

The cyanobacterial lectin, microvirin-N, enhances the specificity and sensitivity of lipoarabinomannan-based TB diagnostics

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Figure S1. Workflow of MVN expression protocol. (1) Transfection of *E. coli* BL21-DE3 competent cells with pET15b vector (ampicillin resistance gene) expressing microvirin lectin with an N-terminal hexa-histidine tag (MVN-His₆); (2) Inoculation of 1 L Terrific Broth culture medium with overnight starter culture of MVN-His₆-expressing *E. coli* BS21-DE3 cells followed by incubation of the bulk culture with ampicillin (100 µg/mL) at 37°C, 22 rpm until OD₆₀₀ ~0.6-0.8 was reached; (3) Cooling of the bulk culture to 18°C, addition of IPTG (1 mM, final concentration), and further overnight incubation (16–18 hrs) of the culture at 18 °C, 22 rpm; (4) Harvesting of the MVN-His₆-expressing bacteria by centrifugation; (5) Resuspension of the bacterial pellet in lysis buffer, and lysis of bacteria by passage through an Emulsiflex-C3 High Pressure Homogenizer (Avestin) maintaining lysis pressure at 15,000 psi (three cycles), followed by clarification of the lysate by centrifugation and passage through 0.22 µm syringe filter; (6) Purification of MVN-His₆ by FPLC using a 5 mL HisTrap HP column and elution of fractions with a linear imidazole gradient (0–500 mM); (7) Fractions containing purified MVN-His₆ were pooled and dialyzed against PBS and either used fresh or stored frozen at -80 °C.

