CLINICAL STUDY PROTOCOL
(Master study protocol)

Protocol Title

Evaluation of the performance of novel rapid diagnostics for Mpox virus at point-of-care

Short title

Performance evaluation of Mpox point-of-care diagnostics

Protocol Version Number: 2.0

Date:

25 M*ay* 2023

Disease Programme:

Pandemic Threats

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Confidentiality Statement:

FIND

Table of Contents

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Study Coordinator:

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*Terms of references and nature of agreements are available from FIND on request.

Signature Page (Sponsor)

We, the undersigned, have developed, reviewed and approved this protocol, including appendices. We will supervise and coordinate the clinical Study according to the principles outlined in the Declaration of Helsinki and Good Clinical Practice and in compliance with applicable regulatory requirements.

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Statement of Principal Investigator

In signing this page, I, the undersigned, agree to conduct the Study according to the protocol and ICH-GCP E6 (R2) guidelines and in compliance with applicable regulations.

I will ensure that the requirements relating to obtaining Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) review and approval are met. I will promptly report to the IRB/IEC any and all changes in the research activities covered by this protocol.

I have sufficient time to properly conduct and complete the Study within the agreed Study period and I have adequate resources (staff and facilities) for the foreseen duration of the Study.

I am responsible for supervising any individual or party to whom I delegate Study related duties and functions conducted at the Study site. Further, I will ensure this individual or party is qualified to perform those Study-related duties and functions.

I certify that key individuals involved with the conduct of this Study, including myself, have completed GCP training and, if applicable, Human Subjects Protection Training.

I understand that all information obtained during the conduct of the Study with regard to the subjects' state of health will be regarded as confidential. No participant's names or personal identifying information may be disclosed. All participant data will be anonymized and identified by assigned numbers on all Case Report Forms, laboratory samples and other Study related information (such as essential documents) forwarded to FIND. Monitoring and auditing by FIND, and inspection by the appropriate regulatory authority(ies), will be permitted.

I will maintain confidentiality of this protocol and all other related investigational materials. Information taken from the Study protocol may not be disseminated or discussed with a third party without the express consent of FIND.

(Site specific) Name of Principal Investigator: _____
(Print)

Signature: _____ Date: _____
DD/MMM/YYYY

Site to add more site staff and signatures, if applicable/required by their Institute

Protocol History/Amendment Summary*

Version number	Release date	Comments
1.0	12 December 2022	Initial version
2.0	25 May 2023	Change in signatory for “Deputy Director, Pandemic Threats”. Updated “date of symptom onset” to “duration of symptom” throughout protocol. Updated study site from “Royal Free Hospital London” to “Liverpool School of Tropical Medicine” throughout protocol. Changed inclusion criteria to "Individuals ≥ 2 years of age…” Added more detail on general blinding, screening, and enrolment procedures. Clarified number of samples collected per sample type. Inclusion of blood as a sample type for exploratory analysis. Added “VTM/UTM” for collection of skin lesion and oropharyngeal swabs. Included interim analysis when enrolment reaches 50 participants to recalculate sample size for screening.

List of Abbreviations and Acronyms

Abbreviation/acronym	Meaning
AE	Adverse Event
Ag	Antigen
CRF	Case Report Form
Ct	Cycle time
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
GDoP	Good Documentation Practice
ICF	Informed Consent Form
ICH	International Council on Harmonisation
IDMC	Independent Data-Monitoring Committee
IEC	Independent Ethics Committee
IRB	Institutional Review Board
INRB	Institut National de Recherche Biomédicale
ISF	Investigator Site File
ISO	International Organisation for Standardization
MPXV	Mpox virus
NPA	Negative percent agreement
PCR	Polymerase chain reaction
POC	Point of care
PPA	Positive percent agreement
QA	Quality Assurance
QC	Quality Control
QMS	Quality Management System
RA	Regulatory Authority
RBM	Risk Based Monitoring
RM	Risk Management
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TMF	Study Master File
UTM	Universal Transport Media
VTM	Viral Transport Media
WHO	World Health Organisation

Protocol Synopsis

Title	Evaluation of the performance of rapid *point-of-care* diagnostics for Mpox virus
Short title	Performance evaluation of MpoX point of care diagnostics
Protocol version and date	Version 2.0 – 25 May 2023 These parts may be adapted in site-specific protocol
Background and rationale	The aim of this study is to independently evaluate the diagnostic performance of rapid, point-of-care (POC) assays for the direct detection of Mpox antigens (Ag) or DNA in comparison to laboratory-based PCR, which is the current gold standard for the confirmatory testing of Mpoxinfection. Mpox is a viral disease of zoonotic origin with symptoms similar to those seen in patients with smallpox, although clinically less severe. Since the eradication of smallpox in the 70s, mpox outbreaks have been reported in multiple countries in West and Central Africa; however, the true burden is unknown (1). There are two known clades of mpox virus, one endemic in Western Africa (clade II) and one in the Congo Basin region (clade I), the latter causing more severe illness. The Democratic Republic of Congo (DRC) reports the highest number of cases per year; since 2010, over 1,500 cases and 150-250 deaths have been reported per year, with a mortality rate around 10%. Cases continue to be regularly reported in the region, sustained by sporadic zoonotic and human-to-human transmissions. In May 2022, multiple cases of mpox were identified in several non-endemic countries. This multi-country outbreak was declared a Public Health Event of International Concern by the IHR Emergency Committee on July 23rd, 2022. As of November 13th, 2022, there have been 79,411 laboratory confirmed cases and 50 deaths reported to WHO from 110 countries/territories in the six WHO regions, with the majority being reported from the WHO region of the Americas. Actual case numbers are likely to be underestimated, due to the lack of early clinical recognition of the disease in non-endemic regions as cases are often atypical to those described in previous outbreaks, limited surveillance mechanisms, and the lack of available laboratory diagnostics, among other factors. PCR on skin lesion material is the recommended method for confirmation of mpox infection. However, in regions with limited PCR testing capacity or rapid access to results, POC tests for mpox, if shown to have sufficient accuracy to aid in clinical decision-making, could contribute substantially to control of disease spread and improved patient management. POC tests might provide the only source of rapid diagnostic testing in resource limited and decentralized settings where reference testing via PCR is a challenge.
Primary objective(s)	**1.1.** To determine the diagnostic accuracy of mpox POC Ag or POC/near-POC molecular tests (index tests) on skin lesion specimens vs. gold-standard PCR (reference test).
Secondary objective(s)	**2.1.** To determine the association of index test sensitivity/positive percent agreement (PPA) results with disease stage (days since onset of symptoms, e.g. acute, early, late) and Ct values (as a proxy for viral load) **2.2.** To compare performance of mpox POC test results o*n skin lesion specimens* by the following subgroups (where information is available): · Sex

