Supplementary Material

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Table S1. STROBE checklist for the syphilis prevalence cross-sectional study of 25,630 adults aged ≥15 years tested at Biolab Diagnostic Laboratories in Jordan from January 21, 2010, to July 27, 2025.

	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	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 designs and analyses')
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods ('Study population and data sources' & 'Study designs and analyses')
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	Methods ('Study population and data sources' & 'Study designs and analyses')
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods ('Study designs and analyses', 'Laboratory methods', & 'Statistical analysis')
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 ('Study population and data sources', 'Study designs and analyses', 'Laboratory methods', & 'Statistical analysis')
Bias	9	Describe any efforts to address potential sources of bias	Methods ('Statistical analysis')
Study size	10	Explain how the study size was arrived at	Methods ('Study designs and analyses') & Fig. 1
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'), Tables 1-2
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 ('Study designs and analyses' & 'Statistical analysis')
		(*c*) Explain how missing data were addressed	Not applicable (see 'Study designs and analyses')
		(*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', 'Syphilis prevalence', & 'Reactivity patterns for RPR and TPHA') & Fig. 1
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Results ('Study population', 'Syphilis prevalence', & 'Reactivity patterns for RPR and TPHA'), & Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable (see 'Study designs and analyses')
Outcome data	15*	Report numbers of outcome events or summary measures	Results ('Study population', 'Syphilis prevalence', & 'Reactivity patterns for RPR and TPHA'), Fig. 2, & Figs. S1-S2 in Supplementary Material
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95%	Results ('Associations with ever testing RPR- or TPHA-reactive'), & Table 2

		confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*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	Results ('Syphilis prevalence') & Fig. 2 & Figs. S1-S2 in Supplementary Material
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 5-14
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 16
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion, paragraphs 5-8
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

Table S2. STROBE checklist for the retrospective cohort study of syphilis incidence among 1,350 adults aged ≥15 years tested at Biolab Diagnostic Laboratories in Jordan from January 21, 2010, to July 27, 2025.

	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	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 designs and analyses')
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods ('Study population and data sources' & 'Study designs and analyses')
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	Methods ('Study population and data sources' & 'Study designs and analyses')
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods ('Study designs and analyses', 'Laboratory methods', & 'Statistical analysis')
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 ('Study population and data sources', 'Study designs and analyses', 'Laboratory methods', & 'Statistical analysis')
Bias	9	Describe any efforts to address potential sources of bias	Methods ('Statistical analysis')
Study size	10	Explain how the study size was arrived at	Methods ('Study designs and analyses') & Fig. 1
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 S4 in Supplementary Material
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 ('Study designs and analyses' & 'Statistical analysis')
		(*c*) Explain how missing data were addressed	Not applicable (see 'Study designs and analyses')
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*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 (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	Results ('Syphilis incidence') & Fig. 3A
Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Results ('Syphilis incidence', paragraph 1) & Table S4 in Supplementary Material
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable (see 'Study designs and analyses')
		(c) Summarise follow-up time (eg, average and total amount)	Results ('Syphilis incidence') & Fig. 3A
Outcome data	15	Report numbers of outcome events or summary measures over time	Results ('Syphilis incidence'), Fig. 3A, & Table S5 in Supplementary Material
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence	Results ('Syphilis incidence')

		interval). Make clear which confounders were adjusted for and why they were included	
		(b) Report category boundaries when continuous variables were categorized	Table S4 in Supplementary Material
		(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	Results ('Syphilis incidence', paragraph 4)
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 5-14
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 16
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion, paragraphs 5-8
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

Table S3. STROBE checklist for the retrospective cohort study of syphilis non-treponemal seroreversion among 10 adults aged ≥15 years tested at Biolab Diagnostic Laboratories in Jordan from January 21, 2010, to July 27, 2025.