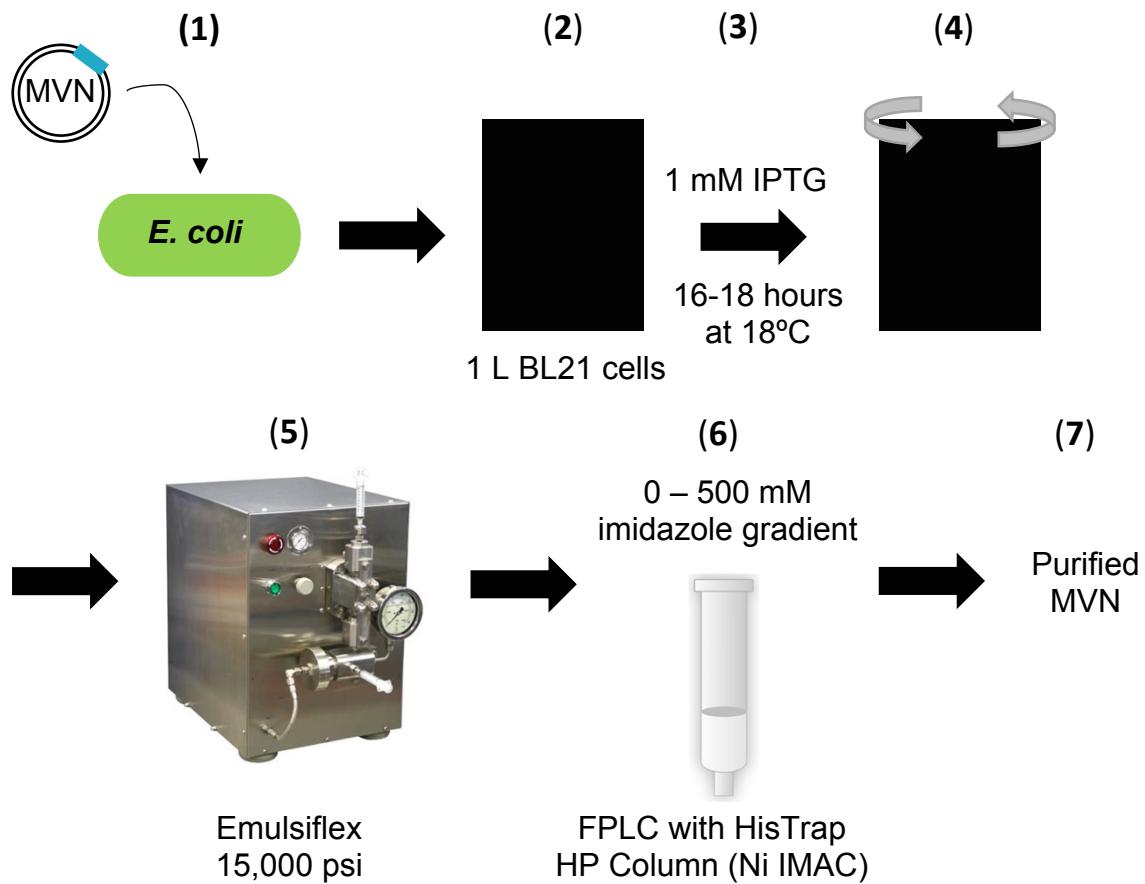


Figure S2. NuPAGE 4-12% gradient gels of the FPLC purification of MVN-His₆ from *E. coli* BL21-DE3 lysate depicting (i) molecular weight marker (gels 1 & 2, lane 1), (ii) lysate of MVN-His₆-expressing *E. coli* BL21-DE3 cells (gel 1, lane 2), (ii) Flow-through from HisTrap HP column (gel 1, lane 3), and (iii) IMAC column fractions (gel 1, lanes 4-15 and continued onto gel 2, lanes 2-15). MVN-His₆ appears as a prominent band at a molecular weight of ~15 kDa in the cell lysate (gel 1, lane 2), but is largely absent in the flow-through (gel 1, lane 3), which indicates the successful binding of MVN-His₆ to the Ni-NTA HisTrap column. Elution of MVN-His₆ under the linear imidazole gradient peaked around the 10th fraction (gel 1, lane 13) and subsequently tapered off as observed by changing intensities of the dark bands at a molecular weight of ~15 kDa. The elution fractions were pooled as follows for further analysis; **Pool 1**: gel 1, lanes 6-9; **Pool 2**: gel 1, lanes 10-13; **Pool 3**: gel 1, lanes 14-15 and gel 2, lanes 2-6; **Pool 4**: gel 2, lanes 7-10. 10 μ L aliquots of each 2 mL fraction was loaded per well.