	· Virus clade · Age group · Vaccination status (e.g. smallpox vaccination)
Exploratory objectives	**3.1** To determine the diagnostic performance of mpox POC and near-POC antigen/molecular tests on other specimen types (e.g. respiratory swabs, *whole* blood/*plasma*) vs. gold-standard PCR on lesion specimens **3.2** To determine the diagnostic performance of mpox POC and near-POC antigen/molecular tests on other specimen types (e.g. respiratory swabs, *whole* blood/*plasma*) vs. gold-standard PCR on the respective specimen.
Primary endpoints (outcomes)	**1.1.** Point estimates of clinical sensitivity/PPA and specificity/negative percent agreement (NPA) of index test, with 95% confidence intervals, using a lab-based PCR as reference standard.
Secondary endpoints (outcomes)	**2.1.** Point estimates of sensitivity/PPA stratified by duration of symptoms, Ct values of the reference PCR test. **2.2.** Point estimates of sensitivity/PPA and specificity/NPA with 95% confidence intervals stratified by the specified subgroups.
Exploratory endpoints (outcomes)	**3.1** Point estimates of sensitivity/PPA and specificity/NPA of index test on alternative specimen types, with 95% confidence intervals, using a lab-based PCR on lesion samples as reference standard **3.2** Point estimates of sensitivity/PPA and specificity/NPA of index test on alternative specimen types, with 95% confidence intervals, using a lab-based PCR on the corresponding sample type as reference standard
Study design	This is a prospective, diagnostic accuracy study. The same study design is applied to each POC evaluation (sub-study) performed under this protocol. Considering that the mpox outbreak situation (incidence) is subject to change, enrichment strategies to meet the study objectives may be discussed with single study sites and adopted at any time during the study, provided that specific go/no go criteria are met, and study validity and data integrity can be preserved. Potential enrichment strategies considered are: 1. Including close symptomatic contacts of a confirmed mpox case through contact tracing procedures 2. Frozen lesion and respiratory samples from FIND or local sample repositories, and/or same-day, fresh left-over specimens available in the local diagnostic laboratory may be used, provided they were collected from patients meeting the eligibility criteria. 3. Additional sites may be considered. The procedures to be followed in case enrichment strategies are implemented will be included in the Study Manual. However, only participants enrolled and/or specimens collected after consent and/or assent will be included in the study. There will be no follow-up visits. Only the reference PCR result will be communicated to the participant. In order to make the information on test performance available timely, a partial analysis will be performed every time the collection of data for each RDT has been completed at one site. This analysis will evaluate the primary outcomes for a given RDT at a specific site.

Study sites/setting	The study will initially include 3 evaluation sites, two in endemic African countries and one in a newly affected European country: - Institut Pasteur de Bangui, Central African Republic - Institut National de Recherche Biomédicale, Democratic Republic of Congo - Liverpool School of Tropical Medicine, United Kingdom Each index test is evaluated at all study sites. Each evaluation site may recruit study participants from one or more associated clinics. Additional sites may be considered should i) other POC devices become available through FIND's selection for clinical performance evaluation and/or ii) mpox outbreak situation change in the above-mentioned sites (see above).
Study population	Individuals ≥ 2 years of age suspected to have mpox (and/or specimens collected from them), as per national or WHO case definitions.
Sample Size	The sample size for this study is at least 30 positive and 30 negative samples for each index test evaluation based on U.S. FDA guidelines. Assuming that both the sensitivity and the specificity of the test are 90% this would result in a full width of 95% confidence interval of ±11%, based on the formula 6.2 in Zhou et al Since the prevalence of the disease in these areas is unknown, an interim analysis will be performed to estimate the prevalence with its 95% confidence interval once the enrolment reaches 50 participants for each site. Following this, the lower bound of the confidence interval will be used to estimate the number of participants to be screened based on the formula 6.3 in Zhou et al. Assuming a lowest possible prevalence of 10%, 5%, and 1%, the number of participants to be screened to reach 30 confirmed cases with an 80% power would be: • 10% prevalence: 348 to screen • 5% prevalence: 697 to screen • 1% prevalence: 3496 to screen
Eligibility criteria	**Inclusion criteria** • Individual ≥ 2years of age • Meet case definition for a suspected case of mpox (as per national or WHO clinical case definitions) • Provide written informed consent (by the participant or legal representative) and/or assent to participate in this study • Provide specimens for the study **Exclusion criteria** • Individual with no visible rash or lesions
Study duration	This study is planned to take approximately 6-9 months to complete.
Time schedule	Study preparation and IRB approvals: 3.5 months Site activation and training: 0.5 months Enrolment and data collection: 1-4 months Data cleaning/data lock and final analysis: 1 month (per site)

Schedule of Activities / Sample Flow

Procedure	Day 1	Day 2+	Notes
Inclusion and exclusion criteria	X		
Informed consent and participant enrolment	X		Consent (by the participant or legal representative) and/or assent
Participant clinical information collection	X		
Sample collection for reference and index test	X		Two (2) lesion swabs, two (2) oropharyngeal swabs collected. *Optional:* whole blood/plasma collected.
Index test performance	X	X	Sample must be processed following manufacturer's instructions (immediately after collection or as per maximum time stated in the IFU).
Reference test performance	X	X	Reference test may be performed at a later date, depending on site's workload and sample collection time.
Reference test communicated to participant	X	X	
Completion of CRFs	X	X	Some data may become available more than 2 days later (e.g., sequencing result)
AE/SAE review	X	X	Limited scope, see Appendix 1

1 Introduction

1.1 Study Rationale

Mpox is a viral disease of zoonotic origin with symptoms similar to those seen in patients with smallpox, although clinically less severe. Since the eradication of smallpox in the 70s, mpox outbreaks have been reported in multiple countries in West and Central Africa; however, the true burden is unknown (1). There are two known clades of mpox virus, one endemic in Western Africa (clade II) and one in the Congo Basin region (clade I), the latter causing more severe illness. The Democratic Republic of Congo (DRC) reports the highest number of cases per year; since 2010, over 1,500 cases and 150-250 deaths have been reported per year, with a mortality rate around 10%. Cases continue to be regularly reported in the region, sustained by sporadic zoonotic and human-to-human transmissions.

In May 2022, multiple cases of mpox were identified in several non-endemic countries (2,3). As of November 13th, 2022, there have been 79,411 laboratory confirmed cases and 50 deaths reported to WHO from 110 countries/territories in the six WHO regions, with the majority being reported from the WHO region of the Americas. This multi-country outbreak was declared a Public Health Event of International Concern by the IHR Emergency Committee on July 23rd, 2022 (4). Actual case numbers are likely to be underestimated, due to the lack of early clinical recognition of the disease in non-endemic regions, as cases are often atypical to those described in previous outbreaks, limited surveillance mechanisms, and the lack of available laboratory diagnostics, among other factors.

The aim of this study is to independently evaluate the performance of novel, rapid, point-of-care (POC) and near-POC assays for the direct detection of mpox antigens (Ag) or DNA in comparison to laboratory-based PCR, which is the current gold standard for confirmatory testing of mpox infection.

1.2 Background

Laboratory-based, real-time or conventional PCR on skin lesion material is the recommended method for confirmation of mpox (5). However, in regions with limited PCR testing capacity or rapid access to results, POC tests for mpox, if shown to have sufficient accuracy to aid in clinical decision-making, could contribute substantially to control disease spread and improved patient management. POC tests might provide the only source of diagnostic testing in resource limited and decentralized settings where reference testing via PCR is a challenge. POC tests may also aid in contact tracing and surveillance during a known mpox outbreak.

Certain real-time PCR assays can discriminate not only mpox virus (MPXV) from other orthopoxviruses but also the two MPXV clades described above. To date, all cases identified in newly affected countries have been identified as being infected with clades IIa and IIb (formerly the West African clade) (6).

1.3 Benefit/Risk Assessment

The IVDs under investigation are considered low risk to study participants as collection of lesion and OP samples are a non-invasive procedure. This procedure will be performed by the health care professionals trained to collect skin lesion samples for the reference standard PCR test.

The probability of an adverse event (AE) or serious adverse event (SAE) occurring to a study participant to be associated with the investigational product is extremely low.

This is a diagnostic accuracy study that does not utilize test results for clinical decision making. All study personnel are made aware that the novel POC tests under study is for research purposes only and cannot be used to determine whether to initiate treatment, nor for any other clinical management decisions.

Post-test counselling and linkage to care will be provided at study sites to enrolled participants with positive mpox status by the PCR reference test.

