Nov 13, 2025

Luminex Magnetic Bead Assay for Assessment of the Immune Response to *Vibrio cholerae*

DOI
dx.doi.org/10.17504/protocols.io.3byl4b1x8vo5/v1

dmslater[1], Jason Harris[1]

[1]Massachusetts General Hospital, Harvard University

dmslater

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.3byl4b1x8vo5/v1

Protocol Citation: dmslater, Jason Harris 2025. Luminex Magnetic Bead Assay for Assessment of the Immune Response to Vibrio cholerae. **protocols.io https://dx.doi.org/10.17504/protocols.io.3byl4b1x8vo5/v1**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: June 08, 2022

Last Modified: November 13, 2025

Protocol Integer ID: 64241

Keywords: antibody responses to vibrio cholerae, luminex magnetic bead assay, luminex magnetic bead assay for assessment, immune response to vibrio cholerae, antibody, assessing antibody response, multiplex bead assay, vibrio cholerae, human blood plasma sample, specific secondary antibody, target antigen, magnetic bead, magnetic microsphere, antigen binding, plasma sample, immune response, blood, pooled plasma standard, bead

Abstract

This protocol provides instructions for assessing antibody responses to *Vibrio cholerae* in human blood plasma samples using a multiplex bead assay (MBA).

Magnetic microspheres (beads) are conjugated with target antigens according to the manufacturer's instructions. Individual bead stocks are added to a buffered solution to create a pooled, master bead stock and dispensed into 384-well plates. Plasma samples are diluted and tested in duplicate. A serial dilution of a pooled plasma standard, positive and negative controls are included on each plate. After an incubation step to allow antibody-antigen binding, magnetic beads are washed, and incubated with fluorescently labeled isotype-specific secondary antibodies. Beads are washed once more and then resuspended and analyzed on a FlexMap3D instrument.

Materials

Materials

Antibody Coupling Kit, Luminex: 40-50016
EDC; Thermo Scientific 77149
Antigen panel (see Table 1, section 7.1.3)
Secondary antibodies, PE-conjugated
- IgG, Southern Biotech Cat # 9040-09
- IgA ,Southern Biotech Cat # 2050-09
- IgM Southern Biotech Cat # 9020-09
- IgG1 Southern Biotech Cat # 9052-09
- IgG2 Southern Biotech Cat # 9070-09
- IgG3 Southern Biotech Cat #9210-09
- IgG4 Southern Biotech Cat #9200-09
- IgA1 Southern Biotech Cat # 9130-09
- IgA2 Southern Biotech Cat # 9140-09

Sample Dilution Buffer; 1% BSA with 0.05% Tween20
Bead Stock Buffer; 0.1% BSA in 1x PBS
Wash Buffer; 1% BSA with 0.05% Tween20 in 1X DPBS
10X sterile DPBS for making 1X wash buffer, Corning, 20-031-CV
Sheath Fluid Plus, Fisher Scientific NC0695082
Bovine Serum Albumin (BSA), for assay buffer and antigen, Sigma A7906-50G
15-mL and 50-mL Falcon tubes
Sterile pipet tips, all sizes
Sterile Reservoirs, VWR 89094-664
1.5 mL Microcentrifuge tubes
384-Well Polystyrene Non-Binding Flat Bottom Microplate, Fisher Scientific Greiner Bio-One™ 07000892
Aluminum foil
Foil Plate Seal, Bio-Rad, MSF 1001
Milli-Q water, or equivalent
U-bottom 96-well plates

Equipment

Dynabead2Mag-Magnet, Thermofisher 12321D
Plate washer, e.g., Tecan Hydrospeed
Microplate Shaker
Sonicating water bath
Vortex mixer
Luminex FlexMap3D instrument
12-channel multichannel pipets
24-channel multichannel pipets
P2, P20, P200 and P1000 single channel pipets

