



Development of a specific immune lateral flow assay for cholera detection

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Introduction

Cholera is an acute diarrheal disease caused by *Vibrio cholerae*, that can be lethal within hours if left untreated. People are usually infected with *V. cholerae* by using contaminated water or food. UNICEF indicate that every year there are as many as 4 million cholera cases globally and as many as 143,000 deaths. Young children, especially those under the age of 5, are at risk. Rapid detection and identification is essential for the most effective response to an infectious disease outbreak. Therefore, our goal is to develop a more specific Point-of-Care test.

Methods

- The following steps were taken for development of a specific Point-of-Care diagnostic test:
- 1) Determine a specific biomarker of the epidemic *V. cholerae* strain.
 - 2) Analyze with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) different *V. cholerae* isolates.
 - 3) Determine if a specific biomarker can be detected.
 - 4) Develop and test mono-clonal antibodies (mABs) against specific biomarker.
 - 5) Currently a prototype immuno-lateral Develop imunno-lateral flow assay.
 - 6) Evaluate its performance.

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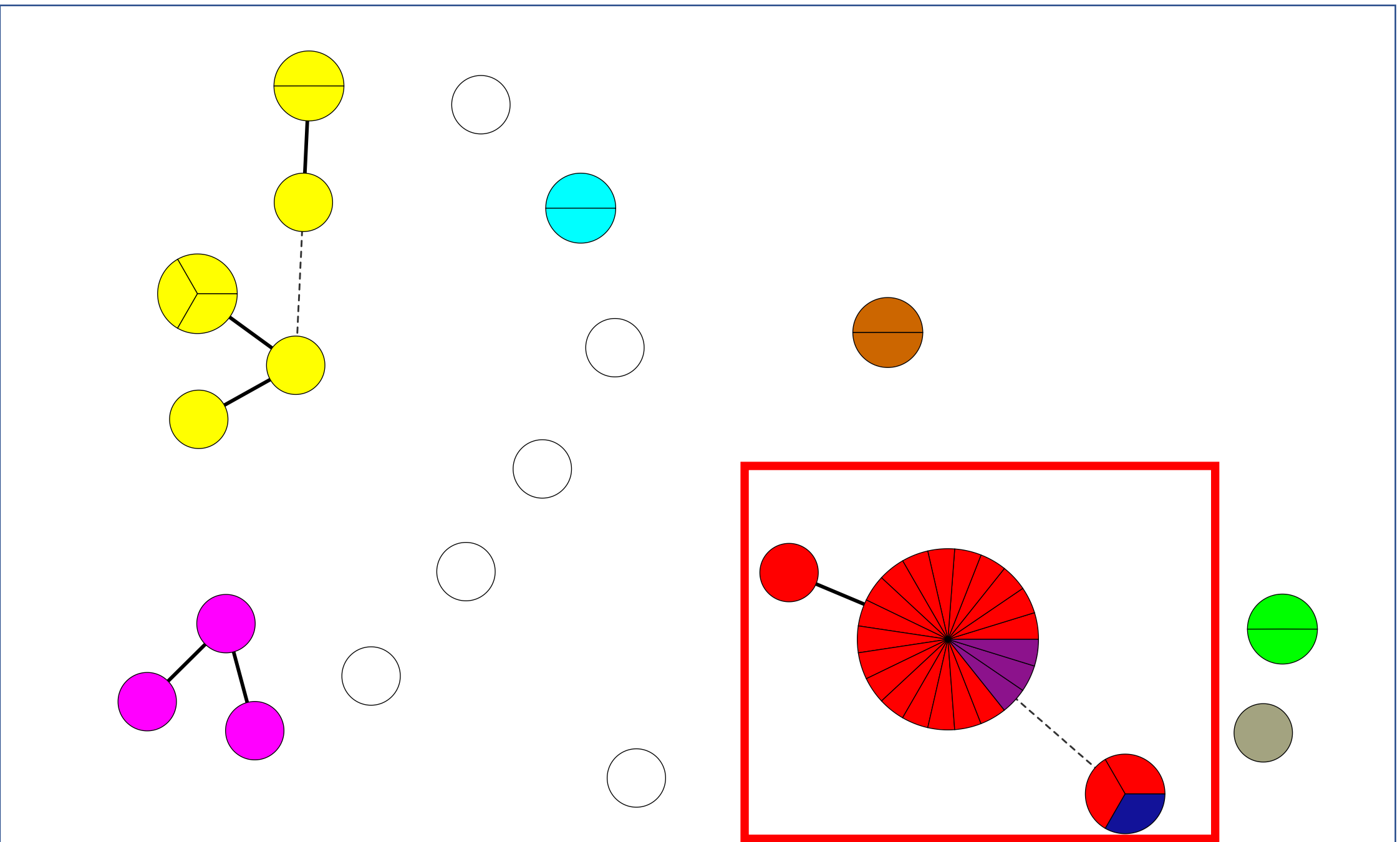