(SITE to add specific details regarding if needed)

Participants do not benefit directly from the study. Participants may be reimbursed for travel, depending on the study site. The study will benefit to society through publication of study results and provide data to inform discussions on mpox POC testing policy and use, particularly in low- and middle-income countries in the future. The development and availability of mpox tests that are accurate and adapted to POC settings will improve diagnosis and treatment of patients, facilitate studies to understand mpox prevalence and natural history, and ultimately lead to effective public health measures, vaccines and therapies.

2 Study Objectives and Endpoints

The objectives and corresponding endpoints of this study are described in Table 1.

Table 1. Study objectives and endpoints.

Objectives	Endpoints
Primary	
1.1 To determine the diagnostic performance of mpox POC or near-POC antigen/molecular tests (index tests) on lesion specimens vs. gold-standard PCR (reference test)	1.1 Point estimates of clinical sensitivity/positive percent agreement (PPA) and specificity/negative percent agreement (NPA) of index test, with 95% confidence intervals, using a lab-based PCR as reference standard.
Secondary	
2.1 To determine the association of index test sensitivity results with disease stage (days since onset of symptoms, e.g. acute, early, late) and Ct values (as a proxy for viral load) 2.2 To compare performance of mpox POC test results o*n skin lesion specimens* by the following subgroups (where information is available): • Sex • Virus clade • Age group • Vaccination status (e.g. smallpox vaccination)	2.1 Point estimates of sensitivity/PPA stratified by duration of symptoms, Ct values of the reference PCR test. 2.2 Point estimates of sensitivity/PPA and specificity/NPA with 95% confidence intervals stratified by the specified subgroups.
Exploratory	
3.1 To determine the diagnostic performance of mpox POC and near-POC antigen/molecular tests on other specimen types (e.g. respiratory swabs, w*hole* blood/*plasma*) vs. gold-standard PCR on lesion specimens	3.1 Point estimates of sensitivity/PPA and specificity/NPA of index test on alternative specimen types, with 95% confidence intervals, using a lab-based PCR on lesion samples as reference standard

3.2 To determine the diagnostic performance of mpox POC and near-POC antigen/molecular tests on other specimen types (e.g. respiratory swabs, whole blood/plasma) vs. gold-standard PCR on the respective specimen.	3.2 Point estimates of sensitivity/PPA and specificity/NPA of index test on alternative specimen types, with 95% confidence intervals, using a lab-based PCR on the corresponding sample type as reference standard

3 Study Design

3.1 General Design

This is a prospective, diagnostic accuracy study. The same study design is applied to each POC evaluation (sub-study) performed under this protocol. The study is conducted at several clinical study sites located in different countries, including two endemic African countries (DRC and CAR) and one newly affected European country (UK). Each index test is evaluated at all study sites. Each evaluation site may recruit study participants from one or more associated clinics.

Considering that the mpox outbreak situation (incidence) is subject to change, enrichment strategies to meet the study objectives may be discussed with single study sites and adopted at any time during the study, provided that specific go/no go criteria are met, and study validity and integrity can be preserved. Potential enrichment strategies considered are:

1. Including close symptomatic contacts of a confirmed mpox case through contact tracing procedures
2. Frozen lesion and respiratory samples from FIND or local sample repositories, and/or same-day, fresh left-over specimens available in the local diagnostic laboratory may be used, provided they were collected from patients meeting the eligibility criteria.
3. Additional sites may be considered.

These strategies will allow to achieve the primary objective of the study and, most likely, to assess the usability of the POC under evaluation. Data collected, though, may not be sufficient/available to perform the subgroup analyses of the secondary objectives.

All study specimens and relevant information will be collected from enrolled participants on the day of recruitment. There will be no follow-up visits. Only PCR results will be communicated to the participant.

Point estimates of sensitivity and specificity, with 95% confidence intervals based on Wilson's score methods, will be calculated using a gold-standard PCR test as reference standard. In order to make the information on test performance available timely, a partial analysis will be performed every time the collection of data for each RDT has been completed at one site. This analysis will evaluate the primary for a given RDT at a specific site.

3.2 Scientific Rationale for Study Design

A prospective design has important advantages over a retrospective design, including a participant sample that is better defined in terms of the participant's clinical characteristics, as well as standardized methods of sample collection and processing across sites and standardized methods of performing and interpreting the test(s) and reference standard procedure. In some circumstances, a prospective design is the only possible approach due to the intended use of the test (i.e. IFU requiring testing on fresh samples, immediately after sample collection) or disease under investigation. A prospective design is also well suited if the study is conducted during an outbreak.

However, given the unpredictability of the mpox outbreak, resulting in reduced disease incidence, a prospective design would be difficult to implement in a timely manner. In such circumstances, the only

possibility to perform this study is through implementing enrichment strategies, such as including close contacts into the study, supplementing specimens collected prospectively with well-characterised specimens available in FIND or local sample archives, and/or including additional sites to conduct the evaluation.

3.3 End of Study Definition

A participant is considered to have completed the study after the specimens needed to perform the reference standard PCR test and the index test under evaluation have been collected and diagnostic results shared with the participant. All mpox DNA positive individuals will be linked to care as per country requirements.

The end of the study is defined as the date the minimum required number of mpox PCR positive and negative cases is reached per site.

3.4 Study Population and Eligibility

Individuals ≥ 2 years of age with symptoms compatible to mpox (and/or specimens collected from them), as per national or WHO guidelines, attending healthcare facilities in Central African Republic, Democratic Republic of Congo, and United Kingdom.

(SITE to add specific setting details, if needed)

If a participant is screened and enrolled but is not able to provide the specimens required for the study, this participant will be withdrawn.

A participant can only be enrolled once in the study. Co-enrolment in multiple studies on the same day is allowed (i.e., where the subject is asked to provide similar specimens in parallel).

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

3.5 Inclusion Criteria

Participants/specimens can be included in the study only if all the following inclusion criteria apply:

- Individual ≥ 2 years of age
- Meet case definition for a suspected case of mpox (as per national or WHO clinical case definitions; see Table 2)
- Provide written informed consent (by the participant or legal representative) and/or assent to participate in this study
- Provide specimens for the study

Table 2. WHO case definitions for mpox (7)

Suspected case
i. A person who is a contact of a probable or confirmed mpox case in the 21 days before the onset of signs or symptoms, and who presents with any of the following: acute onset of fever (>38.5°C), headache, myalgia (muscle pain/body aches), back pain, profound weakness or fatigue.
OR

ii. A person presenting since 01 January 2022 with an unexplained acute skin rash, mucosal lesions or lymphadenopathy (swollen lymph nodes). The skin rash may include single or multiple lesions in the ano-genital region or elsewhere on the body. Mucosal lesions may include single or multiple oral, conjunctival, urethral, penile, vaginal, or ano-rectal lesions. Ano-rectal lesions can also manifest as ano-rectal inflammation (proctitis), pain and/or bleeding.

AND

for which the following common causes of acute rash or skin lesions do not fully explain the clinical picture: varicella zoster, herpes zoster, measles, herpes simplex, bacterial skin infections, disseminated gonococcus infection, primary or secondary syphilis, chancroid, lymphogranuloma venereum, granuloma inguinale, molluscum contagiosum, allergic reaction (e.g., to plants); and any other locally relevant common causes of papular or vesicular rash.

3.6 Exclusion Criteria

Participants are excluded from the study if any of the following exclusion criteria apply:

- Individuals with no visible rash or lesions
- Individuals who are in unstable condition as determined by their treating clinician

3.7 Screen Failures

Screen failures are defined as participants who were eligible to participate in the study but are not subsequently enrolled in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Standards for Reporting of Diagnostic Accuracy Studies (STARD) requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure reasons, eligibility criteria.

