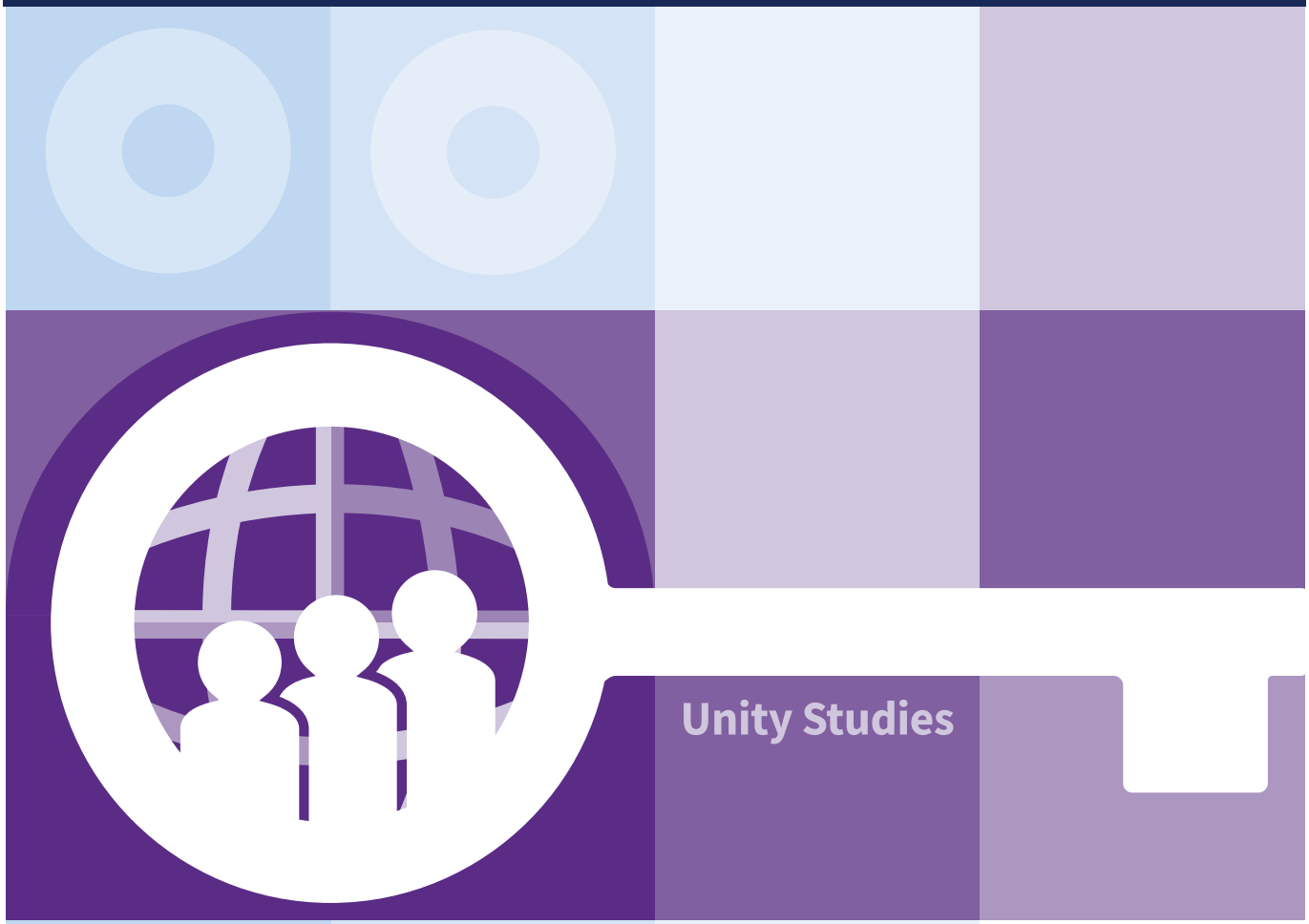
First Few X cases and contacts diagnostic test evaluation for respiratory pathogens with pandemic potential

Template protocol



Unity Studies

World Health Organization

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Protocol template for focused End User Validation of a first In-House Developed Test (IHDT) for molecular detection of Pathogen X by a nationally-designated laboratory and early use to define Pathogen X kinetics and optimal diagnostic sampling strategies in the first few X cases and their close contacts. This protocol will guide the conduct of subsequent FFX studies.

This protocol should be implemented in accordance with guidance from the Responsible Technical Officer (RTO) and relevant national legal requirements and regulations, which take precedence over any procedures described herein.

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Abbreviations

AN swab	Anterior nasal swab
BAL	Bronchoalveolar lavage
CONSISE	Consortium for the Standardization of Influenza Seroepidemiology
COVID-19	Coronavirus disease 2019
EUA	Emergency use authorization
FFP2	Filtering Face Piece 2
FFX	First Few X cases and contacts
FFX-DX	First Few X cases and contacts diagnostic test evaluation
HLIP	High-level Implementation Plan (Pandemic Influenza Preparedness Framework)
IHDT	In-House Developed Test
IHR	International Health Regulations
IRB	Institutional Review Board
LOD	Limit of Detection
LRTI	Lower respiratory tract infection
MERS-CoV	Middle East respiratory syndrome coronavirus
NP swab	Nasopharyngeal swab
OP swab	Oropharyngeal swab
PCR	Polymerase Chain Reaction
PIP	Pandemic Influenza Preparedness
PPE	Personal Protective Equipment
R_0	Basic reproductive number
R_{eff}	Effective reproductive number
RNA	Ribonucleic acid
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SCAR	Secondary Clinical Attack Rate
SIR	Secondary Infection Rate
WHO	World Health Organization

Stages of In-House Developed Test Development and Validation

Pathogen X discovery and early characterization: The very first lab work required to initiate development of a test for Pathogen X. Includes sequencing of Pathogen X and culture to yield an isolate (bacterial, viral).

In-House-Developed Test (IHDT) for molecular detection of Pathogen X: A test that is **developed** and **validated** such that it can be used by a laboratory for clinical (and research) testing prior to receipt of any form of emergency use authorization (EUA) by any relevant regulatory authority, i.e., i) to **diagnose the first cases of Pathogen X** and ii) to use for prospective studies within the FFX-Dx protocol. For the purposes of this protocol, the IHDT is assumed to be a real-time reverse transcription (RT)-PCR or PCR assay.

IHDT Early Development: Work to design and **develop** the molecular test (IHDT) for Pathogen X **prior to** analytical and clinical validation. Includes development of primers, probes, and an initial RT-PCR/PCR assay. This work could be done by the same laboratory involved in Pathogen X discovery/early characterization or by another laboratory or laboratory consortium. **See Annex 4 for details**.

IHDT Early Validation: Rigorous but focused analytical studies performed by the IHDT Developer to **validate** the molecular test (IHDT) for Pathogen X in preparation for use for clinical (and research) testing prior to EUA by any relevant regulatory authority. This work can be done prior to availability of clinical samples from patients infected with Pathogen X and can be done by the same laboratory involved in IHDT Early Development or by another laboratory or laboratory consortium. **See Annex 4 for details**.

IHDT End User Validation (**performed in Part A of the FFX-Dx protocol; see Section 2.2 for details**): A focused validation **to be done by the laboratory implementing the IHDT for clinical testing**. Includes **focused analytical validation** (to verify the analytical test characteristics established by the developing lab) and a **limited clinical validation**, i.e., prospective testing with confirmatory testing to be performed by an independent laboratory. Note that performance of an End User Validation assumes that a fully completed and successful IHDT Early Validation (as defined above) was performed by the IHDT Developer.

Note: different jurisdictions may have alternative rules and requirements for IHDT validation and clinical use.

Beyond the scope of this document is the additional validation required for EUA by any relevant regulatory authority.

Summary

This document sets out the methods to enable the **earliest assessment of human case(s) of a novel or re-emerging respiratory pathogen with pandemic potential and their close contacts, specifically to guide development and early use of pathogen-specific diagnostic tests for clinical use and public health benefit. For the purposes of this protocol, the conceptual respiratory pathogen in question will be referred to as Pathogen X, which causes Disease X.** The First Few X cases and contacts diagnostic test evaluation (FFX-Dx) for respiratory pathogens with pandemic potential: template protocol provides guidance for the End User Validation and early use of the first diagnostic (Dx) test for Pathogen X in a rigorous but focused assessment of the First Few Pathogen X cases and contacts in [Country Y], as part of the Respiratory Investigations and Studies, Unity Studies initiative.

The detection and early spread of a novel or re-emerging pathogen with pandemic potential is accompanied by the need to rapidly develop and deploy pathogen-specific diagnostic tests, requiring both early test validation efforts, sharing of protocols and assay materials (e.g., reagents, QC materials), and early generation of data to inform test use. Development of a reliable molecular diagnostic test followed by direct assessment of early Pathogen X kinetics in relevant sample types to clarify testing approaches with highest yield are needed, both to prevent disease transmission and to define the key epidemiological and clinical characteristics of the novel pathogen. Pathogen X discovery and characterization followed by early development and validation of a molecular In-House Developed test (IHDT) (details in Annex 4) would precede implementation of this FFX-Dx protocol (Figure 1) and can involve laboratories close to or distant from the outbreak. Subsequently, at the site of the outbreak, a focused IHDT End User Validation (FFX-Dx protocol Part A) and subsequent use of the IHDT to quantify the pathogen in matched[1] serial samples from the first few Pathogen X cases and their close contacts (FFX-Dx protocol Part B) will directly inform targeted guidance for early test use and further test development (Figure 1). FFX-Dx study data can provide critical early insight into the pathogen's ability to spread in the human population and its virulence (case-severity), and will also directly inform subsequent studies to better define these epidemiologic parameters. The FFX-Dx study will thus facilitate outbreak control and assist in pandemic prevention and control.

Requirements for the End User Laboratory and clinical team implementing the FFX-Dx protocol (see Annex 5) should be considered in advance for planning purposes. The FFX-Dx protocol is designed to be executed in collaboration with local public health partners and to directly precede and inform a series of enhanced surveillance protocols, developed by the World Health Organization (WHO) in collaboration with technical partners, that are harmonized to help provide detailed insight into the epidemiological characteristics of

1 Matched samples: Samples collected from different body sites at the same time point

respiratory pathogens with pandemic potential. Those protocols build on previous protocols developed by the global Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) *(1)*, and WHO's Influenza Pandemic Special Investigations and Studies, as well as those developed by WHO as part of the Unity Studies for Coronavirus disease 2019 (COVID-19) *(2)* and for Middle East respiratory syndrome coronavirus (MERS-CoV) *(3)*.

All WHO Respiratory Investigations and Studies protocols are available on the WHO website.

Comments for the user's consideration are provided in purple text throughout the document, as there may be a need to modify methods slightly considering the local context in which the investigation will be carried out.

Full title of study	First Few X cases and contacts diagnostic test evaluation for respiratory pathogens with pandemic potential: template protocol [Country Y]
Population	The first few X cases of Pathogen X and their close contacts
Potential output and analysis	End User Validation of a molecular In-House Developed test (IHDT) for Pathogen X followed by characterization of early Pathogen X kinetics and optimal diagnostic sampling strategies through serial collection of matched samples in the population of initial cases of **Pathogen X** and their close contacts. Results will guide subsequent test development and use for achieving clinical and public health goals. Primary objectives include: • Part A: End User Validation (focused analytical and limited clinical validation) of the initial molecular test (IHDT) developed for Pathogen X in focused sample types (NP swab for upper respiratory presentation; NP swab and BAL or sputum for lower respiratory infection). • Part B: quantification of Pathogen X using IHDT (by cycle threshold values or, optimally, pathogen concentration measurements) in matched samples collected serially over the course of infection and post-exposure to assess early Pathogen X kinetics (peak) and optimal sample types for diagnostic testing, including samples relevant to point-of-care and self-testing. Testing of matched samples will also provide validation data for ongoing testing of alternative sample types. • For all cases, gather data on the clinical presentation and course of associated disease to optimize/refine the clinical case definition for Disease X. • For contacts (Part B only), attempt to detect and quantify Pathogen X in asymptomatic or pre-symptomatic infection. Secondary objectives include estimation of the following epidemiological and Pathogen X parameters (epidemiological parameters to be confirmed in the subsequent FFX protocol): • the duration of shedding of Pathogen X; • the symptomatic and asymptomatic proportions of cases; • the serial interval; • the incubation period; • the generation time; • correlation of Ct values and/or Pathogen X nucleic acid concentrations with culture to inform isolation practices; • correlation of Pathogen X antigen concentration with Pathogen X nucleic acid concentration (as soon as a test for quantitative detection of Pathogen X antigen is available). Exploratory objectives (to be defined rigorously in the subsequent FFX protocol) include: • the secondary infection rate (SIR) and secondary clinical attack rate (SCAR) overall, and by key factors such as setting, age, and sex; • possible routes of transmission including possible animal-human transmission; • preliminary case- (i.e., disease) hospitalization and fatality ratios, and infection-hospitalization and fatality ratios.

Study design	Part A of the FFX-Dx Protocol consists of a focused analytical and limited clinical validation of the In-House Developed test (IHDT) by the IHDT End User, including a small prospective clinical investigation involving suspected cases of Pathogen X (Figure 1). Part B includes prospective enrolment of suspected cases and a subset of identified close contacts, including infants and children, that had the most significant exposure to the case (Figure 1). Part B utilizes serial collection of matched samples in confirmed cases and their contacts to characterize early Pathogen X kinetics and define optimal diagnostic sampling strategies (Figure 2). **Part A**: End User analytical and limited clinical validation of a new molecular In-House Developed test (IHDT) for Pathogen X and optimization/refinement of clinical case definition. See Section 2.2 for details of the End User Validation. Note that the focused analytical validation must be completed before utilizing the test for initial clinical testing of samples from patients suspected of having Pathogen X disease. Clinical testing on patient samples to complete Part A should include accrual of 10 probable cases (meeting the clinical case definition **and** testing as a presumptive positive by the IHDT) presenting with upper respiratory infection (test with NP swab only) or lower respiratory infection (test with NP swab and BAL or sputum if feasible), as well as 10 IHDT-negative samples. These 20 IHDT results (10+/10-) should be confirmed by another independent laboratory (either the IHDT Developer laboratory or another laboratory that has completed the End User focused analytical validation of the IHDT). After the first 10+/10- results have been confirmed, individuals meeting the clinical case definition and with positive IHDT results can now be considered confirmed cases. **Part B**: Definition of early Pathogen X kinetics and highest-yield sample types for diagnostic testing. Prospective frequent serial collection of matched samples from confirmed Pathogen X index cases (30–60 adults and, if possible, up to 30 children; see Table 2) and their identified close contacts (up to 3 per case, identified in collaboration with the local public health team); assessment of cycle threshold (Ct values)/Pathogen X nucleic acid concentrations by timing of sample collection and specimen type.
Timing of the investigation	To be initiated in the first days after the initial confirmation of an apparent outbreak of Pathogen X in [Country Y]. FFX-Dx is the first protocol to be initiated in the case of a Pathogen X outbreak/cluster, upon identification of the initial cases of Pathogen X in [Country Y] in the early outbreak phase.
Duration	Recruitment and follow-up of index cases and close contacts for up to 28 days from recruitment (see details for Part B).

Minimum data and specimens to be obtained from participants of clinical parts

The focus of the FFX-Dx protocol is on End User focused analytical/limited clinical validation of the new molecular IHDT (Part A) and determination of Pathogen X kinetics and highest-yield sample types to guide test use and further test development (Part B).

Prospective sample collection, Part A (Form A1, Form C/Annex 3):

Suspect cases: NP swab upon recruitment (Day 1) and on Day 2. If lower respiratory tract infection (LRTI) presentation, also collect BAL/sputum if feasible (note that LRTI sample type requires separate validation, see Section 2.2) If any IHDT result is a presumptive positive, the individual becomes a 'Probable case'. The IHDT results and clinical data yielded in Part A (Form A1) will assist in clarification of the case definition.

Prospective sample collection, Part B (Forms A1/A2 (case), B1/B2 (contact), Form C/Annex 3):

Details of sampling are outlined in Figure 2. Suspect cases: NP swab (priority sample type; see Section 2.7.1) upon recruitment (Day 1) and again on Day 2, in parallel with matched testing (Figure 2). If any validated IHDT result is positive, the individual becomes a confirmed case and matched testing continues as below (Figure 2); if the Day 1/Day 2 validated results are negative, the individual is not a case, and no further sampling is done.

Daily matched testing (AN swab, OP swab, saliva, and if LRTI presentation, sputum) takes place for 10 days from day of enrolment (Day 1), followed by every other day through Day 28 (see Figure 2). NP swab to be collected every other day through Day 14, and at Day 20. If Pathogen X is still detectable at Day 28, continue sampling positive sample types (only) every third day until it is no longer detectable for at least 5 days. An additional NP swab should be collected for viral culture, if possible, every other day through Day 10. BAL (if feasible) at baseline.

Blood collection every third day through Day 28.