	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	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 designs and analyses')
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods ('Study population and data sources' & 'Study designs and analyses')
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	Methods ('Study population and data sources' & 'Study designs and analyses')
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods ('Study designs and analyses', 'Laboratory methods', & 'Statistical analysis')
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 ('Study population and data sources', 'Study designs and analyses', 'Laboratory methods', & 'Statistical analysis')
Bias	9	Describe any efforts to address potential sources of bias	Methods ('Statistical analysis')
Study size	10	Explain how the study size was arrived at	Methods ('Study designs and analyses') & Fig. 1
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 S6 in Supplementary Material
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 ('Study designs and analyses' & 'Statistical analysis')
		(*c*) Explain how missing data were addressed	Not applicable (see 'Study designs and analyses')
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	Methods ('Statistical analysis')
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 (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	Results ('Exploratory analysis of syphilis seroreversion') & Fig. 1
Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Results ('Exploratory analysis of syphilis seroreversion') & Table S6 in Supplementary Material
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable (see 'Study designs and analyses')
		(c) Summarise follow-up time (eg, average and total amount)	Results ('Exploratory analysis of syphilis seroreversion') & Fig. 3B
Outcome data	15	Report numbers of outcome events or summary measures over time	Results ('Exploratory analysis of syphilis seroreversion'), Fig. 3B, & Table S7 in Supplementary 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 ('Exploratory analysis of syphilis seroreversion')
		(b) Report category boundaries when continuous variables were categorized	Table S6 in Supplementary Material
		(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	Results ('Exploratory analysis of syphilis seroreversion')
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 5-14
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 16
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion, paragraphs 5-8
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

Fig. S1. Syphilis prevalence, defined as ever having an RPR- or TPHA-reactive result among 13,473 females and 12,157 males aged ≥15 years tested at Biolab Diagnostic Laboratories in Jordan from January 21, 2010, to July 27, 2025, by A) 2-year time intervals and B) 10-year age groups.