4 Study Intervention

Study Intervention is defined as any investigational intervention(s), marketed product(s), or medical device(s) intended to be used with a Study participant according to the Study protocol.

4.1 Investigational Product

The products under evaluation within this protocol are:

- POC Ag RDTs (up to 3)
- POC/near-POC Molecular assays (up to 3)

The list of potential products for evaluation is listed in Appendix 1.

Other tests may be considered as they become available and vetted through FIND selection process.

Instructions for use of the investigational products will be provided by each manufacturer and shared with sites.

Medical device incidents, including those resulting from malfunctions of the device (or IVD), must be detected, documented, and reported by the Investigator at each site throughout the study (see Appendix 3).

4.2 Preparation/Handling/Storage/Accountability

Acquisition

Procurement of the investigational products will be done through FIND, who will coordinate shipments from the manufacturer. It is the responsibility of each Study site to maintain an updated inventory of the Study materials and to inform FIND immediately if additional materials are required.

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for the investigational product received and any discrepancies are reported and resolved before its use. Further details will be provided in the Study Manual.

Installation and Storage

Procedures for device installation and test kits storage and disposal will be described in the corresponding Instructions for Use (IFU) and manuals. For POC molecular tests, device installation will strictly adhere to manufacturers instruction, meeting all manufacturer and site-specific requirements as well for new equipment installation.

The investigational product must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorized site staff.

Test Handling and Performance

Testing using the investigational products will be performed according to the manufacturer's instructions. Lesion, oropharyngeal, and blood samples, as per index test manufacturer's claim (e.g. lesion swab) from participants enrolled in the study will be processed with the investigational product and only authorized site staff will be responsible for processing.

Accountability

The investigator is responsible for Study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Export and Import Permits

The Investigator at study site is responsible for making import permit applications in a timely manner. FIND logistics team will support providing documentation required from the Sponsor, usually including commercial invoice, packaging list and FIND donation letter. (SITE to amend accordingly)

Quality Control Check for Incoming Shipments

Upon arrival of each new shipment of assays, the sites conduct an incoming quality check following site-specific procedure. New lots may only be used after this quality check is successfully passed.

Local procurement

Sites are responsible for assessing their needs and procuring any supplies, reagents and kits needed for the Study that are locally available and to include these costs in the study budget prior to study initiation.

4.3 Minimisation of Error and Bias

Participant/specimen selection

Spectrum bias will be minimized with the prospective study design. Enrolment is based on clearly defined eligibility criteria applicable to all sites. To ensure the validity and generalizability of study results, descriptive statistics based on participant characteristics (e.g. age, sex, days since symptom onset and PCR Ct values, as surrogate for viral load, etc.) to confirm diagnostic performance across different patient sub-groups will be reported.

In the instance that enrichment strategies are employed to meet the required sample size, there is a risk of bias in the results as the performance of the tests may not reflect their true accuracy in the overall population (eligible for testing) but a subset with increased prevalence of mpox. Therefore, positive and negative predictive values will not be calculated if this was the case.

If frozen or same-day fresh left-over specimens will be used, spectrum bias will be limited by:

- Randomly selecting specimens from biobanks, considering the eligibility criteria and previous PCR qualitative result (positive or negative)
- Testing consecutive fresh left-over specimens from the diagnostic laboratory, considering only the eligibility criteria

Index test

All POC tests

The overall risk of index test results review bias is minimal as the personnel interpreting and recording the results is blind to all other test results.

Proficiency and/or competency assessments will be conducted before initiating and/or during the study at discretion of FIND study team. (SITE-specific procedures may be added.) As part of the training, POC operators are instructed to perform the test and interpret results after all relevant samples are collected and the study participant has left the sample collection/testing area. Additionally, the POC testing is done in the absence of the treating clinician to avoid that the results of the POC are mistakenly used or influence the clinical decision-making process.

In case of an invalid result, the index test may be repeated if there is enough sample volume. Both the initial and re-test results are recorded in the CRF.

POC antigen tests only

For POC Ag tests, to address the subjective nature of results interpretation (e.g. band intensity by visual interpretation) the results will be interpreted by at least 2 readers, within the time period specified in the IFU, for each test, with possibility to include a third reader as a tiebreaker in case of discordant results from the 2 first readers.

The readers for the index test should be blinded to the results of other tests (both index and reference tests).The final result is based on the majority rule. The test results are recorded in the CRFs.

Where possible, immediately after test interpretation by the readers (or within the time reading window, per IFU), a picture of the developed device may be taken for quality control purposes. Harmonization of result interpretation is ensured with proper training at the beginning of the study. Further details will be provided in the Study Manual.

Reference testing

The reference standard PCR is performed at an accredited/certified testing laboratory under routine quality control. Moreover, the results of the reference standard PCR are recorded blind to the index test in the CRFs, eliminating the risk of review bias. In case of an indeterminate result, the test may be repeated from the leftover sample as per local procedure, or sequenced. (SITE to adapt if necessary)

Flow and timing

Samples for the index test are collected in parallel to samples that are used for the reference test (i.e. collected from same lesion), so disease progression bias is not a concern. Samples for PCR reference testing are transported from the collection sites to the reference/testing laboratory. Therefore, control measures must be implemented to ensure the quality of specimens during transport.

If frozen specimens will be used, where possible, to avoid tempering with the integrity of the banked samples, samples will be thawed and used immediately before testing. Similarly, frozen specimens will be retested with the PCR reference test; these results will be used for performance analyses.

Independence of the investigators

All aspects of the study, including specimen collection, testing, data entry, and data analysis, are performed independently of the manufacturer of the POC test being evaluated. All de-identified clinical and laboratory data are analysed by FIND and the study team. Participating sites do not have any financial ties/commercial interests or per conflicts of interest related to any participating test manufacturer.

Handling of discordant results

Discordant results between standard PCR test and index test will not be investigated, as PCR is considered the reference test.

If frozen specimens will be used, discordant results between initial PCR (before freezing and biobanking) and repeated PCR (on thawed specimen selected for the study) may be further investigated. The repeated PCR result will be used for data analysis.

4.4 Randomisation

Not applicable for this study.

4.5 Blinding Procedure

Reference test (PCR) and index test (POC Ag or POC molecular) are operated and read by different users. Such operators are blind to the results of any other tests.

Steps to blind test readers and interpreters

- Index tests will not be run at the same time. One test evaluation will be completed before another index test evaluation can begin. This is aimed to reduce bias in interpretation of tests by results of another test.
- The Reference test and index test may be done on different days by different laboratory technicians in batches.
- The laboratory technician performing and reading the index test should be different from the one who performed the index test.
- Data entry into OpenClinica will be done by staff other than the laboratory technicians.

Laboratory technicians should not have access to OpenClinica data or patient files to minimize access to other test results and resulting bias.

5 Participant Discontinuation/Withdrawal

5.1 Participant Discontinuation/Withdrawal from the Study

A participant may be withdrawn at any time at the discretion of the Investigator for safety, behavioural, compliance, or administrative reasons.

Depending on site-specific informed consent:

- If a participant withdraws consent for disclosure of future information, FIND may retain and continue to use any data collected before such a withdrawal of consent.

- If a participant withdraws from the study, he/she may request destruction of any data collected for the study, and the Investigator must document this in the site study records.
- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.
- If a participant withdraws from the study, he/she may request destruction of any residual samples stored, and the Investigator must document this in the site study records.