Figure 1. Minimal Spanning Tree analysis of *V. cholerae* based on MLST data. Each circle corresponds to a sequence type (ST). The number of partitions in each circle corresponds to the number of isolates. Single locus variants are connected with a solid line, two single variants are connected with a dotted line. Red, *V. cholerae* serogroup O1 serotype Ogawa strains; purple, *V. cholerae* serogroup O139; dark blue, *V. cholerae* serogroup O1 serotype Hikojima. These strains, which all contain the toxin producing genes *ctxAB* and *tcpA*, form together Genotype 1 (GT1, **Red box**). Yellow, *V. cholerae* serogroup O1 serotype Inabe (GT2); pink, *V. cholerae* serogroup O1 serotype Ogawa (2x) and Inabe, lacking *ctxAB* and *tcpA* (GT3). Green (GT4), brown (GT5) and light blue (GT6) are *V. cholerae* non-O1/O139 isolates. Gray, *V. mimicus*.

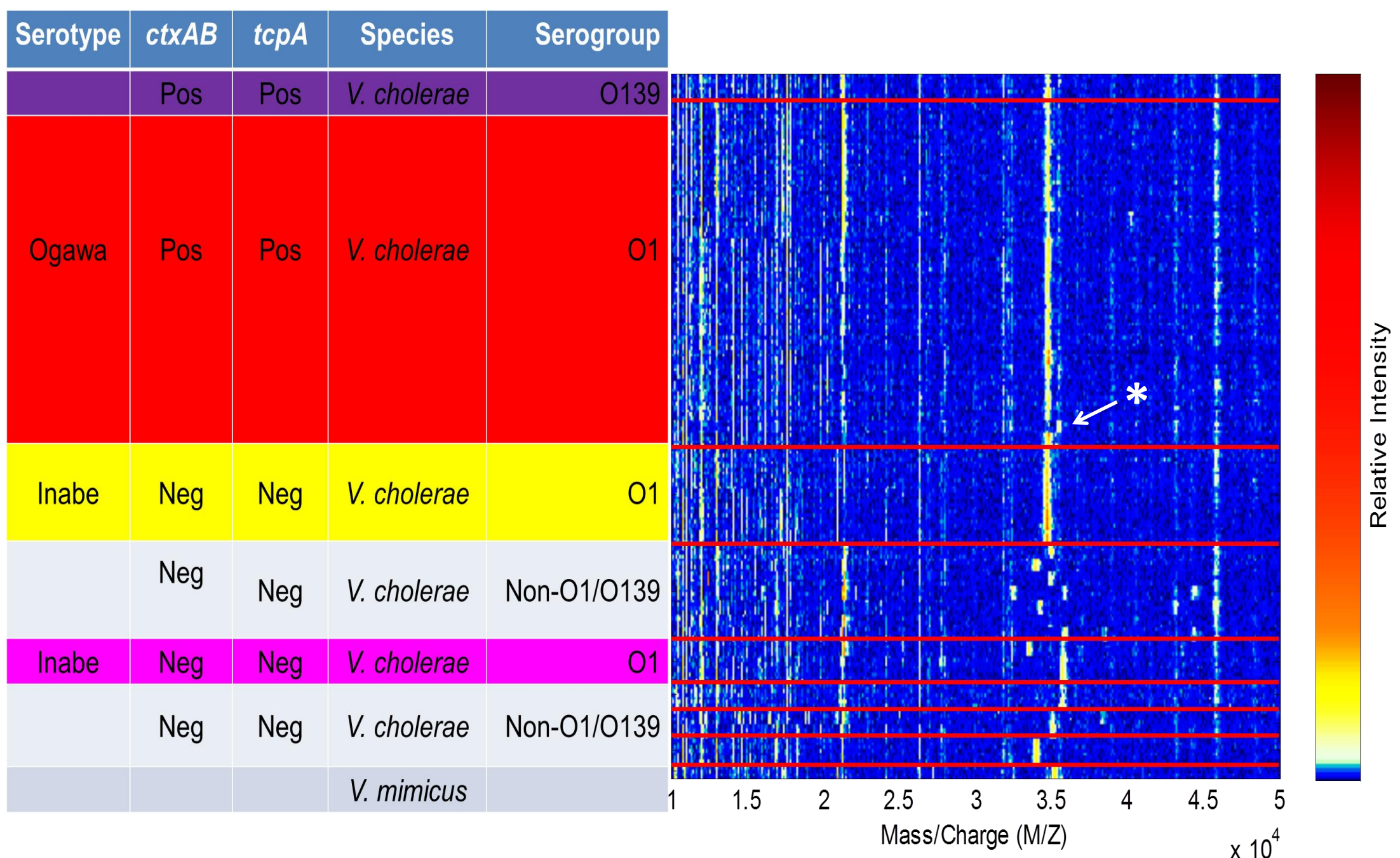


Figure 2. Heatmap (right panel) of 196 MS-spectra from 48 *V. cholerae* and 1 *V. mimicus* isolates. Each horizontal lane depicts one MS spectrum; red lines separate the different genotypes based on MLST, as indicated in the left panel. Purple and red rows; toxigenic and epidemic *V. cholerae* O1 and O139 isolates, producing a specific marker at 34,750 Da. Serogroup O1 serotype Inabe isolates which lack the toxin genes (yellow) produce the marker at 34,675 Da. *V. cholerae* serogroup O1 serotype Ogawa (2x) and Inabe (1x) of GT3 (pink) produce a variant of higher mass (35,671 Da) and non-O1/O139 