Supplementary Information

Fluorescent nanodiamond immunosensors for clinical diagnostics of tuberculosis

Trong-Nghia Le, [a] Mhikee Janella N. Descanzo, [b] Wesley W.-W. Hsiao, [c] Po-Chi Soo, [de] Wen-Ping Peng [b] and Huan-Cheng Chang *[acf]

[a] *Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei 106, Taiwan*

[b] *Department of Physics, National Dong Hwa University, Shoufeng, Hualien 97401, Taiwan*

[c] *Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 106, Taiwan*

[d] *Department of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien 97004, Taiwan*

[e] *Department of Internal Medicine, Buddhist Tzu Chi General Hospital and Tzu Chi University, Hualien 97004, Taiwan*

[f] *Department of Chemistry, National Taiwan Normal University, Taipei 106, Taiwan*

** Email: hchang@gate.sinica.edu.tw*

Table of Contents

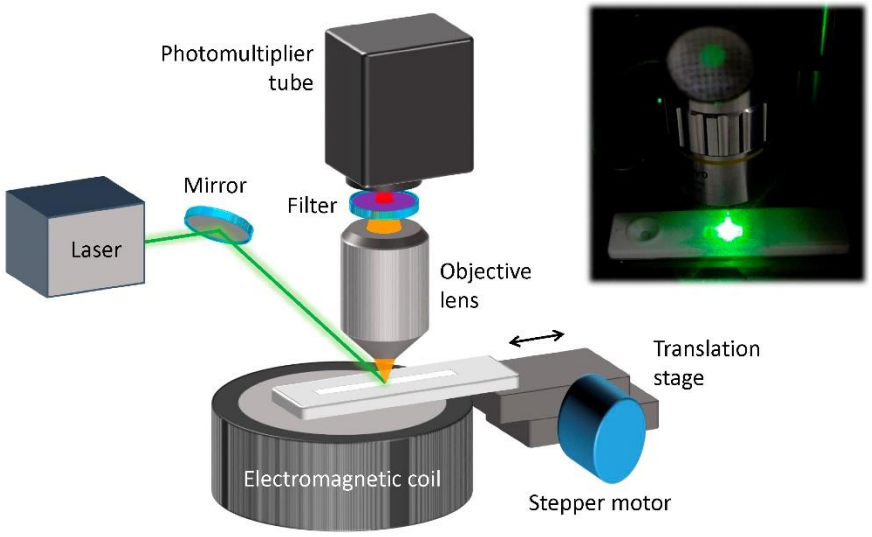

Figure S1. Experimental layout of the SELFIA reader. Inset: Photograph of the excitation region when the instrument is in operation.