Troubleshooting

Safety warnings

Biosafety/PPE

- This protocol requires working with human blood plasma samples that should be handled as potentially infectious.
- Lab staff are required to complete biosafety training prior to performing this protocol.
- Standard lab PPE is recommended for this protocol (e.g., lab coats, gloves, and goggles).
- All solid waste should be discarded in biohazardous waste containers. Liquid waste should be treated with a 10% solution of bleach for 20 minutes before discarding in the sink. Wear eye and/or face protection when disposing liquids in sink.
- Spills should be treated as infectious and cleaned using the lab standard protocol (i.e. cover spill with absorbent material, spray with 10% bleach to soak thoroughly, treat for at least 15 minutes, use tongs to discard material in biohazard waste, change gloves and clean area with 70% ethanol)

Chemical Hazards

- EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) CAS # 25952-53-8
- Acute toxicity: Irritant. Oral, Dermal. Wear protective gloves, lab coat, eye protection/face protection when handling.
- MSDS: https://www.sigmaaldrich.com/US/en/sds/mm/341006

Definitions/Abbreviations

1 **Beads:** Luminex® MagPlex® microspheres
BSA: bovine serum albumin
MFI: mean fluorescent intensity
PBS: phosphate buffered saline
PPE: personal protective equipment
rpm: revolutions per minute
O1 Standard: A high-titer pooled plasma sample prepared from convalescent plasma of patients who have recovered from V. cholerae O1 infection. Serial dilutions are used as a standard curve to normalize MFI data to minimize inter-run variation.
O139 Positive Control: A high-titer pooled plasma sample prepared from convalescent plasma, reactive with the O139 antigen.
Sialidase/TcpA Positive Control: A high-titer pooled plasma sample prepared from convalescent plasma, reactive with the sialidase and tcpA antigens.
Negative Control: A pooled heat-inactivated plasma sample is prepared from North American volunteers without prior exposure to cholera.

Procedure - Antigen Conjugation to MagPlex Beads

2 Antigens are conjugated to beads following the manufacturer's instructions in the Luminex xMAP®Antibody Coupling Kit User Manual, RevE, using a ratio of 5µg of antigen for every 1×10^6 beads. Stocks are quantitated using a cell counter and stored at 4C, wrapped lids in parafilm to prevent evaporation and foil to protect from photobleaching.

The panel consists of the following antigens and bead regions:

	A	B	C	D
	Antigen	**Antigen Source**	**Bead Region**	**Bead Catalog #**
	V. cholerae O1 Inaba OSP-BSA	Edward Ryan, MGH	44	MC10042-01
	V. cholerae holotoxin (CTh)	List Biological, 100B	77	MC10044-01
	V. cholerae toxin, B subunit (CtxB)	Sigma, C9903-1MG	38	MC10077-01
	V. cholerae sialidase, N16961 strain	Richelle Charles, MGH	35	MC10038-01

	A	B	C	D
	V. cholerae toxin-regulated pilus (TcpA) N16961 strain	Harris Lab, MGH	49	MC10035-01
	V. cholerae O1 Ogawa OSP-BSA	Edward Ryan, MGH	42	MC10049-01
	V. cholerae O139 OSP-BSA	Edward Ryan, MGH	61	MC10061-01
	V. cholerae cytolysin (VCC)	Richelle Charles, MGH	25	MC10025-01
	Influenza A HA antigen HA1(A/California/07/2009) (H1N1)	Immune Technology, IT-003-SW12p	18	MC10018-01
	E. coli heat-labile toxin, human isoform (LTh)	Dennis Clements,Tulane University	55	MC10055-01
	E. coli heat-labile toxin, B subunit (LT-B)	Dennis Clements, Tulane University	20	MC10020-01

V. cholerae **MagPlex Bead Panel**

Preparation of Standards and Controls

3 Standard and controls should be prepared in 1.5-mL microcentrifuge tubes, to allow for thorough mixing using a vortex mixer.

