



I.R.C.C.S. Ospedale
San Raffaele

CLINICAL STUDY PROTOCOL

Study Code: HIV-HOTE (HIV Hospital Test)

Title: HIV testing: bringing to light the hidden burden in the intra-hospital setting


Principal Investigator: Silvia Nozza, MD

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Study Identifier	HIV-HOTE (HIV Hospital Test)
RSO number	NA
Other Protocol Codes	NCT06282328
Version	1.0
Date	27-Nov-2023


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Principal Investigator	Silvia Nozza, MD

	HIV-HOTE	Date: 27 Nov 2023 Version: 1.0
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VERSION HISTORY

Protocol version n.	Reason of changes	Date issued
1.0	First Version submitted to the Ethics Committee (EC)	27 Nov 2023

PROTOCOL SIGNATURE PAGE

Study Title: HIV testing: bringing to light the hidden burden in the intra-hospital setting

Study Code: HIV-HOTE (HIV Hospital Test)

Protocol Version and Date: 1.0 27 Nov 2023

The undersigned has read and understood all the aspects of the protocol detailed within this document and agrees to supervise and conduct the study in accordance with the protocol, the Declaration of Helsinki, Guideline for Good Clinical Practice ICH E6 (R2), and all applicable regulatory requirements.

Antonella Castagna



U.O. Infectious
Diseases,
IRCCS Ospedale San
Raffaele

09 Feb 2026

**Authorized Sponsor
Representative Name**

Signature

Affiliation

Date

Silvia Nozza



U.O. Infectious Diseases,
IRCCS Ospedale San
Raffaele

09 Feb 2026

**Principal Investigator
Name**

Signature


Affiliation

Date

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1. KEY STUDY CONTACTS

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2. SYNOPSIS

Study Identifier	HIV-HOTE (HIV Hospital Test)
Study Title	HIV testing: bringing to light the hidden burden in the intra-hospital setting

Protocol Version and Date	1.0 27 Nov 2023
Sponsor	IRCCS Ospedale San Raffaele
Principal Investigator	Silvia Nozza, MD Infectious Diseases Unit, IRCCS Ospedale San Raffaele, Milan Via Stamira D'Ancona, 20 20127 Milan, Italy
Study Description	<p>Considering that 1 out of 3 HIV diagnoses occur among people with symptoms or diseases attributable to the infection, the number of late presenters could be reduced by implementing new testing strategies also at the hospital level (intra-hospital setting). HIV testing is already regularly performed for people with AIDS-defining clinical conditions that are strictly associated with HIV infection; however, there are further clinical conditions that, although not directly associated with an AIDS stage, can be considered indicators of HIV infection.</p> <p>The "HIV indicator conditions" (hereafter HIV-IC) are a series of clinical, radiological and laboratory conditions, which include, in addition to all AIDS-defining conditions, many non-AIDS-defining conditions.</p> <p>HIV-IC can be used as a warning sign for performing HIV testing; these are associated with HIV infection because they share the same risk factors (as for example in the case of infection with hepatotropic viruses) or develop following early/late immunosuppression (as for example herpes zoster). Moreover, the use of HIV-IC, in combination with behavioural risk factors, represents a valid strategy to apply to hospital clinical practice. In the extra-hospital setting, access to testing is based only on behavioural risk factors and is therefore often limited to key populations, with a disparity in access to testing. The intra-hospital setting instead ensures the possibility of combining information on risk behaviours with numerous clinical, radiological and laboratory data, available during standard clinical practice. This strategy could allow access to HIV testing to be expanded to new populations, while still ensuring an evidence-guided strategy, individualized to the single person. The purpose of this study is to evaluate the prevalence of HIV infection, previously unknown, in the hospital setting (in the different inpatient wards of medical or surgical areas) in people</p>