(SITE to adapt and clarify what happens to samples and data after participant's withdrawal in agreement with ICF)

5.2 Lost to Follow Up

Not applicable for this study.

6 Study Procedures

Study procedures and their timing are summarized in the Schedule of Activities. Protocol waivers or exemptions are not allowed.

Adherence to the study design requirements, including those specified in the Schedule of Activities, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants/specimens meet all eligibility criteria.

Further details will be provided in the Study Manual

6.1 Screening potential study participants

Screening patients for the study may happen at the study site or peripheral screening locations, e.g. other health facilities, community etc. The study team may identify potential participants through the following strategies (non-exhaustive list), and refer them to the study site for detailed screening and consenting before enrolment into the study;

- Screening at hospitals or clinics. Patients admitted or attending outpatient clinics may be screened for eligibility.
- Community-based (field) pre-screening. Study staff going into the community may identify patients eligible for the study.
- Contact tracing of patients with confirmed Mpox. Contacts of patients with confirmed Mpox are screened for eligibility.
- Patients from the FIND biobanking (PP007) will be included in the test evaluations (PP008) projects.

In cases of declining Mpox cases (i.e. reduced disease incidence), stored lesion, oropharyngeal, blood samples may be identified from FIND or another repository.

6.2 Specimen Collection, Handling, Transport and Storage

Participants will be recruited at the study sites (SITE to add specific details where participants will be recruited). Participants who meet all inclusion and none of the exclusion criteria and showing interest in participating in the study will be informed by an authorized study site team member. All elements of the consent and/or assent form will be carefully, patiently, and clearly explained to the prospective subject, providing him/her sufficient time to ask questions and to make his/her decision. If he/she decides to participate, the participant and the authorized study site team member, responsible for conducting the informed consent/assent process, will sign the consent/assent form. Participants will only be enrolled to the study after signing the informed consent/assent.

Selected sites have established procedures for skin lesion sample collection for mpox diagnostic testing. Health care workers collecting samples are already trained specifically in lesion sample collection and handling techniques and are wearing appropriate personal protective equipment (PPE) at the time of sample collection. A lesion sample for reference testing is collected on the day of enrolment as per clinical routine, as first specimen. One additional lesion sample is collected and used to perform the POC testing immediately after collection (Table 3). Similarly, an additional 2 oropharyngeal swab (OP) can be taken – 1 OP swab will be used for PCR reference testing, while the other OP swab will be used for index testing (Table 3). *Optional*: an additional 1 tube (3-5 mL) of whole blood/plasma may be collected in addition and used for both PCR reference testing and index testing (Table 3).

All novel POC tests are performed within designated isolation zones for participants suspected of having mpox directly at recruitment/sampling location or in dedicated laboratory facilities close to participants recruitment/sampling location, ensuring safety for everyone in that area and timely processing of the index test, as per IFU. (SITE to add specific details on POC location). Operators running the index test and lab personnel shall wear appropriate PPE.

Participants will be diagnosed based on PCR results performed on the routine diagnostic sample (lesion) and clinical signs and symptoms by their treating physician; results of the POC tests will not be used for clinical management.

Table 3: **Sample types and testing**

Sample Type	Testing	Additional Notes
Skin lesion (surface, exudate, crust) swab for reference testing (n=1)	Molecular testing for mpox by PCR for diagnosis as per routine practice (placed in standard viral transport medium/universal transport medium (VTM/UTM).	Transported to reference/testing laboratory according to routine site practice. Leftover sample after PCR testing can be kept at -70°C or lower until the study ends. Leftover samples may be used for repeating PCR testing and/or genomic sequencing following positive PCR results. If using alternative approaches (e.g. frozen remnant sample): Remnant samples will be retested by PCR. SITE to clarify whether the remaining specimen may be stored in biobank for future studies.
Skin lesion (surface, exudate, crust) swab for index testing (n=1 or multiple, depending on	POC Ag or POC molecular testing for study purposes (placed in proprietary media, as recommended by test	Sample must be processed following manufacturer's instructions (immediately after collection or as per maximum time stated in the IFU).

test manufacturer's claim)	manufacturer in the IFU or in standard VTM/UTM).	
Oropharyngeal swab for reference test (n=1)	Molecular testing for mpox by PCR for study purposes (placed in standard VTM/UTM).	Transported to reference/testing laboratory according to routine site practice. Leftover sample after PCR testing can be kept at -70°C or lower until the study ends. Leftover samples may be used for repeating PCR testing and/or genomic sequencing following positive PCR results.
Oropharyngeal swab for index testing (n=1)	POC Ag or POC molecular testing for study purposes (placed in proprietary media, as recommended by test manufacturer in the IFU or in standard VTM/UTM).	Sample must be processed following manufacturer's instructions (immediately after collection or as per maximum time stated in the IFU).
Blood (e.g. serum, plasma, whole blood) tube for index and reference testing (n=1)	One blood tube (e.g. SST, EDTA) for collection. One aliquot for molecular testing of mpox by PCR for study purposes. Additional aliquot for POC Ag or POC molecular testing by study purposes (placed in proprietary media, as recommended by the test manufacturer in the IFU).	Transported to reference/testing laboratory according to routine site practice. Leftover sample after PCR testing can be kept at -70°C or lower until the study ends. Leftover samples may be used for repeating PCR testing and/or genomic sequencing following positive PCR results. POC Ag or POC molecular test: Sample must be processed following manufacturer's instructions (immediately after collection or as per maximum time stated in the IFU).

Note: Detailed procedures for each index test will be included in the Study Manual.

If frozen or same-day fresh left-over specimens will be used, specimen type and collection/storage medium must be validated by FIND for evaluation with the specific POC test. The device manufacturer will be consulted to this purpose. Where possible, to avoid tempering with the integrity of biobanked samples by continuous freezing and thawing, frozen specimens will be retrieved from the freezers once and batch tested.

6.3 Reference Standard Test and Index Test Procedures

Reference test

The reference test for this study will be one of the PCR kits evaluated through the WHO (8), unless importation or supply chain issues prevent the use of any of such tests in specific settings selected for the evaluation. In this case, other comparable PCR tests might be implemented throughout the study provided they are considered equivalent in respect to the study and is a country-approved method.

The diagnostic sample for the reference test is processed by trained routine personnel according to the specific standard operating procedure (SOP) in place at the site. For remnant samples, all samples included in the study will be retested by the select PCR kit (8). The test result will be recorded in the corresponding CRF. The operator is blind to the index test result.

Index tests

The samples for the index test (POC Ag or molecular) are processed by personnel trained by the test manufacturer directly, or FIND team trained by the manufacturer, at the testing location following the specific IFU provided by the manufacturer. The test result is recorded in the corresponding CRF alongside the picture of the developed POC Ag test as source data where applicable. The operator is blind to the reference test and other tests results, including other Ag RDTs that may be evaluated in parallel during the study.

If possible, all subjects enrolled for testing with a given novel POC test will have their PCR testing done with the same reference test done in the same institution.

Participants are diagnosed by their treating physician based on the reference PCR results performed on the diagnostic sample and clinical signs and symptoms; results of the POC tests are not communicated to the participants and not used for clinical management.

6.4 Genetic Analysis

Individual genetic data will not be evaluated in this Study.

6.5 Experimental Biomarkers

Experimental biomarkers are not evaluated in this Study.

6.6 Other Tests (Sequencing)

Leftover specimens which tested positive in the reference assay will undergo sequencing for identification of MPXV clades. Where possible, sequencing will be performed following the procedure established in the sequencing laboratory/facility situated in the same organisation as the study site conducting the evaluation. (SITE to add specific details on their sequencing facility and procedure).