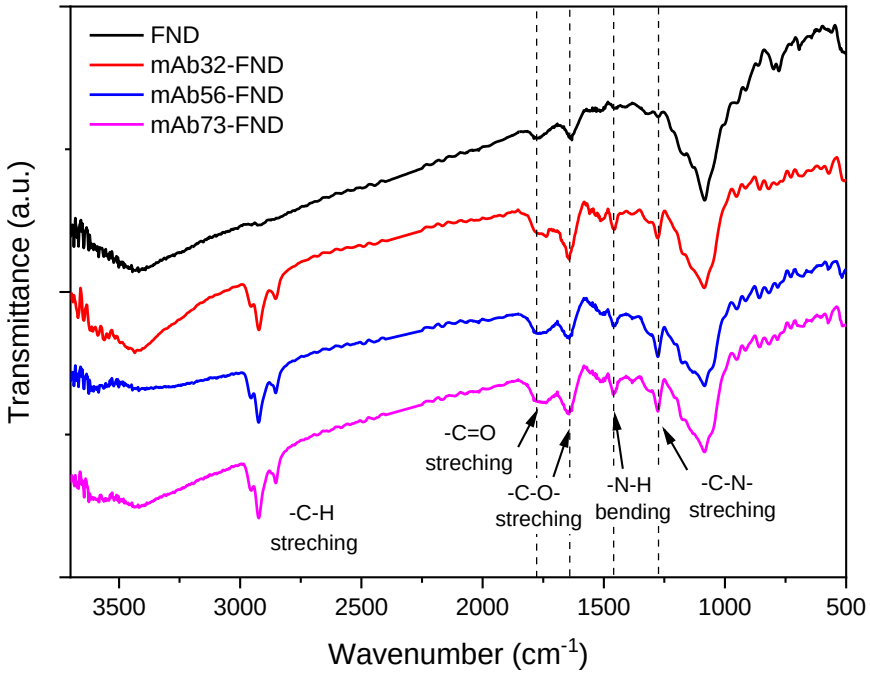

Figure S2. Fourier-transform infrared (FTIR) spectra of FNDs before and after conjugation with antibodies (mAb32, mAb56, and mAb73). The spectra are shifted vertically for clarity.

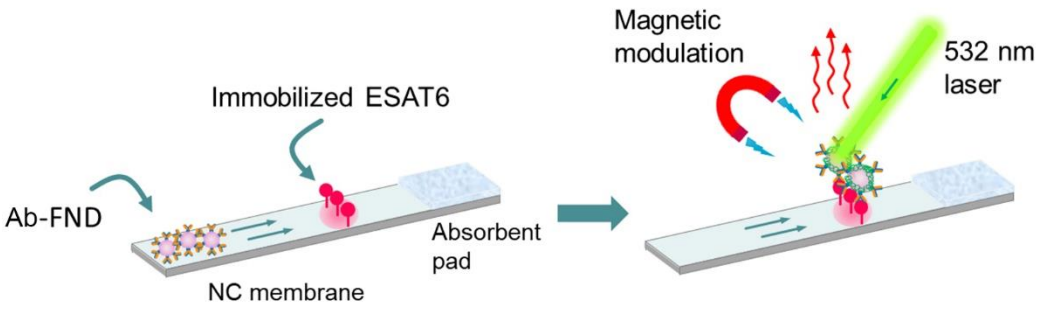

Figure S3. Illustration of direct SELFIA. An excess amount of ESAT6 is first immobilized on a nitrocellulose membrane strip. Next, a known amount of antibody-conjugated FNDs is added to the strip, followed by fluorescent intensity measurement by using the SELFIA platform after running the sample solution for 30 min.