	with the presence of at least one HIV-IC and/or risk behaviours. The prevalence will be estimated in relation to the different HIV-IC and risk behaviours and, finally, the predictive performance of the HIV-IC and risk strata for the presence of HIV infection will be assessed.	
Study Design	.Cross-sectional, single-center study, with an additional procedure. The study provides for the collection of clinical and laboratory data on people with the presence of at least one HIV-IC and/or behavioural risk factor for HIV infection, admitted to a Hospital Operating Unit of San Raffaele Hospital in Milan, for any reason (Appendix B).	
Primary Objective To estimate the prevalence of HIV infection previously unknown (new HIV infection) in the hospital setting among people with the presence of at least one HIV-IC and/or risk behaviours.	Primary Endpoint The primary endpoint will be assessed by calculating the proportion of people with newly diagnosed HIV infection previously unknown in the hospital setting, confirmed (positive HIV test), out of the total number of people included in the study and tested for HIV.	Time point(s) Primary endpoint detection times At the end of the planned enrollment procedure
Secondary Objectives . <ul style="list-style-type: none"> Assess the association between the presence of newly diagnosed HIV infection and the different HIV-IC categories. Assess the association between the presence of newly diagnosed HIV infection and the different behavioural risk factors. 	Secondary Endpoints The secondary objectives will be assessed by calculating: <ul style="list-style-type: none"> Proportion of people with newly diagnosed HIV infection in relation to the presence/absence of the different HIV-IC (class 1, 2 or 3). Proportion of people with newly diagnosed HIV infection in relation to the 	Time point(s) Secondary endpoints detection times At the end of the planned enrollment procedure

<ul style="list-style-type: none">Assess the association between the presence of newly diagnosed HIV infection and the number of HIV-IC and behavioural risk factors present.Assess the number of new HIV infections according to the Hospital Operating Unit (medical or surgical).Assess the association between the stage of newly diagnosed HIV infection, assessed on the basis of HIV-RNA and CD4+ lymphocyte level, and the different HIV-IC and behavioural risk factors.	<p>presence/absence of the different behavioural risk factors.</p> <ul style="list-style-type: none">Mean number of "HIV-IC and/or behavioural risk factors" present in people with newly diagnosed HIV infection.Proportion of people with newly diagnosed HIV infection according to the Hospital Operating Unit.Mean HIV-RNA value and CD4+ lymphocytes at HIV diagnosis (proportion of late presenters).	
Study Population	People admitted to different Hospital Operating Units of San Raffaele Hospital (medical or surgical area) will be included.	
Inclusion Criteria	<ul style="list-style-type: none">People older than 14 years.People with at least one of the following criteria:Presence of at least one HIV-IC.Presence of behavioural risk factors for HIV infection.People who provide specific written informed consent for the study, or if minors whose parents or legal guardians provide specific written informed consent for the study	
Exclusion Criteria	<ul style="list-style-type: none">People who do not provide specific written informed consent for the study.People with known HIV infection.	
PROCEDURE		
Procedure(s)	Patients will be actively recruited into the study in the different Hospital Operating Units (medical or surgical) of San Raffaele Hospital. After the patient has provided and dated/signed their informed consent to participate in the study, the clinical and	

	laboratory information relevant to the study, as well as study-specific blood samples, will be collected. The assessment of clinical, radiological and laboratory parameters already available during the hospital admission will allow the presence of at least one HIV-IC to be established
Additional procedure	<p>Immediately after the patient has provided and dated/signed their informed consent to participate in the study, the hospitalized patient will be asked some questions (Appendix C) aimed at identifying the presence of behavioural risk factors for HIV infection. In case of the presence of at least one HIV-IC or a behavioural risk factor, the person will be eligible for inclusion in the clinical study and the capillary rapid HIV test will be performed.</p> <p>If the capillary blood rapid HIV test is positive, on the same day, according to clinical practice, the antigen/antibody test (using the Cobas 6800® method) and HIV Western Blot on venous blood will be performed for confirmation of infection. HIV tests and Western Blot will be performed at the virology laboratory of San Raffaele Hospital in Milan.</p> <p>In case of confirmed HIV infection (positive HIV test and positive Western Blot test), according to good clinical practice, an infectious diseases specialist will perform a patient evaluation, within 48 hours of diagnosis and before discharge, in order to:</p> <ul style="list-style-type: none"> request the determination of: viral load (HIV-RNA), CD4+ lymphocyte count, presence of other co-infections with hepatitis viruses (in particular HBV and HCV), according to clinical practice; refer the person to an outpatient follow-up pathway, after discharge, according to clinical practice, at the Infectious Diseases Unit of San Raffaele Hospital. <p>In case of a negative capillary HIV test, the patient will be informed of the negative result of the test.</p> <p>Signing the informed consent, collection of clinical and laboratory data, questions on behavioural risk factors and performance of the HIV test will be carried out on the same day.</p>
Sample Size	It is planned to enrol 1000 people admitted to the different Hospital Operating Units of San Raffaele Hospital. An HIV infection finding is estimated in 3% of people tested on the basis of HIV-IC and risk behaviours. Under the hypothesis that the prevalence of HIV is 3%, a sample size of 1000 people allows a