4 **Standard Dilutions**
Using sample dilution buffer as a diluent, prepare an 8-step dilution series of the O1 Standard, beginning with a 1:10 dilution of the stock and then performing 7 × 4-fold additional dilutions. The resulting series will represent the following dilutions of standard: 1:10, 1:40, 1:160, 1:640, 1:2560, 1:10240, 1:40960 and 1:163840.

Adjust the volume for dilutions based on the number of plates and/or antibody isotypes to be tested. 5 µL of each dilution will be used per replicate (384-well plate).

5 **Controls**
Prepare Positive and Negative Controls by diluting 1:100 in sample dilution buffer. Factor in any previous dilutions of the plasma stock. 5 µL of each dilution will be used per replicate (384-well plate).

Prepare an additional aliquot of sample dilution buffer in a 1.5 mL centrifuge tube to use as a blank (plate background measurement).

Sample Preparation

6 Sample dilutions are prepared in low-protein binding round-bottom 96-well plates to allow for use of multi-channel pipet when transferring samples to the 384 well plate.

Prepare plasma samples by diluting 1:100 in sample dilution buffer, factoring in any previous dilutions of the plasma sample.

Using a multichannel pipet, add 148.5 µL of sample dilution buffer to 96-well plate.

Mix samples briefly by pulsing on a vortex mixer and then using a P2 pipet, transfer 1.5 µL of plasma into buffer to make the 1:100 dilution.

Seal plate with a foil plate seal and place on a microplate shaker at room temperature to mix at 550 rpm while preparing bead stocks (approximately 30 minutes).

Preparation of Pooled Bead Stock

7 A 10x concentrated bead stock is prepared and mixed well before diluting to the final 1x stock concentration dispensed into the 384 well plate. The final 1x stock contains 810 beads for each bead region (antigen type) in a 45 µL volume. An overage of 20% is included to account for loss during pipetting.

The table below provides an example calculation.

	A	B	C
	Step	**Example**	**Formula**
	number of samples	24	24
	number of controls	12	12
	replicate number	2	2
	number of secondary Abs	1	1
	total number of wells	72	=(B2+B3)*B4*B5
	number of 384-well plates	0.2	=B6/384
	number of beads/region/well	810	810

	A	B	C
	total number of beads + 20%	69984	=B6*B8*1.2
	volume beads per well (µL)	45	45
	total volume of 1x bead stock + 20% (mL)	3.888	=B6*B10*1.2/1000
	total volume of 10x bead stock (mL)	0.389	=B11/10

Bead Stock Calculation Example

8 Prepare bead pool in a 50-mL centrifuge tube. Protect tube from light by wrapping in foil.

Vortex bead stocks for 1 minute to resuspend beads and then sonicate for 2 minutes in an ultrasonic water bath at a frequency of 46 KHz.

Add the predetermined volume of Bead Stock Buffer to the 50-mL tube for the 10x pooled bead stock, then add beads for each of the antigens in the panel. Vortex each bead stock for 5-10 seconds to mix immediately before making the transfer.

Vortex 10x pooled bead stock for 1 minute, then add the remaining Bead Stock buffer to dilute bead stock to final 1x concentration. Vortex 1x pooled bead stock again for 1 minute to mix.

Incubation Steps

9 Transfer half of the 1x pooled bead stock to a reagent reservoir and use a multichannel pipet to transfer 45 µL to the wells of the 384-well plate. Ensure that the bead pool in the reservoir remains mixed by pipetting up and down 3-5 times before each transfer. Mix the 50-mL tube for 1-minute before transferring the remaining portion of 1x pooled bead stock to the reservoir.

10 Using a 12-channel pipettor, transfer 5 µL of diluted sample from the 96-well plates to the 384-well plate. Cover plate with an opaque foil seal and incubate for 2 hours at room temperature on a microplate shaker set to 850 rpm.

11 Prepare a 1:150 dilution of each secondary antibody in sample dilution buffer at the tail end of the incubation step and protect from light. Prepare sufficient volume to cover

addition of 40 µL per well plus 15-20% overage for loss during pipetting and vortex gently for 10 seconds to mix.