isolates produce different variants, all with masses distinguishable from that of the toxin producing isolates. *V. cholerae* O1 serotype Hikojima, which produces the toxin, is an exception as it does not show the discriminatory peak at 34,750 (*).

Results

1. Cholera is caused by a pandemic strain of *Vibrio cholerae*. The strain can have a O1 or a O139 serotype and produces the cholera toxin (Figure 1). Other *V. cholerae* isolates aren't O1 or O139 or are O1 but don't produce the cholera toxin. The complexity is to detect a biomarker that discriminates the pandemic strain from other *V. cholerae* isolates.
2. Using MALDI-TOF MS a biomarker was found, which is specific for the pandemic *V. cholerae* strain. Moreover, by using Ferulic Acid as a MALDI matrix a specific protein for the *V. cholerae* pandemic strain was discovered (Figure 2 and 3).
3. After a band at 35kDa was cut out of a SDS-PAGE gel and analyzed with LC-MS/MS the protein was identified as OmpU. A abundantly expressed outer membrane protein of *V. cholerae* (Figure 4).
4. Monoclonal antibodies (mABs) were developed and tested. A dotblot of mABs binds specific to the pandemic *V. cholerae* strain Figure 4)
5. The development of the immuno-lateral flow assay is ongoing.
6. After the prototypes is developed its performance will be evaluated in a laboratory against different bacteria. Next, if successful immuno-lateral flow assay's will be validate in the field, in an area where cholera is endemic.