	two-sided 95% confidence interval for this prevalence between 1.9% and 4.1% to be obtained.
Statistical Design	<p>Statistical analyses will be carried out internally within the Infectious Diseases Unit of San Raffaele Hospital.</p> <p>The median (first and third quartile) and frequency (percentage) will be used to describe the characteristics of people with or without HIV infection, HIV-IC and risk behaviours.</p> <p>The assessment of the primary endpoint involves estimating the proportion of people with previously unknown HIV infection and the corresponding two-sided 95% confidence interval.</p> <p>Characteristics of subjects with and without HIV infection will be compared with Fisher's exact/chi-square test or the Mann-Whitney test.</p> <p>The assessment of HIV-IC and/or risk behaviours as risk factors for HIV infection will be evaluated through a univariate logistic regression.</p> <p>The presence of a linear trend in the proportion of people with HIV infection and the number of HIV-IC and/or risk behaviours will be evaluated through the Cochran-Armitage test for trend.</p> <p>Two-sided p-values <0.05 will be considered statistically significant.</p> <p>Statistical analyses will be performed with SAS software, version 9.4 (SAS Institute, Cary, NC).</p>
Duration of the Study	<p>Overall study duration: 24 months.</p> <p>Enrolment duration: 20 months.</p> <p>Duration of extraction and review of clinical and biochemical data: 2 months.</p> <p>Duration of statistical analysis: 1 month.</p> <p>Duration of results writing: 1 month.</p>

3. ABBREVIATIONS AND DEFINITIONS

3.1. Abbreviations

CIOMS	Council for International Organization of Medical Science
CRF	Case Report Form
CRO	Contract Research Organization
CTC	Clinical Trial Center
DPIA	Data Protection Impact Assessment
EC	Ethics Committee
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonization
LVLP	Last Visit of Last Patient
MedDRA	Medical Dictionary for Regulatory Activities
OSR	Ospedale San Raffaele
PI	Principal Investigator
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure

4. BACKGROUND AND RATIONALE

According to the Italian bulletin of 2020 (COA ISS), 60% of new HIV diagnoses were found among people with advanced infection or late presenters (people with a CD4+ lymphocyte count below 350 cells/microL), reaching 67% among heterosexual women. Reducing the number of late diagnoses is crucial to achieve better clinical outcomes and reduce secondary transmission.

The proportion of late presenters has been increasing since 2015 despite screening efforts both in hospital and in territorial (community-based) settings aimed at the general population (extra-hospital setting). These strategies mainly target people at high risk of HIV infection, based on sexual behaviours (such as men who have sex with men, MSM) or use of narcotic

substances (people who use substances intravenously, IDU), while not reaching those who do not belong to key populations. Consequently, strategies to strengthen access to HIV testing are needed.

Considering that 1 out of 3 HIV diagnoses occur among people with symptoms or diseases attributable to the infection, the number of late presenters could be reduced by implementing new testing strategies also at the hospital level (intra-hospital setting). HIV testing is already regularly performed for people with AIDS-defining clinical conditions that are strictly associated with HIV infection; however, there are further clinical conditions that, although not directly associated with an AIDS stage, can be considered indicators of HIV infection.

The "HIV indicator conditions" (hereafter HIV-IC) are a series of clinical, radiological and laboratory conditions, which include, in addition to all AIDS-defining conditions, many non-AIDS-defining conditions.

The indicators are grouped into 3 different categories:

- AIDS-defining conditions;
- Conditions associated in the literature with HIV infection in >0.1% of cases (non-AIDS-defining);
- Conditions in which undiagnosed HIV infection would lead to serious adverse conditions (non-AIDS-defining).

The complete list of HIV-IC is presented in Appendix A.

HIV-IC can be used as a warning sign for performing HIV testing; these are associated with HIV infection because they share the same risk factors (as for example in the case of infection with hepatotropic viruses) or develop following early/late immunosuppression (as for example herpes zoster).

Moreover, the use of HIV-IC, in combination with behavioural risk factors, represents a valid strategy to apply to hospital clinical practice. In the extra-hospital setting, access to testing is based only on behavioural risk factors and is therefore often limited to key populations, with a disparity in access to testing. The intra-hospital setting instead ensures the possibility of combining information on risk behaviours with numerous clinical, radiological and laboratory data, available during standard clinical practice. This strategy could allow access to HIV testing to be expanded to new populations, while still ensuring an evidence-guided strategy, individualized to the single person.