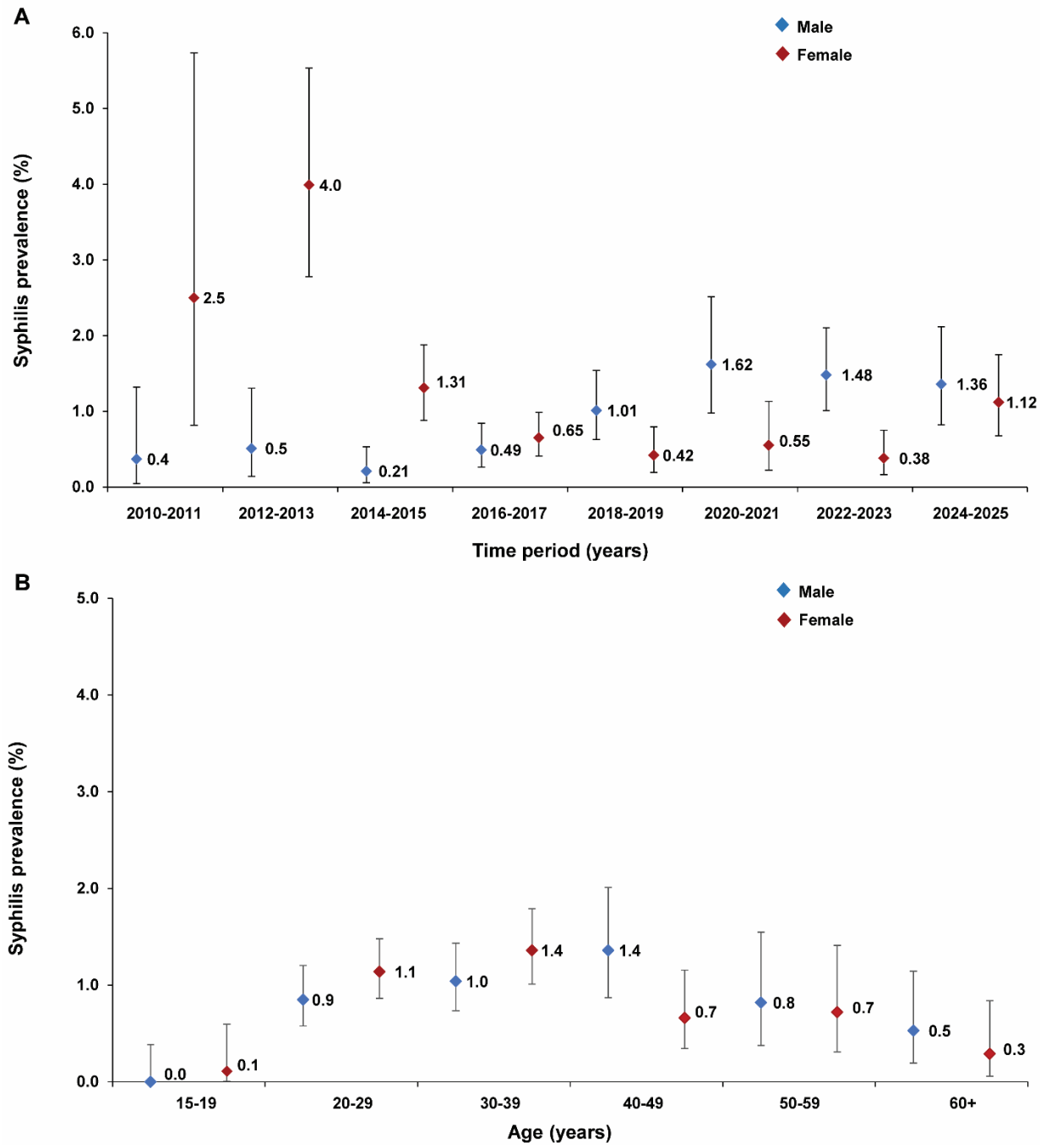
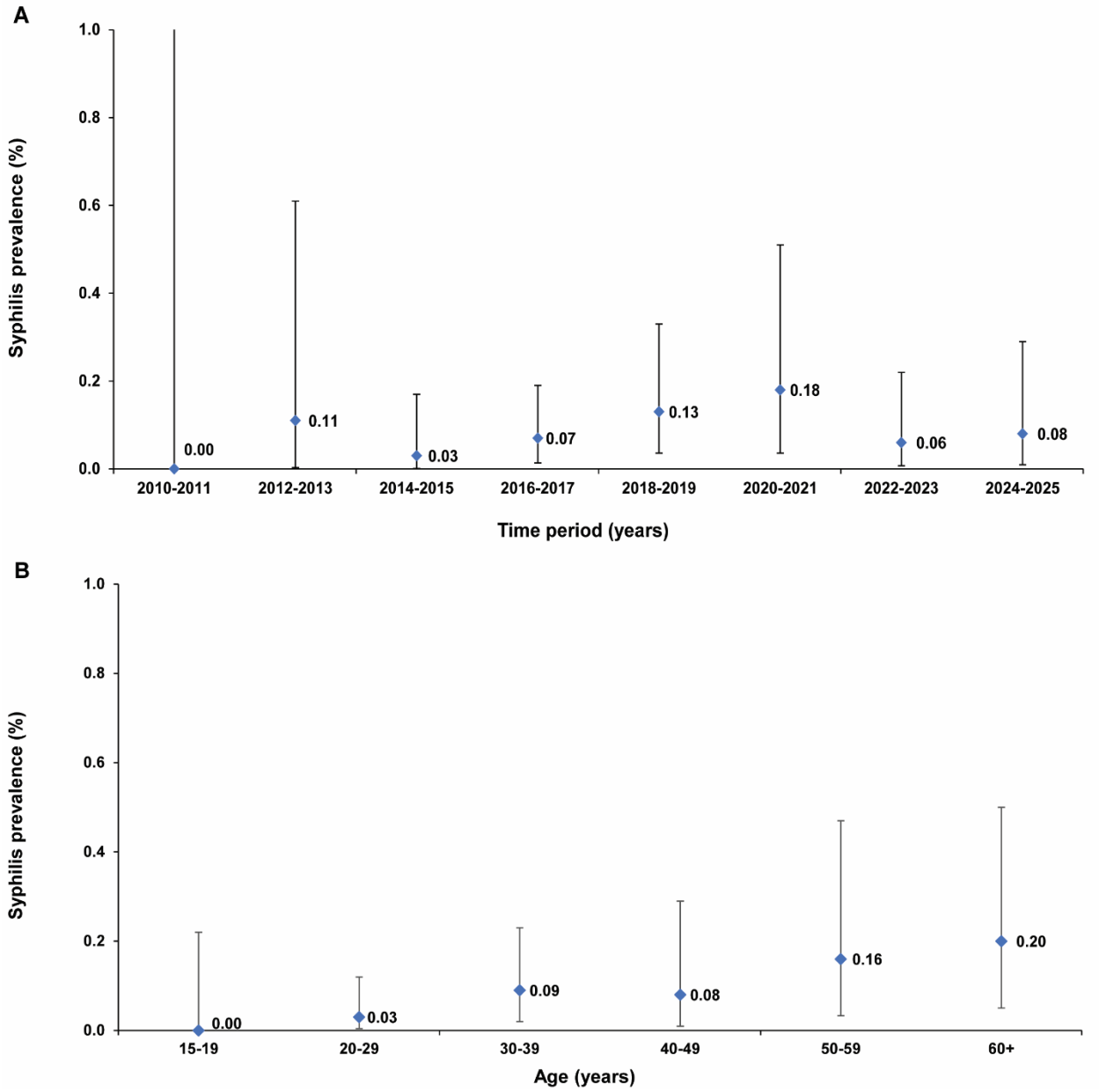


