# Supplemental Material

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**Section S1.** Methods of rapid plasma regain testing

The positive specimens for *Treponema pallidum* antibodies, identified using the Mindray CL-900i Chemiluminescence Immunoassay Analyzer,[1 2] were subjected to rapid plasma reagin (RPR) testing at the Laboratory Section of the Medical Commission Department of the Ministry of Public Health. This laboratory conducts about 150-200 RPR tests daily due to different screening requirements. The methods used for RPR testing at this laboratory are described below.

The Rapid Plasma Reagin card test (Fortress Diagnostics Ltd, Antrim Technology Park, Antrim, UK)[3] is used for RPR syphilis serological detection. This test is a qualitative and semi-quantitative non-treponemal flocculation assay designed to detect reagin antibodies in serum specimens. The principle of this assay is based on a modified VDRL antigen containing carbon particles to enhance result visualization. When reagin antibodies in the specimen bind with the cholesterol/cardiolipin/lecithin complex in the reagent, the reaction can be observed macroscopically as black clumps. The absence of visual flocculation indicates a negative result.

The testing using this assay is performed according to the manufacturer's protocol. Briefly, 50 μL of the patient's specimen and 50 μL each of the positive and negative controls are placed into separate circles on the RPR test card. Then, 16 μL of the RPR carbon antigen (provided by the manufacturer) is added to each test circle and mixed with a disposable pipette/stirrer, spreading the mixture over the entire circle. A new stirrer is used for each specimen. The card is then rotated at 100 rpm for 8 minutes on an automatic rotator. The formation of black clumps under light indicates a positive result.

The positive specimens are then subjected to semi-quantitative analysis. Briefly, 50 μL of isotonic saline is added to circles 2, 3, 4, and 5. Then, 50 μL of the patient's specimen is added to circles 1 and 2. The specimen and saline are mixed in circle 2. Serial dilutions are prepared by

transferring 50 μL from circle 2 to the saline in circle 3, continuing in the same manner through to the last circle, discarding 50 μL at the end. Using a stirrer, the diluted specimens are spread over the entire circle, starting from circle 5 and working backwards to the specimen in circle 1.

Next, 16 μL of the RPR carbon antigen is added to each diluted test circle, mixed with a disposable pipette/stirrer, and spread over the entire circle. A new stirrer is used for each specimen. The card is then rotated at 100 rpm for 8 minutes on an automatic rotator. The result is interpreted qualitatively as reactive if medium or large clumps are observed and non-reactive if no clumping is visible. The result is also interpreted semi-quantitatively as a titer, representing the last dilution that produces a reactive result.[3]

The following quality control and quality assurance measures are implemented by the lab:

**Standard operating procedures**

All laboratory personnel are trained and adhere to standardized procedures for specimen collection, handling, storage, testing, and interpretation.

**Internal quality control**

On a daily basis, the laboratory tests positive and negative control materials provided by the assay manufacturer before testing patient specimens. This practice ensures that the controls yield the expected results.

**Lot-to-lot verification**

When a new lot of reagent is received, its performance is verified through parallel testing with the current lot using validation specimens with known positive results (10 specimens) that cover a range of reactivity titer levels. The same operator performs the parallel testing to ensure consistency. According to laboratory protocol, the accepted criterion is that the positive titer

results should not differ by more than one dilution step (a twofold difference). Additionally, parallel testing is conducted with 10 validation specimens with known negative results to ensure that both lots yield negative results.

**Reproducibility of positive results**

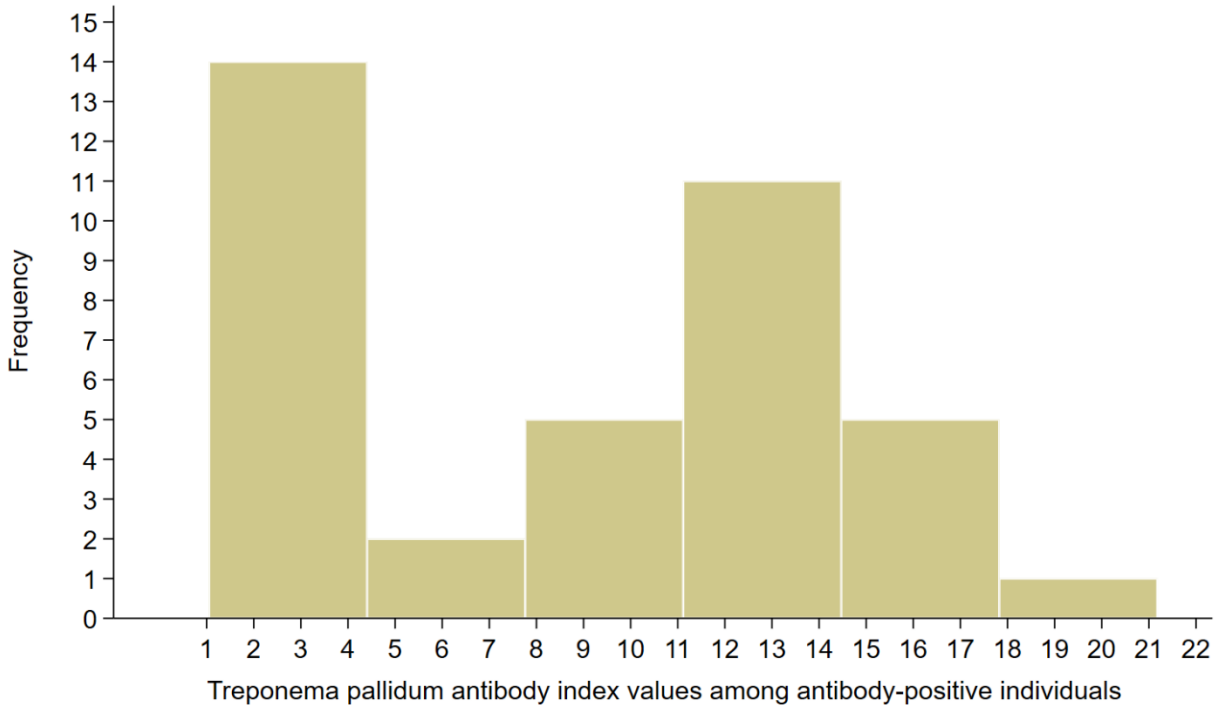
According to monitoring and control protocols, all reactive specimens in RUN 1 are re-checked by another technician for titer in RUN 2 without knowing each other's titer results. However, this protocol was discontinued after a one-year trial since the reproducibility was 100%.[4]

