nature portfolio

Corresponding author(s): Chloe L. Thio

Last updated by author(s): Jul 22, 2026

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	ELISA and neutralization-assay readouts were acquired using a microplate reader and the associated instrument software. HBsAg immunocomplex assay data were acquired using the proprietary operating software of the Abbott ARCHITECT platform. No custom software was used for data collection.
Data analysis	Statistical analyses were performed using R version 4.4.1 (R Core Team) for unadjusted analyses and SAS version 9.4 (PROC MIXED) for multivariable linear mixed-effects models. Figures were generated in R version 4.4.1 using standard statistical and plotting functions. Study-specific analysis scripts were used, but no custom or previously unpublished software or algorithms central to the study conclusions were developed.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Individual-level data used in this study were obtained from the MACS/WIHS Combined Cohort Study (MWCCS). Because these data contain sensitive participant information, including HIV status, they are not publicly available. Access may be requested through the established MWCCS concept-sheet and data-request process. Requests are reviewed by the MWCCS, and approved external investigators are required to execute a third-party data use agreement before receiving data. Information about the application process is available through the MWCCS website, and inquiries may be directed to the MWCCS Data Analysis and Sharing Center at mwccs@jhu.edu. Study-generated laboratory data underlying the reported results are available through the same controlled-access process, subject to MWCCS approval.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	All 136 participants were men enrolled in the MACS component of the MWCCS, including 99 participants without HIV and 37 with HIV. Because the analytic cohort included only one recorded sex/gender category, sex- or gender-based comparative analyses were not performed. Overall numbers are reported here; individual-level participant data are available only through the controlled-access MWCCS data-request process.
Reporting on race, ethnicity, or other socially relevant groupings	Race/ethnicity were self-reported by participants at cohort enrollment. Race was included to describe the demographic composition of the cohort and was not used as a proxy for genetic ancestry, socioeconomic status or another socially constructed variable. It was not included as an analytic covariate. For presentation in this study, categories were summarized as White and Black/Other.
Population characteristics	The analytic cohort comprised 136 men with documented incident HBV infection followed by spontaneous control, including 99 participants without HIV and 37 with HIV. The median age was 33 years (IQR, 27–40 years). Among participants with HIV, the median HIV RNA level at the first HBsAg-seroclearance visit was 8,040 copies/mL, the median CD4 T-cell count was 539 cells/mm^3, and eight participants were receiving antiretroviral therapy, including four receiving HBV-active therapy. Additional population characteristics are reported in Table 1.
Recruitment	Participants were originally enrolled in the Multicenter AIDS Cohort Study, a multicenter prospective U.S. cohort of men who have sex with men recruited at four sites during three enrollment periods between 1984 and 2003. The present analytic sample comprised cohort participants with documented incident HBV infection followed by spontaneous control and at least one of three prespecified stored plasma samples available for testing. Selection depended on participation in the original cohort and availability of archived specimens. Because all participants were men from a U.S. cohort of men who have sex with men, the findings may not be generalizable to women or to populations in other epidemiological settings.
Ethics oversight	The study was approved by the institutional review boards at all participating MWCCS sites, and all participants provided written informed consent.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical method was used to predetermine sample size. The analytic sample comprised all MACS/MWCCS participants who met the prespecified eligibility criteria, documented incident HBV infection followed by spontaneous control and availability of at least one of three prespecified stored plasma samples, yielding 136 participants (99 without HIV and 37 with HIV). Sample size was therefore determined by the number of eligible participants and specimen availability within the source cohort.
Data exclusions	No participants or measurements were excluded after application of the prespecified eligibility and assay-specific availability criteria. Analyses used all available measurements for each outcome. Sample availability varied by assay and study visit; anti-HBc analyses were restricted to participants with samples available at all three timepoints, and the exact sample sizes for each assay and visit are reported in the text and figure legends. No additional post hoc data exclusions were applied.