Fig. S2. Syphilis prevalence, defined as ever having an RPR- or TPHA-reactive result in the subgroup of 18,577 adults aged ≥15 years who had at least one visa-related medical screening test at Biolab Diagnostic Laboratories in Jordan from January 21, 2010, to July 27, 2025, by A) 2-year time intervals and B) 10-year age groups.



Abbreviations: CI, confidence interval; RPR, rapid plasma reagin; TPHA, *Treponema pallidum pallidum* hemagglutination assay.
*The upper bound for the 95% CI was truncated because the CI was too wide.

Table S4. Characteristics of 1,350 adults aged ≥15 years included in the retrospective cohort study of syphilis incidence and tested at Biolab Diagnostic Laboratories in Jordan from January 21, 2010, to July 27, 2025.

Characteristics	RPR	TPHA	Total
	N (%)*	N (%)*	N (%)*
Number of individuals	1,340 (100)	10 (100)	1,350 (100)
Median age (interquartile range)—years	33.0 (26.0-46.0)	38.5 (25.0-45.0)	33.0 (26.0-46.0)
Age (years)			
15-19	96 (7.2)	0 (0.0)	96 (7.1)
20-29	417 (31.1)	3 (30.0)	420 (31.1)
30-39	364 (27.2)	3 (30.0)	367 (27.2)
40-49	188 (14.0)	3 (30.0)	191 (14.1)
50-59	149 (11.1)	1 (10.0)	150 (11.1)
60-69	76 (5.7)	0 (0.0)	76 (5.6)
70+	50 (3.7)	0 (0.0)	50 (3.7)
Sex			
Female	613 (45.7)	2 (20.0)	615 (45.6)
Male	727 (54.3)	8 (80.0)	735 (54.4)
Governorate			
Amman	1,331 (99.3)	9 (90.0)	1,340 (99.3)
Aqaba	0 (0.0)	1 (10.0)	1 (0.1)
Balqaa	2 (0.1)	0 (0.0)	2 (0.1)
Irbid	7 (0.5)	0 (0.0)	7 (0.5)
Year of first non-reactive test			
2010	15 (1.1)	1 (10.0)	16 (1.2)
2011	13 (1.0)	1 (10.0)	14 (1.0)
2012	53 (4.0)	0 (0.0)	53 (3.9)
2013	58 (4.3)	0 (0.0)	58 (4.3)
2014	94 (7.0)	1 (10.0)	95 (7.0)
2015	77 (5.7)	4 (40.0)	81 (6.0)
2016	73 (5.4)	0 (0.0)	73 (5.4)
2017	89 (6.6)	0 (0.0)	89 (6.6)
2018	327 (24.4)	0 (0.0)	327 (24.2)
2019	169 (12.6)	2 (20.0)	171 (12.7)
2020	110 (8.2)	0 (0.0)	110 (8.1)
2021	51 (3.8)	0 (0.0)	51 (3.8)
2022	80 (6.0)	0 (0.0)	80 (5.9)
2023	95 (7.1)	0 (0.0)	95 (7.0)
2024	36 (2.7)	1 (10.0)	37 (2.7)
Insurance			
No	1,335 (99.6)	9 (90.0)	1,344 (99.6)
Yes	5 (0.4)	1 (10.0)	6 (0.4)
Encounter type			
Outpatient/walk-in	249 (18.6)	6 (60.0)	255 (18.9)
Doctor referral	61 (4.6)	4 (40.0)	65 (4.8)
Visa-related medical screening	1,029 (76.8)	0 (0.0)	1,029 (76.2)
Laboratory referral	1 (0.1)	0 (0.0)	1 (0.1)
Proportion with			
2 tests	1,222 (91.2)	8 (80.0)	1,230 (91.1)
3 tests	91 (6.8)	2 (20.0)	93 (6.9)
4 tests	17 (1.3)	0 (0.0)	17 (1.3)
5+ tests	10 (0.7)	0 (0.0)	10 (0.7)

Abbreviations: RPR, rapid plasma reagin; TPHA, *Treponema pallidum pallidum* hemagglutination assay.
*Percentages may not always sum precisely to 100% for a given variable because of rounding.