12 After the 2-hour incubation, wash beads on a magnetic plate washer (e.g. Tecan HydroSpeed) using instrument dispense and aspiration rates suitable for magnetic beads. Use 6 cycles of 50 µL of Wash Buffer.

An example wash protocol for a Tecan HydroSpeed Instrument is provided below:

Grenier 384-well plate
Aspiration Rate: 1

Stage 1
1. Cycles: 1
2. Soak
3. Intensity: Off Shake
4. Time: 90 seconds

Stage 2
1. Cycles: 6
2. Aspirate
3. Z-position: Bottom, Normal
4. Set: 8.0 [mm]
5. Time: 1 second
6. Head speed: 5 [mm/s]
7. Dispense
8. Z-position: Bottom
9. Set: 8.0 [mm]
10. Dispense rate: 50 [µl/s]
11. Channel: 2
12. Volume: 50 [µl]
13. Soak
14. Intensity: Medium Shake
15. Time: 15 seconds
16. Soak
17. Intensity: Off Shake
18. Time: 60 seconds
19. Cycles: 1
20. Aspirate
21. Z-position: Bottom, Normal
22. Set: 8.0 [mm]
23. Time: 1 second
24. Head speed: 5 [mm/s]

13 Using a reagent reservoir and a multichannel pipet, add 40 µL of secondary antibody to each well. Cover plate with a foil seal and incubate at room temperature for 1 hour on a microplate shaker set at 850 rpm.

14 After the 1-hour incubation, wash beads on a magnetic plate washer (e.g. Tecan HydroSpeed) using instrument dispense and aspiration rates suitable for magnetic beads. Using 6 cycles of 50 µL of Luminex Sheath Buffer and a final resuspension step with 60 µL of Luminex Sheath Buffer.

An example wash protocol for a Tecan HydroSpeed Instrument is provided below:

Grenier 384-well plate
Aspiration Rate: 1

Stage 1
1. Cycles: 1
2. Soak
3. Intensity: Off Shake
4. Time: 90 seconds

Stage 2
1. Cycles: 6
2. Aspirate
3. Z-position: Bottom, Normal
4. Set: 8.0 [mm]
5. Time: 1 seconds
6. Head speed: 5 [mm/s]
7. Dispense
8. Z-position: Bottom
9. Set: 8.0 [mm]
10. Dispense rate: 50 [µl/s]
11. Channel: 1
12. Volume: 50 [µl]
13. Soak
14. Intensity: Medium Shake
15. Time: 15 seconds
16. Soak
17. Intensity: Off Shake
18. Time: 60 seconds
19. Cycles: 1
20. Aspirate
21. Z-position: Bottom, Normal
22. Set: 8.0 [mm]
23. Time: 1 second

24. Head speed: 5 [mm/s]

Stage 3

1. Dispense
2. Z-position: Overflow
3. Set: 8.0 [mm]
4. Dispense rate: 50 [µl/s]
5. Channel: 1
6. Volume: 60 [µl]

15 Cover plate with a foil seal and sonicate in an ultrasonic water bath for 1 minute to disperse beads well before removing foil seal and loading on the Luminex instrument.

Luminex Run Protocol

16 **Luminex acquisition settings:**
Volume: 50 microliters
Timeout: 200 seconds
Bead Type: MagPlex
Plate Name: Greiner Luminex
DD Gating: 7500 to 15000
Reporter Gain: Enhanced PMT (High)

Assign the antigens to their corresponding beads regions (see table in step 2). Set the target bead count to a minimum of 50 for each antigen.

We also typically add 4-8 alcohol flush steps at regular intervals throughout the 384-well plate, depending on how many samples are being tested.

17 **Run QC:**

- Retest plates if over half of the dilutions of standards had 5 or more antigens with a coefficient of variation greater than 20% (calculated from triplicate MFI measurements)
- Exclude any measurements with a bead count less than 30