6.7 Safety Assessments

The study complies with all biosafety precautions appropriate to the study procedures, including lesion specimen collection, handling, processing, and disposal, to protect study participants and personnel. The index test should be performed at the testing site within designated isolation zones for participants suspected of having mpox and processing areas or in a designated laboratory facility. The reference test is performed in laboratories designated for safe handling of these specimen and following laboratory's safety protocols. All testing is performed by appropriately trained personnel wearing all required PPE. The probability of (serious) adverse events (AE, SAE) occurring to a study participant while collecting the clinical sample is deemed to be extremely low, as this procedure is considered to be at very low risk. Nevertheless, should such event occur, this will be managed following the procedures described in section 7.

7 Safety and Incident Reporting

Given that this is a diagnostic accuracy study of mpox diagnostic tests, the probability of an AE or SAE occurring to a study participant to be associated with the investigational products is extremely low. Nevertheless, safety and incident reporting are described as follows.

This study does not have predefined termination criteria being an accuracy study.

SITE to add details on specific safety reporting requirements by the IEC/IRB

7.1 Adverse Events and Serious Adverse Events

The definitions of an Adverse Event (AE) and Serious Adverse Event (SAE) can be found in Appendix 2.

Given the nature of this Study AE reporting is limited in scope to:

- SAEs that may be associated with lesion sample collection
- SAEs that occur at the testing sites using the investigational product
- Any other serious events that affect the rights, safety, or welfare of subjects.

There is minimal to absent likelihood of AEs for the current protocol evaluating an external *in vitro* medical diagnostic device. The AE is not associated with the diagnostic device itself but could be associated with the performance of the sample collection procedure (procedure-related AE).

7.1.1 Time Period for Collecting SAE Information

Information will be collected at the specimen collection and in the testing of the investigational product. SAEs will be recorded and reported to the sponsor or designee within 24 hours of the investigator aware of the event.

All SAEs will be collected from the signing of the informed consent form (ICF) until completion of the procedures to collect the POC test sample and participant is discharged from the study.

The investigator will submit any updated SAE data to the sponsor within 24 hours of being made aware of the event.

Investigators are not obligated to actively seek SAEs after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study participation, the investigator must promptly notify FIND.

7.1.2 Reporting and Follow up of SAEs

All SAEs will be followed until resolution, stabilization, or the event is otherwise explained.

Prompt notification by the investigator to FIND of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants are met.

FIND will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

An investigator who receives a safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from FIND will review and then file it in the Investigator Site File (ISF) and will notify the IRB/IEC, if appropriate according to local requirements.

7.1.3 Medical Device Incidents (including Malfunctions)

Medical devices are being provided for use in this study. In order to fulfil regulatory reporting obligations worldwide, the investigator is responsible for the detection and documentation of events meeting the definitions of incident or malfunction that occur during the study with such devices.

An incident is any event/malfunction/deterioration in characteristics and/or performance of a device that causes, or has the potential to cause, unexpected or unwanted effects involving the health and safety of participants, users, or other persons. This study focuses on POC tests where the medical device itself does not come in contact with the study participant; if any, incident could only be associated with the performance of the clinical sample collection procedure (procedure-related incident).

The majority of POC tests investigated are instrument-free; moreover, this is not an interventional study, therefore, device malfunctions are of limited concern for this study. Nevertheless, should any

incident/malfunction arise due to inadequate IFU, inappropriate user practice or inappropriate environment in which the device is used or stored, this will be reported by the investigator to FIND using a dedicated issue/incident log.

7.1.4 Time Period for Detecting Medical Device Incidents

Medical device incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.

If the investigator learns of any incident at any time after a participant has been discharged from the study, and such incident is considered reasonably related to a medical device provided for the study, the investigator will promptly notify FIND.

Any medical device incident occurring to participant during the study will be documented in the participant's medical records, in accordance with the investigator's normal clinical practice, and on the CRF.

Any medical device incident/malfunction occurring to/detected by user during the study will be documented in accordance with the investigator's normal practice. FIND may also provide the site with an issue/incident log and a Medical Device Incident Report Form, where applicable.

7.1.5 Follow-up of Medical Device Incidents

All medical device incidents involving an SAE will be followed and reported in the same manner as other SAEs (see Section 7.1.2). This applies to all participants.

The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the incident.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator. It is very important that the investigator provides his/her assessment of causality (relationship to the medical device provided by FIND) and describes any corrective or remedial actions taken to prevent recurrence of the incident. A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of an incident. This includes any amendment to the device design to prevent recurrence.

7.1.6 Reporting of Medical Device Incidents to FIND

Medical device incidents will be reported to FIND within 24 hours after the investigator determines that the event meets the protocol definition of a medical device incident also by email. Where applicable, FIND will promptly inform the manufacturer.

7.1.7 Regulatory Reporting Requirements for Medical Device Incidents

The investigator will promptly report all incidents occurring with any medical device provided for use in the Study for FIND to fulfil the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

The investigator, or responsible person according to local requirements (e.g., the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of incidents to the IRB/IEC.

8 Statistical Considerations

8.1 Statistical Hypotheses

No statistical hypotheses will be tested in this study, as the goal is to evaluate the performance of tests versus a defined reference standard.

8.2 Sample Size Determination

The sample size for this study per site is at least 30 positive and 30 negative samples for each index test evaluation based on U.S. FDA guidelines (9). Assuming that both the sensitivity and the specificity of the test are 90% this would result in a full width of 95% confidence interval of ±11%, based on the formula 6.2 in Zhou et al (10).

Since the prevalence of the disease in these areas are unknown, an interim analysis will be performed to estimate the prevalence with its 95% confidence interval once the enrolment reaches 50 participants for each site. Following this, the lower bound of the confidence interval will be used to estimate the number of participants to be screened based on the formula 6.3 in Zhou et al. Assuming a lowest possible prevalence of 10%, 5%, and 1%, the number of participants to be screened to reach 30 confirmed cases with an 80% power would be:

- 10% prevalence: 348 to screen
- 5% prevalence: 697 to screen
- 1% prevalence: 3496 to screen

8.3 Populations for Analyses

For purposes of analysis, the following populations are defined in Table 5:

Table 5: Populations for analysis

Population	Description
Enrolled/intention-to-test (ITT)	All participants who sign the ICF
Evaluable/Per Protocol Population (PP)	All participants in ITT with valid reference and index test results

8.4 Statistical Analysis Plan

The statistical analysis plan (SAP) will be developed and finalized before the start of enrolment and will provide detailed explanation on the methods and techniques to be used for the analysis of each endpoint. This section is a brief summary of the planned statistical analyses of the primary and secondary endpoints.

8.4.1 General Methodology

Point estimates of sensitivity and specificity, with 95% confidence intervals based on Wilson's score methods, will be calculated based on the following definitions, using a gold-standard PCR test as reference test:

Table 6: Case predictions

	Reference standard classification			
Case prediction		Positive	Negative	Total
	Predicted positive	a	b	(a + b)

	Predicted negative	c	d	(c + d)
	Total	(a + c)	(b + d)	(a + b + c + d)

a = True Positives b = False Positives c = False Negatives d = True Negatives	Sensitivity = a / (a + c) Specificity = d / (b + d)

Endpoints: See Table 1 listing objectives and endpoints

The primary and secondary endpoints will be also analysed separately for the following subgroups:

- By sex (where the information is available)
- By duration of symptoms (as a categorical value, e.g. 1-3 days, 4-7 days, >7 days from symptoms onset)
- By comparator Ct value (as a categorical variable, e.g. Ct < 35, Ct >= 35)
- By virus clade (i.e Clade I, IIa, IIb, where the information is available)
- By age group (as categorical variable, i.e. ≤17, 18-45, >45)
- By vaccination status (e.g. smallpox vaccination, where the information is available)

A final analysis will be performed per each POC test evaluated once the enrolment has been completed for all sites evaluating this test.