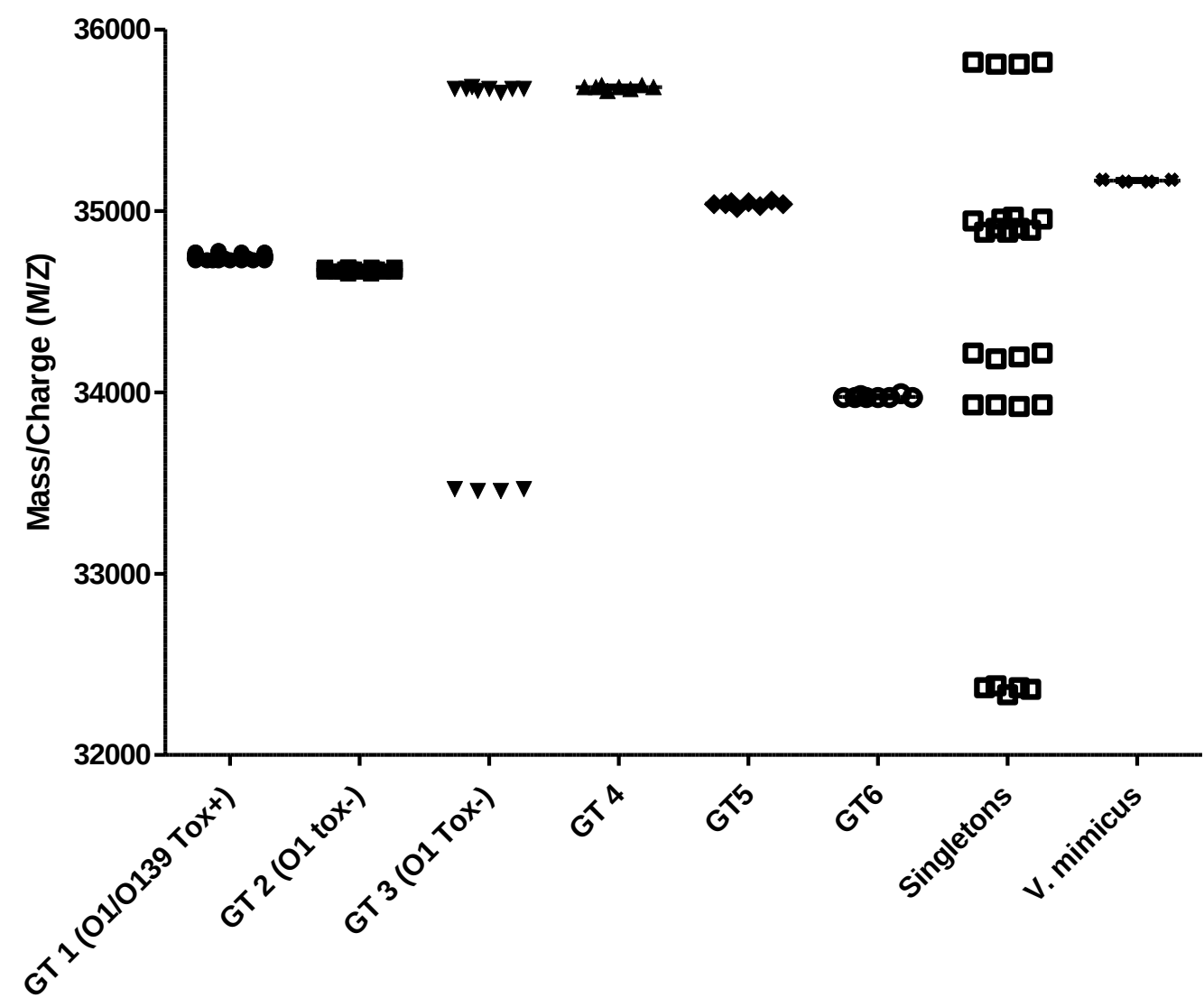


Figure 3. Distribution of observed peak positions in the 32 – 36 kDa range among different genotype groups. Each isolate is represented by 4 peak positions. GT1 (O1/O139 Tox+) comprises 100 peak positions of 25 isolates; GT2 (O1, Tox-) 32 peak positions of 8 isolates; etc. GT3 (O1 Tox-) shows 12 peak positions of 3 isolates with the same genotype but different serotypes. Singletons show 26 peak masses of 7 isolates with unique genotypes (2 MS-spectra from one isolate failed).

