Abstract

Development of a double-antibody sandwich ELISA for sensitive and specific detection of *Yersinia pestis*

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Yersinia pestis is a highly pathogenic gram-negative, rod-shaped bacterium that causes plague. Y. pestis is classified as a high-risk pathogen, due to its severe threat to public health and safety. Antibody-based commercial kits for the detection of Y. pestis are available but these kits are not sensitive enough to identify plague patients in the early stage of infection. Therefore, this study developed a biotin-streptavidin-based sandwich enzyme immunoassay to perform and allow sensitive and specific detection of Y. pestis. In this assay, F1 capsular protein and Y. pestis were captured by anti-F1 mouse monoclonal antibody followed by detection with biotinylated-anti-F1 rabbit polyclonal antibody and horseradish peroxidase- conjugated streptavidin. The F1 sandwich enzyme-linked immunosorbent assay (ELISA) could detect not only the F1 protein up to 17 pg/mL but also Y. pestis up to 129.2 CFU/mL in human serum. In addition, the F1 ELISA did not show any cross-reactivity with various proteins and bacterial strains. This study concluded that F1 ELISA could be utilized as a tool to help confirm the diagnosis of plague patients.

Keywords: *Yersinia pestis*, diagnostics, F1 capsule, sandwich ELISA

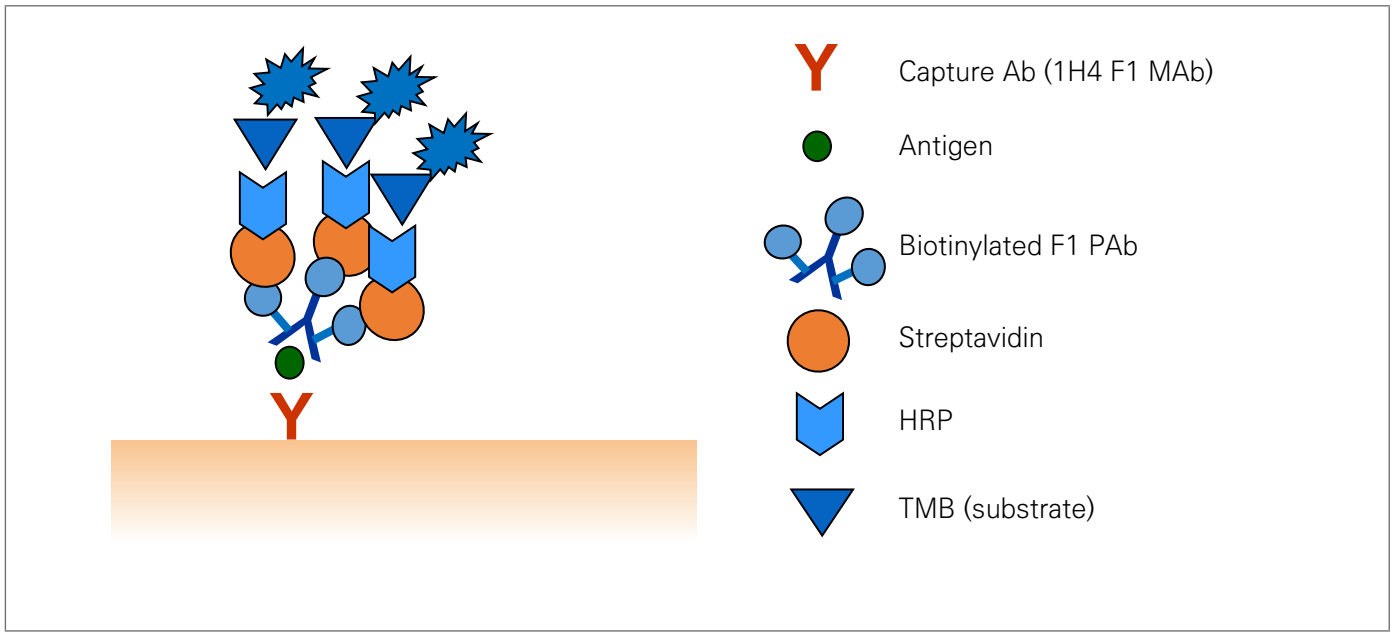

Figure 1. Schematic representation of the F1 sandwich ELISA

Table 1. Detection sensitivity of the F1 sandwich ELISA

Sample type	Sample matrix	Limit of detection	%CV
F1 protein	PBS	29 ± 4.7 pg/ml	9.46
Yersinia pestis		177.8 ± 30.2 CFU/ml	9.8
F1 protein	Human serum	17 ± 2.6 pg/ml	8.92
Yersinia pestis		129.2 ± 25.3 CFU/ml	11.3

Table 2. Specificity of the F1 sandwich ELISA using various proteins (1 μg/ml) and bacterial strains (1 × 10^7 CFU/ml)

Proteins and bacterial strains	Mean OD ± SD		Results	Source or reference
	PBS	Human serum		
Recombinant F1 (rF1)	1.626 ± 0.027	1.653 ± 0.034	+	KCDC
Recombinant V	0.093 ± 0.003	0.066 ± 0.010	−	KCDC
Botulinum toxin A	0.085 ± 0.003	0.060 ± 0.002	−	Meta biologics
Botulinum toxin B	0.090 ± 0.005	0.059 ± 0.002	−	Meta biologics
Botulinum toxin E	0.086 ± 0.005	0.058 ± 0.001	−	Meta biologics
Tetanus toxin	0.087 ± 0.004	0.058 ± 0.002	−	Sigma-Aldrich
Ricin A chain	0.082 ± 0.001	0.062 ± 0.004	−	Sigma-Aldrich
Staphylococcal enterotoxin B	0.087 ± 0.003	0.058 ± 0.002	−	Sigma-Aldrich
Yersinia pestis ATCC19428	1.121 ± 0.021	1.058 ± 0.059	+	ATCC
Yersinia pseudotuberculosis (1)	0.094 ± 0.003	0.063 ± 0.002	−	KCDC
Yersinia pseudotuberculosis (2)	0.096 ± 0.004	0.063 ± 0.005	−	KCDC
Yersinia enterocolitica (1)	0.115 ± 0.002	0.065 ± 0.003	−	KCDC
Yersinia enterocolitica (2)	0.090 ± 0.005	0.061 ± 0.003	−	KCDC
Klebsiella pneumoniae	0.098 ± 0.003	0.074 ± 0.019	−	KCDC
Escherichia coli	0.116 ± 0.001	0.066 ± 0.003	−	KCDC
Bacillus cereus	0.112 ± 0.001	0.062 ± 0.003	−	KCDC
Bacillus mycoides	0.098 ± 0.002	0.066 ± 0.001	−	KCDC
Pseudomonas aeruginosa	0.098 ± 0.002	0.065 ± 0.007	−	KCDC

Abbreviations: KCDC, Korea Centers for Disease Control and Prevention; OD, optical density at wavelength 450 nm.