Table S5. Individuals who tested RPR- or TPHA-reactive after a baseline non-reactive result in the retrospective cohort study of syphilis incidence.

Patient	First (non-reactive) test at baseline		Reactive test associated with incidence of infection		Differences between tests (days)
	Test date*	Test result	Test date*	Test result	
1	Mar 2011	TPHA non-reactive	May 2011	TPHA reactive	74
2	Aug 2019	TPHA non-reactive	Jun 2021	TPHA reactive	655

Abbreviations: RPR, rapid plasma reagin; TPHA, *Treponema pallidum pallidum* hemagglutination assay.
*Specific dates were not reported to preserve patient confidentiality.

Table S6. Characteristics of 10 adults aged ≥15 years included in the retrospective cohort study of syphilis non-treponemal seroreversion and tested at Biolab Diagnostic Laboratories in Jordan from January 21, 2010, to July 27, 2025.

Characteristics	RPR
	N (%)*
Number of individuals	10 (100)
Median age (interquartile range)—years	35.5 (24.0-46.0)
Age (years)	
20-29	3 (30.0)
30-39	3 (30.0)
40-49	2 (20.0)
70+	2 (20.0)
Sex	
Female	4 (40.0)
Male	6 (60.0)
Governorate	
Amman	8 (80.0)
Balqaa	1 (10.0)
Zarqa	1 (10.0)
Year of first reactive test	
2014	1 (10.0)
2015	0 (0.0)
2016	0 (0.0)
2018	1 (10.0)
2019	3 (30.0)
2020	2 (20.0)
2021	0 (0.0)
2022	1 (10.0)
2023	1 (10.0)
2024	0 (0.0)
2025	1 (10.0)
Insurance	
No	10 (100)
Encounter type	
Outpatient/walk-in	6 (60.0)
Doctor referral	2 (20.0)
Visa-related medical screening	2 (20.0)
Proportion with	
2 tests	7 (70.0)
3 tests	0 (0.0)
4 tests	2 (20.0)
5+ tests	1 (10.0)

Abbreviations: RPR, rapid plasma reagin.

*Percentages may not always sum precisely to 100% for a given variable because of rounding.

Table S7. Individuals who tested RPR-non-reactive after a baseline RPR-reactive result in the retrospective cohort study of syphilis non-treponemal seroreversion.

Patient	First (reactive) test at baseline		Non-reactive test associated with seroreversion		Differences between tests (days)
	Test date*	Test result	Test date*	Test result	
1	Feb 2019	RPR reactive	Mar 2019	RPR non-reactive	3†
2	Feb 2020	RPR reactive	Feb 2021	RPR non-reactive	372
3	Sep 2020	RPR reactive	Jan 2023	RPR non-reactive	861
4	Aug 2022	RPR reactive	Oct 2022	RPR non-reactive	31
5	Apr 2023	RPR reactive	Sep 2023	RPR non-reactive	149
6	Feb 2025	RPR reactive	Mar 2025	RPR non-reactive	35

Abbreviations: RPR, rapid plasma reagin.

*Specific dates were not reported to preserve patient confidentiality.

†Excluded from the seroreversion cohort because the non-reactive result occurred within 30 days of a preceding reactive RPR test. The ≥30-day threshold was used because seroreversion occurring very soon after a reactive result may indicate a false outcome in one of the two tests, rather than true serologic clearance[1-4]. In routine clinical and epidemiologic settings, genuine RPR seroreversion is typically observed only after several months[1-4].

References

1 Papp, J. R. *et al.* CDC Laboratory Recommendations for Syphilis Testing, United States, 2024. *MMWR Recomm Rep* **73**, 1-32, doi:10.15585/mmwr.rr7301a1 (2024).

2 Workowski, K. A. *et al.* Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep* **70**, 1-187, doi:10.15585/mmwr.rr7004a1 (2021).

3 Sena, A. C. *et al.* Predictors of serological cure and Serofast State after treatment in HIV-negative persons with early syphilis. *Clin Infect Dis* **53**, 1092-1099, doi:10.1093/cid/cir671 (2011).

4 Ghanem, K. G., Ram, S. & Rice, P. A. The Modern Epidemic of Syphilis. *N Engl J Med* **382**, 845-854, doi:10.1056/NEJMra1901593 (2020).