The purpose of this study is to evaluate the prevalence of HIV infection, previously unknown, in the hospital setting (in the different inpatient wards of medical or surgical areas) in people with the presence of at least one HIV-IC and/or risk behaviours. The prevalence will be estimated in relation to the different HIV-IC and risk behaviours and, finally, the predictive performance of the HIV-IC and risk strata for the presence of HIV infection will be assessed.

5. OBJECTIVES AND ENDPOINTS

Primary objective.

The primary objective of the study is to estimate the prevalence of HIV infection previously unknown (new HIV infection) in the hospital setting among people with the presence of at least one HIV-IC and/or risk behaviours.

Primary endpoint.

The primary endpoint will be assessed by calculating the proportion of people with newly diagnosed HIV infection previously unknown in the hospital setting, confirmed (positive HIV test), out of the total number of people included in the study and tested for HIV.

Secondary objectives.

- Assess the association between the presence of newly diagnosed HIV infection and the different HIV-IC categories.
- Assess the association between the presence of newly diagnosed HIV infection and the different behavioural risk factors.
- Assess the association between the presence of newly diagnosed HIV infection and the number of HIV-IC and behavioural risk factors present.
- Assess the number of new HIV infections according to the Hospital Operating Unit (medical or surgical).
- Assess the association between the stage of newly diagnosed HIV infection, assessed on the basis of HIV-RNA and CD4+ lymphocyte level, and the different HIV-IC and behavioural risk factors.

Secondary endpoints.

The secondary objectives will be assessed by calculating:

- Proportion of people with newly diagnosed HIV infection in relation to the presence/absence of the different HIV-IC (class 1, 2 or 3).
- Proportion of people with newly diagnosed HIV infection in relation to the presence/absence of the different behavioural risk factors.
- Mean number of "HIV-IC and/or behavioural risk factors" present in people with newly diagnosed HIV infection.

- Proportion of people with newly diagnosed HIV infection according to the Hospital Operating Unit.
- Mean HIV-RNA value and CD4+ lymphocytes at HIV diagnosis (proportion of late presenters).

Objectives	Endpoints	Time point(s)
Primary Objective To estimate the prevalence of HIV infection previously unknown (new HIV infection) in the hospital setting among people with the presence of at least one HIV-IC and/or risk behaviours	Primary Endpoint The primary endpoint will be assessed by calculating the proportion of people with newly diagnosed HIV infection previously unknown in the hospital setting, confirmed (positive HIV test), out of the total number of people included in the study and tested for HIV.	Primary endpoint detection times At the end of the planned enrollment procedure
Secondary Objectives Assess the association between the presence of newly diagnosed HIV infection and the different HIV-IC categories. <ul style="list-style-type: none"> • Assess the association between the presence of newly diagnosed HIV infection and the different behavioural risk factors. • Assess the association between the presence of newly diagnosed HIV infection and the number of HIV-IC and behavioural risk factors present. • Assess the number of new HIV infections according to 	Secondary Endpoints The secondary objectives will be assessed by calculating: <ul style="list-style-type: none"> • Proportion of people with newly diagnosed HIV infection in relation to the presence/absence of the different HIV-IC (class 1, 2 or 3). • Proportion of people with newly diagnosed HIV infection in relation to the presence/absence of the different behavioural risk factors. • Mean number of "HIV-IC and/or behavioural risk factors" present in people with newly diagnosed HIV infection. • Proportion of people with newly diagnosed HIV infection 	Secondary endpoint detection times At the end of the planned enrollment procedure

the Hospital Operating Unit (medical or surgical). <ul style="list-style-type: none"> Assess the association between the stage of newly diagnosed HIV infection, assessed on the basis of HIV-RNA and CD4+ lymphocyte level, and the different HIV-IC and behavioural risk factors. 	according to the Hospital Operating Unit. <ul style="list-style-type: none"> Mean HIV-RNA value and CD4+ lymphocytes at HIV diagnosis (proportion of late presenters). 	
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6. STUDY DESIGN

Cross-sectional, single-center study, with an additional procedure.

The study provides for the collection of clinical and laboratory data on people with the presence of at least one HIV-IC and/or behavioural risk factor for HIV infection, admitted to a Hospital Operating Unit of San Raffaele Hospital in Milan, for any reason (Appendix B).