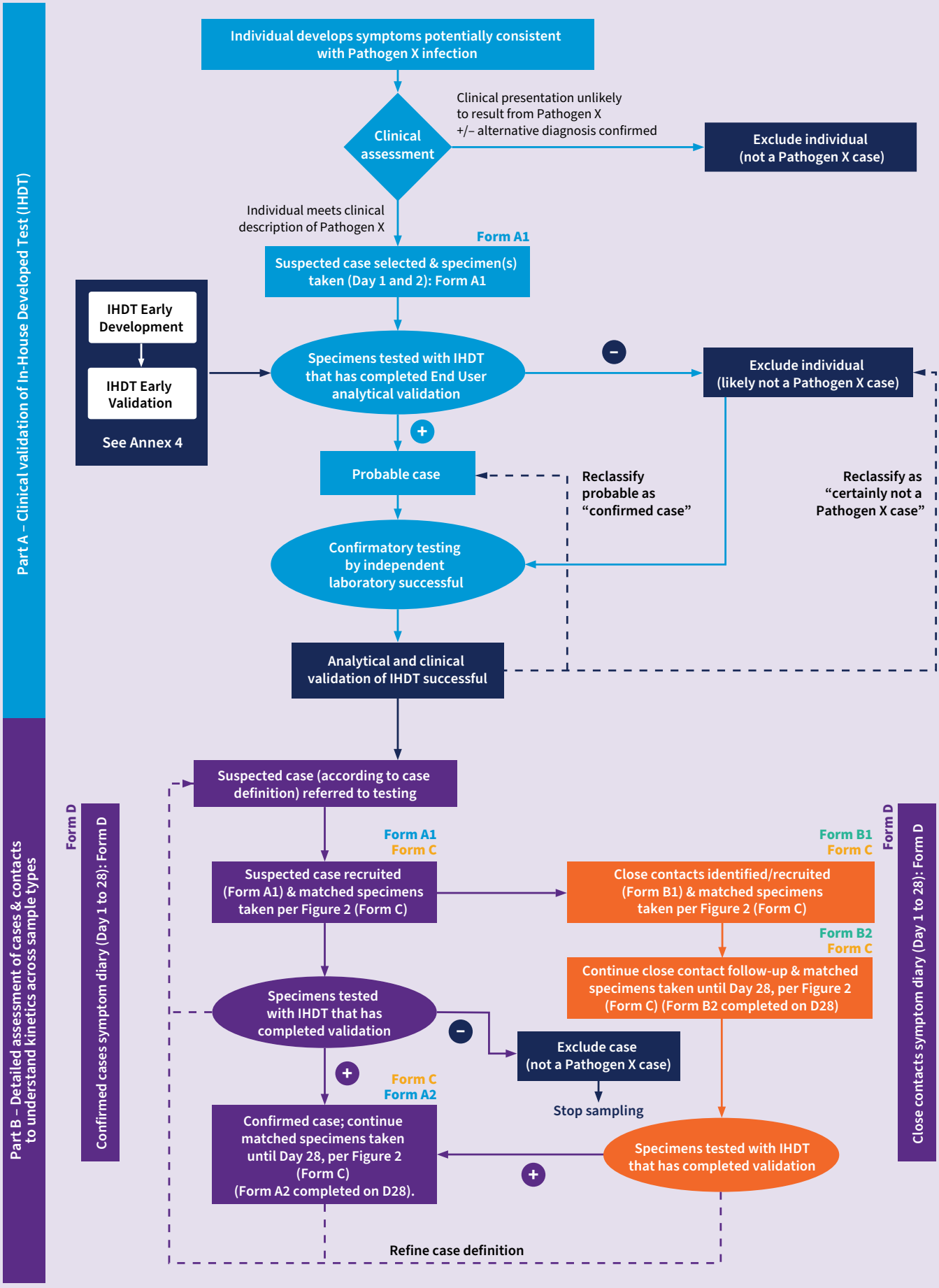
Sequencing should be performed from the priority sample type (e.g., NP).

Urine and stool collected as per Figure 2. For contacts and children, overall sampling is reduced (Figure 2).

Symptom diaries will be kept for each suspected and confirmed case (Part B only) from the day of enrolment (Day 1) through Day 28 to assist in refinement/optimization of the early case definition and to clarify the association of clinical symptoms with sampling site yield. Contacts will also complete symptom diaries. Epidemiological data will be gathered to assist in identifying close contacts and potential transmission modes.

All samples (Part A and Part B) will be collected and handled using best practice procedures (see Annex 3 for details) and aliquoted prior to freezing to facilitate confirmatory testing (Part A only) and provide resources for test validation in other laboratories.

Figure 1: Case investigation algorithm and summary of data-collection tools



1. Background and objectives

1.1 Background to Pandemic Investigations and Studies

Following a review of the global response to the last influenza pandemic (2009 influenza pandemic H1N1) *(4)*, the global Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) *(1)*, and World Health Organization's (WHO) Influenza Pandemic Special Investigations and Studies initiative were established to develop a suite of standardized early investigation protocols, supported by the Global Influenza Programme *(5)* and the Pandemic Influenza Preparedness (PIP) Framework *(6)*. Standardized protocols were also implemented following the emergence of Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 *(3)* and Zika virus in 2016 *(7)*.

In January 2020, the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the coronavirus disease 2019 (COVID-19), was declared a public health emergency of international concern by WHO. This required rapid implementation of early investigations to inform appropriate national and global public health actions. Adaptation of existing protocols was initiated from the first weeks of the detection onwards and further developed under the WHO initiative, Unity Studies *(2)*. The Unity Studies protocols standardized methods and facilitated rapid generation of local data for public health action and comparison of key epidemiological parameters across regions and globally *(2, 8–10)*. However, early diagnostic test development and use in many parts of the world were relatively uncoordinated, limiting efforts to prevent disease transmission and synthesis of diagnostic data to guide test utilization. In some cases, early test development and validation were expeditious and well-coordinated, based on preparedness research over many years prior. Nevertheless, there was substantial delay in defining pathogen kinetics, clinical presentations, optimal sample types for testing, and testing timing.

Based on the learnings from the COVID-19 response, a series of standardized template protocols have been developed to support the implementation of standardized, high-quality investigations (both disease-specific, e.g., influenza, and for any novel respiratory pathogen of pandemic potential) to ensure readiness for a future pandemic. These have been developed as part of the WHO Investigations and Studies, Unity Studies initiative. The initiative aims to provide an 'at the ready' international framework for preparedness and response to future pandemics, providing a suite of enhanced surveillance and investigational activities that are harmonized to help provide not only diagnostic development and use guidance but also detailed insight into the epidemiological characteristics of (re-) emerging respiratory pathogens of pandemic potential. They are included in both the Global Influenza Strategy *(11)* [pillar 6: "Number of sites (or geographical coverage) primed to conduct at least one of the WHO Pandemic Influenza Special Investigations in case of a pandemic with target for 2023: At least 2 operationally ready sites in each WHO region (so 12 sites)"] and the third version of the High-Level Implementation Plan (HLIP III) for pandemic preparedness (PIP) *(12)* for the 2024-2030 period under Output Indicator 2.11: "Number of sites participating in the WHO Investigations and studies network (Unity Studies)".

Investigations and studies are also included in the Mosaic Respiratory Surveillance Framework *(13)*. The Mosaic framework presents respiratory virus surveillance systems in a collaborative context where they are each focused on the objectives to which they are best suited. As it is impossible to address the many complex needs of respiratory virus surveillance with a single system, multiple fit-for-purpose surveillance approaches and complementary investigations must fit together as tiles in a "mosaic". Only together will these approaches provide a complete picture of respiratory virus infections and the impact of associated illnesses and interventions at the country level *(13).* Discrete studies and early investigations like the Unity Studies can address certain public health objectives that are not efficiently met by existing systems, such as to rapidly assess transmissibility, estimate population susceptibility/ immunity and infection severity, aid identification of population groups in need to target interventions, and estimate burden of disease and vaccine effectiveness *(13)*. **The FFX-Dx protocol aims to support outbreak response and prevent an early outbreak from turning into a pandemic by providing a clear pathway to validate a new In-House Developed test (IHDT) for molecular detection of Pathogen X and to define Pathogen X kinetics, highest-yield sample types, and timing for diagnostic testing in the First Few X cases and contacts (FFX).**

The detection and spread of a respiratory Pathogen X with pandemic potential is accompanied by scientific uncertainty with regards to optimal methods for detection of infection, epidemiological and serological characteristics, transmissibility (i.e., ability to spread in a population), and virulence (i.e., severity). Looking ahead, leveraging smart diagnostic and surveillance approaches through protocol implementation and standardization will be key to prepare for future potential pan-respiratory pathogen threats *(14)*. These types of enhanced diagnostic and surveillance investigations can be used to inform public health responses to respiratory pathogens of pandemic potential. Pathogen X might be a novel pathogen (e.g., SARS-CoV-2 in late 2019 or a novel drug-resistant bacterial pathogen) or a re-emerging existing pathogen (e.g., novel strains of influenza).

1.2 Introduction to FFX studies and their approach

As with many novel respiratory pathogens, key diagnostic, epidemiological, clinical, and virological (if applicable, i.e., if Pathogen X is a virus) parameters and the outbreak dynamics are unknown at the beginning. This is the situation for Pathogen X, first detected in [Country Y] in [mm/yyyy].

At this stage, key questions exist around the case definition and the optimal sampling and testing strategies for diagnosis, screening, and scaled use. As a result, it is essential to immediately develop and validate a working diagnostic test, use it to characterize the kinetics of Pathogen X, and define optimal diagnostic sample types as well as timing of sample collection in the first few X cases and their close contacts (FFX-Dx protocol) to guide diagnostic use and further diagnostic development. It is also important to begin to

understand the epidemiological, clinical, and virological characteristics of the first cases of Pathogen X and their close contacts in order to inform targeted guidance and measures for the [Country Y] public health response. As such, the FFX-Dx protocol will help inform the design of subsequent FFX protocols.

Each country may need to tailor some aspects of this protocol to align with local public health, laboratory, and clinical systems according to their capacity and availability of resources, as well as the cultural appropriateness of the protocol. The protocol should also align with country plans for clinical and public health management, including infection prevention and control measures for health workers, laboratorians, and the cases and contacts. Different jurisdictions may have alternative rules and requirements for IHDT validation and clinical use.

Toolkit item

The timely and standardized implementation of this protocol should be supported by a toolkit developed by WHO and implementing partners. The toolkit will comprise components to support different elements of the protocol. These will be highlighted throughout the protocol and discussed in Section 5. When available, Toolkit components will be available on the WHO website.

Toolkit items to support this section may include:

- Terms of Reference for Unity Studies network sites.

See additional 'Toolkit item' boxes throughout this document.

This **FFX-Dx protocol** outlines the components of an End User Validation of the new IHDT for molecular detection of Pathogen X and the process for rapid data and sample collection from early cases of Disease X and their close contacts to guide diagnostic test development and use, as well as helping to refine an early case definition and exploring information on transmissibility and severity. This protocol **should be the first investigation conducted for Pathogen X.**

FFX investigations are resource intensive and have been designed for maximal yield of information from the smallest number of participants. The FFX-Dx protocol is expected to be followed by the three Unity Studies transmission and severity protocols (of the three, the FFX protocol would likely be the first to be executed) to produce additional setting-specific estimates of key transmissibility and severity parameters including the effect of interventions in reducing the risk of infection, the risk of secondary infection, and the asymptomatic fraction.

The table in Annex 1 summarizes the different features and complementarity of the four protocols. All protocols are available on the WHO website.

1.3 Objectives

By using the standardized protocol described here, an early test for Pathogen X can be efficiently validated and implemented by the End User, and biological samples can be systematically collected and tested to assess Pathogen X kinetics and highest-yield testing approaches to guide further test development and use. Clinical and testing data generated can also guide refinement of an early case definition and clarification/confirmation of asymptomatic and pre-symptomatic infection. Samples and data can then be shared rapidly across many different settings globally. The FFX-Dx study will thus inform public health responses and policy decisions as well as facilitate subsequent implementation of the Unity Studies transmission and severity protocols to provide timely estimates of the infection-severity and transmissibility of Pathogen X infection.

The overall objective of the FFX-Dx study is to perform an End User Validation of a molecular In-House Developed test (IHDT; assumed for this protocol to be an RT-PCR/PCR test) for Pathogen X followed by characterization of early Pathogen X kinetics and optimal diagnostic sampling strategies through serial collection of matched samples in the population of initial cases of Pathogen X and their close contacts. Results will guide subsequent test development and use for clinical and public health goals.

Primary objectives include:

- Part A: End User Validation (Focused analytical and limited clinical validation) of the initial molecular test (In-House Developed test, or IHDT) developed for Pathogen X in focused sample types (NP swab for upper respiratory presentation; NP swab and BAL or sputum for lower respiratory infection).
- Part B: quantification of Pathogen X using IHDT (by cycle threshold values or, optimally, pathogen concentration measurements) in matched samples collected serially over the course of infection and post-exposure to assess early Pathogen X kinetics (peak) and optimal sample types for diagnostic testing, including samples relevant to point-of-care and self-testing. Testing of matched samples will also provide validation data for ongoing testing of alternative sample types.
- For all cases, gather data on the clinical presentation and course of associated disease to optimize/refine the clinical case definition for Disease X.
- For contacts (Part B only), attempt to detect and quantify Pathogen X in asymptomatic or pre-symptomatic infection.

Secondary objectives include estimation of the following epidemiological and Pathogen X parameters (epidemiological parameters to be confirmed in the subsequent FFX protocol):

- the duration of shedding of Pathogen X;
- the symptomatic and asymptomatic proportions of cases;
- the serial interval;
- the incubation period;
- the generation time;
- correlation of Ct values and/or Pathogen X nucleic acid concentrations with culture to inform isolation practices;

- correlation of Pathogen X antigen concentration with Pathogen X nucleic acid concentration (as soon as a test for quantitative detection of Pathogen X antigen is available).

Exploratory objectives (to be evaluated further in the subsequent FFX protocol) include:

- the secondary infection rate (SIR) and secondary clinical attack rate (SCAR) overall, and by key factors such as setting, age, and sex;
- possible routes of transmission including possible animal-human transmission;
- preliminary case- (i.e., disease) hospitalization and fatality ratios, and infection-hospitalization and fatality ratio.

For definitions of the key epidemiological terms used in the secondary and exploratory objectives, please see the FFX-protocol, which is meant to follow the FFX-Dx protocol. These epidemiological secondary and exploratory objectives will be evaluated further in the subsequent FFX protocol.

1.4 Overview of methodology

Section 2 of this protocol will describe in detail the methodology for this investigation including the End User Validation, case/contact/participant definitions, clinical study design, start and duration of the investigation, data collection, data management, specimen collection and transport, laboratory testing, and ethical considerations.

Coordination of investigations and sharing of information from the FFX-Dx protocol in real-time will be needed at both country and global levels. Epidemiologists, modelers, virologists, statisticians, clinicians, and public health experts must all assist in developing early estimates of key clinical, epidemiological, and virological parameters of Pathogen X. Table 1 shows the roles and responsibilities involved for [Country Y].

Table 1: Coordination matrix of roles and responsibilities in [Country Y]

What?	Who?
Overall coordination of the investigation	[Cite institution/body/person(s)]
End User Validation	[Cite institution/body/person(s)]
Case detection and investigation	[Cite institution/body/person(s)]
Contact identification and follow-up	[Cite institution/body/person(s)]
Analysis of data	[Cite institution/body/person(s)]
Data management	[Cite institution/body/person(s)]
IT management	[Cite institution/body/person(s)]
[add more roles, as per country context]	[Cite institution/body/person(s)]

The FFX-Dx system will be maintained centrally by [cite institution/body/person(s)]. Centralized coordination will require development of a "command and control" plan, to allow for prioritization of investigations.

Undertaking this kind of enhanced investigation will require resources and implementation plans to be developed in advance.

Toolkit item

Toolkit items to consider developing to support this section may include:

- Funding guidance
- How to access support
- Project management/budgeting guidance

When available Toolkit items developed by WHO and partners will be available on the WHO website.

2. Methods

2.1 Summary of studies

Part A of the FFX-Dx Protocol consists of an End User focused analytical and limited clinical validation of the In-House Developed test (IHDT), including a small prospective clinical investigation involving suspected cases of Pathogen X. Part B includes prospective enrolment of suspected cases and a subset of identified close contacts, including infants and children, that had the longest exposure to the case. Part B utilizes serial collection of matched samples in confirmed cases and their contacts to characterize early Pathogen X kinetics and define optimal diagnostic sampling strategies.

2.2 Part A: End User Validation of IHDT for Pathogen X

The **End User Validation** of the IHDT is a focused validation **to be performed by the lab implementing the IHDT for clinical testing**. **Note that performance of an End User Validation assumes that a fully completed and successful Early Validation of the IHDT (Annex 4) was already performed by the IHDT Developer.** Note: if the IHDT Developer is also the End User, only the limited clinical validation (which includes confirmatory testing by an independent laboratory) and stability testing below need be performed.

