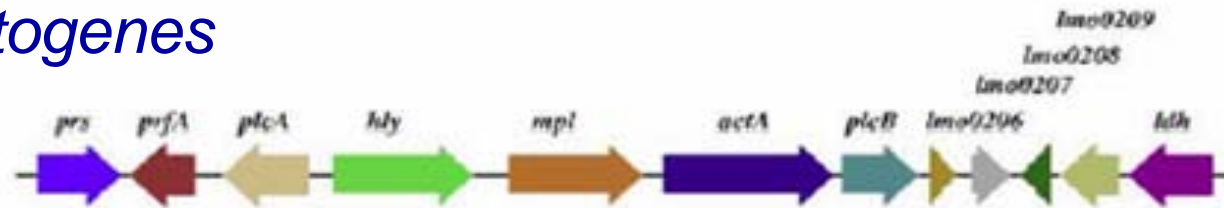
# *Antibodies for Listeria monocytogenes*

- Requirements for an Antibody to *Listeria monocytogenes*
  - Broadly reactive against *L. monocytogenes* serotypes
  - Low cross-reactivity against other *Listeria* species

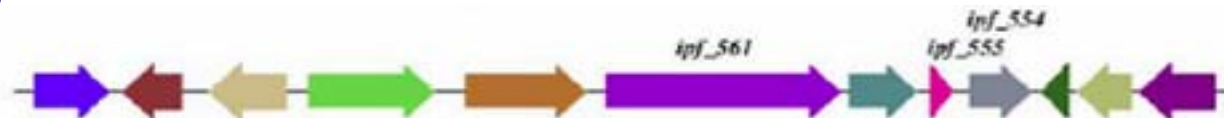


# Similarity between *Listeria* genomes

## *L. monocytogenes*



## *L. ivanovii*



# Summary

- Goat anti-*E. coli* O157:H7 and goat anti-Salmonella spp. are available for use in research and diagnostic applications
  - Can be customized for size
  - Conjugates available
  - Subtype-specific antibodies may be available on a custom basis
  - “Molecular grade” (DNA-free) option available
- Antibodies to *Listeria spp.* and *Listeria monocytogenes* are in development
  - Samples may be available for evaluation upon request
- ISO9001:2000 registered company



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