The endpoints on accuracy will be calculated on the PP population. Based on the distribution of the results and on the heterogeneity of results across sites, a meta-analysis approach could be used in order to estimate the pooled values of sensitivity and specificity. Details on the methodology will be provided in the SAP.

8.5 Planned Interim Analyses

In order to make the information on test performance available timely, a partial analysis will be performed every time the collection of data for each test has been completed at one site. This analysis will evaluate the primary and secondary outcomes for a given test at a specific site.

Similarly, an interim analysis will be performed at each site when the enrolment reaches 50 participants (i.e. PCR results available for 50 participants), to determine the disease prevalence at that site. Once the prevalence is determined (with 95% confidence intervals according to Wilson's score method) the lower bound will be used to recalculate the sample size for screening at each site. Should the updated target (i.e. number of participants to screen) change, FIND will inform the site about next steps following a detailed assessment of the feasibility of reaching the revised target.

8.5.1 Data Monitoring Committee (DMC)

A DMC is not applicable for this Study is this is a low-risk study.

9 Regulatory and Ethical Considerations

9.1 Regulatory and Ethics Approvals

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki
- Applicable Good Clinical Practice Guidelines: ICH GCP E6 (R2)
- Applicable laws and regulations *(SITE to amend accordingly)*

The protocol, protocol amendments, ICF and other relevant documents (e.g. advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the Study is initiated. A copy of the IRB/IEC approval letter will be filed in the investigator site file. *(SITE to amend accordingly)*

FIND-approved versions of an amended Study protocol must be signed by the Investigator(s). Any substantial amendments to the protocol will require IRB/IEC approval before implementation of changes made to the Study design, except for changes necessary to eliminate an immediate hazard to Study participants. Protocol amendments restricted to clerical edits only will be provided to the Study sites and submitted to the IRB/IEC for informational purposes.

The investigator will be responsible for the following:

- Providing written summaries of the status of the Study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the Study at the site and adherence to requirements of ICH guidelines, the IRB/IEC, the WHO Good Clinical Laboratory Practice (GCLP), and with applicable national regulations.

(SITE to amend accordingly)

9.2 Financial Disclosure

Investigators and sub-investigators will provide FIND with sufficient, accurate financial information as requested to allow FIND to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities.

9.3 Informed Consent Process

Eligible participants will be approached at the time of sample collection for MPX testing per clinical routine. The investigator or his/her representative trained in human subject's protections explains the nature of the study to the participant in a language understandable to him/her and answers all questions about the study. In addition to oral explanations, a written information sheet describing the study purpose and procedures, risks and benefits, as well as confidentiality regulations is given to each subject.

Participants must be informed that their participation is voluntary, and that they are free to refuse to participate and may withdraw their consent at any time. Clinical personnel/study staff must explain that in this case the same patient care will be provided. Subjects willing to participate in the study are required to sign and date a statement of informed consent that meets the requirements of ICH-GCP guidelines, and the IRB/IEC or study centre.

There must be evidence that written informed consent was obtained before the participant was enrolled in the study, and ample time was given to participant to consent. The date the written consent was obtained (as well as the time, ideally) must be recorded. The authorised person obtaining the informed consent must also sign and date the ICF.

Illiterate participants must provide a thumbprint on the ICF and further the ICF must signed and dated by an impartial witness.

A signed original of this informed consent should be provided to the participant and a second original should be attached to the subject's study record.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study when new information, which might affect the participants decision about whether to continue in the research, becomes available and when study procedures are added, modified or removed from the study. In case of non-substantive changes/minor changes, re-consent is not necessary, as this does not increase risk or decrease benefit for participant.

There is no payment for participation in this research study, although participants may be reimbursed for time and transport a fee of 5-10 USD max (SITE to amend accordingly, where necessary). There will be no costs to the subject for participating in this research study.

9.4 Data Protection

Participants will be assigned a unique identifier generated by FIND. Any participant records or datasets that are transferred to FIND will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant will be informed that his/her personal Study-related data will be used by FIND in accordance with local data protection law. The level of disclosure must also be explained to the participant.

The participant will be informed that his/her medical records may be examined by quality assurance auditors or other authorized personnel appointed by FIND, by appropriate IRB/IEC members, and by inspectors from regulatory authorities (i.e., the sensitive data must be blacked out to preserve confidentiality and privacy of study participants). This holds true also in case of remote visits, including remote Source Data Verification activities.

10 Data Handling and Record Keeping

In general, FIND is responsible for the data management of this study including quality control checks of the data and assessment of overall protocol compliance, except for the site in Central African Republic, which will be responsible for data management for their study via RedCap All participant data relating to the study can be recorded in paper CRF by study site staff and entered from the paper CRF into FIND's online clinical study platform (OpenClinica Enterprise Edition version 4.0), unless transmitted to FIND electronically (e.g. transfer of laboratory electronic datasets) or entered directly in OpenClinica. The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the CRF.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 10 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of FIND. No records may be transferred to another location or party without written notification to FIND.

10.1 Source Data and Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. The investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the site's study participants (source data). The investigator may need to request previous medical records or reports (if available), depending on the study. Source documents are filed at the investigator's site.

Source data should follow the ALCOA plus i.e Attributable, Legible, Contemporaneous, Original, Accurate, Complete, Consistent, Enduring and Available Changes to source data should be traceable, should not obscure the original entry and should be explained if necessary.

Source documents are the following:

- Raw data on PCR from the local lab
- POC test CRF (paper or electronic)
- Device report (for POC molecular tests)
- All lab notebooks/lab files and patient medical charts where any data would be recorded in the first place.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to participant medical records and source documents used for this study.

To support the evaluation of assay performance, demographic and clinical data for each participant will be collected. Laboratory results of the novel POC tests, including positive/negative, valid vs. invalid, reason for invalid, are recorded on-site. Also, the ambient temperature and humidity in the testing location may be recorded directly at the testing site on a laboratory worksheet. Results of the routine PCR tests, including Ct values, are also obtained from reference/testing laboratories. Operator feedback on the ease of test operation and results interpretation using a standardized questionnaire may also be collected.

10.2 Data Management

Data Management procedures at FIND, including the setup of the database, programming edit and range checks and querying, are described in the Data Management Plan.

Results for testing each sample are collected first on paper (using the data collection form), using a separate sheet for each test and then recorded on an EDC system, or recorded directly on OpenClinica, or alternative EDC system in in place at study site and deemed suitable for the purpose by FIND. Results are qualitative, according to manufacturer's instructions. Data from each paper form are entered daily into OpenClinica or alternative EDC system. An electronic, site-specific database on OpenClinica platform can be used to capture all clinical information associated with each sample. Data quality control is performed daily by the Investigator or project team manager. Index and reference test results are recorded with the specimen/participant unique identifier (ID) in paper CRF and then entered to OpenClinica database, or directly in OpenClinica or alternative EDC system.

Whenever possible, clinical data and laboratory results are captured directly onto an electronic CRF designed by FIND in the OpenClinica EDC system. Site staff members are responsible for entering their data into OpenClinica where direct capture into EDC is not possible. Timelines for data transfer are provided by FIND study team. Data are cleaned of errors by FIND throughout the study as it is captured electronically.

The investigator is responsible for verifying that data entries are accurate and correct. Data entered in the OpenClinica database must be consistent with the source documents or the discrepancies must be explained.