6.1. Study duration

Overall study duration: 24 months.

Enrolment duration: 20 months.

Duration of extraction and review of clinical and biochemical data: 2 months.

Duration of statistical analysis: 1 month.

Duration of results writing: 1 month.

7. STUDY POPULATION

7.1. Study Participants

People admitted to different Hospital Operating Units of San Raffaele Hospital (medical or surgical area) will be included.

7.2. Inclusion Criteria

- People older than 14 years.

- People with at least one of the following criteria:
- Presence of at least one HIV-IC.
- Presence of behavioural risk factors for HIV infection.
- People who provide specific written informed consent for the study, or if minors whose parents or legal guardians provide specific written informed consent for the study.

7.3. Exclusion Criteria

- People who do not provide specific written informed consent for the study.
- People with known HIV infection.

8. STUDY VARIABLES

The information collected during the hospital admission that will be verified and recorded is (Appendix D):

- Gender and age;
- General clinical assessment (e.g., symptoms, new clinical events, including adverse events, vital signs);
- Results of the most recent laboratory tests;
- Abdominal circumference and weight;
- Systolic and diastolic blood pressure;
- HIV test result;
- If HIV infection is confirmed (according to clinical practice): viral load (HIV-RNA), CD4+ lymphocyte count, co-infection with hepatitis viruses (in particular HBV and HCV), history of AIDS-defining events;
- Smoking habit, alcohol consumption;
- Comorbidities (including cancers, diabetes, cardiovascular, renal and liver diseases, psychiatric disorders (with a focus on depression), sexually transmitted diseases);
- Risk behaviours (e.g., use of substances intravenously, sexual habits);
- Concomitant medications.

9. STUDY PROCEDURES

Patients will be actively recruited into the study in the different Hospital Operating Units (medical or surgical) of San Raffaele Hospital. After the patient has provided and dated/signed their informed consent to participate in the study, the clinical and laboratory information relevant to the study, as well as study-specific blood samples, will be collected. The assessment of clinical, radiological and laboratory parameters already available during the hospital admission will allow the presence of at least one HIV-IC to be established.

ADDITIONAL PROCEDURES OR ANALYSES: Immediately after the patient has provided and dated/signed their informed consent to participate in the study, the hospitalized patient will be asked some questions (Appendix C) aimed at identifying the presence of behavioural risk factors for HIV infection. In case of the presence of at least one HIV-IC or a behavioural risk factor, the person will be eligible for inclusion in the clinical study and the capillary rapid HIV test will be performed.

If the capillary blood rapid HIV test is positive, on the same day, according to clinical practice, the antigen/antibody test (using the Cobas 6800® method) and HIV Western Blot on venous blood will be performed for confirmation of infection. HIV tests and Western Blot will be performed at the virology laboratory of San Raffaele Hospital in Milan.

In case of confirmed HIV infection (positive HIV test and positive Western Blot test), according to good clinical practice, an infectious diseases specialist will perform a patient evaluation, within 48 hours of diagnosis and before discharge, in order to:

- request the determination of: viral load (HIV-RNA), CD4+ lymphocyte count, presence of other co-infections with hepatitis viruses (in particular HBV and HCV), according to clinical practice;
- refer the person to an outpatient follow-up pathway, after discharge, according to clinical practice, at the Infectious Diseases Unit of San Raffaele Hospital.

In case of a negative capillary HIV test, the patient will be informed of the negative result of the test.

Signing the informed consent, collection of clinical and laboratory data, questions on behavioural risk factors and performance of the HIV test will be carried out on the same day.

Procedures	Day 0
Informed consent	X
Demographics	X
Survey on behaviours	X
Assessment of HIV-IC	X
HIV rapid test	X

9.1. Informed Consent

The study will be conducted in accordance with the ethical principles deriving from the Declaration of Helsinki and with the current regulations on Observational Studies.

The investigators involved will conduct the study in accordance with this protocol and the applicable GCP.

Before the formal activation of the study, approval by the Ethics Committee of IRCCS Ospedale San Raffaele is planned.

All patients will be informed as exhaustively as possible about all aspects concerning the study in a language and in terms totally understandable to them. Before the patient's participation, specific written informed consent for the study will be obtained.

The signed and dated informed consent form will be filed in the Trial Master File; a copy of the consent will be delivered to each participating subject.