Note: optimally, the End User laboratory will receive assay reagents (primers and probes) and Pathogen X control material (including quantified target nucleic acid needed for LOD determination) from the IHDT Developer, with assistance for reagent development and transfer potentially provided by WHO. If this is not feasible, the End User laboratory can generate their own reagents/control material with guidance from the IHDT Developer.

A. Focused analytical validation

1. Determine the Limit of Detection (LOD), preferably using the same quantified material used in the developing laboratory.

Note that NP swab matrix is the top priority for LOD determination; subsequently, sputum or BAL matrix can be validated.

NP swab: If the End User has access to the same quantified material used by the IHDT Developer for LOD determination in NP swab matrix, an abbreviated LOD confirmation can be performed, as follows:

Perform a targeted 2- or 3-fold dilution series in pooled NP swab matrix, spanning the expected LOD and including at least one concentration below the LOD, with each concentration tested in triplicate. If 3/3 samples at the LOD are detected, the LOD

is considered confirmed. If this testing fails to confirm the LOD, the LOD must be re-established (see Annex 4).

If the End User does not have access to the same quantified material used by the IHDT Developer for LOD determination in NP swab matrix, the conservative approach is to fully re-establish the LOD (see Annex 4). However, if the dilution series above (range finding) indicates that the preliminary LOD matches the expected value, it may be reasonable to perform fewer replicates at dilutions above and below the preliminary LOD during confirmatory testing.

A major discrepancy between LODs is defined as >3-fold above or below the expected value. If major discrepancies with the LOD obtained by the IHDT Developer are observed after an attempt to re-establish the LOD, this should be discussed with the developing laboratory.

Sputum: Note that the LOD in sputum matrix must be established separately from the LOD in NP swab matrix. If sputum LOD has not yet been established by the IHDT Developer, a full LOD determination in pooled sputum should be performed by the End User (see Annex 4). If sputum LOD has already been established by the IHDT Developer, the process for LOD confirmation/determination will depend on whether the same quantified material used by the IHDT Developer is available to the End User, or not (as above).

Note: validation of sputum also covers the BAL sample type; it is not necessary to also determine LOD in BAL matrix.

2. Perform a small performance evaluation:

Positive: n = 10 contrived samples (10 NP or 5 NP/5 sputum) created by spiking control material (inactivated/quantified target nucleic acid, or isolate) into individual clinical specimens (target matrix), ideally at no more than 3x LOD. The clinical specimens used for spiking can be the negative samples below.

Negative: n = 10 negative samples (10 NP or 5 NP/5 sputum) from individual patients who predate Pathogen X (alternative: collect prospectively from patients who have no known connection to patients with Pathogen X).

In all cases appropriate ethical approval as needed must have been obtained for use of these samples for the intended purposes.

These positive and negative samples should be combined as a set of 20 samples and tested blindly.

Acceptability criteria: 95% agreement with expected results (19/20)

Note: the focused analytical validation above is sufficient even if the End User laboratory is using different platforms for extraction and amplification than those used by the IHDT Developer lab (Annex 4).

B. Limited clinical validation

After completing the focused analytical validation of the IHDT in Section A, above, the End User laboratory can initiate clinical testing of patients suspected to be infected with Pathogen X, with results considered "presumptive" and cases considered "probable". However, in order to complete the validation and allow patients with positive IHDT results to be considered confirmed cases, confirmatory testing should be performed by an independent laboratory, as follows: The End User lab should send at a minimum the first 10 IHDT-positive and 10 IHDT-negative patient specimens to either the IHDT Developer laboratory or to another End User laboratory that has successfully performed the focused analytical validation above (Note: if more than one IHDT is available in the area, confirmation with an alternative assay is also helpful). **During the window prior to completion of confirmatory testing, IHDT-positive cases are considered probable cases within the FFX-Dx protocol. After the confirmatory testing has been successfully completed, IHDT-positive cases are considered confirmed within the FFX-Dx protocol.**

Note: a confirmed case from Part A can be included in FFX-Dx protocol Part B (see Section 2.10).

Note: if the window prior to confirmatory testing is expected to be prolonged, a consensus decision can be made in collaboration with public health partners for probable cases to proceed to Part B; see Section 2.4. This should consider trade-offs between the extensive sampling in Part B and the timeline, presumed number of cases, and presumed associated morbidity and mortality of Pathogen X.

Acceptability criteria: 100% qualitative agreement between laboratories (any discordant results should be fully investigated by both laboratories, including having both laboratories retest the specimen, and will ultimately be arbitrated by the director of the End User Laboratory).

Note: the End User laboratory should follow national regulations, as applicable, for considering the test acceptable for clinical use.

C. Sample stability

Testing should be conducted to demonstrate sample stability throughout the real-world conditions in which samples are to be collected, tested, and stored by the End User, using actual clinical samples. If the IHDT Developer has already performed stability studies with actual positive clinical specimens, only new conditions relevant to the End User sample

handling need to be tested by the End User (e.g., different transport conditions). If initial stability studies performed by the IHDT Developer utilized contrived samples, the End User can initiate testing utilizing that stability data but would optimally repeat the stability studies with actual clinical samples once available.

Fresh versus frozen studies should be performed in preparation for the clinical study (Part B).

Minimum conditions:
One of the following: Saline, VTM/UTM (as relevant to the laboratory),
RT (in area of use), 4°C, –20°C, –80°C (as relevant to the laboratory),
Time (consider transport time to laboratory and consider extended delays)
Minimum # of replicates at each condition: n=3
Expectation: </= 3 Ct change between baseline and other condition (e.g., time 0 vs time X, fresh vs frozen)

D. Analytical validation of alternative specimen type samples collected in the FFX-Dx protocol

Note: NP, AN, and OP swabs will be collected in transport media (see Section 2.7.1).
AN swab: No additional analytical validation needed (NP swab validation sufficient).
OP swab: No additional analytical validation needed (NP swab validation sufficient).

Validation of stool, urine, saliva, and/or conjunctival samples will require focused analytical validation in the specific matrix (see A1 and A2). The requirement for full LOD determination versus abbreviated LOD confirmation will again depend on what work has been done by the Developer.

2.3 Definitions used in the clinical parts of the FFX-Dx protocol

2.3.1 Case definitions

Case definitions are necessary to standardize the identification of cases. A case definition should **outline all criteria determining how an individual is identified as a case**.

For Pathogen X, cases must be reported as *Confirmed, Probable,* or *Suspected* (Box 1) cases based on a series of epidemiological, clinical, and laboratory criteria to ensure data specificity and comparability.

A summary of relevant definitions that will be used to form the case definitions *(15)* for Pathogen X are included in Box 1.

Box 1. Case definitions in the FFX-Dx protocol

Confirmed case:

A person with laboratory confirmation [positive result from the molecular IHDT that has successfully completed the full End User Validation testing in FFX-Dx Part A] of Pathogen X infection, irrespective of meeting the clinical description (i.e., irrespective of clinical signs and symptoms).

Probable case:

A person meeting the clinical description and having a presumptive positive IHDT result [i.e., the analytical portion of the IHDT End User Validation has been completed but the limited clinical validation (requiring confirmatory testing) has not yet been completed].

Suspected case:

A person meeting the clinical description, with no laboratory confirmation or testing results.

A Clinical Description is a description of the illness – signs, symptoms (e.g., cough, sore throat, fever), severity, and individual characteristics where relevant (e.g., international travel history, contact with animals) – associated with the outbreak.

COMMENT: In the early stages of an outbreak of Pathogen X, the appropriate identification and enrolment of index cases contributes to the internal validity of the FFX-Dx and Unity Studies transmission and severity studies *(16)*.

The case definitions for Pathogen X reporting will be published on the WHO website when available.

In Part A of the FFX-Dx protocol, individuals with a clinically compatible presentation will be considered to be “suspected”; a positive IHDT result in this individual should be considered “presumptive” and the individual with that positive result a “probable case”. For Part B, the IHDT will have passed the full End User Validation, and a laboratory-confirmed case will be considered “confirmed”.

These definitions will be subject to change as more information becomes available during the course of the investigative response and additional diagnostics become available. For instance, the case definition may be adapted to incorporate point of care or rapid antigen tests with proven sufficient sensitivity and specificity as they become available.

2.3.2 Contact definitions

Contacts are defined as all individuals who are associated with some sphere of activity of the case and may have similar, or other, exposures as the case. Contacts can include household members, social or health workers, other family contacts, visitors, neighbours, colleagues and co-workers, teachers, classmates, and members of a social group.

As with case definitions, contact definitions for Pathogen X will be available on the WHO website. These definitions will be subject to change as more information becomes available about Pathogen X.

FFX-Dx Part B investigations focus on **close contacts (in addition to cases)**, which are a subset of contacts (3 per case) who have had the highest level of interaction with the case with regards to proximity to the case and duration of contact. Identification of these close contacts should be done in collaboration with public health teams.

The definition of close contact should consider:

- plausible modes of transmission (e.g., airborne/inhalation, direct deposition, direct/indirect contact)
- duration of contact
- setting of contact (i.e., indoor or outdoor)
- plausible infectiousness of Pathogen X relative to symptom onset
- the period of time from symptom onset in an index case (see 2.3.1) to testing, notification, and recruitment into the FFX-Dx investigation

For the purpose of this protocol, interim and generic **close contact** definitions and classification for Pathogen X are proposed in Box 2. Note: should FFX-Dx Part B cases become part of the FFX protocol, all remaining contacts for each case will be enrolled in the FFX protocol.

COMMENT: Implementing countries may wish to consider more detailed definitions of close contacts if appropriate. Note also that these definitions are context dependent and may change over time.

Box 2. Contact definitions in the FFX-Dx protocol

Contact:

Any person who has had contact *(17)*:

- with a symptomatic case during their symptomatic period, 2 days before symptom onset, and in the 10 days after the onset of symptoms, or;
- with a confirmed asymptomatic case, including 2 days before and the 10 days after the date on which the sample was taken which led to confirmation.

Classification of close contacts (for use in contact questionnaires):

- **Health worker contact:** Any social or health worker* who provided direct or indirect personal or clinical care, handling specimens from, or examination of a case of Pathogen X, **OR** who was within the same indoor space when an aerosol-generating procedure was implemented, **AND** who were not wearing recommended personal protective equipment (PPE)^ at the time or with a possible breach of PPE.

  * Health workers at risk of infection with Pathogen X include ambulance staff, reception staff, health assistants, nurses, doctors, laboratory workers, and cleaners.

  ^ Full PPE is defined as correctly fitted high filtration mask (FFP2), gown, gloves, and eye protection.

- **Household contact:** Any person who resides or resided in the same household† as the Pathogen X case**

  † Household is defined as a group of people (2 or more) living in the same residence. In practice, the technical definition may vary due to social, political, and cultural practices *(18)*. Definitions may be (but are not limited to):

  - Two or more people living together in a domestic residence (residential institutions, such as boarding schools, dormitories, hostels, or prisons will be excluded).
  - One possible generic definition of a household is a dwelling or group of dwellings with a shared kitchen or common opening onto a shared household space.

  **Household contact definitions may vary and implementing countries may wish to consider more detailed definitions if appropriate (see below).

  A household contact may also be:

  - a person who commonly resides in the same household as the case
  - any person who had resided in the same household as the case for at least one night during the exposure period (two days before to 10 days after onset of illness in the primary case)

- **Other close contacts:** Any person who has had contact (within 2 meters and for more than 5 minutes):
  - with a symptomatic case during their symptomatic period, 2 days before symptom onset, and in the 10 days after the onset of symptoms, or;
  - with a confirmed asymptomatic case, including 2 days before and the 10 days after the date on which the sample was taken which led to confirmation.

Comment: It is important that the definitions are consistently applied throughout the investigation and well detailed in any reporting of the investigation for the purposes of comparability between investigations.

Comment: Note that restricting follow-up to a subset of contacts may limit detecting infections for all possible routes of transmission.

2.3.3 Classification of FFX-Dx participants

During the course of the FFX-Dx investigation, transmission events associated with an index case will be observed (or inferred) through laboratory testing and symptom monitoring of close contacts. These observations will help identify the chains of transmission within clusters and allow for final classification of all participants, as described in Box 3 below.

Box 3. Classification of FFX-Dx participants

A. Index case: The first case of Pathogen X identified within a cluster according to the case definition (see Box 1). It is the identification of the index case that leads to recruitment into the study*.

B. Primary case: An individual who has the first evidence of infection/disease within the cluster (See Box 1), i.e., the case with the earliest symptom onset date and/or positive IHDT result within a cluster.

C. Co-primary case(s): Of the first cases identified within a cluster, two or more cases identified within the same 24-hour period are considered to be **"co-primary" cases**.

COMMENT: Cases in A), B) and C) must also be classified further as *confirmed*, *probable*, or *suspected* cases according to Box 1. The relevant clinical and laboratory criteria for Pathogen X must be added into the definitions above when available.

* In some instances, the index case is also the primary case but this is not always the case. Following investigation, index cases may be classified as a primary, co-primary, or secondary case *(19)*.

D. Imported case: An index, primary, or co-primary case with a history of travel from an affected area [define "affected area"] in the 14 days before disease onset.

E. Secondary cases: Close contacts meeting the case definition between 24 hours to 28 days after the positive IHDT result or symptom onset of the primary and/or co-primary case(s) if direct transmission from the primary or co-primary case is considered likely.

Note: close contacts meeting the case definition are not necessarily a secondary case. Depending on the chains of transmission, an individual meeting these criteria may also be an unrelated case (see F below). It is important to consider the timing of exposure and virological characteristics (if appliable) of Pathogen X when classifying close contacts as secondary cases. Testing protocols, symptom information, and genomic sequencing data may help to distinguish between secondary cases and unrelated cases.

COMMENT: Cases in E), may also be classified as confirmed, probable, or suspected secondary cases according to Box 1. The relevant clinical and laboratory criteria for Pathogen X must be added into the definitions above.

F. Unrelated case: Includes other cases for the purposes of the FFX-Dx investigation such as tertiary cases (those with evidence of being infected by a secondary case) and cases infected from other external sources (i.e., not the primary case)

2.4 Design, duration, and population of interest (Part A, Part B)

2.4.1 Study design and sample size

After completion of the End User focused analytical validation work in Part A of this protocol, the conclusion of Part A will include a small clinical validation including at least 10 probable cases of Pathogen X, i.e., participants meeting the clinical case definition and having a positive (at this stage, presumptive) IHDT result. After completion of the confirmatory testing (by another laboratory) of 10 IHDT+ and 10 IHDT- clinical samples, individuals meeting the clinical case definition and with positive IHDT results can now be considered confirmed cases. The number of participants (suspected cases) required to yield 10 IHDT+ individuals will depend on the prevalence of disease X and the specificity of the clinical case definition at the time.

Part B will also be prospective and include cases confirmed with the IHDT (that has completed the limited clinical validation in Part A) and for each case, 3 of their identified close contacts. The required sample size in adults depends on the accuracy of the IHDT, which will be informed by the Part A evaluation. A sample size should be chosen that allows for a 95% confidence interval with a lower bound of ideally ≥90% when considering positive agreement with the matched NP swab (e.g., 60 confirmed adult cases satisfy this criterion if the point estimate is an agreement of ≥98.3%, i.e., 59 or 60 confirmed out of 60; Table 2). For calculation of agreement, we will consider the denominator positive (i.e., the individual) if at least one sample of a validated matched sample type is positive, and then calculate the percentage positive for each matched sample type at a specific time point. If, e.g., only NP has been validated, we will calculate i) the percentage of individuals positive by alternative sample over those positive by the NP specimen; ii) the percentage of individuals negative by alternative sample over those negative by the NP specimen; iii) the percentage of dual positive (percentage of individuals positive for both the alternative specimen and NP specimen); and iv) the percentage of dual negative. In addition, efforts should be made to enroll enough infants and children (ideally at least 30), if possible. Given the complexity of serial sampling, a higher sample size in children, while considered optimal, is unlikely feasible.

For each suspected case, 3 of their identified close contacts will be enrolled. As incidence is expected to be low in this early phase of the outbreak, it is expected that many suspected cases will be IHDT test negative.

Note: a person can only participate in Part B as a case and/or contact once.

Table 2: Sample size considerations

Agreement at enrolment

N = 30		
Positives	Agreement	95% CI
26/30	86,67%	69%, 97%
27/30	90%	73%, 98%
28/30	93,30%	77%, 100%
29/30	96,67%	82%, 100%
30/30	100%	88%, 100%

N = 50		
Positives	Agreement	95% CI
46/50	92%	80%, 98%
47/50	94%	83%, 99%
48/50	96%	86%, 100%
49/50	98%	89%, 100%
50/50	100%	92%, 100%

N = 60		
Positives	Agreement	95% CI
56/60	93,30%	83%, 99%
57/60	95%	86%, 99%
58/60	96,67%	88%, 100%
59/60	98,33%	91%, 100%
60/60	100%	94%, 100%

MEDIUM BLUE BOXES Acceptable criteria reached (lower-bound of 95% CI ≥ 80%)

LIGHT GREEN BOXES Desirable criteria reached (lower-bound of 95% CI ≥ 90%)

Note: the sample size calculation does not consider the correlation between two samples from the same patient.