The site is provided with individual password-protected accounts to access OpenClinica, following a training session given by FIND.

OpenClinica provides an audit trail system recording all data entries/changes and queries between FIND and the site. Data entry training will be provided by FIND, either on site or remotely sharing screen through Skype or any other similar system.

11 Quality Management

Quality Management for this Study consists of Quality Control activities, training and capacity building provided by FIND (or designee) to the investigational sites and laboratories.

Training on the protocol, GCP and the use of the IVDs and laboratory tests will be provided by FIND. Training on the EDC system will be provided by FIND Data Management prior to first participant enrolment.

11.1 Quality Control (monitoring)

Quality control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly. The investigational site is responsible for performing regular Quality Control checks on the data they generate.

FIND will perform risk-based monitoring of this Study, and associated Quality Control checks, as described in the Monitoring Plan. Study monitors will perform source data review and source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the Study is being conducted in accordance with the currently approved protocol and any other Study agreements, ICH GCP, and all applicable regulatory requirements.

11.2 Quality Assurance (auditing)

As part of routine Quality Assurance, FIND or designee may conduct an audit of the investigational site.

11.3 Study and Site Closure

FIND reserves the right to close the study site or terminate the study at any time for any reason at its sole discretion. Investigational sites will be closed upon Study completion. A study site is considered closed when all required documents and study supplies have been collected.

The investigator may initiate Study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for early closure of a Study site by FIND are described in the contractual agreement.

12 Publication Policy

Data obtained from participation in this study are considered confidential. The investigators must adhere to the non-disclosure requirements set forth in the contractual agreement.

Participants of the study can be informed about the general outcome of the study. To do so, they are asked to contact an investigator.

The investigator is obligated to provide FIND or its designee with complete test results and all data obtained in this study. The information obtained during this study can be made available to other investigators and third parties, including regulatory agencies, only with written permission from FIND.

Authorship for scientific publication of the study results will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements, as described in the publication policy section of the contractual agreement.

13 References

1. Brown K, Leggat P. Human Monkeypox: Current State of Knowledge and Implications for the Future. Trop Med Infect Dis. 2016 Dec 20;1(1):8.

2. Al-Tammemi AB, Albakri R, Alabsi S. The Outbreak of Human Monkeypox in 2022: A Changing Epidemiology or an Impending Aftereffect of Smallpox Eradication? Front Trop Dis [Internet]. 2022 [cited 2022 Aug 5];3. Available from: https://www.frontiersin.org/articles/10.3389/fitd.2022.951380

3. Multi-country monkeypox outbreak: situation update [Internet]. [cited 2022 Jun 23]. Available from: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON393

4. Wenham C, Eccleston-Turner M. Monkeypox as a PHEIC: implications for global health governance. The Lancet [Internet]. 2022 Aug 1 [cited 2022 Aug 12];0(0). Available from: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(22)01437-4/fulltext?rss=yes

5. World Health Organization. Laboratory testing for the monkeypox virus: interim guidance, 23 May 2022 [Internet]. 2022. Available from: https://apps.who.int/iris/handle/10665/354488

6. Monkeypox: experts give virus variants new names [Internet]. [cited 2022 Aug 14]. Available from: https://www.who.int/news/item/12-08-2022-monkeypox--experts-give-virus-variants-new-names

7. Clinical management and infection prevention and control for monkeypox: Interim rapid response guidance, 10 June 2022 [Internet]. [cited 2022 Nov 21]. Available from: https://www.who.int/publications-detail-redirect/WHO-MPX-Clinical-and-IPC-2022.1

8. Michel J, Targosz A, Rinner T, Bourquain D, Brinkmann A, Sacks JA, et al. Evaluation of eleven commercially available PCR kits for the detection of Monkeypox virus DNA [Internet]. medRxiv; 2022 [cited 2022 Oct 24]. p. 2022.10.14.22281096. Available from: https://www.medrxiv.org/content/10.1101/2022.10.14.22281096v1

9. Center for Devices and Radiological Health. Monkeypox Emergency Use Authorizations for Medical Devices: Templates for EUA Submissions [Internet]. FDA. FDA; 2022 [cited 2022 Nov 15]. Available from: https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-emergency-use-authorizations-medical-devices

10. Zhou X hua, McClish DK, Obuchowski NA. Statistical methods in diagnostic medicine. 2nd ed. Hoboken, N.J: Wiley; 2011. 545 p. (Wiley series in probability and statistics).

14 Appendices

Appendix 1: Investigational products for evaluation

Appendix 2: Safety Definitions and Reporting

Appendix 3: Incident Definition and Reporting

Appendix 1: Potential investigational products for evaluation

1) POC Ag tests

Test manufacturer	Test name
ACON Biotech(Hangzhou) Co., Ltd.	Flowflex Mpox Virus Antigen Rapid Test
Assure Tech. (Hangzhou) Co., ltd.	Mpox Antigen Rapid Test
Tianjin Bioscience Diagnostic Technology Co., ltd	Mpox virus antigen rapid test (colloidal gold)
Nanjing Synthgene Medical Technology Co., Ltd.	Mpox Virus Antigen Rapid Test Kit
SD BIOSENSOR INC	STANDARD Q Mpox Ag Test
Getein Biotech, Inc.	Mpox Virus Antigen Rapid Self-Test Kit

2) POC molecular tests

Test manufacturer	Test name
SD Biosensor	STANDARD M10 MPX/OPX
Getein Biotech, Inc.	Akso Mpox Virus Real-time PCR Kit
Cepheid	Xpert Mpox

Appendix 2: Safety Definitions and Reporting

Adverse Event (AE) Definition
• An AE is any unfavourable and unintended sign (including abnormal laboratory finding), symptom or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

Serious Adverse Event (SAE) Definition:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d. Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect
f. Other situations: • Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include intensive treatment in an emergency room or at home for allergic bronchospasm or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

The Protocol Author is responsible for verifying safety reporting requirements. SAE Reporting to FIND may differ than described below. If that is the case, describe the procedure in detail.

SAE Reporting to FIND
• The SAE Report must be sent to the FIND Senior Scientist (Devy Emperador, devy.emperador@finddx.org) and Study Manager (Juvenal Nkeramahame, juvenal.nkeramahame@finddx.org) via e-mail, marked High Priority, with a follow up call to ensure receipt. • Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE Report within the designated reporting time frames. • Contacts for SAE reporting can be found on the front of the protocol.

Appendix 3: Incident Definition and Reporting

Medical Device/IVD Incident Definition
• A medical device incident is any malfunction or deterioration in the characteristics and/or performance of a device or IVD as well as any inadequacy in the labelling or the instructions for use which, directly or indirectly, might lead to or might have led to the death of a participant/user/other person or to a serious deterioration in his/her state of health. • Not all incidents lead to death or serious deterioration in health. The non-occurrence of such a result might have been due to other fortunate circumstances or to the intervention of health care personnel.

It is sufficient that:

- An incident associated with a device happened.

  AND

- The incident was such that, if it occurred again, might lead to death or a serious deterioration in health.

A serious deterioration in state of health can include any of the following:

- Life-threatening illness
- Permanent impairment of body function or permanent damage to body structure
- Condition necessitating medical or surgical intervention to prevent one of the above
- Foetal distress, foetal death, or any congenital abnormality or birth defects

Medical Device Incident Documenting
• For medical device incidents fulfilling the definition above, complete the SAE Report Form. Further details will be provided in the Study Manual. • It is very important that the investigator provides his/her assessment of causality (relationship to the medical device provided by FIND) and describes any corrective or remedial actions taken to prevent recurrence of the incident. • A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of an incident. This includes any amendment to the device design to prevent recurrence.