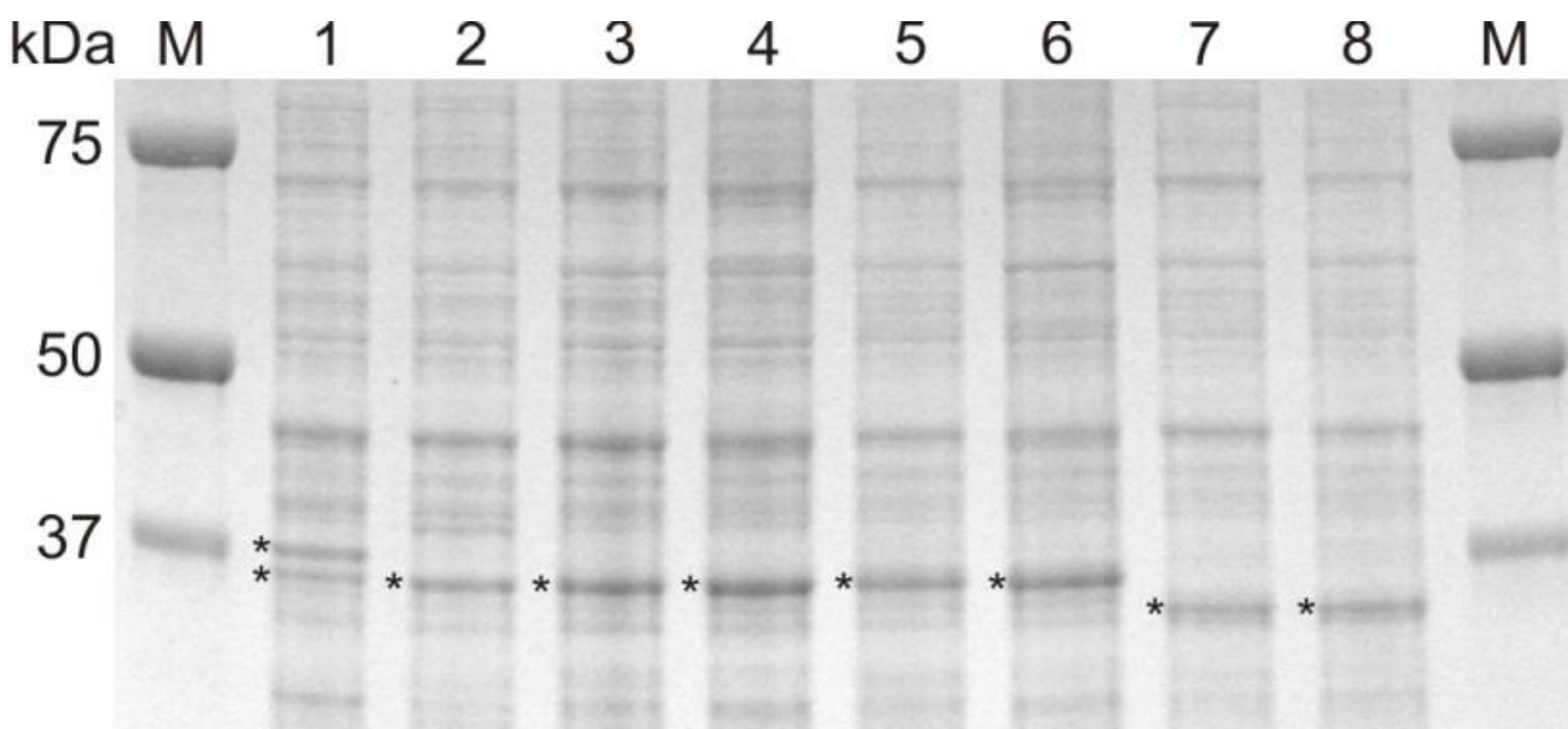


Figure 4. SDS-PAGE analysis of whole cell fractions of eight *V. cholerae* isolates: 1, O1 serotype Hikojima Tox+ (GT1); 2, O139 Tox+ (GT1); 3, O1 serotype Ogawa Tox+ (GT1); 4, O1 Tox+ (GT1); 5, O1 Tox- (GT2); 6, serogroup Inabe Tox- (GT2); 7, non-O1/O139 Tox- (singleton); 8, non-O1/O139 Tox- (GT6). Bands indicated with an asterisk were excised from the gel, in-gel digested with trypsin and analyzed by LC-MS/MS. All bands were identified as OmpU homologues, except the upper band of strain *V. cholerae* O1 serotype Hikojima Tox+ GT1, which was identified as OmpT.