10. SAMPLE HANDLING

No ad hoc collection of biological samples is planned for this study. Capillary rapid HIV tests will be destroyed immediately after the test is performed. The venous blood drawn in case of a possible positive rapid HIV test will be destroyed at the end of the study.

The venous blood HIV test and the possible Western Blot will be performed on venous blood already available and drawn for hematochemical tests prescribed according to clinical practice by the ward treating physician. No storage of samples beyond the usual laboratory practice in force at San Raffaele Hospital is planned.

11. PATIENT SAFETY

The participant may feel intimidated or uncomfortable in answering some personal questions such as those related to their sexual habits or substance use. The possibility will be given to answer the questions autonomously on a paper sheet. On the paper sheet, the participant's name, nor any other information that could identify them, will be reported, but only an identification number that will allow the data to be identified within the study.

Blood sampling: The HIV test will be performed on capillary blood and, in case of a positive test, the HIV antigen/antibody test and the HIV test on venous blood will be performed. For this reason, there are no additional risks resulting from the procedures of this study beyond those related to blood draws performed during admission and which are:

- there is the possibility of experiencing discomfort if bleeding, swelling, dizziness or bruising occurs when the needle is inserted into the arm;

- fainting or infections may rarely occur;
- very rarely, vascular or nerve lesions may occur which can result in permanent damage in individual cases.

Social risks: The study staff undertakes to implement every action to protect participants' privacy. Following confirmation of HIV infection, regular follow-up of the patient at an Infectious Diseases outpatient center for the treatment of the infection will be necessary after the study: this could reveal problems in the social sphere. When a participant becomes aware of being HIV positive, they may feel depressed and suicidal. During follow-up outpatient visits, the patient's infectious diseases specialist will try to assess these aspects and, when necessary, support the patient also through counselling.

12. DATA MANAGEMENT

The data recorded in medical records and the study variables will be collected in an eCRF and reported in pseudonymized form, and used in this way for statistical analysis. Such data will be processed in compliance with the patient's rights and the Sponsor's right to rework and publish them for scientific purposes, after appropriate anonymization or pseudonymization, depending on the case and on the scientific needs to be met.

Responsibility for data collection

Data collection will be the responsibility of the staff involved in the clinical trial under the supervision of the Principal Investigator of the study. During the conduct of the study, the Investigator will maintain complete and accurate documentation.

Clinical documents and data archiving

Data collection will be the responsibility of the staff involved in the clinical trial under the supervision of the Principal Investigator of the study. The data will be reported in an accurate, complete and legible manner, exactly as recorded in the original documents (medical records, reports, etc.).

The entered data may be modified by the Principal Investigator or by a person delegated by them for data entry, in accordance with the modalities provided by GCP.

All data and documents related to the research will be kept and protected from unauthorized access in the Study Trial Master File.

12.1. Documentation of data in Case Report Forms (CRFs)

All relevant data collected during the study for all of the patients enrolled in the study shall be entered in the CRF by the principal investigator or someone authorized by the investigator in a timely manner (as soon as possible after the information is collected) to ensure that they are clear and legible. The physician shall confirm the completeness, correctness, plausibility, and compliance with the ICH guidelines and the institutional SOPs of the data by dated signature. An explanation must be provided for any and all missing data. The entries shall be made with black ballpoint pen.

The properly filled in CRF will remain in the study site; in case of electronic CRF, a copy of all pages will be conserved in the study site.

12.2.Data Recording and Record Keeping

The investigator shall arrange for the retention of Essential Documents for the Conduct of a Clinical study (e.g., patient files, other source data, and the Trial Master File/Investigator Site File) after the completion or discontinuation of the study according to institutional procedures and applicable laws.

12.3.Data Protection

Information related to study subjects will be kept confidential and managed according to the relevant provisions (D.L. 196/30 June 2003 and subsequent amendments and additions and EU Regulation 679/2016; Privacy Authority Guidelines of 24/07/2008).

Each patient will be assigned an identification code and the data collected during the Study, except for the name, will be recorded, processed and stored together with this code, age, sex, weight, height and all clinical data relating to health status. Only the study physician and authorized persons may link the code to the name and will not provide third parties with the data of patients included in the study. Therefore, all those who conduct research activities on the information collected within the study will treat the information in coded form (use of the code for each patient).

13. STATISTICS

It is not performed by a CRO.