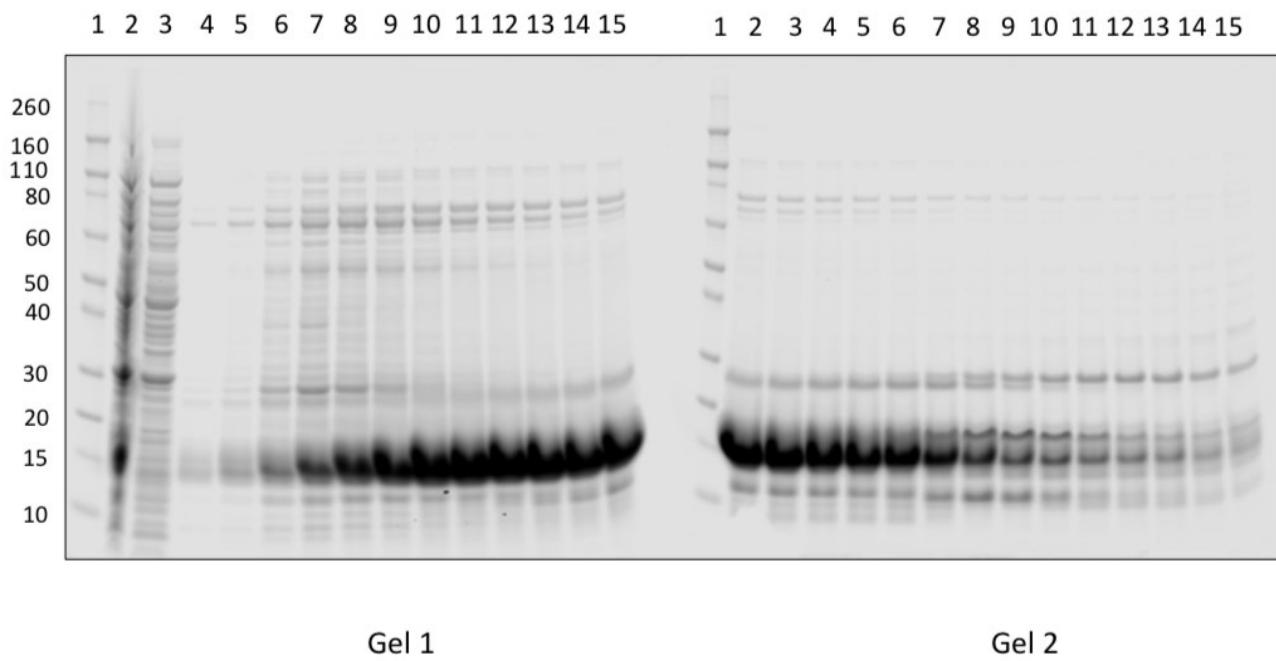


Figure S3. NuPAGE 4-12% gradient gel of pooled FPLC elution fractions. **(A)** Pools 2 and 3 were diluted ten-fold prior to being loaded onto the gel. **(B)** Pools 1 and 4 were loaded undiluted onto the gel. All pools were loaded in three lanes with 2.5, 5, and 10 μ L sample, left to right. BSA standards (100, 250, 500, 750, and 1000 ng) were also loaded onto the gel to enable densitometric quantification of MVN-His₆ in the various elution fractions.