2.4.2 Timing of the study

This FFX-Dx investigation should be established as soon as possible after the identification of the first cases of Pathogen X infection in [Country Y]. It is intended to be conducted before widespread community transmission occurs, that is, within the early phases of the Pathogen X epidemic.

2.4.3 Population of interest and study representativeness

The study population is the first few cases of Pathogen X and their close contacts in [Country Y]. Index cases will be identified through national line listings/initial case register or other relevant surveillance systems (dependent on country).

However, it is important to note that the early cases are unlikely to be broadly representative of the general population, as identified cases are likely to be persons with more severe disease that present for healthcare and are more easily detected by a surveillance system. Furthermore, early cases need to satisfy the clinical case definition including the presence of particular exposures (such as international travel, contact with another case) and this may exclude other cases if the pathogen is already circulating in [Country Y].

In a situation where there are more eligible index cases than can be recruited due to capacity constraints, recruitment should be consecutive to reduce the potential for ascertainment biases. It is critical that recruitment complexity does not delay the execution of the FFX-Dx protocol.

A **screening log** should be used to collect basic information on recruited and non-recruited cases to understand and describe the representativeness of the FFX-Dx sample.

2.5 Eligibility criteria of cases for the study

Inclusion criteria:

- The **first few cases of pandemic Pathogen X in** [Country Y] should be enrolled according to the case definitions provided in Box 1 (suspected for Part A and Part B).
- Participating individuals give appropriate informed consent (see Section 2.10).
- At least 7 days of follow-up is expected to be feasible.

Exclusion criteria:

- No consent.
- Medical reason that prohibits sampling scheme (e.g., excessive nose bleeds, recent nasal or brain surgery).
- If a case tests positive (early in clinical course) for other respiratory pathogen(s) that can explain symptoms (e.g., influenza).

2.6 Data collection

This FFX-Dx protocol calls for the recruitment and follow-up of index cases and their close contacts. Note that for the purpose of data collection, Day 1 will be the day of recruitment. For analysis to ensure alignment between cases, Day 0 will be standardized to the day of symptom onset (cases) or presumed contact (contacts; if multiple or ongoing exposures it would the day of first exposure).

Epidemiological, clinical, and virological data will be collected from each participant in Part B at multiple times during the study – including demographic and epidemiological survey (**Forms A1/A2** and **B1/B2**) at baseline and Day 28, symptom diaries from Days 1-28, and specimen collection (as per Section 2.6 and Figure 2). This information can be obtained through a combination of methods, including face-to-face or telephone interviews/consults with participants (or family members if the case is too ill to be interviewed), self-reporting, interview of health workers and/or review of medical records, and professional/medical specimen collection services. After Day 28, any additional follow up needed would be the responsibility of local medical authorities.

Toolkit item

Toolkit items to consider developing to support this section may include:

- Data dictionaries
- Data quality checklist
- Study team training template

When available, Toolkit items developed by WHO and partners will be available on the WHO website.

2.6.1 Clinical and epidemiological data collection

A summary of the data and specimen collection forms can be found in Table 3 and Figure 2.

Part A

Suspected cases will be identified based on meeting the clinical case definition; **Form A1** will be completed; initial sample(s) will be collected on Day 1 and Day 2. Participants who test presumptive positive by IHDT will be considered 'Probable cases'.

Part B

Form D – symptom diaries will be provided for all Part B cases and close contacts to complete (throughout Day 1 to Day 28), to record the presence or absence of various signs or symptoms. A proxy may fill out the symptom diaries on behalf of those unable to complete the form themselves.

Once a suspected case has been recruited into Part B of the investigation (Day 1), **Form A1** will be completed (if not already completed in Part A):

- The symptom diary (**Form D**) will be initiated.
- Sample(s) will be collected per Figure 2 (**Form C**) and the three close contacts will be identified and recruited into the study.

Note: a contact line list in collaboration with the public health department should be used to record close contacts identified at the time of first encounter (see Annex 2, Contact Line List).

Afterwards, IHDT testing will be performed on the suspected case:

- If the IHDT result for the NP swab (i.e., priority sample) from the suspected case is positive on either Day 1 or Day 2 (or, if BAL/sputum has also been validated by the

laboratory, BAL/sputum is positive on Day 1 or Day 2), the cases will be reclassified as confirmed cases and will continue with sample collection (per Figure 2) and symptom diaries through Day 28, before completing **Form A2 at Day 28**.
- If the validated samples taken on Day 1 and Day 2 are negative by IHDT, the patient will not be a case and will not continue with sampling.

If an alternate diagnosis is made, irrespective of the IHDT result or the suspected case having symptoms consistent with the clinical case definition, the participant will be excluded (per Section 2.5). However, individuals with IHDT+ results should still be managed as cases for clinical and infection control purposes, despite study exclusion.

Close contacts of the suspected case will complete **Form B1** once recruited to the study:

- The symptom diary (**Form D**) will be initiated.
- Samples will be collected per Figure 2 (**Form C**).

Note: sampling of the contacts might have to be initiated before a case is confirmed (dependent on turnaround time of test); if the suspected case is not confirmed by Day 1 or Day 2 testing, the contacts will stop sampling and samples will be discarded.

If a contact is subsequently confirmed to be a case before Day 28 (i.e., IHDT positive, irrespective of symptoms), they should:

- be sampled per confirmed cases in Figure 2, where the date of the sample taken that yielded the positive IHDT becomes Day 1 of their sampling/ participation as a case;
- continue to fill out **Form D**, and complete **Form B2** at Day 28 of their participation as a case.

Note: if a contact has symptoms at the time of enrolment **AND meets the clinical case criteria**, sampling of this participant should utilize strategy shown in Figure 2 per suspected cases (i.e., they should not be recruited as a contact, but rather as a suspected case). If the IHDT is negative, the participant will be excluded.

If the contact becomes symptomatic after enrolment (per the clinical case definition) but IHDT is negative, **Form D** should be continued, and the contact will continue to be sampled per the contact sampling strategy (Figure 2):

- **Form B2** will be completed at Day 28, if the IHDT remains negative.
- If an alternate diagnosis has been made, the participant will be excluded (per Section 2.5).

Note: NP samples (i.e., priority sample) from the contacts will be tested in real time to detect incident infection and exclude other pathogens (other samples will be tested as soon as possible).

Form C will be used for **all** specimen collection visits (throughout Day 1 to Day 28) for cases and close contacts. Each specimen will be accompanied by its designated **Form C**.

All **investigation questionnaires can be found in Annex 2** of this document. Note that the questionnaires may need to be adapted based on the local setting of implementation.

Table 3: Summary of data collection tools

Form number	Purpose of form	Collecting from whom?	When should it be collected?
Cases			
Form A1	Case **initial** report form	For suspected (Part A) or confirmed (Part B) Pathogen X cases	Day of recruitment into the FFX-Dx investigation **(Note: day of recruitment = Day 1)**
Form A2	Case **follow-up** form	For confirmed (Part B) Pathogen X cases: Final outcome	**28 days** after day 1
Contacts			
Form B1	Contact **initial** reporting form	For contacts of Pathogen X suspected/confirmed cases	Day of recruitment into the FFX-Dx investigation (ideally within 24 hours after identification of the index case) **(Note: day of recruitment = Day 1)**
Form B2	Contact **follow-up** form	For contacts of Pathogen X confirmed cases: Final outcome	**28 days** after day 1
Cases & contacts			
Form C	Track and summarize **all laboratory results (and methods used)**	For Pathogen X suspected/ probable/confirmed cases and contacts (Parts A and B)	Form C should be completed for **each specimen at each collection time point**
Form D: Symptom diary	Record the presence or absence of various signs or symptoms	For Pathogen X suspected/ confirmed cases and contacts (Part B only)	Between **Day 1 and 28**

2.7 Specimen collection and transport

The following section is intended to guide specimen collection from index cases and their close contacts. Careful planning should be done to ensure arrangements are in place for specimen collection, how to access required PPE, and how to arrange safe sample collection and transport of specimens. Sample collection and transport should follow national guidelines.

Toolkit item

Toolkit items to consider developing to support this section may include:

- Standard operating procedure for sample collection
- Advice for specimen types to be collected

When available, Toolkit items developed by WHO and partners will be available on the WHO website.

2.7.1 Sampling rationale

This section outlines the rationale for the collection of respiratory and serological specimens from cases and close contacts in Part B.

For the purpose of this protocol, the priority sample type is considered to be the most important sample type to be collected for detection of Pathogen X. This is the sample type in which presence of Pathogen X is most likely expected based on prior experience. This is most likely going to be a NP swab or BAL/sputum sample depending on the clinical presentation (i.e., upper or lower respiratory tract infection and clinical status). Priority samples from cases and contacts should be prioritized for testing in real-time. Other samples should be tested as soon as possible, and ideally in real-time as well if capacity allows. The collection of specimens from cases and close contacts will consider:

The **timing and frequency of sample collection (Figure 2):** Sampling of cases will occur for a minimum of 14 days, assuming a positive IHDT result on one of the first two consecutive priority samples. If a suspect participant is negative on the first two consecutive priority samples, then sampling for them and their contacts will be stopped, and the case will be considered not a Pathogen X case. Repeat negative testing in suspects is required to avoid exclusion based on clinical sampling or laboratory errors. After Day 14, sampling can be discontinued if the pathogen is no longer detectable for at least two consecutive priority samples. If Pathogen X is still detectable at Day 28, sampling should continue every third day until the pathogen is no longer detectable for at least two consecutive samples. At least two consecutive negative samples are necessary in this case to account for potential fluctuations in pathogen levels and avoid decisions based on laboratory errors.

For contacts of confirmed cases, more frequent sampling will occur for 10 days from day of enrolment given the most likely incubation time of a relevant pathogen, followed by less frequent sampling through Day 28.

Infants and children will have a less frequent sampling interval to reduce the burden of sampling.

The **types of respiratory specimens** collected will include priority samples (i.e., NP swab and BAL/sputum if an LRTI is present). More accessible sample types (AN swab, OP swab, saliva) will be collected more frequently. An additional NP swab should be collected to enable viral culture to be done at baseline (on site or in referral laboratory if feasible) to inform an understanding of culturability (and related transmissibility). Subsequently, additional NP swabs for culture are optional. If additional samples are collected, duration of sampling for viral culture should depend on Ct value trajectory over time. BAL (if feasible) will be done at baseline or at any other time if the case transitions to being ventilated.

For contacts, AN swab, OP swab, and saliva will be collected. NP swab will be collected at baseline and every other day after enrolment. NP samples are considered priority samples. If a contact becomes a confirmed case (whether symptomatic or asymptomatic), sampling from Day 1 onward as described for cases will start.

If a participant (case or contact) refuses NP swabs, testing should default to AN swab for both Ct value and culture.

For children, sampling will focus on AN and saliva given difficulty to collect NP swabs in children.

Non-respiratory samples: Blood will be collected in adult cases and contacts throughout the follow-up period. Blood collection could be for blood culture (e.g., bacterial pathogen) or serological response (any pathogen). For bacterial pathogens, intensified blood sampling for culture should be considered within the first three days of presentation. If serologic testing is not available at the time of the study, the samples should be stored for future testing. Stool should be collected within the first three days for all to have an additional means to detect possible co-primary cases.

For children 0–3 years of age stool should be collected every 3rd day thereafter as it is a more readily available sample type. Depending on the clinical syndrome or pathogen identified, urine may be collected. Unique clinical presentations (e.g., conjunctivitis) should also prompt consideration of alternative specimen types (conjunctival swab).

How the specimens will be collected: All samples will be collected and handled using best practice procedures (see Annex 3 for details) and aliquoted before freezing to allow sharing with other laboratories. All samples will be collected by professional specimen collection service / trained healthcare workers. For swab sampling, a flocked swab should be used if available. Should supply chain issues preclude using a flocked swab, then it should be guaranteed that at least the same swab is used throughout the study for sampling. If Pathogen X is a virus, the swab sample should be collected using UTM/VTM as transport media.

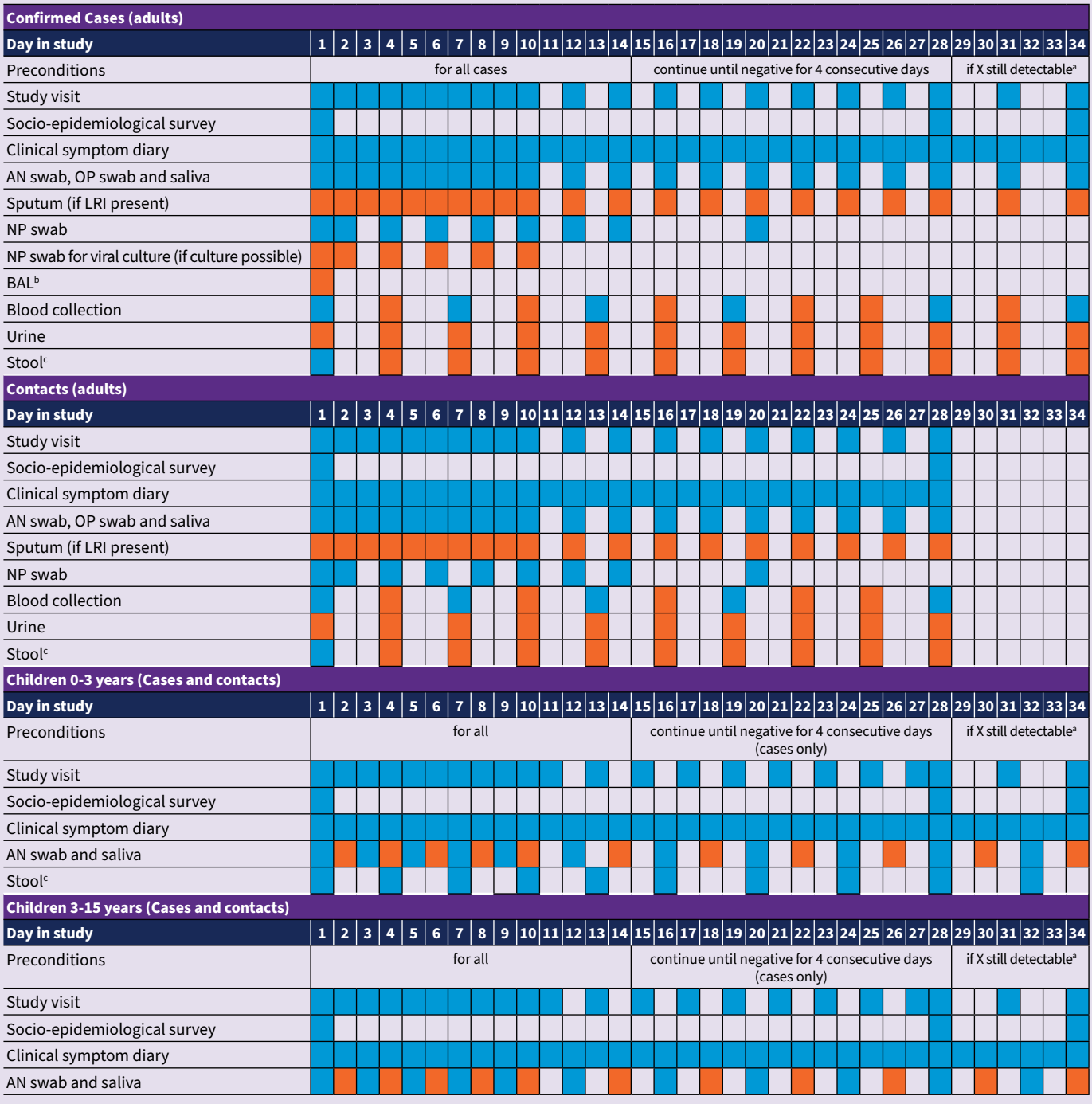
Matched samples will be collected at each visit to allow for comparisons to be made between the demonstrated yield of each sample type for detecting Pathogen X with the IHDT (Annex 3). Data from matched samples can also be utilized to validate alternative samples for ongoing clinical testing and ultimately for EUA (from any relevant regulatory authority).

How the specimens will be tested: If capacity is not available to test all samples in real-time, then focus should be on priority samples, particularly in contacts (to detect incident infection).

2.7.2 Sampling strategy, Part B

The sampling strategy for the investigation is presented in Figure 2.

Figure 2: Sampling strategy for cases and contacts in Part B



[a] continue sampling until pathogen is no longer detectable for at least 4 days or 2 consecutive samples.
[b] if transition to ventilation: collection at any alternative time point.
[c] collect sample within 3 days of the indicated time point.

Note: if the participant had a bronchoalveolar lavage (BAL) prior to study entry, this does not need to be repeated
NP, nasopharyngeal ; BAL, Bronchoalveolar lavage ; LRI, lower respiratory infection ; AN, anterior nares ; OP, oropharyngeal (throat).

BLUE BOXES indicate activities that are **desirable** for the investigation of Pathogen X.

ORANGE BOXES indicate where additional specimen types could be collected to increase the information available.