13.1. Description of Statistical Methods

Statistical analyses will be carried out internally within the Infectious Diseases Unit of San Raffaele Hospital.

The median (first and third quartile) and frequency (percentage) will be used to describe the characteristics of people with or without HIV infection, HIV-IC and risk behaviours.

The assessment of the primary endpoint involves estimating the proportion of people with previously unknown HIV infection and the corresponding two-sided 95% confidence interval.

Characteristics of subjects with and without HIV infection will be compared with Fisher's exact/chi-square test or the Mann-Whitney test.

The assessment of HIV-IC and/or risk behaviours as risk factors for HIV infection will be evaluated through a univariate logistic regression.

The presence of a linear trend in the proportion of people with HIV infection and the number of HIV-IC and/or risk behaviours will be evaluated through the Cochran-Armitage test for trend.

Two-sided p-values < 0.05 will be considered statistically significant. Statistical analyses will be performed with SAS software, version 9.4 (SAS Institute, Cary, NC).

13.2. Sample Size Determination

It is planned to enrol 1000 people admitted to the different Hospital Operating Units of San Raffaele Hospital. An HIV infection finding is estimated in 3% of people tested on the basis of HIV-IC and risk behaviours. Under the hypothesis that the prevalence of HIV is 3%, a sample size of 1000 people allows a two-sided 95% confidence interval for this prevalence between 1.9% and 4.1% to be obtained.

14. ETHICAL AND REGULATORY CONSIDERATIONS

This clinical study will be conducted in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments established by the World Medical Assemblies, and the ICH guidelines for Good Clinical Practice.

This clinical study will be conducted in compliance with all international laws and regulations; national laws and regulations of the country in which the clinical study is performed; as well as any other applicable guidelines.

14.1. Responsibilities of the Investigator(s)

The Investigator(s) undertake(s) the responsibility to perform the study in accordance with this Protocol, Good Clinical Practice, and the applicable regulatory requirements. The Investigator is required to ensure compliance with the investigational product schedule, visits schedule, and

procedures required by the protocol. The Investigator agrees to provide all information requested in the Case Report Form (CRF) in an accurate and legible manner. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to study subjects without prior EC approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted. The investigator must have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the study to conduct the study properly and safely.

14.2. Ethics Committee (EC) Approvals

This clinical study protocol as well as the Informed Consent are to be submitted to the appropriate Ethics Committee, and it is mandatory to obtain the written and dated approval, signed by the chairman with Ethics Committee(s) composition.

The clinical study the documents reviewed, the list of voting members and their qualifications, and the date of the review should be clearly stated on the written Ethics Committee approval.

15. QUALITY ASSURANCE AND QUALITY CONTROL

As this is an observational study that provides for the execution of the following additional procedure: capillary HIV test, a study “surveillance” activity will be carried out.

To guarantee study quality, the Sponsor will implement the following operating modalities:

- The Sponsor’s CTC-Quality Unit sets up the TMF/ISF with the related forms (delegation log, subject log, AE/SAE log, etc.) using its own templates;
- The PI organizes a study kick-off meeting (also via telematics) with the coordinating center team. The PI presents the protocol and CTC-Quality explains document management, the Informed Consent collection process, and patient safety management. CTC-Quality will minute and share the meeting minutes with participants.
- The PI must communicate to CTC-Quality any SAE and major deviations from the protocol and GCP;
- CTC-Quality may request periodic updates from the PI and conduct audits of the study to verify correct application of the protocol, applicable regulations and institutional procedures.

16. FINANCE AND INSURANCE

16.1. Patient Insurance

Given the observational nature of the proposed study, insurance coverage is not necessary.

The study is non-profit and does not receive dedicated funding. No additional costs are expected for administering questionnaires on sexual behaviours and for collecting and performing data analysis. Expenses for any resources required (capillary rapid HIV tests) will be covered by funds available to the Infectious Diseases Unit.

17. END OF CLINICAL STUDY

In accordance with applicable regulation, ICH GCP and SOPs, the PI shall notify the end of the clinical study within 15 days from the end of the clinical study and the reasons for such action.

17.1. Summary of the results of the clinical study

Irrespective of the outcome of a clinical study, within one year from the end of a clinical study, the PI shall submit a summary of the results of the clinical study.

18. INTELLECTUAL PROPERTY

The data and results generated by the study are the property of IRCCS Ospedale San Raffaele.

