# Microfluidic Platform for the Enzymatic Pretreatment of Human Serum for the Detection of the Tuberculosis Biomarker Mannose-Capped Lipoarabinomannan

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## ELISA for ManLAM

Our ELISA for ManLAM has been reported previously.[1,2] The assay is a sandwich-type ELISA utilizing a polyclonal rabbit antibody to *Mycobacterium tuberculosis* purified protein derivative (Virostat #4601) as a capture antibody and a biotinylated human monoclonal antibody to ManLAM (A-194-01[3]) as the label. This assay exhibits good performance with a limit of detection consistently near 10 pg/mL for ManLAM in an innocuous buffered matrix (MPBS+BSA, see below).

*Materials, Reagents, and Equipment*

BupH™ modified Dulbecco's PBS packs (MPBS), StartingBlock™ PBS blocking buffer, and 1-Step™ Ultra TMB-ELISA (TMB) were from Thermo Scientific. Tween® 20 was from Fisher Scientific. Bovine serum albumin (BSA) and sulfuric acid were from Sigma-Aldrich. Streptavidin-conjugated horseradish peroxidase (STV-HRP) was from R&D Systems. A-194-01 antibody was generously provided by Dr. Abraham Pinter (Rutgers University) and biotinylated in-house as described elsewhere.[2] COSTAR 3590 high-binding polystyrene 96-well plates were from Corning Incorporated.

MPBS was reconstituted with ultrapure water per package directions. MPBS with 0.05% v/v Tween® 20 was used as washing buffer for the ELISA and is referred to as MPBST. MPBS with 1% w/v bovine serum albumin was used as a diluent for ManLAM standards, label antibody, and STV-HRP, and is referred to as MPBS+BSA.

Labnet Vortemp incubators were used to incubate the plates during the assay. A BioTek Multiflo FX microplate washer was used for plate washing steps and a BioTek ELx800 microplate reader was used to measure the absorbance in each well.

*Assay protocol*

1. Incubate plate with 100 µL/well capture antibody (10 µg/mL in MPBS) overnight at 4 °C.
2. Equilibrate plate in incubator for 30 min (30 °C, 250 rpm; these conditions used for all subsequent incubations).
3. Wash plate 3× with 300 µL/well MPBST (422 µL/sec dispense rate; these conditions used for all subsequent washes).
4. Incubate plate 1 h with 200 µL/well StartingBlock™.
5. Wash plate.
6. Incubate plate 2 h with 100 µL/well sample.
7. Wash plate.
8. Incubate plate 2 h with 100 µL/well biotinylated label antibody (200 ng/mL in MPBS+BSA).
9. Wash plate.
10. Incubate plate 25 min with STV-HRP (diluted 1:200 from stock in MBPS+BSA).
11. Wash plate.
12. Incubate plate 30 min with 100 µL/well TMB.
13. Add 50 µL/well 2 N sulfuric acid.
14. Read absorbance.

Absorbance of the TMB substrate is measured at 450 nm and a background reading at 630 nm is subtracted to account for well-to-well variations in the microplate. The limit of detection is calculated as the average absorbance of the blank plus three times its standard deviation,[4,5] which is converted to a ManLAM concentration by interpolation on a linear least squares fit to the calibration data. All samples are run in triplicate. Comparisons between samples run on separate plates are made by referencing to the calibration standards run on each plate.

**Bill of Materials**

| *Material* | *Vendor* | *Catalog number* |
|---|---|---|
| Aluminum plates, type 6061 | McMaster-Carr | 8975K513 |
| PMMA sheet, 1/4 in | McMaster-Carr | 8560K355 |
| Sylgard 184 PDMS kit | Ellsworth Adhesives | 184 SIL ELAST KIT 3.9KG |
| PMMA sheet, 1 mm | Uxcell | a19123000ux0199 |
| Silicone membrane, 240 µm | Rogers Corporation | BISCO-HT-6210 |
| Silicone rubber insulation | Home Depot | 304267928 |
| Thermocouple | Omega Engineering, Inc. | SA1-J |
| Ceramic cartridge heater tatoko 502 | Amazon | B07Q5R7GV6 |
| Tygon tubing, 0.060” O.D. × 0.020” I.D. | Saint Gobain Performance Plastics | AAD04103 (Fisher Scientific 15670211) |
| PID controller | Omega Engineering, Inc. | CN4216-R1-R2 |
| MH1 valve manifold | Festo USA | 197334 |
| Programmable logic controller | WAGO USA | 750-881 |
| Silicone adhesive 1 | Adhesive Research | ARclad IS-8026 |
| Silicone adhesive 2 | FLEXcon | SA6104 LR |
| Loctite 34931 light cure adhesive | Amazon | B005TPGT4M |
| J-B Weld Original 2-part epoxy | Amazon | B0B5VNG2YT |