2.7.3 Specimen collection and transport

Appropriate PPE should be worn when specimens are being collected from index cases and all contacts; samples should be collected by professionals/health-care specimen collection services *(20)*. All those involved in collecting and transporting specimens should be trained in the safe handling of infectious substances and infectious spill decontamination procedures. For details regarding the collection and transport of samples, please refer to Annex 3 and the case management algorithm and laboratory guidance in the country, and to WHO laboratory guidance *(21)*.

For each biological sample collected, the time of collection, the conditions for transportation, and the time of arrival at the laboratory should be recorded. Specimens should be transported using cold chain (4°C) and reach the laboratory as soon as possible after collection. If the specimen is not likely to reach the laboratory and to be processed within 48 hours, it should be frozen, preferably at –80°C, and shipped on dry ice as per applicable WHO guidance *(21)*. It is, however, important to aliquot each sample, if possible, prior to freezing to avoid the deleterious effects of such handling on the pathogen's nucleic acid integrity (and infectivity, relevant for culture). The storage of specimens in domestic frost-free freezers should be avoided, owing to their wide temperature fluctuations. Further detail on specimen type, transportation, and storage is provided in Annex 3.

Transport of specimens within national borders should comply with applicable national regulations. International transport of specimens should follow applicable international regulations as described in the WHO Guidance on regulations for the transport of infectious substances 2021–2022 *(22)*.

2.8 Laboratory evaluations

2.8.1 Laboratory analysis

Details of the End User focused analytical and limited clinical validation of the IHDT are in Section 2.2. Following successful validation, clinical testing for Pathogen X using the IHDT will be performed per clinical laboratory routine. In order for the End User laboratory to test other specimen types than those initially validated (NP and/or BAL/sputum), an additional focused validation will need to be performed (Section 2.2).

For Part B, all collected samples will be tested with the analytically and clinically validated IHDT. If testing capacity is limited, only priority sample types will be tested in real time, and testing of contacts will be prioritized to detect incident infection (only results from validated sample types can be reported). In case of an invalid/indeterminate result, the test should be repeated if sample volume allows. Viral culture from NP samples is optional. If not possible within the End User laboratory, dedicated NP samples (see Figure 2) collected within the

first 10 days can be sent to a referral laboratory for viral culture. Similarly, whole genome sequencing of the pathogen is optional and could be done in a referral laboratory if not possible at the End User laboratory.

Toolkit item

Toolkit items to consider developing to support this section may include:

- Protocols/standard operating procedures

When available, Toolkit items developed by WHO and partners will be available on the WHO website.

2.9 Data management generalities

The FFX-Dx data system will be maintained centrally by [cite institution/body/person(s) in the country].

Toolkit item

Toolkit items to consider developing support this section may include:

- Data collection/entry guidance
- Data transfer
- Data quality checklist
- Data dictionaries
- Data cleaning
- Data security

When available, Toolkit items developed by WHO and partners will be available on the WHO website.

2.10 Ethical considerations

The FFx-Dx protocol aims to produce significant social value in the event of the emergence of a novel or re-emerging pathogen with pandemic potential. The likely social value in terms of public health benefits from these investigations includes the development of a reliable molecular diagnostic test enabling effective diagnosis and identification of cases, and informing public health practice regarding: (i) appropriate isolation and

quarantine policy, (ii) the use of less invasive testing strategies (e.g., nasal swabs or saliva for self-collection rather than nasopharyngeal sampling), (iii) other effective and ethically appropriate infection control measures. Other sources of social value include the potential for these investigations to produce important scientific benefits via the secondary and exploratory objectives of the investigations (e.g., regarding infection and transmission dynamics), and hence generate information to help formulate policy and guidelines for the public health response.

The FFX-Dx protocol is designed to involve collaboration between appropriate national health authorities (e.g. ministry of health), microbiology laboratories (public and/or private), research institutions, and international partners (e.g., via the Unity Studies initiative). The main output of FFX-Dx, i.e., a validated diagnostic test, will be shared to improve diagnostic capacity with local laboratories as well as with international partners.

National and local ethical requirements must be followed. Ethical approval should be sought as per individual country requirements since ethical requirements will vary by country. WHO Guidelines on Ethical Issues in Public Health Surveillance *(23)* specifies that informed consent is not ethically required for activities classified as surveillance where "reliable, valid, complete data sets are required" and "relevant protection[s]" are in place. Relevant protections include transparency, e.g., the FFX-Dx protocol are publicly available online via the WHO website, as well as appropriate data protections (discussed below).

In some countries, Part A of this investigation may fall under public health surveillance (emergency response) acts and may not require ethical approval from an institutional review board (IRB). For Part B a review by an IRB is likely needed. It will be upon national authorities to advise on the specific requirements for the investigation in [Country Y]. In all cases, it is essential to ensure that participants participate voluntarily and in appropriate situations following an explicit process of informed consent (see section 2.10.1).

Data sharing and data protection

The WHO Guidelines on Ethics Issues in Public Health Surveillance states that it is imperative that "all parties involved in surveillance share data in a timely fashion" and that it is also crucial that clinical and research data for emergency response is also shared *(23)*.

Both guidance documents also state that as part of preparedness efforts, "countries should review their laws, policies, and practices regarding data sharing to ensure that they adequately protect the confidentiality of personal information and address other relevant ethical questions." *(23,24)* Consent may also be sought for shipping samples outside of the country for additional testing, and/or the storage and use of samples for future public health needs. The primary risks to individuals relate to the unwanted disclosure of personal information *(24)*. This can be minimized by protecting the confidentiality of individuals' identities *(24)*, for instance by only sharing data when needed for specific/appropriate purposes (i.e., the investigation objectives) and sharing only the minimal data required.

It is important that, wherever possible, ethics pre-approval is sought in advance of an epidemic or pandemic to reduce the time to activate Respiratory Investigations and Studies in accordance with local, regional, and national authorities. In addition to ethics approvals, material transfer agreements such as those used by the WHO BioHub should be reviewed by appropriate legal representatives in advance to facilitate transparent and efficient shipment of biological materials that will accelerate confirmatory testing and validation of Pathogen X diagnostics. Additionally, countries should consider how they will facilitate access to the validated test post-evaluation.

Further information on the ethical considerations of importance to public health surveillance can be found in key WHO guidance:

- WHO guidelines on ethical issues in public health surveillance *(23)*
- Guidance for managing ethical issues in infectious disease outbreaks (who.int) *(24)*

Toolkit item

Toolkit items to consider developing to support this section may include:

- Ethical exemption/clearance letter templates
- Links to key WHO guidance on ethical considerations of importance to public health surveillance
- Template consent and assent forms, including long term storage and use of biological material

When available, Toolkit items developed by WHO and partners will be available on the WHO website.

2.10.1 Informed consent and assent

The purpose of the investigation will be explained to all individuals willing to participate before the start of the investigation. For all investigation activities not included in routine public health management, informed consent and assent may be required. This will depend on the country's national ethical requirements. Informed consent will seek relevant approvals for the collection of all data and specimens for the purposes of this investigation, as well as use of leftover diagnostic samples as determined by [Country Y].

Whether Part A participants should provide informed consent should be considered in advance, and will be country- and context-dependent. Options for Part A include IRB exemption (i.e., activities are considered part of public health investigation), formal waiver of informed consent, or full informed consent.

Consent will be sought from adults in Part B given the intense sampling regime necessary for the participation in the study. Whether consent is required for Part A participants will depend on country requirements. For children under the legal age of consent (usually 18 years but will vary from country to country) consent will be sought from a parent or legal guardian. An **Assent** will be sought from children and adolescents under the legal age of consent but who can understand the implications of informed consent and go through the necessary procedures. This is usually for children over the age of 12 to 13 years, but this will vary by country. A consent form from a parent or legal guardian will be collected in addition.

Template informed consent and assent forms will be included in the supporting FFX-Dx investigation toolkit.

All eligible individuals, regardless of whether they are well or unwell, should be able to participate in the investigation. For individuals who lack the decisional capacity to consent at the time of the investigation, consent/assent by proxy (parent/ legal guardian/ spouse/ family member) may be considered so as to not unduly exclude individuals from participating in the investigation.

The processes related to withdrawal of a participant need to be described both in the protocol and in the information given to the participant at the time of enrolment. In this description it must be made clear that a participant can withdraw from the investigation, without justification, at any time by informing one of the members of the investigation team. The contact details of one of the members of the investigation need to be provided in the information for the participant. If any participant decides to withdraw from the investigation, the samples collected and data should be discarded, except if the participant indicates that these can be kept for the purpose of conducting the investigation, or for future studies of other infectious pathogens.

Informed consent will seek approval to collect blood and respiratory samples (and other optional samples, e.g., urine, stool) and data for the intended purpose of this investigation. It may also seek approval that samples may be shipped outside of the country for additional testing (including, if necessary, testing like sequencing and culture); and that samples may be stored and used for future public health needs, in accordance with national laws and regulations. More specifically, information about where samples will be stored (e.g., which biobank), for how long, and who will have access to them should be included if samples will be used for commercial assay development. Additional detail in the consent / assent forms may be needed, according to national laws and regulations, if the investigation calls for storage and future use of samples.

2.10.2 Risks and benefits for subjects

This investigation poses minimal risk to participants, involving the collection of a small amount of blood and respiratory specimens. However, consideration should also be given, if relevant to local laws and regulations on infectious diseases, to whether specific measures

may be taken such as: isolation/quarantine, mandatory reporting, further surveillance of contacts and family members, detention, etc.

The direct benefit to the participant is the possibility for early detection of pandemic Pathogen X infection, which would allow for appropriate monitoring and treatment for themselves and their close contacts. If relevant, this should be extended to in kind compensation for available clinical care/treatment or vaccination. The primary benefit of the investigation is indirect, in that data collected will facilitate access to validated testing in the country and help improve and guide efforts to understand transmission of pandemic Pathogen X and inform appropriate public health responses.

In terms of treatment of cases, case management may be facilitated by early detection of the infection/disease and will follow national guidance, but the investigators may or may not be directly involved in clinical management of patients. Processes on how cases will be referred for medical care, as well as details on provisions of care as part of the investigation will need to be detailed.

Incentives are not suggested for participating in this investigation, but participants will be provided relevant and updated health advice to reduce the risk of transmission and severe outcomes where possible.

2.10.3 Reporting of serious adverse events, including death of a participant

Any serious adverse event,[2] including death, of a participant during the investigation period needs to be immediately (within 24h) reported to the Principal Investigator and the institution responsible for the investigation. The contact details for reporting serious adverse events needs to be provided to each member of the investigation team.

In accordance with national regulations, any serious adverse event may also have to be reported to the local ethical review committee if the adapted protocol was not deemed exempt from local ethical review.

2.10.4 Confidentiality

Participant confidentiality will be maintained throughout the investigation. All subjects who participate in the investigation will be assigned a unique identification number by the investigation team, for the labelling of questionnaires and clinical specimens. The link of this identification number to individuals will be maintained by the investigation team and will not be disclosed elsewhere.

Data and specimens must be securely stored. If the data and samples are shared by the implementing organization with WHO or any agency or institution providing support for

2 An adverse event can be defined as: An injury related to medical management, in contrast to complications of disease *(26)*.

data analysis and laboratory testing, the shared data and samples will include only the investigation identification number and not any personally identifiable information. Data sharing outside [Country Y] must be managed according to national laws and regulations, as appropriate.

COMMENT: The investigators will need to describe how data and specimens will be securely stored, the duration of storage, and the destruction of data and specimens at the end of the duration of storage, in accordance with national laws and regulations.

Article 45 of the International Health Regulations (IHR) describes the "treatment of personal data" *(25)*. Personally identifiable data collected under the IHR should be kept confidential and processed anonymously, as required by national law. However, such data may be disclosed for assessments and management of public health risks, provided the data are processed fairly and lawfully.

2.10.5 Future use of samples

The investigators may decide on storage and potential future use of specimens and the time frame for their destruction, and seek appropriate approvals for this. Biorepositories should comply with Guideline 11 in the International Ethical Guidelines for Health-related Research Involving Humans. The investigators will need to provide specific information on potential future use of specimens (including sharing with other laboratories and potentially with commercial diagnostic manufacturers) and the time frame for their destruction, including in the information for the participant and the informed consent/assent form. Additional consent forms may need to be developed by the country, to comply with national laws and regulations.

2.10.6 Prevention of Pathogen X infection among investigation and laboratory personnel

All personnel involved in the investigation visits must be trained in procedures for infection prevention and control (as determined by national or local guidelines) *(27)* when collecting data or specimens in proximity to cases and close contacts. These procedures should include proper hand hygiene and the correct use of surgical or respiratory face masks to minimize their risk of infection.

Laboratorians conducting clinical and study testing should follow their laboratory's procedures for training and infection prevention.

WHO technical guidance on infection prevention and control specific to Pathogen X will be published on the WHO website. For more general guidance on biorisk management, countries should refer to *(28)*.

3. Statistical analyses

3.1 Plan of analysis

FFX-Dx investigations will contribute key data in the early stages of an epidemic, which will inform development and use of novel diagnostics for Pathogen X as well as an early understanding of clinical and epidemiological parameters. Other protocols (e.g., FFX protocol) for investigations for Pathogen X will provide further data to help with the calculation of key epidemiological parameters.

An analysis plan incorporating the study objectives, definitions, and planned analysis to address each objective is available. For the purpose of analysis, all data will be adjusted to Day 0 being the first day of symptoms (cases) or exposure (contacts). Key components included in the analysis plan are:

A descriptive analysis of the FFX-Dx data should provide preliminary insight into the operational characteristics of the novel IHDT, as well as comparative performance of different sample types over the course of infection. An analysis of semiquantitative data (Ct values) or quantitative pathogen nucleic acid concentration data generated using the IHDT will provide initial estimates of Pathogen X characteristics (especially duration of detection and peak of viral load or equivalent). Separately, an analysis of demographics of individuals infected with Pathogen X, parameters relevant for transmission (e.g., generation time), and the spectrum of clinical presentations and course of disease will be performed.

Additional complex analyses can be conducted using the FFX-Dx forms/questionnaires and specimens generated (including culture of Pathogen X) and can potentially inform early mathematical modelling approaches.

Toolkit item

Toolkit items to consider developing to support this section may include:

- Template statistical analysis plans
- Data analysis scripts
- SOP for interpretation
- Reporting guidelines for outcomes

When available, Toolkit items developed by WHO and partners will be available on the WHO website.

4. Data sharing and reporting of findings

4.1 Data and sample sharing

Data and samples from these WHO Respiratory Investigations and Studies may be **pooled and/or aggregated across multiple sites** by WHO if the data and samples are collected in a consistent manner, to increase analytic power and improve precision in estimates. The pooling of some of this data may depend on how transmission dynamics vary between countries. If the data and samples are shared by the implementing organization or country, with WHO or with any agency or institution providing support for data and sample analysis, data and samples shared will include only the investigation identification number and not any personally identifiable information.

Any data and sample sharing must also be covered by ethical approvals, where relevant, or national regulations.

Toolkit item

Toolkit items to support this section may include:

- Data sharing agreement templates
- Platforms for data sharing
- Standardized formats/templates for data sharing

When available, Toolkit items developed by WHO and partners will be available on the WHO website.

4.2 Reporting of findings

Reporting should follow standard practices:

Timely dissemination of the results of this investigation is critical to update guidance and inform local, national, and international public health responses and policies for diagnostics development and use. WHO needs to be able to adapt their global recommendations in a timely manner. Countries are strongly encouraged to share with WHO, in line with IHR requirements and in a confidential manner, any early findings, especially if they will impact WHO global guidance. Terms for sharing data with other stakeholders including national centres for disease control and other bodies should be agreed upon in advance.

It is also important to fully **document the investigation design**, the approach to ascertainment of cases and contacts in the different parts of the study, the duration of follow-up, the timing and method of sample collection, and the laboratory methods used

to ensure that data can be pooled (e.g., to increase the precision of peak or epidemiological parameter estimates).

Information should be collected in a **standardized format** according to the questionnaires and tools in this generic protocol to assist with data harmonization and comparison of results (see forms in Annex 2).

Toolkit item

Toolkit items to consider to support this section may include:

- Standard criteria for reporting results
- Templates for presentations and publications
- Training on messaging to different audiences
- Technical briefs

When available, Toolkit items developed by WHO and partners will be available on the WHO website.

4.3 Science translation for decision makers

Clear and timely reporting and communication of results is necessary to support policy/decision makers.