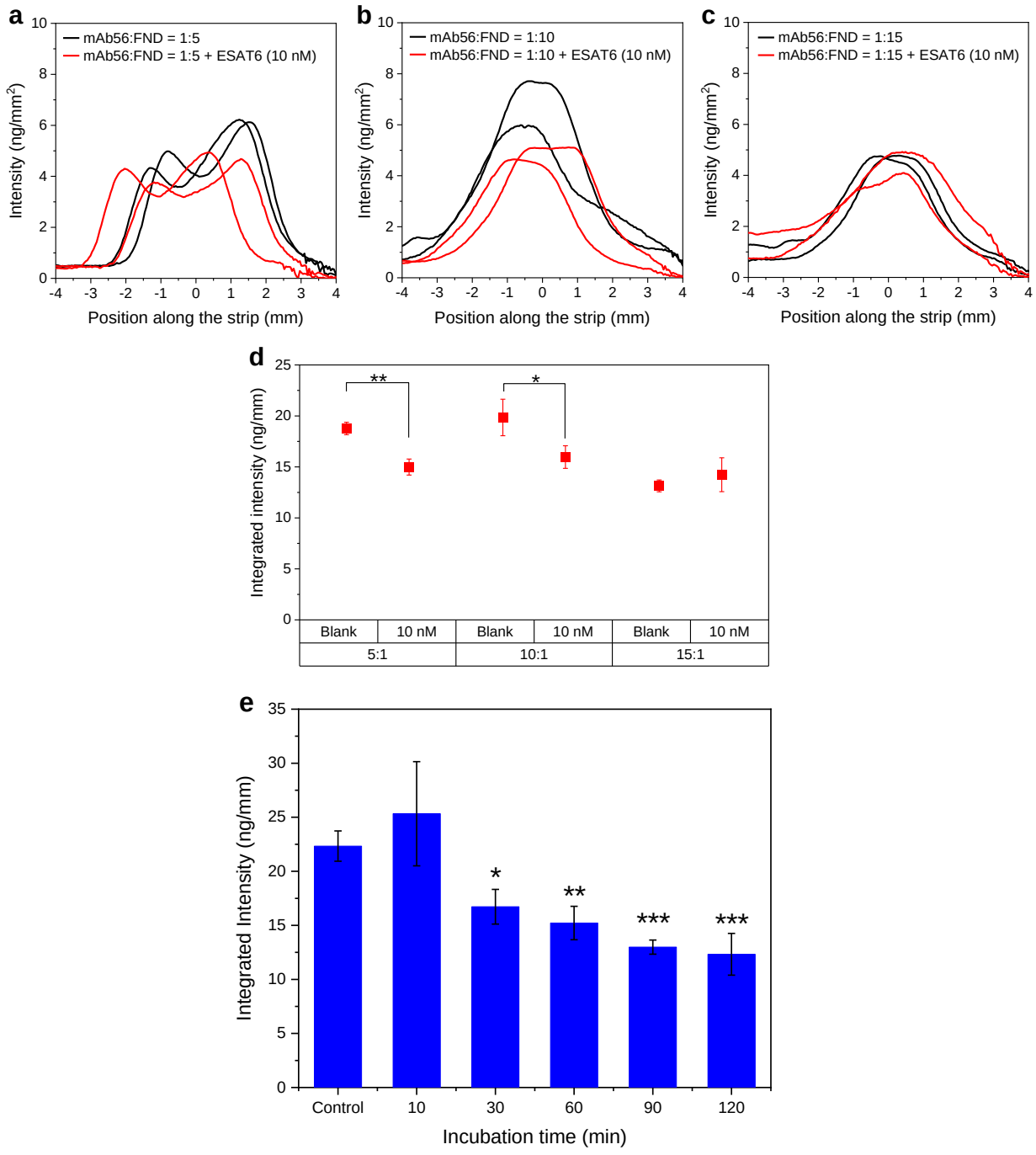

Figure S4. (a-c) Fluorescence intensity line profiles and (d) integrated fluorescence intensities of competitive SELFIA for ESAT6 with mAb56-FNDs prepared at different weight ratios of mAb56:FND = 1:5 (a), 1:10 (b), 1:15 (c). (e) Optimization of the incubation time of mAb56-FND with ESAT6. The control consisted of PBS, and the sample solution was 10 nM ESAT6. Data are presented as mean ± standard deviation, $n = 3$ and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

