

# A New Diagnostic Francisella Peptide Chip Assay Allowing Quantitative and Specific Detection of *F. Tularensis*

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## INTRODUCTION

To develop and validate a protein chip technology that couples an antibody-based protein chip to mass spectrometric detection and quantitation of *Francisella tularensis* and other potential bioterrorist agents.

## OBJECTIVE

*Francisella tularensis* (F.t.), a small, nonmotile, aerobic Gram-negative coccobacillus, causes the disease tularemia, and has been designated by the CDC as one of the ten organisms most likely to be engineered for bioterrorism. The symptoms of tularemia in human are non-specific and make the disease difficult to diagnose. For this reason, methods for early diagnosis of tularemia are of critical importance.

We are developing a diagnostic *Francisella* peptide chip assay allowing the rapid, sensitive, safe, quantitative and specific detection of F.t. This method utilizes antibodies immobilized on beads for the capture of target peptides, instead of proteins which are captured in conventional protein chip assays.

## METHODS

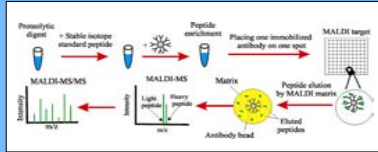
Two proteins specific to F.t. (a 43 kDa lipoprotein (FopA), and a 23 kDa protein characteristic of tularemia infection), were examined.

The 23-kDa protein was obtained as a recombinant protein from the Kawula laboratory, and was in-solution digested with trypsin. The digest was examined on the MALDI-TOF/TOF, and the four peptides with the highest sensitivity from each protein were selected as target peptides against which antibodies are generated. The 43-kDa protein was obtained by in-gel tryptic digestion of gel bands from a F.t. bacterial extract. The extracts were analyzed by MALDI-TOF/TOF to determine the most sensitive and specific peptides from each protein.

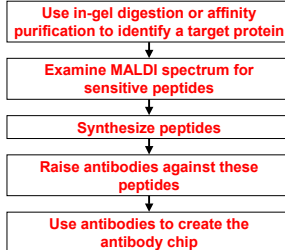
For the F.t. diagnostic chip, the three or four peptides with the highest sensitivity from each protein were selected as target peptides against which antibodies were generated. Peptides were synthesized by Sigma-Genosys, and antibodies were raised against these peptides. The specificities of the antibodies were checked, and the most specific antibody was selected for work on the diagnostic chip.

This study was funded by a gift from an anonymous donor to support research in proteomics at UNC, by the Lineberger Comprehensive Cancer Center (P30 CA 16086-25), by a Duke SERCEB Developmental Project Award, by an SBIR grant from NCI (HHSN261200433014C), and by grant 5U54HD035041-07 from NIH.

## Scheme of Diagnostic Chip



## Analytical Approach

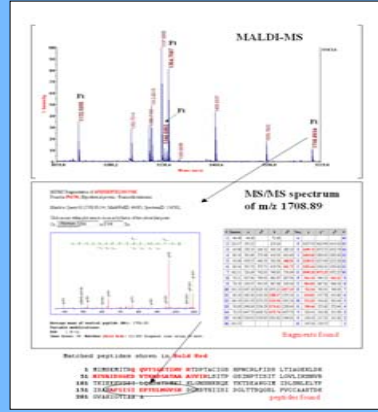


## Acknowledgements

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## RESULTS

### Application to F.t. p23



MALDI-MS and MS/MS analysis of recombinant F.t. p23 purified from an E-coli extract. A) MALDI-MS; B) MALDI-MS/MS; C) sequence coverage of F.t. p23

### Selection of target F.t. p23 Peptides

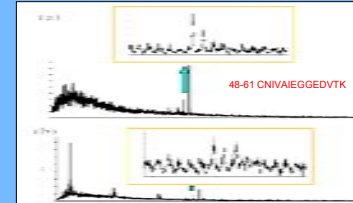
The 23 kDa recombinant F. tularensis protein, provided by Dr. Kawula, was digested in solution, and analyzed by MALDI-MS/MS. Mascot search of the data identified F. tularensis. The relative sensitivities of the different tryptic peptides were determined by MALDI-MS (Figure 1A). On the basis of their MS sensitivities, and their molecular weights which are suitable for sequencing, four peptides were selected as target peptides for the peptide chip. The sequences of these 4 peptides from p23 are:

8-19	QQVTSGETHVR	m/z 1353.70
49-61	NIVAIEGGEDVTK	m/z 1343.69
62-73	ADSATAAASVIR	m/z 1131.59
153-168	AFSISIEPTELMGVSK	m/z 1707.89

### Evaluation of Anti-F.t. peptide Abs

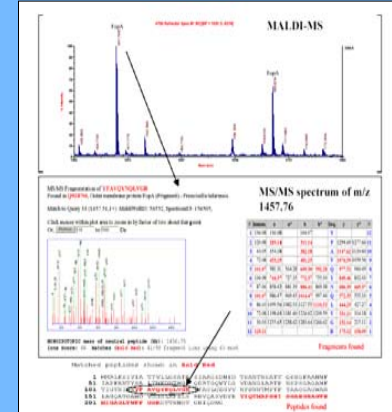
Specificity testing of F.t. antibodies by Sigma-Genosys		
F.t. Antibodies ELISA Results		Response
Description		
1	153-167 AFSISIEPTELMGVSK	1/50,000
2	8-19 QQVTSGETHVR	1/3,000
3	49-61 NIVAIEGGEDVTK	1/500,000
4	62-73 ADSATAAASVIR	1/10,000
	1/20,000	Specificity is "marginal"
	1/100,000	Specificity is "optimal"
	1/500,000	Maximum measurable

### Detection Limit for F.t. p23 peptide



MALDI-MS spectrum of (a) 150 amol (b) ~15 amol of peptide (48-61 CNVAIEGGEDVTK) affinity-bound to anti-F.t. antibody beads. The singly-charged, epitope-containing, F.t. peptide is observed at m/z = 1447.7

### Application to F.t. FopA



### Selection of target FopA Peptides

Two peptides were selected as target peptides for the peptide chip. The sequences of these 2 peptides from FopA are: 109-120 YFAVQYNQLVGR m/z 1457.51 and 181-196 YQTMAPSINISGANR m/z 1695.63

## CONCLUSIONS

Previous experiments have demonstrated that this peptide chip approach was capable of detecting peptides bound to a single antibody bead at fmol levels. These current experiments have demonstrated that with proper selection of the anti-peptide antibody and the target peptide, detection limits can be in the low amol range.

The use of several peptides from different F.t. proteins, coupled with the ability to obtain amino acid sequence data on any affinity-captured peptides, will provide a specific and sensitive diagnostic technique. The ability to obtain sequence information avoids problems from "false positives" which could result from the non-specific binding that can occur with any affinity-based technique. Quantitation can be achieved through the use of stable-labeled versions of the target peptides, using deuterated lysine or arginine.