Toolkit item

Toolkit items to consider developing to support this section may include:

- Training on messaging to different audiences
- Policy makers questions that will be answered by study objectives
- Science translation materials

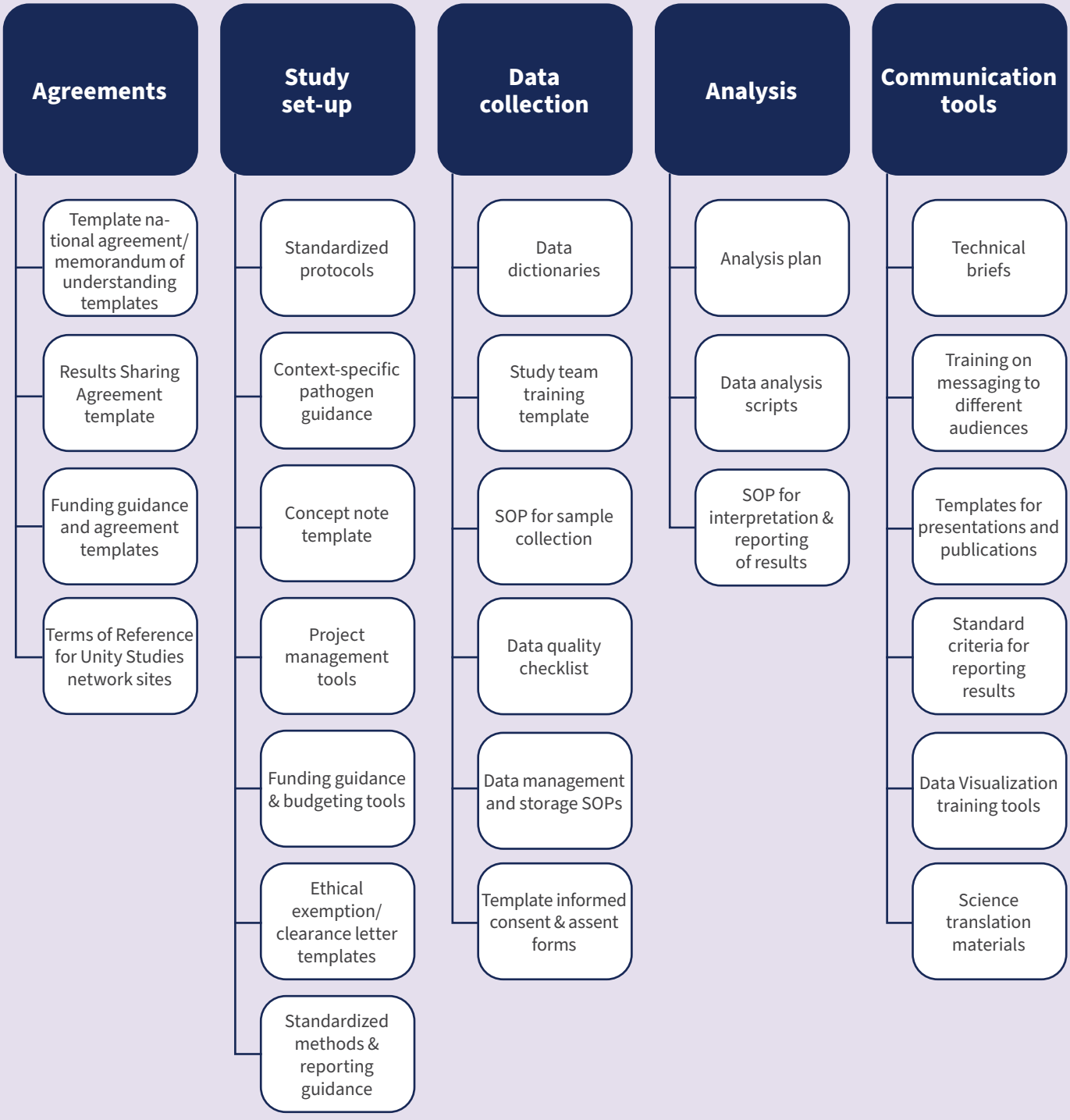
When available, Toolkit items developed by WHO and partners will be available on the WHO website.

5. Protocol Toolkit

5.1 Protocol Toolkit

The timely and quality implementation of this protocol should be supported by the use of toolkits developed by WHO and implementing partners. These will include components such as pre-planned agreements, study set-up resources, data collection, analysis and communication tools as shown in Figure 3 and highlighted throughout the protocol. When available, Toolkit items developed by WHO will be available on the WHO website.

Figure 3: Proposed toolkit components to support quality implementation of Investigations and Studies protocols



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7. Annexes

Annex 1: Comparison between the features and complementarity of the main respiratory pathogens of pandemic potential investigation protocols

	First Few X cases and contacts Diagnostic Test Evaluation (FFX-Dx) for respiratory pathogens with pandemic potential: template protocol	The First Few X cases and contacts (FFX) investigation protocol for respiratory pathogens of pandemic potential	Household transmission investigation protocol for respiratory pathogens of pandemic potential	Closed setting transmission investigation protocol for respiratory pathogens of pandemic potential
Population	The first few X cases of Pathogen X and their close contacts in the general population	The first few X cases of Pathogen X and their close contacts in the general population	Household close contacts of index cases of Pathogen X (smaller epidemiological unit than FFX)	Contacts of index cases in the closed setting (larger epidemiological unit)
Aim	End User In-House Developed Test (IHDT) validation followed by characterization of early Pathogen X kinetics and optimal diagnostic sampling strategies	Transmission dynamics, severity, and clinical spectrum, in a proxy of the general population	Transmission dynamics, severity, and clinical spectrum in household settings	Transmissibility-chains of infection in closed settings such as schools, hospitals, and army barracks
Potential output and analysis	Primary objectives: • Part A: End User Validation (Focused analytical and limited clinical validation) of the initial molecular test (IHDT) developed for Pathogen X in focused sample types (NP swab for upper respiratory presentation; NP swab AND BAL or sputum for lower respiratory infection); • Part B: Quantification of Pathogen X using IHDT in matched samples collected serially over the course of infection and post-exposure to assess early Pathogen X kinetics (peak) and optimal sample types for diagnostic testing, including samples relevant to point-of-care and self-testing. Testing of matched samples will also provide validation data for ongoing testing of alternative sample types; • For all cases, gather data on the clinical presentation and course of associated disease to optimize/refine the clinical case definition for Disease X; • For contacts (Part B only), attempt to detect and quantify Pathogen X in asymptomatic or pre-symptomatic infection.	Transmission dynamics, severity, and clinical spectrum, through estimates of, primarily: • the secondary infection rate (SIR) and secondary clinical attack rate of Pathogen X, and by key factors such as setting age and sex • the clinical presentation of Pathogen X infection and course of associated disease • the symptomatic & asymptomatic proportions of Pathogen X cases • preliminary case and infection hospitalization and fatality ratios	Key data **to complement and reinforce the findings of FFX,** through estimates of, primarily: • the secondary infection rate (SIR) and secondary clinical attack rate (SCAR) of Pathogen X in households, and by key factors such as age and sex • the clinical presentation of Pathogen X infection and course of associated disease • the symptomatic & asymptomatic proportions of Pathogen X cases • preliminary case and infection hospitalization ratios	Key data **to complement and reinforce the findings of FFX,** through estimates of, primarily: • the overall infection and clinical attack rate of Pathogen X, and by key factors such as age and sex • the secondary infection rate (SIR) and secondary clinical attack rate of Pathogen X infection overall, and by key factors such as setting age and sex • the clinical presentation of Pathogen X infection and course of associated disease • the symptomatic and asymptomatic proportions of Pathogen X cases • preliminary case and infection hospitalization and fatality ratios

	First Few X cases and contacts Diagnostic Test Evaluation (FFX-Dx) for respiratory pathogens with pandemic potential: template protocol	The First Few X cases and contacts (FFX) investigation protocol for respiratory pathogens of pandemic potential	Household transmission investigation protocol for respiratory pathogens of pandemic potential	Closed setting transmission investigation protocol for respiratory pathogens of pandemic potential
Potential output and analysis (cont.)	Secondary objectives include estimation of the following epidemiological and Pathogen X parameters (epidemiological parameters to be confirmed in the subsequent FFX protocol): • the duration of shedding of Pathogen X; • the symptomatic and asymptomatic proportions of cases; • the serial interval; • the incubation period; • the generation time; • correlation of Ct values and/or Pathogen X nucleic acid concentrations with culture to inform isolation practices; • correlation of Pathogen X antigen concentration with Pathogen X nucleic acid concentration (as soon as a test for quantitative detection of Pathogen X antigen is available).	and secondarily: • the serial interval of Pathogen X • duration of viral shedding (if samples are taken at higher frequency and adequate resources are available) • identification of possible routes of transmission including possible animal/human transmission • risk and/or protective factors for transmission or severe disease Advance related objectives: • the basic reproduction number (R_0) of Pathogen X • the effective reproductive number (R_{eff}) of Pathogen X • the incubation period of Pathogen X • the generation interval of Pathogen X	and secondarily: • the serial interval of Pathogen X • duration of viral shedding (if samples are taken at higher frequency and adequate resources are available) • risk and/or protective factors for transmission or severe disease Advance related objectives: • the incubation period of Pathogen X	and secondarily: • the serial interval of Pathogen X • risk and/or protective factors for transmission or severe disease Advance related objectives: • the basic reproduction number (R_0) of Pathogen X • the effective reproductive number (R_{eff}) of Pathogen X • the incubation period of Pathogen X • the generation interval of Pathogen X
Duration	Part A: completion of analytical validation and identification and testing of suspected Pathogen X cases on two consecutive days Part B: recruitment of Pathogen X cases and close contacts with serial sampling for up to 28 days (or longer if IHDT still positive; see Section 2.7.2), and collection of clinical and epidemiological data for at least 28 days.	Recruitment and follow-up of index cases and their close contacts for a maximum period of 28 days from identification of the index case.	Recruitment and follow-up of index cases and their household contacts for a maximum period of 28 days from identification of the index case. Study enrolment could be extended as far as desired; however, the most valuable period in order to use data for targeted public health action is in the early phases of the epidemic (first 2–3 months).	Recruitment and follow-up of index cases and their close contacts in a closed setting for a maximum period of 28 days from laboratory confirmation of the index case. Enrolment could be extended as far as desired, however the most valuable period in order to use data for targeted public health action is in the early phases of the epidemic/ pandemic (first 2–3 months).
Start of the investigation	To be initiated once the first Pathogen X IHDT is available for clinical use by an end-user laboratory, with identification of participants initiated in the first days after the identification of a case of Pathogen X in [Country Y].	To be initiated in the first days after the identification of a case of Pathogen X in [Country Y].	Prospective study, ideally before widespread community transmission occurs, within 2–3 months after identification of initial cases.	Prospective study, ideally before widespread community transmission occurs, within 2–3 months after identification of initial cases.

	First Few X cases and contacts Diagnostic Test Evaluation (FFX-Dx) for respiratory pathogens with pandemic potential: template protocol	The First Few X cases and contacts (FFX) investigation protocol for respiratory pathogens of pandemic potential	Household transmission investigation protocol for respiratory pathogens of pandemic potential	Closed setting transmission investigation protocol for respiratory pathogens of pandemic potential
Recruitment	The first few cases of pandemic Pathogen X in [Country Y] and their contacts (Part B) should participate.	The first few cases of Pathogen X in [Country Y], and their close contacts, will be first few participants to be recruited.	Household contacts of index cases of Pathogen X.	Contacts within closed settings will be enrolled.
Minimum data and specimens to be obtained from participants	Epidemiological, clinical, virological (if applicable), and serological data (if available) will be collected from each participant at multiple times during the study – including surveys at baseline and day 28, symptom diaries from day 2–28 and specimen collection.			

Annex 2: Questionnaires and guidance

First Few X cases and contacts Diagnostic Test Evaluation (FFX-Dx) for respiratory pathogens with pandemic potential: template protocol

For cases

- **Contact Line List**
- **Form A1**: Case initial report form – for Pathogen X index cases (Day 1)
- **Form A2**: Case follow-up form – for Pathogen X index cases (Day 28)

For contacts

- **Form B1**: Contact initial reporting form – for close contacts of Pathogen X cases (Day 1)
- **Form B2**: Contact follow-up reporting form – for close contacts of Pathogen X cases (Day 28)

For cases and contacts

- **Form C**: Specimen collection and laboratory results (one form per sample; all participants)
- **Form D**: Symptom diary for all participants –Pathogen X index cases and close contacts (latter Part B only)

Contact Line List

Please copy and transpose the following fields into an Excel spreadsheet and use to populate the Contact Line List. Alternatively use appropriate electronic methods to capture this information. To be completed at the initial visit with an index case.

Index Case ID/Cluster number
Contact ID Number (C…)
First Name
Family Name
Sex (M/F/Unknown)
DOB (dd/mm/yyyy)
Telephone number
Type of contact (Household/health worker/other)

First Few X cases and contacts (FFX-Dx) investigation for respiratory pathogens with pandemic potential: template protocol

1. For cases

Form A1: Case initial reporting form – for Pathogen X suspected/probable/confirmed index cases (Day 1)

Unique Case ID/Cluster number (if applicable):

1. Current status

☐ Alive ☐ Dead

2. Data collector information

Name of data collector	
Data collector institution	
Data collector telephone number	
Data collector email	
Form completion date (dd/mm/yyyy)	____/____/____

3. Interview respondent information (if the person providing the information is not the index case)

First name	
Family name	
Sex	☐ Male ☐ Female ☐ Non-binary ☐ Not known
Date of birth (dd/mm/yyyy)	____/____/____ ☐ Unknown
Relationship to case (select all that are relevant)	☐ Household member ☐ Immediate family member ☐ Extended family member ☐ Healthcare worker looking after case ☐ Friend ☐ Co-worker ☐ Teacher ☐ Carer ☐ Acquaintance ☐ Unknown
Respondent address	
Telephone (mobile) number	

Form A1: Case initial reporting form – for Pathogen X suspected/probable/confirmed index cases **(Day 1)** (continued)

4. Patient identifier information	
First name*	
Family name*	
Sex	☐ Male ☐ Female ☐ Non-binary ☐ Not known
Date of birth (dd/mm/yyyy)*	____/____/____ ☐ Unknown
Age (years, months)	____ years ____ months ☐ Unknown
Telephone (mobile) number	
Email	
Address*	
National social number/identifier (if applicable)*	
Country of residence	
Nationality	
Case occupation (specify location/facility)	☐ Health care worker ☐ Working with animals ☐ Health laboratory worker ☐ Other, specify: For each occupation, please specify location or facility: _____
Ethnicity (optional)	☐ Arab ☐ Black ☐ East Asian ☐ South Asian ☐ West Asian ☐ Latin American ☐ White ☐ Aboriginal/First Nations ☐ Mixed Race ☐ Other: _____ ☐ Unknown
Is this case part of an institutional outbreak (e.g., aged care facility, hospital, group home)?	☐ Yes ☐ No ☐ Unknown If Yes, specify: _____
Name of institution involved in outbreak if appropriate	

* These identifiers are commonly accepted as personally identifiable information and must be kept confidential, however, these may vary by country and should be updated by Country Y according to national guidelines.