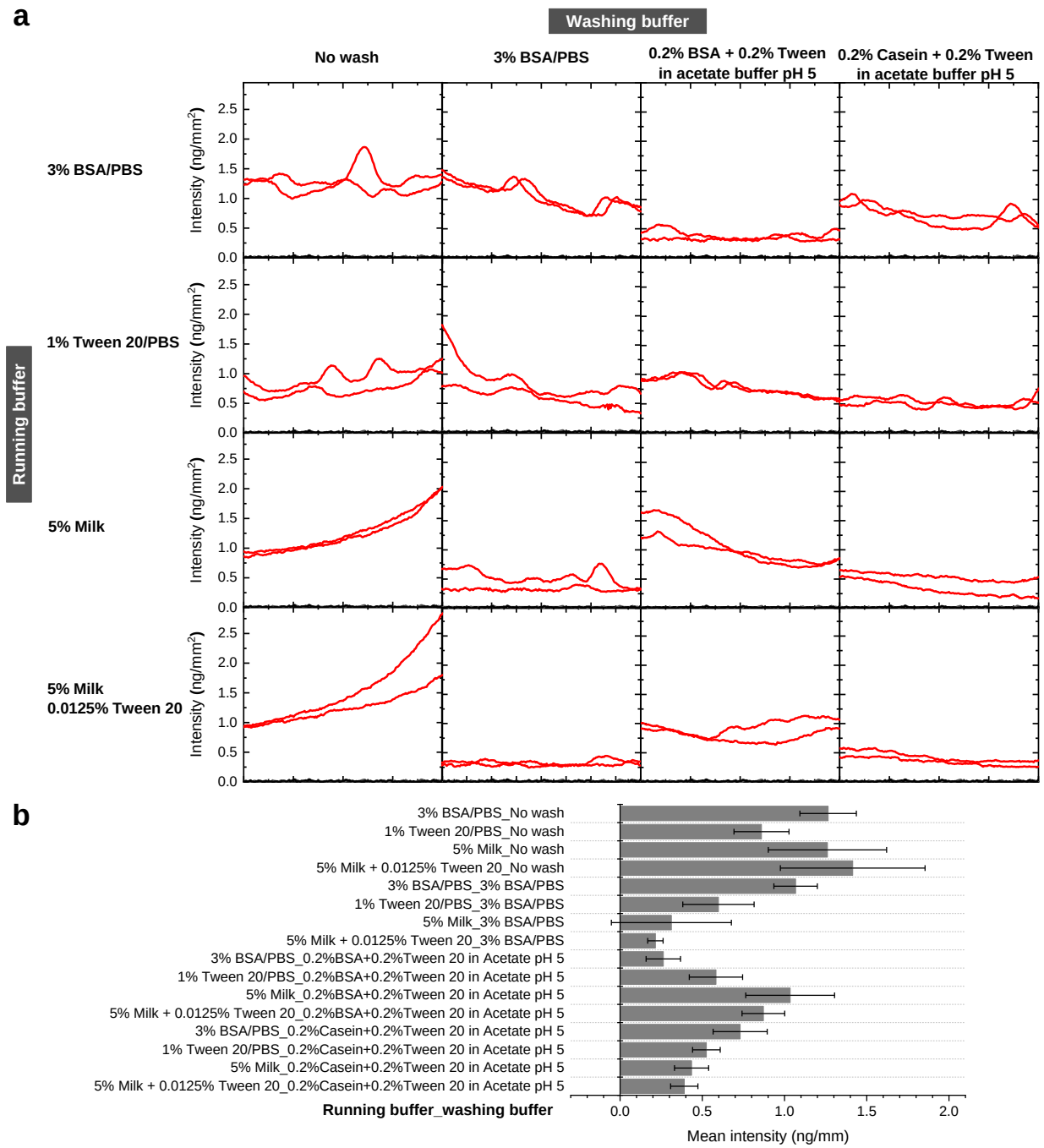

Figure S5. Optimization of the running and washing buffers for competitive SELFIA of ESAT6. (a) Fluorescent intensity profiles and (b) mean intensities of mAb56-FNDs flowing through the nitrocellulose membrane strips using different combinations of running buffer and washing buffer. Data are presented as mean ± standard deviation, n = 2.

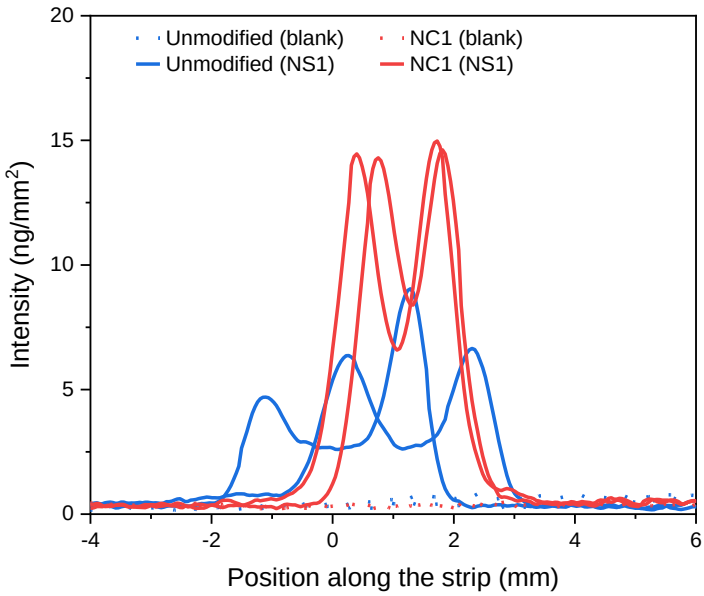

Figure S6. Validation of narrow channel strips using the non-structural protein NS1 of the DV1 Dengue virus at the concentrations of 0 (blank) and 10 µg/mL (NS1).