**Table S1.** STROBE checklist for cross-sectional studies.

| | Item No | Recommendation | Main text |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | Title & Abstract |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | Abstract |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | Introduction |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | Introduction |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | Methods ('Study design and sampling') |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | Methods ('Study design and sampling' & 'Sample collection and handling') |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants | Methods ('Study design and sampling') |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | Methods ('Laboratory methods' & 'Statistical analysis') & Table 1 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | Methods ('Sample collection and handling', 'Laboratory methods' & 'Statistical analysis') & Table 1 |
| Bias | 9 | Describe any efforts to address potential sources of bias | Methods ('Study design and sampling' & 'Statistical analysis') |
| Study size | 10 | Explain how the study size was arrived at | Methods ('Study design and sampling') |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | Methods ('Laboratory methods' & 'Statistical analysis') & Table 1 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | Methods ('Statistical analysis') |
| | | (*b*) Describe any methods used to examine subgroups and interactions | Methods ('Statistical analysis') |
| | | (*c*) Explain how missing data were addressed | Footnote of Table 1 |
| | | (*d*) If applicable, describe analytical methods taking account of sampling strategy | Methods ('Statistical analysis') |
| | | (*e*) Describe any sensitivity analyses | Not applicable |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | Results ('Study population', paragraph 1) |
| | | (b) Give reasons for non-participation at each stage | Results ('Study population', paragraph 1) |
| | | (c) Consider use of a flow diagram | Not applicable |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | Results ('Study population', paragraph 2), Table 1 & Figure 1 |
| | | (b) Indicate number of participants with missing data for each variable of interest | Results ('Lifetime syphilis infection' & 'Recent syphilis infection'), footnote of Table 1 & Table 3 |
| Outcome data | 15* | Report numbers of outcome events or summary measures | Results ('Lifetime syphilis infection' & 'Recent syphilis infection'), Figure 1, & Figure S1 in Supplemental Material |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | Results ('Lifetime syphilis infection' & 'Recent syphilis infection') & Table 2 |
| | | (*b*) Report category boundaries when continuous variables were categorized | Tables 1 & 2 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | Not applicable |

| | | | |
|---|---|---|---|
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | Not applicable |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | Discussion, paragraphs 1-4 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | Discussion, paragraphs 5-10 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | Discussion, paragraph 11 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | Discussion, paragraphs 8-9 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | Funding |

**Figure S1.** Distribution of *Treponema pallidum* antibody index values among antibody-positive individuals as measured using Mindray CL-900i Chemiluminescence Immunoassay Analyzer.



**References**


1. Mindray. Mindray CL-900i Chemiluminescence Immunoassay Analyzer. *Cat No Anti-TP112, Shenzen, China* 2015
2. Xia CS, Yue ZH, Wang H. Evaluation of three automated Treponema pallidum antibody assays for syphilis screening. *J Infect Chemother* 2018;24(11):887-91. doi: 10.1016/j.jiac.2018.07.017 [published Online First: 2018/09/11]
3. Fortress Diagnostics. The Fortress Diagnostics Rapid Plasma Reagin. Available at: https://www.fortressdiagnostics.com/products/syphilis-tests/rpr. Accessed on July 19, 2024. 2024
4. Nasrallah GK, Al-Buainain R, Younes N, et al. Screening and diagnostic testing protocols for HIV and Syphilis infections in health care setting in Qatar: Evaluation and recommendations. *PLoS One* 2023;18(2):e0278079. doi: 10.1371/journal.pone.0278079 [published Online First: 2023/02/08]