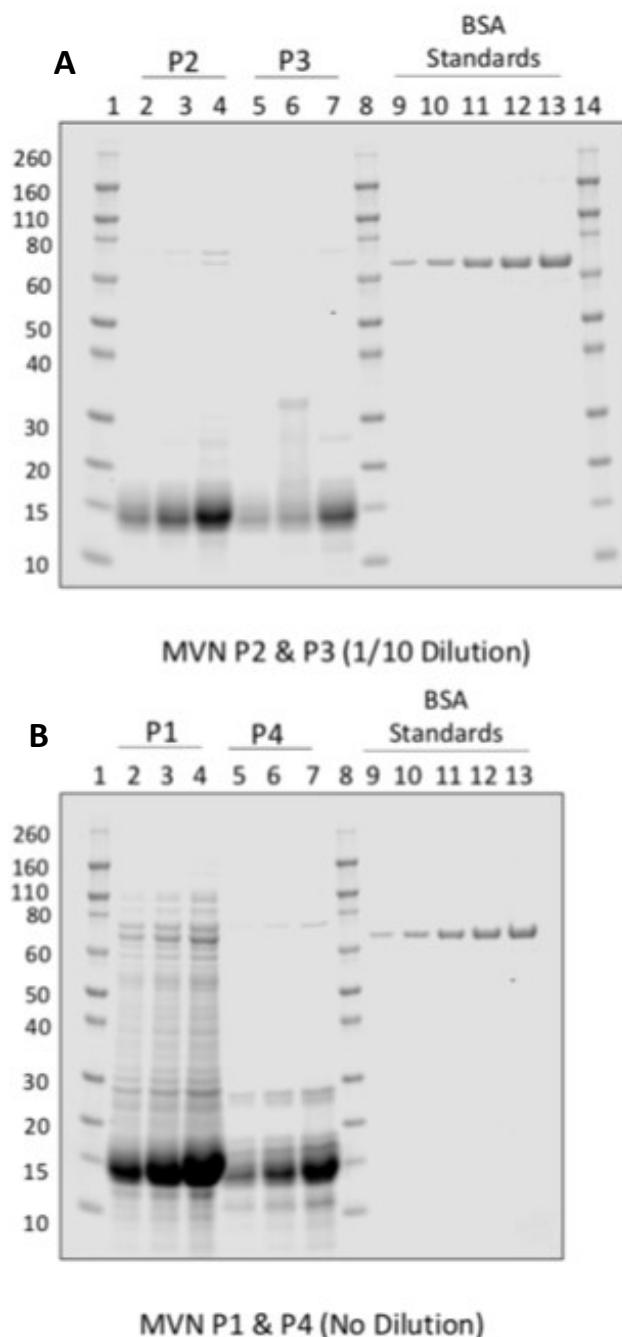


Table S1. Kinetic binding analysis of pooled fractions containing MVN-His₆ to *M.tb* H37Rv ManLAM. The combined fraction Pools 2 and 3 exhibited higher binding affinity toward *M.tb* H37Rv ManLAM and higher purity compared to Pools 1 and 4 (Figure S3). Therefore, fraction Pools 2 and 3 were combined, and used exclusively for further assay development.

Fraction	Binding Affinity (K _D) to ManLAM
1	Not tested
2	1.18 nM
3	61.3 pM
4	0.487 nM

Figure S4. 100 μ L of ManLAM or PILAM in pooled human urine is added to a clear flat-bottom 96-well plate. A 100 μ L aliquot of 2 μ g/mL Ab28 conjugated to HRP in 0.5% BSA PBST is then added to the wells. Lastly, 4 μ L MVN-functionalized magnetic Dynabeads is added to each well and the plate is incubated on a shaker for 30 minutes. The beads are magnetically separated from the supernatant using a 96-well magnetic separation rack and washed three times with 200 μ L PBST. A 100 μ L aliquot of TMB One is then added to each well and the plate is incubated on a shaker for 10 minutes while protected from light. Lastly, 100 μ L 2 M H_2SO_4 is added to each well to stop the reaction and the signal was measured by absorbance at 450 nm.