19. PUBLICATION POLICY

Study results will be made public in anonymous form through conference presentations and scientific publications. In no case is the publication of data that allow recognition of the patient envisaged.

The results of this study will be submitted to national and international journals; posters and oral communications will be submitted to national and international congresses. All publications and communications regarding the study will be approved in advance by the PI.

The Sponsor/Principal Investigator of the study is responsible for preparing an annual report on the clinical study to be sent to the Ethics Committee and for preparing a final report on the clinical study. After the data have been completely analyzed, they will be communicated, in anonymous form, to all researchers involved in the study.

20. REFERENCES

1. O'Byrne P. HIV self-testing: A review and analysis to guide HIV prevention policy. Public Health Nurs. 2021 Sep;38(5):885–891. doi: 10.1111/phn.12917. Epub 2021 May 27. PMID: 34043831.
2. Bogers SJ, Schim van der Loeff MF, Davidovich U, Boyd A, van der Valk M, Brinkman K, de Bree GJ, Reiss P, van Bergen JEAM, Geerlings SE; HIV Transmission Elimination Amsterdam (H-TEAM) Consortium. Promoting HIV indicator condition-guided testing in hospital settings (PROTEST 2.0): study protocol for a multicentre interventional study. BMC Infect Dis. 2021 Jun 2;21(1):519. doi: 10.1186/s12879-021-06183-8. PMID: 34078315; PMCID: PMC8173796.
3. Gómez-Ayerbe C, Martínez-Sanz J, Muriel A, Pérez Elías P, Moreno A, Barea R, Polo L, Cano A, Uranga A, Santos C, Casado JL, Quereda C, Robledillo G, Díaz-de Santiago A, Vivancos MJ, Dronda F, Navas E, Moreno S, Pérez Elías MJ. Impact of a structured HIV testing program in a hospital emergency department and a primary care center. PLoS One. 2019 Aug 1;14(8):e0220375. doi: 10.1371/journal.pone.0220375. PMID: 31369594; PMCID: PMC6675098.
4. COA/ISS HIV/AIDS 2020 Bulletin. Accessible at: <https://www.epicentro.iss.it/aids/notiziario-coa>
5. Lazarus JV, Hoekstra M, Raben D, Delpech V, Coenen T, Lundgren JD; HIV in Europe Initiative Steering Committee. The case for indicator condition-guided HIV screening. HIV Med. 2013 Aug;14(7):445–8. doi: 10.1111/hiv.12022. Epub 2013 Mar 10. PMID: 23480600.
6. Cerini C, Casari S, Donato F, Porter E, Rodella A, Terlenghi L, Compostella S, Apostoli A, Brianese N, Urbinati L, Salvi A, Rossini A, Agabiti Rosei E, Caruso A, Carosi G, Castelli F. Trigger-oriented HIV testing at Internal Medicine hospital Departments in Northern Italy: an observational study (Fo.C.S. Study). Infect Dis (Lond). 2016 Nov-Dec;48(11-12):838–43. doi: 10.3109/23744235.2016.1169551. Epub 2016 Aug 11. PMID: 27622515.
7. Horsley Downie J, Pegler M, Widdrington J, Price DA, Premchand N, Chadwick DR. Late HIV diagnosis and missed opportunities for testing: piloting a standardised, multi-source review process. Int J STD AIDS. 2020 Mar;31(3):208–213. doi: 10.1177/0956462419868637. Epub 2020 Jan 22. PMID: 31969058.
8. D'Arminio Monforte A. et al. Milan fast track city: study on HIV prevalence in two different settings. Presented at ICAR 2021 Congress.
9. Picarelli C. May administering routine HIV test be useful to uncover the iceberg in a University Hospital setting? Our experience. Presented at ICAR 2020 Congress.
10. Galli M, Borderi M, Viale P. HIV policy in Italy and recommendations across the HIV care continuum. Infez Med. 2020 Mar 1;28(1):17–28. PMID: 32172257.
11. HIV Indicator Conditions. Accessible at: https://eurotest.org/Portals/0/Indicator%20diseases/CHIP_Guidance%20in%20short_UK_updated%20JUN2016_mlj.pdf

12. Bull L, Rayment M. HIV-indicator-condition-driven HIV testing: clinically effective but still rarely implemented. Clin Med (Lond). 2016 Apr;16(2):175-9. doi: 10.7861/clinmedicine.16-2-175. PMID: 27037389; PMCID: PMC4952973.