5a. Patient symptoms (from onset of symptoms)	
Date of first symptom onset (dd/mm/yyyy)	____/____/____ ☐ No symptoms ☐ Unknown
Fever (≥38°C) or history of fever since disease onset	☐ Yes ☐ No ☐ Unknown If Yes, date (dd/mm/yyyy): ____/____/____

Form A1: Case initial reporting form – for Pathogen X suspected/probable/confirmed index cases (Day 1) (continued)

5b. Respiratory symptoms	
Sore throat	☐ Yes ☐ No ☐ Unknown If Yes, date (dd/mm/yyyy): ____/____/____
Runny nose	☐ Yes ☐ No ☐ Unknown If Yes, date (dd/mm/yyyy): ____/____/____
Cough	☐ Yes ☐ No ☐ Unknown If Yes to cough, productive, ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry, ☐ Yes ☐ No ☐ Unknown If Yes, date (dd/mm/yyyy): ____/____/____
Shortness of breath	☐ Yes ☐ No ☐ Unknown If Yes, date (dd/mm/yyyy): ____/____/____
5c. General symptoms	
Chills	☐ Yes ☐ No ☐ Unknown
Anosmia (loss of smell)	☐ Yes ☐ No ☐ Unknown
Ageusia (loss of taste)	☐ Yes ☐ No ☐ Unknown
Loss of appetite	☐ Yes ☐ No ☐ Unknown
Vomiting	☐ Yes ☐ No ☐ Unknown
Nausea	☐ Yes ☐ No ☐ Unknown
Diarrhoea	☐ Yes ☐ No ☐ Unknown
Headache	☐ Yes ☐ No ☐ Unknown
Rash	☐ Yes ☐ No ☐ Unknown
Fatigue	☐ Yes ☐ No ☐ Unknown
Muscle aches	☐ Yes ☐ No ☐ Unknown
Joint ache	☐ Yes ☐ No ☐ Unknown
Conjunctivitis	☐ Yes ☐ No ☐ Unknown
Nose bleed	☐ Yes ☐ No ☐ Unknown
Chest pain	☐ Yes ☐ No ☐ Unknown
5d. Neurological symptoms	
Seizures	☐ Yes ☐ No ☐ Unknown
Altered level of consciousness	☐ Yes ☐ No ☐ Unknown
Other neurological signs	☐ Yes ☐ No ☐ Unknown If Yes, specify: _____
5e. Other symptoms	
Other symptoms	☐ Yes ☐ No ☐ Unknown If Yes, specify: _____

Form A1: Case initial reporting form – for Pathogen X suspected/probable/confirmed index cases (Day 1) (continued)

6. Primary health-care center/treating physician's details (interactions that have already occurred by the time of study enrolment)	
Date of first primary health facility visit (including traditional care) (dd/mm/yyyy)	____/____/____ ☐ Not applicable (NA) ☐ Unknown
Total health facilities visited to date	☐ NA ☐ Unknown Specify: _____
Visit to primary healthcare (PHC; GP, etc) (repeat for as many visits as required)	☐ Yes ☐ No ☐ Unknown
If yes, date of first PHC contact (dd/mm/yyyy)	____/____/____ ☐ Unknown ☐ NA
If yes, Name of health-care center	
If yes, Name of treating physician	
If yes, Telephone number	
If yes, Fax	
If yes, Address	

7. Other health-care interactions (that have already occurred by the time of study enrolment)	
Contact with emergency number/hotline	☐ Yes ☐ No ☐ Unknown
Date of emergency contact (dd/mm/yyyy)	____/____/____ ☐ Unknown
Visited emergency department (A&E) (repeat for as many contacts as required)	☐ Yes ☐ No ☐ Unknown
Date of first A&E contact (dd/mm/yyyy)	____/____/____ ☐ Unknown ☐ NA
Hospitalization (repeat for as many admissions as required)	☐ Yes ☐ No ☐ Unknown
Date of admission to hospital (dd/mm/yyyy)	____/____/____ ☐ Unknown ☐ NA
Name of hospital	
Location of hospital	
Date of discharge from hospital (dd/mm/yyyy)	____/____/____
Reason for hospitalization	☐ Isolation/quarantine ☐ Clinical management ☐ Other If Other, specify: _____
ICU (intensive care unit) admission	☐ Yes ☐ No ☐ Unknown
Date of ICU admission (dd/mm/yyyy)	____/____/____ ☐ Unknown ☐ NA
Date of discharge from ICU (dd/mm/yyyy)	____/____/____ or ☐ Ongoing ☐ Unknown ☐ NA
Mechanical ventilation	☐ Yes ☐ No ☐ Unknown
Dates of mechanical ventilation (dd/mm/yyyy)	Start: ____/____/____ Stop: ____/____/____ or ☐ Ongoing ☐ Unknown ☐ NA
Length of ventilation (days)	

Form A1: Case initial reporting form – for Pathogen X suspected/probable/confirmed index cases (Day 1) (continued)

8. Case symptoms: complications (that have already occurred by the time of enrolment)	
Acute respiratory distress syndrome (ARDS)	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy): ____/____/____
Acute renal failure	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy): ____/____/____
Cardiac failure	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy): ____/____/____
Consumptive coagulopathy	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy): ____/____/____
Pneumonia by chest X-ray	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy): ____/____/____
Other complications	☐ Yes ☐ No ☐ Unknown If Yes, secondary bacterial infection* ☐ Yes ☐ No ☐ Unknown Specify infection: _____ If other complication, specify: _____ * Fill out relevant laboratory information in specimen collection form
Hypotension requiring vasopressors	☐ Yes ☐ No ☐ Unknown
Extracorporeal membrane oxygenation (ECMO) required	☐ Yes ☐ No ☐ Unknown
Outcome (current)	☐ Alive ☐ Dead, if Yes, specify date of death (dd/mm/yyyy): ____/____/____ ☐ Unknown/lost to follow-up

9. Treatment with antivirals	
Did the case receive an antiviral treatment in the last 14 days?	☐ Yes ☐ No ☐ Unknown (If no or unknown skip to next question) If Yes Specify which antiviral was received? _____ Date started (dd/mm/yyyy): ____/____/____ Date stopped (dd/mm/yyyy): ____/____/____ Dosage (specify): What were antivirals prescribed for? ☐ Treatment ☐ Prophylaxis

Form A1: Case initial reporting form – for Pathogen X suspected/probable/confirmed index cases (Day 1) (continued)

10. Patient pre-existing condition(s)	
Pregnancy	☐ Yes ☐ No ☐ Unknown If Yes, specify trimester: ☐ First ☐ Second ☐ Third ☐ Unknown
Obesity	☐ Yes ☐ No ☐ Unknown
Cancer	☐ Yes ☐ No ☐ Unknown
Diabetes	☐ Yes ☐ No ☐ Unknown
HIV/other immune deficiency	☐ Yes ☐ No ☐ Unknown
Heart disease	☐ Yes ☐ No ☐ Unknown
Asthma	☐ Yes ☐ No ☐ Unknown
Chronic lung disease (non-asthma)	☐ Yes ☐ No ☐ Unknown
Chronic liver disease	☐ Yes ☐ No ☐ Unknown
Chronic haematological disorder	☐ Yes ☐ No ☐ Unknown
Chronic kidney disease	☐ Yes ☐ No ☐ Unknown
Chronic neurological impairment/disease	☐ Yes ☐ No ☐ Unknown
Hypertension	☐ Yes ☐ No ☐ Unknown
TB	☐ Yes ☐ No ☐ Unknown
Organ or bone marrow recipient	☐ Yes ☐ No ☐ Unknown
Other pre-existing condition(s)	☐ Yes ☐ No ☐ Unknown If Yes, specify: _____

11. Vaccination	
Case was vaccinated for seasonal influenza in the 12 months prior to onset of symptoms	☐ Yes ☐ No ☐ Unknown If Yes, date of vaccination, country of vaccination (dd/mm/yyyy): ____/____/____ Country: _____
Case was vaccinated for SARS-CoV-2 in the 12 months prior to onset of symptoms	☐ Yes ☐ No ☐ Unknown If Yes, date of vaccination, country of vaccination (dd/mm/yyyy): ____/____/____ Country: _____ Date of last infection (dd/mm/yyyy): ____/____/____ or ☐ Unknown
Case was vaccinated with pneumococcal vaccine (either conjugate or polysaccharide)	☐ Yes ☐ No ☐ Unknown If Yes, date (dd/mm/yyyy) ____/____/____

Form A1: Case initial reporting form – for Pathogen X suspected/probable/confirmed index cases (Day 1) (continued)

12. Human exposures in the days before symptom onset (in the past 14 days)	
Case travelled within the last 14 days domestically?	☐ Yes ☐ No ☐ Unknown If Yes, dates of travel (dd/mm/yyyy): ____/____/____ to ____/____/____ Regions visited: Cities visited:
Case travelled within the last 14 days internationally?	☐ Yes ☐ No ☐ Unknown If Yes, dates of travel (dd/mm/yyyy): ____/____/____ to ____/____/____ Regions visited: Cities visited:
Has the case had contact with anyone with suspected or confirmed Pathogen X infection in the past 14 days?	☐ Yes ☐ No ☐ Unknown If Yes, dates of last contact (dd/mm/yyyy): ____/____/____
If yes, location of exposure	☐ Home ☐ Hospital ☐ Workplace ☐ Tour group ☐ School ☐ Unknown ☐ Other, specify:
Has the case attended a festival or mass gathering in the past 14 days?	☐ Yes ☐ No ☐ Unknown If Yes, specify:
Has the case visited or been admitted to an inpatient health facility in the past 14 days?	☐ Yes ☐ No ☐ Unknown If Yes, specify:
Has the case visited an outpatient treatment facility in the past 14 days?	☐ Yes ☐ No ☐ Unknown If Yes, specify:
Has the case visited a traditional healer in the past 14 days?	☐ Yes ☐ No ☐ Unknown If Yes, specify type:

13. Status of form completion	
Form completed	☐ Yes (Date ____/____/____) ☐ Partially (Date ____/____/____) If partially, reason: ☐ Missed ☐ Not attempted ☐ Not performed ☐ Refusal ☐ Other, specify:

Form A1: Case initial reporting form – for Pathogen X suspected/probable/confirmed index cases **(Day 1)** (continued)

Additional information to collect regarding potential routes of transmission

Please note there is no need to collect this information if the pathogen is known to sustainably transmit between humans. Only collect this information if there is suspected ongoing zoonotic transmission. Note: cases with zoonotic exposure can't be included in the same analysis of transmission parameters as human acquired cases.

14. Human exposures to animals in the days before illness onset (in the past 14 days)		
Direct handling of animals		
A	Case handled animals	☐ Yes ☐ No ☐ Unknown If No or Unknown, skip to F If Yes, specify:
B	Types of animals handled	☐ Pigs ☐ Chicken ☐ Ducks ☐ Other If other, specify:
C	Nature of contact (e.g., feed, groom or slaughter)	Specify:
D	Location of animal contact (select all that are relevant)	☐ Home ☐ Workplace ☐ Neighbourhood ☐ Live animal market ☐ Agricultural fair/zoo group ☐ Farm ☐ Other, specify:
E	Within 2 weeks before or after this contact, were any animals sick or dead in the location of animal contact?	☐ Yes ☐ No ☐ Unknown If Yes, specify type and number, and proportion from flock or herd:
Other contact (not direct handling)		
F	Case had no direct contact with animals (e.g., in neighborhood, farm, zoo, at home, agricultural fair or work)	☐ Yes ☐ No ☐ Unknown If No or Unknown, skip to J If Yes, specify:
G	Types of animals in that environment	☐ Pigs ☐ Chicken ☐ Ducks ☐ Other If other, specify:
H	Location of exposure	☐ Home ☐ Workplace ☐ Neighbourhood ☐ Live animal market ☐ Agricultural fair/zoo group ☐ Farm ☐ Other, specify:
I	Within 2 weeks before or after exposure, were any animals sick or dead in the location of possible exposure?	☐ Yes ☐ No ☐ Unknown If Yes, specify type and number, and proportion from flock or herd:
J	Case exposed to animal by-products (e.g., bird feathers) or animal excreta	☐ Yes ☐ No ☐ Unknown If Yes, specify:
K	Case consumed raw or unpasteurized animal products (if yes, specify products)	☐ Yes ☐ No ☐ Unknown If Yes, specify:
L	Case consumed health or traditional remedies with raw or unpasteurized animal products (if yes, specify products)	☐ Yes ☐ No ☐ Unknown If Yes, specify:

Form A2: Case follow-up reporting form – for Pathogen X confirmed cases only (Day 28)

Unique Case ID/Cluster number (if applicable):

1. Data collector information

Name of data collector	
Data collector institution	
Data collector telephone number	
Data collector email	
Form completion date (dd/mm/yyyy)	____/____/____

2. Interview respondent information (if the person providing the information is not the index case)

First name	
Family name	
Sex	☐ Male ☐ Female ☐ Non-binary ☐ Not known
Date of birth (dd/mm/yyyy)	____/____/____ ☐ Unknown
Relationship to case (select all that are relevant)	☐ Household member ☐ Immediate family member ☐ Extended family member ☐ Health-care worker looking after case ☐ Friend ☐ Co-worker ☐ Teacher ☐ Carer ☐ Acquaintance ☐ Unknown
Respondent address	
Telephone (mobile) number	

3. Outcome/status

Status	☐ Alive ☐ Dead, if Yes, specify date of death (dd/mm/yyyy): ____/____/____ ☐ Unknown/lost to follow-up
Hospitalization ever required?	☐ Yes ☐ No ☐ Unknown

Form A2: Case follow-up reporting form – for Pathogen X confirmed cases only (Day 28) (continued)

(NB: If the information below is not currently available, please leave blank and send through an update as soon as results are available)	
If dead, contribution of Pathogen X to death:	☐ Underlying/primary ☐ Contributing/secondary ☐ No contribution to death ☐ Unknown
If dead, was a post mortem performed?	☐ Yes ☐ No ☐ Unknown
If dead, results of postmortem's report where available:	
If dead, cause of death on Death certificate (specify)	

4. Hospital health-care interactions since baseline

Hospitalization since baseline	☐ Yes ☐ No ☐ Unknown
Date of admission to hospital (dd/mm/yyyy)	____/____/____ ☐ Unknown
Name of hospital	
Location of hospital	
Date of discharge from hospital (dd/mm/yyyy)	____/____/____ or ☐ Ongoing
Reason for hospitalization	☐ Isolation/quarantine ☐ Clinical management ☐ Other If Other, specify: _____
ICU (intensive care unit) admission since baseline	☐ Yes ☐ No ☐ Unknown
Date of ICU admission (dd/mm/yyyy)	____/____/____ ☐ Unknown
Date of discharge from ICU (dd/mm/yyyy)	____/____/____ or ☐ Ongoing ☐ Unknown ☐ NA
Mechanical ventilation since baseline	☐ Yes ☐ No ☐ Unknown
Dates of mechanical ventilation (dd/mm/yyyy)	Start: ____/____/____ Stop: ____/____/____ or ☐ Ongoing ☐ Unknown ☐ NA
Length of ventilation (days)	

5. Case symptoms: complications

Acute respiratory distress syndrome (ARDS)	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy): ____/____/____
Acute renal failure	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy): ____/____/____
Cardiac failure	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy): ____/____/____
Consumptive coagulopathy	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy): ____/____/____
Pneumonia by chest X-ray	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy): ____/____/____

Form A2: Case follow-up reporting form – for Pathogen X confirmed cases only (Day 28) (continued)

Other complications	☐ Yes ☐ No ☐ Unknown If Yes, secondary bacterial infection* ☐ Yes ☐ No ☐ Unknown Specify infection: _____ If other complication, specify: _____ * Fill out relevant laboratory information in specimen collection form
Hypotension requiring vasopressors	☐ Yes ☐ No ☐ Unknown
Extracorporeal membrane oxygenation (ECMO) required	☐ Yes ☐ No ☐ Unknown

6. Patient pre-existing condition(s)

Pregnancy	☐ Yes ☐ No ☐ Unknown If Yes, specify trimester: ☐ First ☐ Second ☐ Third ☐ Unknown

7. Treatment with antivirals

Did the case receive an antiviral treatment in the last 28 days?	☐ Yes ☐ No ☐ Unknown (If No or Unknown, skip to next question) If Yes, specify which antiviral was received? _____ Date started (dd/mm/yyyy) ____/____/____ Date stopped (dd/mm/yyyy) ____/____/____ Dosage (specify): What were antivirals prescribed for? ☐ Treatment ☐ Prophylaxis

8. Final case classification

Final case classification (select one from each category that apply)	☐ Confirmed & ☐ Primary ☐ Co-primary ☐ Secondary ☐ Other, specify: (e.g., tertiary case) & (optional) ☐ Imported & (optional) ☐ Unrelated

Form A2: Case follow-up reporting form – for Pathogen X confirmed cases only (Day 28) (continued)

9. Status of form completion	
Form completed	☐ Yes ☐ No ☐ partially If No or partially, reason: ☐ Missed ☐ Not attempted ☐ Not performed ☐ Refusal ☐ Other, specify:

First Few X cases and contacts (FFX-Dx) investigation for respiratory pathogens with pandemic potential: template protocol

2. For contacts

Form B1: Contact initial reporting form (Day 1) – for close contacts of Pathogen X index cases

Unique Index Case ID/Cluster number (if applicable):

Contact ID Number (C…):
Note: contact ID numbers should be issued at the time of completion of Form A1/Contact Line List.

1. Current status

☐ Alive ☐ Dead ☐ Unknown/lost to follow-up

2. Data collector information

Name of data collector	
Data collector institution	
Data collector telephone number	
Data collector email	
Form completion date (dd/mm/yyyy)	____/____/____

3. Interview respondent information (if the person providing the information is not the contact)

First name	
Family name	
Sex	☐ Male ☐ Female ☐ Non-binary ☐ Not known
Date of birth (dd/mm/yyyy)	____/____/____ ☐ Unknown
Relationship to contact (select all that are relevant)	☐ Household member ☐ Immediate family member ☐ Extended family member ☐ Health-care worker looking after contact ☐ Friend ☐ Co-worker ☐ Teacher ☐ Carer ☐ Acquaintance ☐ Unknown
Respondent address	
Telephone (mobile) number	

Form B1: Contact initial reporting form (Day 1) – for close contacts of Pathogen X index cases (continued)

4. Contact details (details of the contact)	
First name*	
Family name*	
Sex	☐ Male ☐ Female ☐ Non-binary ☐ Not known
Date of birth (dd/mm/yyyy)*	____/____/____ ☐ Unknown
Age (years, months)	____ years ____ months ☐ Unknown
Relationship to case (select all that are relevant)	☐ Household member ☐ Immediate family member ☐ Extended family member ☐ Health-care worker looking after contact ☐ Friend ☐ Co-worker ☐ Teacher ☐ Carer ☐ Acquaintance ☐ Unknown
Telephone (mobile) number	
Email	
Address (village/town, district, province/region)*	
National social number/identifier (optional)*	
Nationality	
Country of residence	
Preferred mode of contact	☐ Mobile ☐ Work ☐ Home ☐ Email
Ethnicity (optional)	☐ Arab ☐ Black ☐ East Asian ☐ South Asian ☐ West Asian ☐ Latin American ☐ White ☐ Aboriginal/First Nations ☐ Mixed race ☐ Other: _____ ☐ Unknown
Is this contact part of an institutional outbreak (e.g., aged care facility, hospital, group home)?	☐ Yes ☐ No ☐ Unknown If Yes, specify: _____
Name of institution involved in outbreak if appropriate	

* These identifiers are commonly accepted as personally identifiable information and must be kept confidential, however these may vary by country and should be updated by Country Y according to national guidelines.