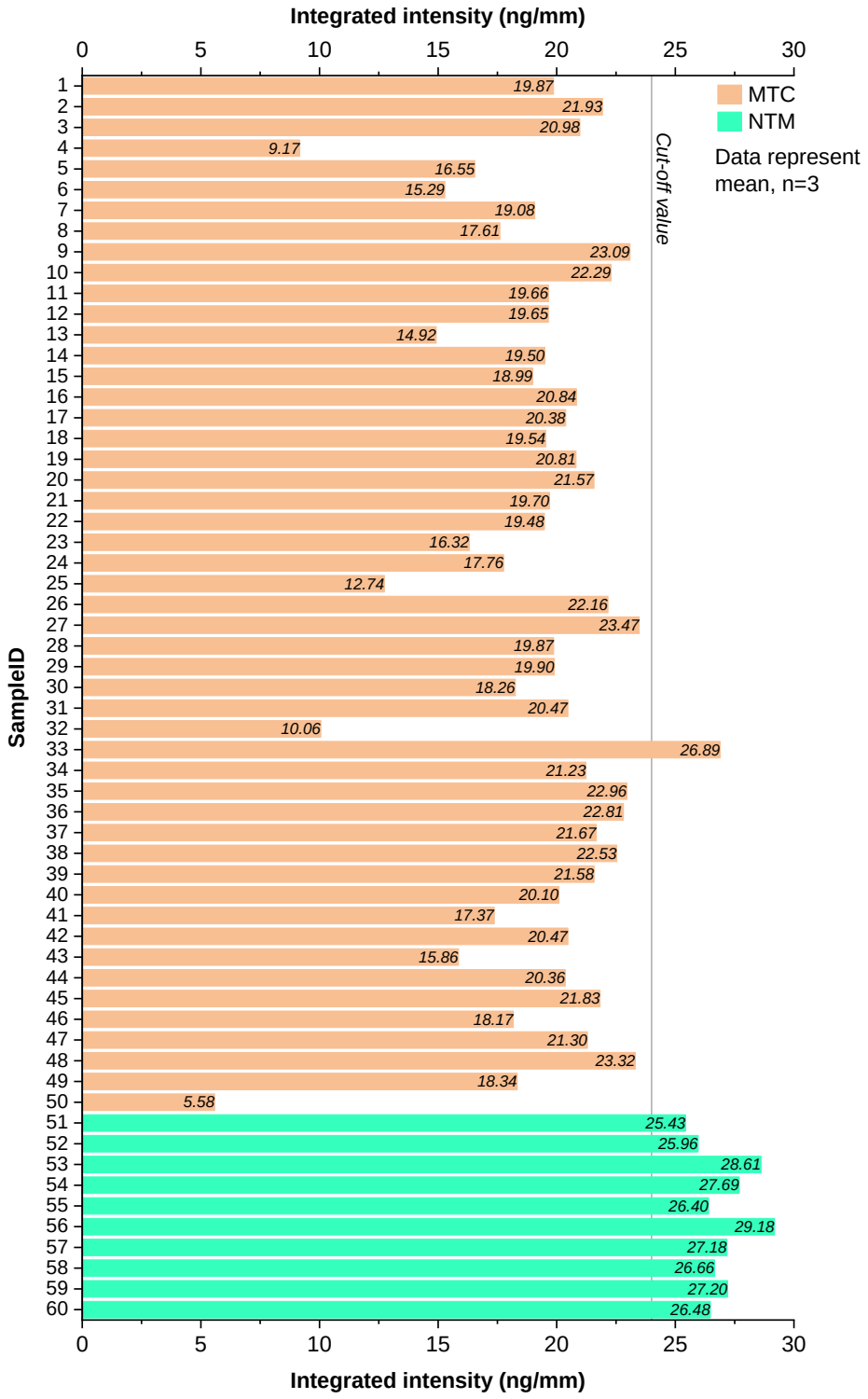

Figure S7. Detection of ESAT6 in culture mediums of 60 clinical samples using competitive SELFIA. With the cut-off value set at 24.0 ng/mm (denoted by the vertical line), the sensitivity and specificity of the assays were 98% and 100%, respectively.