**ECPMD Principle of Operation**

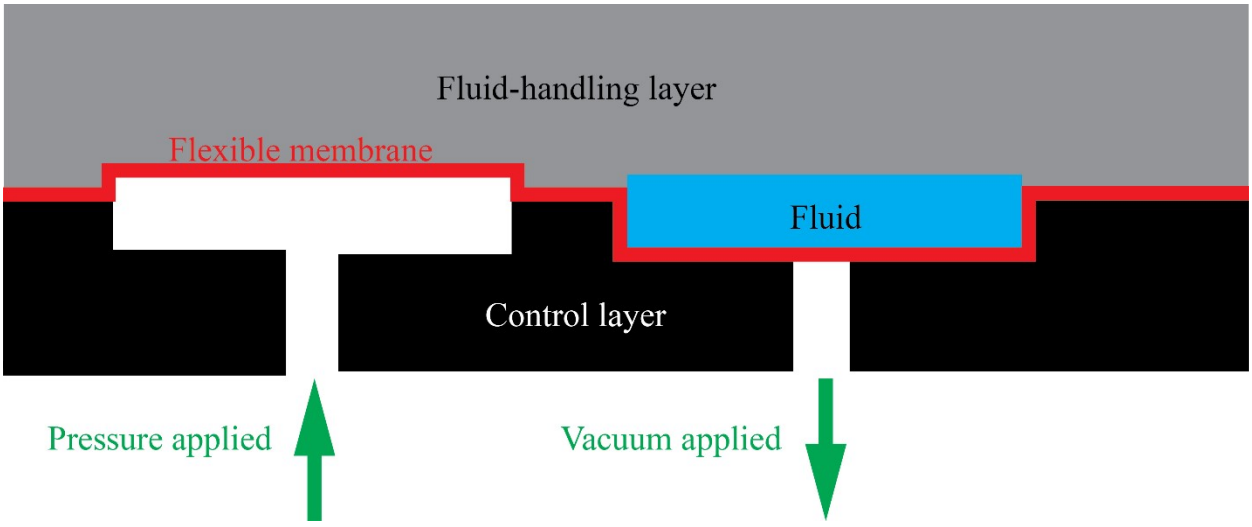


**Figure S-1.** Operation of the membrane-controlled fluid chambers. When positive pressure is applied to the control layer, the membrane flexes up, closing the chamber and expelling its contents. When vacuum is applied to the control layer, the membrane flexes down, opening the chamber and taking in liquid from any connected chamber or reservoir.

## Performance Comparison

**Table S-1.** Raw data and paired t-test calculations for the comparison of ManLAM ELISA dose response between on-chip and on-bench sample pretreatment (accompanying **Figure 3A**).

| *[ManLAM] (pg/mL)* | *Signal from on-chip pretreatment (A.U.)* | *Signal from on-bench pretreatment (A.U.)* | *Difference (chip-bench)* |
|---|---|---|---|
| 0 | 0.060 | 0.045 | 0.015 |
| 10. | 0.080 | 0.075 | 0.005 |
| 50. | 0.225 | 0.204 | 0.021 |
| 100. | 0.411 | 0.402 | 0.009 |
| 500. | 2.055 | 1.834 | 0.221 |
| | | Mean difference | 0.054 |
| | | Standard deviation | 0.093 |
| | | $t_{calculated}$ | 1.301 |
| | | $t_{table}$ | 2.776 |

**Table S-2.** Raw data and paired t-test calculations for the comparison of determined ManLAM concentrations following pretreatment of blinded samples on bench and on chip.

| *Spiked [ManLAM] (pg/mL)* | *[ManLAM] determined following on-chip pretreatment (pg/mL)* | *[ManLAM] determined following on-bench pretreatment (pg/mL)* | *Difference (chip-bench)* |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 25. | 29 | 24 | -6 |
| 75. | 75 | 89 | 17 |
| 300. | 279 | 355 | 88 |
| | | Mean difference | 25 |
| | | Standard deviation | 43 |
| | | $t_{calculated}$ | 1.157 |
| | | $t_{table}$ | 3.182 |

**Calibration Curve for Determination of ManLAM Concentration**

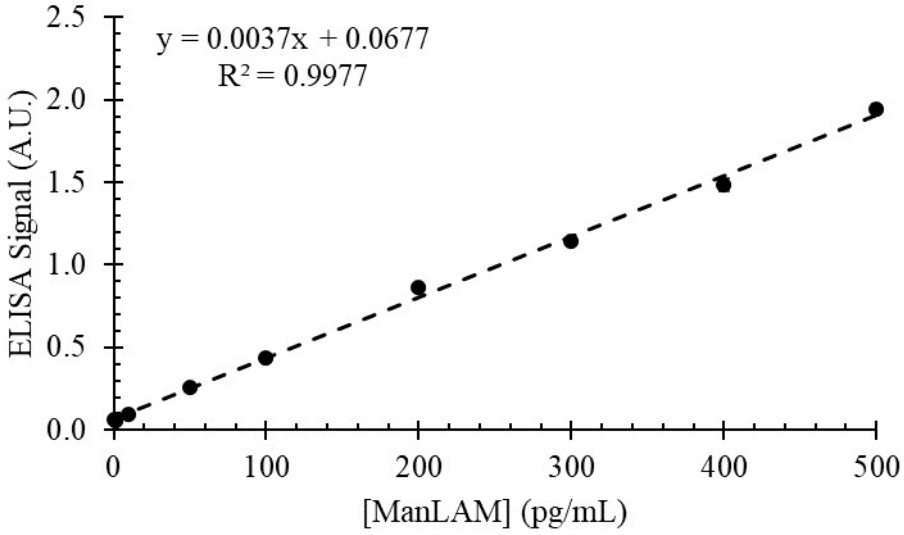


**Figure S-2.** Calibration curve for ManLAM ELISA used to determine the concentration of ManLAM in the blinded samples. The samples for this curve were prepared in MPBS+BSA. Error bars representing the standard deviation of three replicates are on par with the size of the data points. The limit of detection for this calibration curve was 7 pg/mL.

**References**

1 N. A. Owens, C. C. Young, L. B. Laurentius, P. De, D. Chatterjee and M. D. Porter, *Anal. Chim. Acta*, 2019, **1046**, 140–147.

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