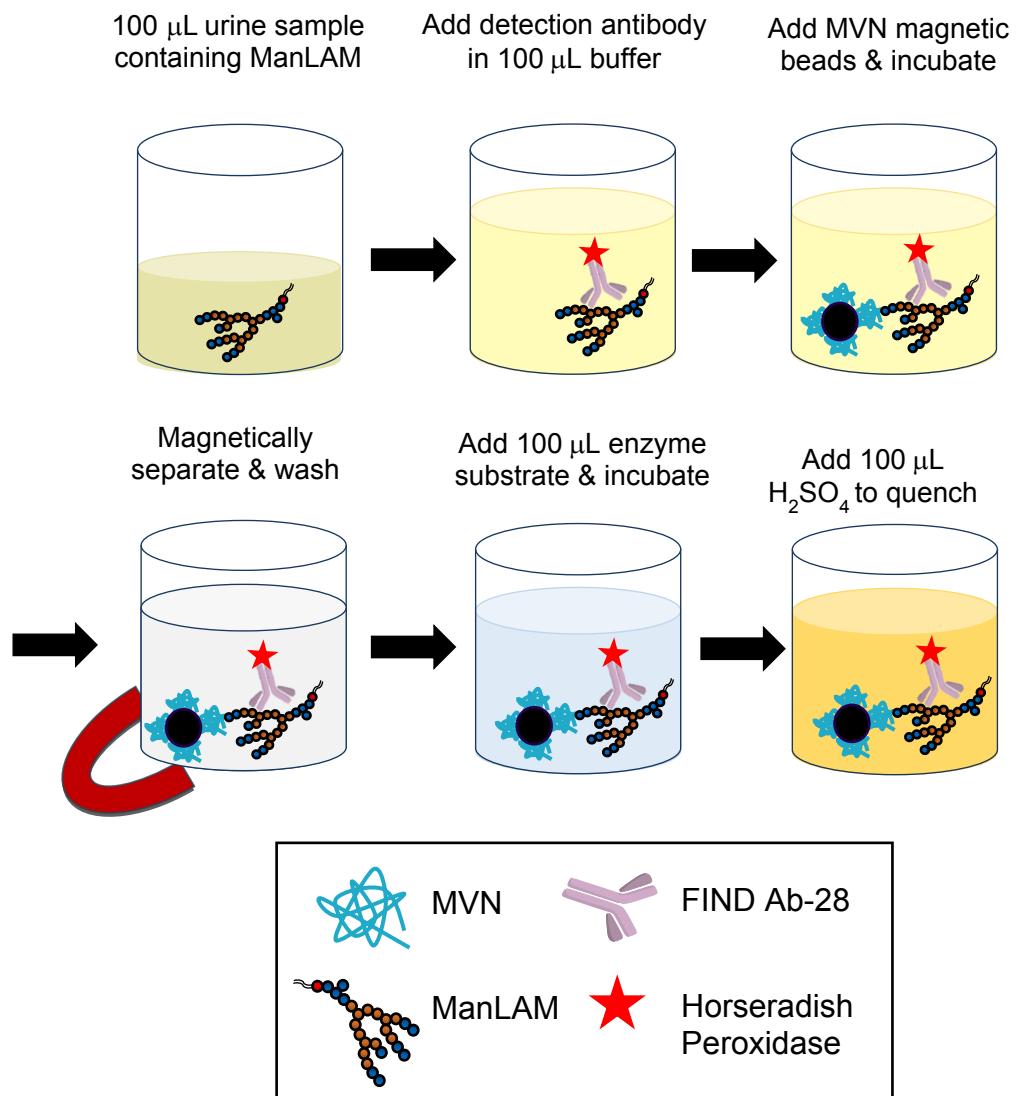


Figure S5. Development of the OB-ELISA. **(A)** Signal-to-noise ratios for sequential addition vs all-in-one addition format for OB-ELISA showing no significant different (p-value = 0.21). **(B)** Optimization of detection antibody concentration. **(C)** Optimization of bead number and type where Bead 1 was functionalized with 60 μ g MVN per 100 μ L beads and Bead 2 was functionalized with 30 μ g MVN per 100 μ L beads.

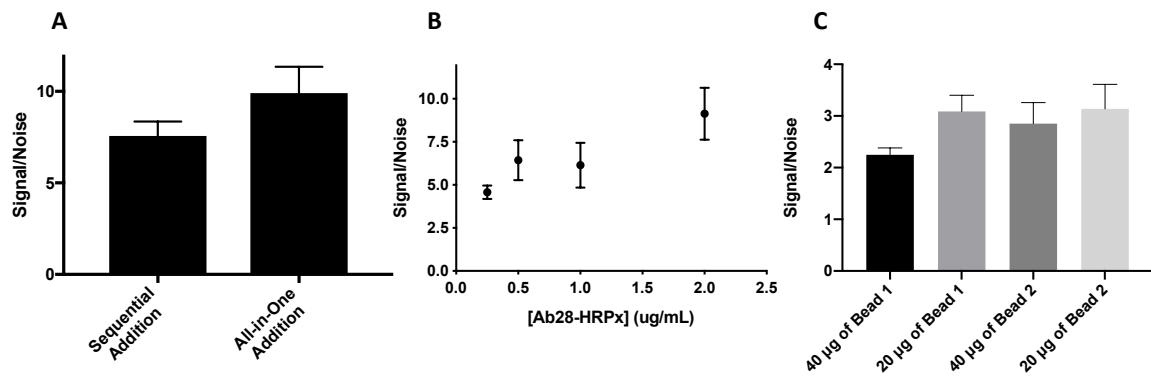


Figure S6. An antibody-based ELISA was developed. **(A)** The concentration of capture antibody Ab170 was optimized and 4 $\mu\text{g}/\text{mL}$ was chosen. **(B)** The percentage of BSA in the blocking buffer was optimized and 1.25% was chosen. **(C)** The concentration of detection antibody Ab28 conjugated to HRPx was optimized and 0.5 $\mu\text{g}/\text{mL}$ was chosen. **(D)** Standard curves of ManLAM and PILAM spiked into urine were used to determine the LODs of 729 pg/mL and 360 pg/mL, respectively.