Table S1. Performance comparison between competitive SELFIA and other reported assays for ESAT6 from *Mycobacterium tuberculosis*.

Method	LOD	Sensitivity	Specificity	Assay time	Ref.
Competitive SELFIA	0.02 ng/mL	98% (sputum)	100%	50 min	This work
Aptamer-based qPCR	2.56 nM	50% (serum)	91.7%	4 h	[1]
Immunochromatographic strips	6 ng/mL	100% (sputum) 34.1% (plasma)	91.2%	10 min	[2]
NanoDisk-MS	197 pM	91.6% (blood)	95.8%	2.5 h	[3]
ELSIA	60 pg/mL	95.4% (sputum)	100%	6 h	[4]
Electrochemiluminescence immunoassays	-	65% (urine) 46% (serum)	97%	-	[5]
Electrochemical immunosensors	1.5 ng/mL	100% (sputum)	100%	2 h	[6]
Electrochemical immunosensors	1.5 ng/mL	100% (sputum)	91.7%	2 h	[7]
Electrochemical immunosensors	1 ng/mL	-	-	-	[8]
Electrochemical immunosensors	0.15 ng/mL	-	-	6 h	[9]

References

[1] B. Kil, E. Kim, M. Kang, J. Jeon, Y. Chang, C. L. Chang and C. Ban, *Sens. Actuators. B Chem.*, 2023, **381**, 133427.

[2] X. Wu, Y. Wang, T. Weng, C. Hu, F. X. C. Wang, Z. Wu, D. Yu, H. Lu and H. Yao, *Medicine*, 2017, **96**, e9350.

[3] C. Liu, C. J. Lyon, Y. Bu, Z. Deng, E. Walters, Y. Li, L. Zhang, A. C. Hesseling, E. A. Graviss and Y. Hu, *Clin. Chem.*, 2018, **64**, 791.

[4] T. T. Feng, C. M. Shou, L. Shen, Y. Qian, Z. G. Wu, J. Fan, Y. Z. Zhang, Y. W. Tang, N. P. Wu, H. Z. Lu and H. P. Yao, *Int. J. Tuberc. Lung Dis.*, 2011, **15**, 804.

[5] T. Broger, M. Tsionksy, A. Mathew, T. L. Lowary, A. Pinter, T. Plisova, D. Bartlett, S. Barbero, C. M. Denkinger, E. Moreau, K. Katsuragi, M. Kawasaki, P. Nahid and G.B. Sigal, *PLoS One*, 2019, **14**, e0215443.

[6] U. Z. M. Azmi, N. A. Yusof, J. Abdullah, F. Mohammad, S. A. A. Ahmad, S. Suraiya, N. H. A. Raston, F. N. M. Faudzi, S. K. Khiste and H. A. Al-Lohedan, *Nanomaterials* 2021, **11**, 2446.

[7] U. Z. Mohd Azmi, N. A. Yusof, J. Abdullah, S. A. Alang Ahmad, F. N. Mohd Faudzi, N. H. Ahmad Raston, S. Suraiya, P. S. Ong, D. Krishnan and N. K. Sahar, *Microchim. Acta*, 2021, **188**, 20.

[8] R. A. Omar, N. Verma and P. K. Arora, *Front. Immunol.*, 2021, **12**, 653853.

[9] N. Mohd Bakhori, N. A. Yusof, J. Abdullah, H. Wasoh, S. K. Ab Rahman and S. F. Abd Rahman, *Materials*, 2020, **13**, 149.