Replication	The principal findings were not independently replicated in a separate cohort. Assays were performed using predefined procedures with appropriate standards and run-specific positive and negative controls. ELISA concentrations were calculated using multiple dilution points within the reliable quantitative range. Neutralization assays included virus-only wells, HBIg, purified human IgG and pooled anti-HBs-negative plasma controls. Endpoint neutralization titer was evaluated as a sensitivity measure derived from the same dilution-series data and produced findings consistent with the primary AUC measure.
Randomization	This was an observational cohort study; participants were not allocated to experimental groups, and randomization was therefore not relevant. Comparisons were based on pre-existing HIV status. Longitudinal models adjusted for HIV status, time since the first HBsAg-seroclearance visit, the HIV-status-by-time interaction and age group. Additional models among participants with HIV included HIV RNA level or CD4 T-cell count.
Blinding	This observational study did not involve randomized group allocation. Laboratory personnel performing the anti-HBs ELISA were blinded to participant HIV status at the time of testing. HIV status was subsequently disclosed before the HBV neutralization and anti-HBc assays were performed, and these assays were therefore conducted without blinding to HIV status. Abbott laboratory personnel performing the HBsAg–anti-HBs immunocomplex assay were blinded to participant HIV status. Laboratory data analyses and statistical modeling were conducted with knowledge of HIV status because HIV status defined the prespecified comparison groups. All assays used predefined procedures, objective instrument-generated readouts, and prespecified standards and controls.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☐	☒ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Serum anti-HBs was measured using the XpressBio quantitative anti-HBs ELISA kit (catalog no. IM-219H), and serum anti-HBc was measured using the Wantai quantitative anti-HBc ELISA kit (catalog no. WB-26SQ96). HBsAg in neutralization-assay supernatants was measured using the Abnova qualitative HBsAg ELISA kit (catalog no. KA0286). The Abbott Research Use Only HBsAg–anti-HBs immune-complex assay used a mouse anti-HBs monoclonal capture antibody and an acridinium-labeled monoclonal anti-human IgG detection antibody.
Validation	Commercial immunoassay kits were used according to the manufacturers' instructions with kit-provided standards and controls. The XpressBio anti-HBs and Wantai anti-HBc assays were evaluated using their respective calibration standards, and concentrations were calculated from dilution points within the reliable quantitative range. The Abnova HBsAg assay was used as a qualitative readout for the neutralization assay. The Abbott Research Use Only HBsAg–anti-HBs immune-complex assay was previously described in Bazinet et al. and included assay-specific controls. No custom antibodies were generated for this study.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HepG2-NTCP cells, derived from the male-donor HepG2 cell line, were provided by Dr. Charles M. Rice at The Rockefeller University. The exact HepG2-NTCP subline identifier and RRID are being confirmed. HepAD38 cells, a male-donor HepG2-derived cell line, were obtained from the American Type Culture Collection (ATCC CRL-3561; RRID:CVCL_M177).
Authentication	HepAD38 cells were obtained directly from ATCC (CRL-3561). ATCC provides an STR profile for this cell line; no additional in-house STR authentication was performed. HepG2-NTCP cells were obtained directly from the originating laboratory and were not independently authenticated by STR profiling in our laboratory. Their expected functional phenotype was confirmed by susceptibility to NTCP-dependent HBV infection; this functional assessment was not used as a substitute for cell-line authentication.
Mycoplasma contamination	The HepG2-NTCP working cultures tested negative for mycoplasma contamination. The HepAD38 working cultures were not tested for mycoplasma contamination in our laboratory. ATCC reports mycoplasma as not detected for its distributed HepAD38 stock; the specific lot documentation should be consulted to confirm the status of the stock originally received.

Commonly misidentified lines (See ICLAC register)	Neither HepG2-NTCP nor HepAD38 is listed in the ICLAC Register of Misidentified Cell Lines (version 14, accessed 20 July 2026).

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	This was an observational cohort study using previously collected participant data and archived biological specimens. Participants were not prospectively assigned to a health-related intervention; therefore, clinical trial registration was not required.
Study protocol	This study was conducted as an ancillary observational analysis within the MACS/WIHS Combined Cohort Study under approved cohort protocols and a study-specific MWCCS concept sheet. No separate clinical trial protocol was applicable.
Data collection	Participants were recruited into the Multicenter AIDS Cohort Study at four U.S. sites during three recruitment periods between 1984 and 2003 and were followed semiannually through 2019. Clinical data and biological specimens were collected during cohort visits. This analysis used archived specimens from the first HBsAg-seroclearance visit and visits approximately 6 and 30 months later.
Outcomes	This observational study did not have registered clinical trial endpoints. Study outcomes included anti-HBs concentration, serum HBV neutralization measured as area under the neutralization curve, endpoint neutralization titer, anti-HBc concentration, and HBsAg–anti-HBs immune-complex signal. The assays and outcome definitions are described in the Methods and Supplementary Information.

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	This study did not involve seed stocks or other plant material.
Novel plant genotypes	No novel plant genotypes were generated or analyzed in this study.
Authentication	No plant materials or plant genotypes were used; therefore, plant authentication procedures were not required.