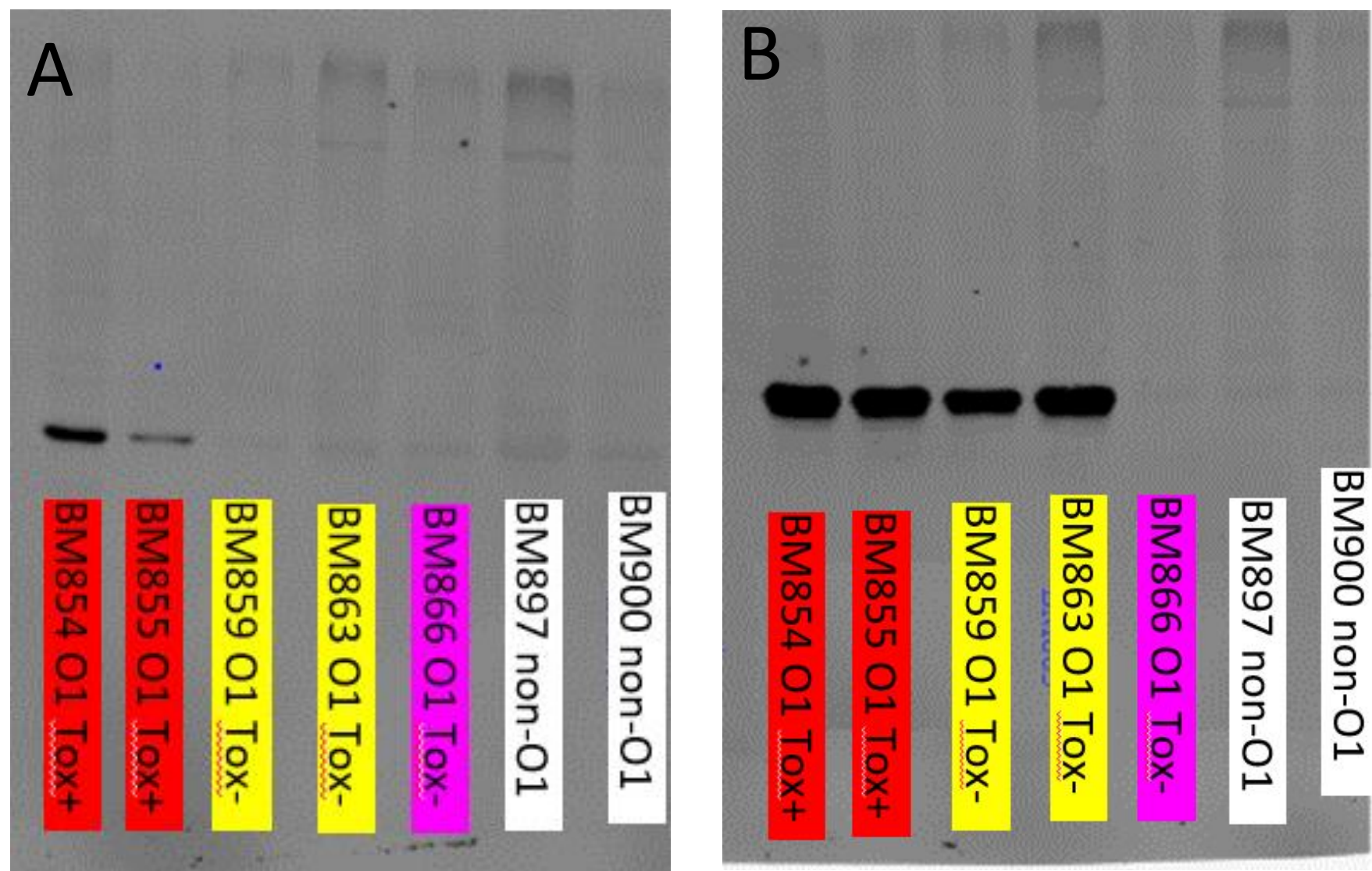


Figure 5. 3 Dotblots with 2 different mABs against Ompu of pandemic *V. cholerae* strain.
A. Monoclonal antibody 1 is binds specific to OmpU of the pandemic *V. cholerae* strain
B. Monoclonal antibody 3 is binds to OmpU of pandemic *V. cholerae* strain but also to one other *V. cholerae* O1 isolate that isn't able to produce cholera-toxin.

Conclusions

Using MALDI-TOF MS a specific biomarker for the pandemic *V. cholerae* strain was detected. The abundantly expressed outer membrane protein OmpU is a promising candidate to develop a imunno-later-flow assay. Therefore, specific monoclonal antibodies were developed against OpmU. The specific mABs are now used to develop a LFA-prototype. Next, the LFA will be evaluated in the lab and if successful field tests will be conducted in areas where cholera is endemic.