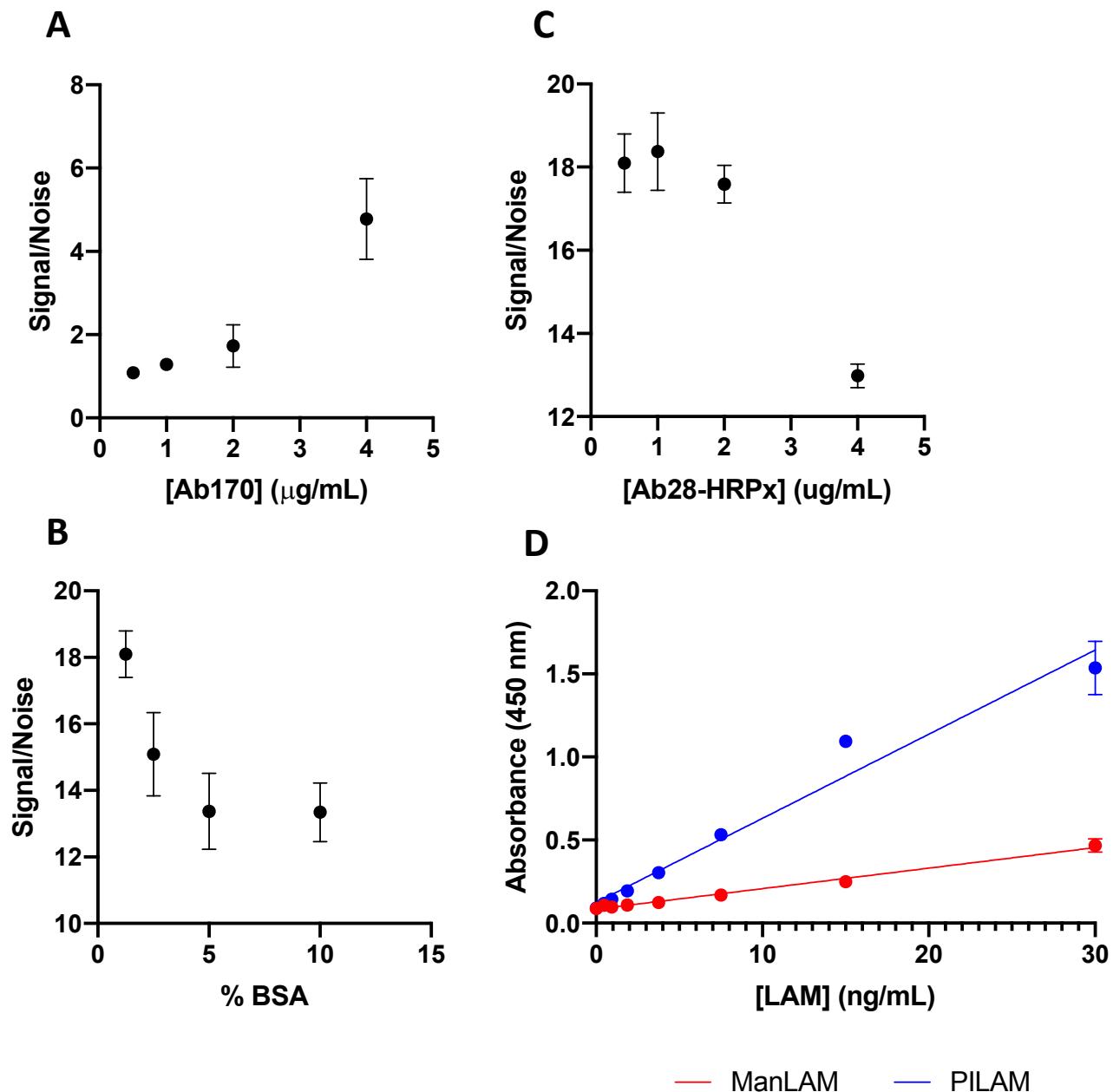


Figure S7. Titration curves of *M. tb* ManLAM or *M. smegmatis* PILAM on the Alere LFA. The visual LODs were found to be 1.25 ng/mL ManLAM and 0.625 ng/mL PILAM.