5. General exposure information	
Have you travelled within the last 14 days domestically?	☐ Yes ☐ No ☐ Unknown If Yes, dates of travel (dd/mm/yyyy): ____/____/____ to ____/____/____ Regions visited: Cities visited:

Form B1: Contact initial reporting form (Day 1) – for close contacts of Pathogen X index cases (continued)

Have you travelled within the last 14 days internationally?	☐ Yes ☐ No ☐ Unknown If Yes, dates of travel (dd/mm/yyyy): ____/____/____ to ____/____/____ Countries visited: Cities visited:
In the past 10 days, have you had contact with any suspected or confirmed Pathogen X cases (i.e., other cases that are not the index case in their recruited cluster)?	☐ Yes ☐ No ☐ Unknown If Yes, dates of last contact (dd/mm/yyyy): ____/____/____
If yes, location of exposure	☐ Home ☐ Hospital ☐ Workplace ☐ Tour group ☐ School ☐ Unknown ☐ Other, specify: _____
Have you attended a festival or mass gathering in the past 14 days?	☐ Yes ☐ No ☐ Unknown If Yes, specify: _____
Have you visited or been admitted to an inpatient health facility in the past 14 days?	☐ Yes ☐ No ☐ Unknown If Yes, specify: _____
Have you visited an outpatient treatment facility in the past 14 days?	☐ Yes ☐ No ☐ Unknown If Yes, specify: _____
Have you visited a traditional healer in the past 14 days?	☐ Yes ☐ No ☐ Unknown If Yes, specify: _____
Occupation (specify location/facility)	☐ Health worker ☐ Working with animals ☐ Health laboratory worker ☐ Other, specify: For each occupation, please specify location or facility: _____

For additional exposure questions if relevant to complete (human exposures to animals in the days before illness onset (14 days) please see Q13 on Form A1.

Note for next 2 sections:

- **Complete Section 6** if the contact is a health worker (HW).
- **Complete Section 7** if the contact is NOT a health worker.

6. Exposure information (if the close contact is a Health Worker [HW])

Job title (specify)		
Place of work		
Direct physical contact with the index case (e.g., hands-on physical contact)	☐ Yes ☐ No	
If yes, date of exposure	First contact: Date (dd/mm/yyyy): ____/____/____	Last contact: Date (dd/mm/yyyy): ____/____/____ ☐ Still in contact with case

Form B1: Contact initial reporting form (Day 1) – for close contacts of Pathogen X index cases (continued)

Has the HW had prolonged face-to-face contact (>15 minutes) with a case in a health facility?	☐ Yes ☐ No If Yes, what type of protective equipment was used by the HW? (select all that are relevant) ☐ Gown ☐ Gloves ☐ Eye protection ☐ Surgical/medical mask ☐ NIOSH-certified N95 or an EU standard FFP2 mask ☐ FFP3 mask
Was the contact present while any aerosol-generating procedures took place? (Add as many procedures and their dates as required)	☐ Yes ☐ No If Yes, specify procedure and date (dd/mm/yyyy) Procedure: ____/____/____ Procedure: ____/____/____ Was the contact wearing any type of a mask at this/these procedures? (select all that are relevant) ☐ Surgical/medical mask ☐ NIOSH-certified N95, an EU standard FFP2 mask ☐ FFP3 mask ☐ None

7. Exposure information (if the close contact is NOT a Health Worker)

Is the contact a household contact?	☐ Yes ☐ No If Yes, specify household ID/ number: _____	
Specify characteristics of contact with the case from first contact, while the case was **symptomatic**, until the last unprotected contact (Add as many dates as required)	First contact: Date (dd/mm/yyyy) ____/____/____	Last contact: (dd/mm/yyyy) ____/____/____ ☐ Still in contact with case
	Duration	____(minutes)
	Setting	☐ Home/household ☐ Hospital/health care ☐ Workplace ☐ Tour group ☐ Other, specify:

Form B1: Contact initial reporting form (Day 1) – for close contacts of Pathogen X index cases (continued)

8a. Symptoms in contact (Note: if the contact has symptoms at the time of enrolment, they should be enrolled as a suspected case. If the contact develops symptoms subsequently, sampling of this participant should utilize strategy shown for cases in Figure 2, starting at Day 1. Symptoms and date of onset can be recorded on this form. If the contact is confirmed as a case (symptomatic or asymptomatic) based on a positive IHDT result, they should newly follow the confirmed case sampling pathway (for asymptomatic cases, day of positive IHDT = Day 1 of study) but complete form B2 and continue with existing Form D.	
Has the contact experienced any respiratory symptoms (sore throat, runny nose, cough, shortness of breath) in the period from 10 days **before** symptom onset in the index case until the present?	☐ Yes ☐ No
Has the contact experienced any respiratory symptoms (sore throat, runny nose, cough, shortness of breath) in the period up to 10 days **after** the last contact or until the present date, whichever is the earlier?	☐ Yes ☐ No
Date (dd/mm/yyyy) and time of first symptom onset	____/____/____ _____ ☐ am ☐ pm
Fever (>38°C) or history of fever since disease onset	☐ Yes ☐ No ☐ Unknown If Yes, date ____/____/____
8b. Respiratory symptoms	
Sore throat	☐ Yes ☐ No ☐ Unknown If Yes, date ____/____/____
Runny nose	☐ Yes ☐ No ☐ Unknown If Yes, date ____/____/____
Cough	☐ Yes ☐ No ☐ Unknown If Yes to cough, productive, ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry, ☐ Yes ☐ No ☐ Unknown If Yes, date ____/____/____
Shortness of breath	☐ Yes ☐ No ☐ Unknown If Yes, date ____/____/____

8c. General symptoms	
Chills	☐ Yes ☐ No ☐ Unknown
Anosmia (loss of smell)	☐ Yes ☐ No ☐ Unknown
Ageusia (loss of taste)	☐ Yes ☐ No ☐ Unknown
Loss of appetite	☐ Yes ☐ No ☐ Unknown
Vomiting	☐ Yes ☐ No ☐ Unknown
Nausea	☐ Yes ☐ No ☐ Unknown
Diarrhoea	☐ Yes ☐ No ☐ Unknown
Headache	☐ Yes ☐ No ☐ Unknown
Rash	☐ Yes ☐ No ☐ Unknown

Form B1: Contact initial reporting form (Day 1) – for close contacts of Pathogen X index cases (continued)

Fatigue	☐ Yes ☐ No ☐ Unknown
Muscle aches	☐ Yes ☐ No ☐ Unknown
Joint ache	☐ Yes ☐ No ☐ Unknown
Conjunctivitis	☐ Yes ☐ No ☐ Unknown
Nose bleed	☐ Yes ☐ No ☐ Unknown
Chest pain	☐ Yes ☐ No ☐ Unknown
8d. Neurological symptoms	
Seizures	☐ Yes ☐ No ☐ Unknown
Altered level of consciousness	☐ Yes ☐ No ☐ Unknown
Other neurological signs	☐ Yes ☐ No ☐ Unknown If Yes, specify:
8e. Other symptoms	
Other symptoms	☐ Yes ☐ No ☐ Unknown If Yes, specify:

9. If ill, health-care center/treating physician's details	
Date of first health facility visit (including traditional care) (dd/mm/yyyy)	____/____/____ ☐ NA ☐ Unknown
Total health facilities visited to date	☐ NA ☐ Unknown Specify:
Visit to primary healthcare (PHC; GP, etc) (repeat for as many visits as required)	☐ Yes ☐ No ☐ Unknown
If Yes, date of first PHC contact (dd/mm/yyyy)	____/____/____ ☐ Unknown ☐ NA
If Yes, Name of health-care center	
If Yes, Name of treating physician	
If Yes, Telephone number	
If Yes, Fax	
If Yes, Address	

10. If ill, other health-care interactions	
Contact with emergency number/hotline	☐ Yes ☐ No ☐ Unknown
Date of emergency contact (dd/mm/yyyy)	____/____/____ ☐ Unknown
Visited emergency department (A&E) (repeat for as many contacts as required)	☐ Yes ☐ No ☐ Unknown
Date of first A&E contact (dd/mm/yyyy)	____/____/____ ☐ Unknown ☐ NA

Form B1: Contact initial reporting form (Day 1) – for close contacts of Pathogen X index cases (continued)

Hospitalization (repeat for as many admissions as required)	☐ Yes ☐ No ☐ Unknown
Date of admission to hospital (dd/mm/yyyy)	____/____/____ ☐ Unknown ☐ NA
Name and place of hospital	
Date of discharge from hospital (dd/mm/yyyy)	____/____/____ or ☐ Ongoing ☐ Unknown ☐ NA
Reason for hospitalization	☐ Isolation/quarantine ☐ Clinical management ☐ Other If Other, specify: _____
ICU (intensive care unit) admission	☐ Yes ☐ No ☐ Unknown
Date of ICU admission (dd/mm/yyyy)	____/____/____ ☐ Unknown
Date of discharge from ICU (dd/mm/yyyy)	____/____/____ or ☐ Ongoing ☐ Unknown ☐ NA
Mechanical ventilation	☐ Yes ☐ No ☐ Unknown
Dates of mechanical ventilation (dd/mm/yyyy)	Start: ____/____/____ Stop: ____/____/____ or ☐ Ongoing ☐ Unknown
Length of ventilation (days)	

11. If ill, contact symptoms: complications (that have already occurred by the time of enrolment)

Acute respiratory distress syndrome (ARDS)	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy) ____/____/____
Acute renal failure	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy) ____/____/____
Cardiac failure	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy) ____/____/____
Consumptive coagulopathy	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy) ____/____/____
Pneumonia by chest X-ray	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy) ____/____/____
Other complications	☐ Yes ☐ No ☐ Unknown If Yes, secondary bacterial infection* ☐ Yes ☐ No ☐ Unknown Specify infection: If other complication, specify: *Fill out relevant laboratory information in specimen collection form
Hypotension requiring vasopressors	☐ Yes ☐ No ☐ Unknown
Extracorporeal membrane oxygenation (ECMO) required	☐ Yes ☐ No ☐ Unknown

Form B1: Contact initial reporting form (Day 1) – for close contacts of Pathogen X index cases (continued)

Outcome	☐ Alive ☐ Dead, if Yes, specify date of death (dd/mm/yyyy): ____/____/____ ☐ Unknown/lost to follow-up
Outcome current as of date (dd/mm/yyyy)	____/____/____ ☐ Unknown ☐ NA

12. Treatment with antivirals

Did the contact receive an antiviral treatment in the last 14 days?	☐ Yes ☐ No ☐ Unknown (If no or unknown skip to next question) If Yes, Specify which antiviral was received? _____ Date started (dd/mm/yyyy) ____/____/____ Date stopped (dd/mm/yyyy) ____/____/____ Dosage (specify): _____ What were antivirals prescribed for? ☐ Treatment ☐ Prophylaxis

Form B1: Contact initial reporting form (Day 1) – for close contacts of Pathogen X index cases (continued)

13. Contact pre-existing condition(s)

Pregnancy	☐ Yes ☐ No ☐ Unknown If Yes, specify trimester: ☐ First ☐ Second ☐ Third ☐ Unknown
Obesity	☐ Yes ☐ No ☐ Unknown
Cancer	☐ Yes ☐ No ☐ Unknown
Diabetes	☐ Yes ☐ No ☐ Unknown
HIV/other immune deficiency	☐ Yes ☐ No ☐ Unknown
Heart disease	☐ Yes ☐ No ☐ Unknown
Asthma (requiring medication)	☐ Yes ☐ No ☐ Unknown
Chronic lung disease (non-asthma)	☐ Yes ☐ No ☐ Unknown
Chronic liver disease	☐ Yes ☐ No ☐ Unknown
Chronic haematological disorder	☐ Yes ☐ No ☐ Unknown
Chronic kidney disease	☐ Yes ☐ No ☐ Unknown
Chronic neurological impairment/disease	☐ Yes ☐ No ☐ Unknown
Hypertension	☐ Yes ☐ No ☐ Unknown
TB	☐ Yes ☐ No ☐ Unknown
Organ or bone marrow recipient	☐ Yes ☐ No ☐ Unknown
Other pre-existing condition(s)	☐ Yes ☐ No ☐ Unknown If Yes, specify: _____

Form B1: Contact initial reporting form (Day 1) – for close contacts of Pathogen X index cases (continued)

14. Vaccination	
Contact was vaccinated for seasonal influenza in the 12 months prior to onset of symptoms	☐ Yes ☐ No ☐ Unknown If Yes, date of vaccination, country of vaccination (dd/mm/yyyy): ____/____/____ Country: _____
Contact was vaccinated for SARS-CoV-2 in the 12 months prior to onset of symptoms	☐ Yes ☐ No ☐ Unknown If Yes, date of vaccination, country of vaccination (dd/mm/yyyy): ____/____/____ Country: _____ Date of last infection (dd/mm/yyyy): ____/____/____ or ☐ Unknown
Contact was vaccinated with pneumococcal vaccine	☐ Yes ☐ No ☐ Unknown If Yes, date (dd/mm/yyyy) ____/____/____

15. Status of form completion	
Form completed	☐ Yes (Date dd/mm/yyyy): ____/____/____ ☐ Partially (Date dd/mm/yyyy): ____/____/____ If partially, reason: ☐ Missed ☐ Not attempted ☐ Not performed ☐ Refusal ☐ Other, specify:

Form B2: Contact follow-up reporting form – for close contacts of Pathogen X index cases (Day 28)

Unique Index Case ID/Cluster number (if applicable):

Contact ID Number (C…):
Note: contact ID numbers should be issued at the time of completion of Form A1/Contact Line List.

1. Data collector information

Name of data collector	
Data collector institution	
Data collector telephone number	
Data collector email	
Form completion date (dd/mm/yyyy)	____/____/____

2. Interview respondent information (if the person providing the information is not the contact)

First name	
Family name	
Sex	☐ Male ☐ Female ☐ Non-binary ☐ Not known
Date of birth (dd/mm/yyyy)	____/____/____ ☐ Unknown
Relationship to contact (select all that are relevant)	☐ Household member ☐ Immediate family member ☐ Extended family member ☐ Health-care worker looking after contact ☐ Friend ☐ Co-worker ☐ Teacher ☐ Carer ☐ Acquaintance ☐ Unknown
Respondent address	
Telephone (mobile) number	

3. Exposure information (update from Day 1)

Specify characteristics of contact with the confirmed case from first contact, while the primary case was **symptomatic**, until the last unprotected contact (Add as many dates as required)	Date (dd/mm/yyyy)	____/____/____
	Duration	____(minutes) ☐ Still in contact with case
	Setting	☐ Home/household (Household ID number:___) ☐ Hospital/health care ☐ Workplace ☐ Tour group ☐ Other, specify:

Form B2: Contact follow-up reporting form – for close contacts of Pathogen X index cases (Day 28) (continued)

4. Outcome/status	
Status	☐ Alive ☐ Dead, if Yes, specify date of death (dd/mm/yyyy): ____/____/____ ☐ Unknown/lost to follow-up
Hospitalization ever required?	☐ Yes ☐ No ☐ Unknown
(NB. If the information below is not currently available, please leave blank and send through an update as soon as results are available)	
If dead, was a post mortem performed?	☐ Yes ☐ No ☐ Unknown
If dead, contribution of Pathogen X to death:	☐ Underlying/primary ☐ Contributing/secondary ☐ No contribution to death ☐ Unknown
If dead, results of postmortem's report where available:	
If dead, cause of death on Death certificate (specify)	

5. Hospital health-care interactions since Day 1	
Hospitalization since baseline	☐ Yes ☐ No ☐ Unknown
Date of first hospitalization (dd/mm/yyyy)	____/____/____ ☐ Unknown ☐ NA
Name and place of hospital	
Date of discharge from hospital (dd/mm/yyyy)	____/____/____ or ☐ Ongoing ☐ Unknown ☐ NA
Reason for hospitalization	☐ Isolation/quarantine ☐ Clinical management ☐ Other If Other, specify: _____
ICU (intensive care unit) admission since baseline	☐ Yes ☐ No ☐ Unknown
Date of ICU admission (dd/mm/yyyy)	____/____/____ ☐ Unknown
Date of discharge from ICU (dd/mm/yyyy)	____/____/____ or ☐ Ongoing ☐ Unknown ☐ NA
Mechanical ventilation since baseline	☐ Yes ☐ No ☐ Unknown
Dates of mechanical ventilation (dd/mm/yyyy)	Start: ____/____/____ Stop: ____/____/____ or ☐ Ongoing ☐ Unknown
Length of ventilation (days)	

Form B2: Contact follow-up reporting form – for close contacts of Pathogen X index cases (Day 28) (continued)

6. Contact symptoms: complications since Day 1	
Acute respiratory distress syndrome (ARDS)	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy) ____/____/____
Acute renal failure	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy) ____/____/____
Cardiac failure	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy) ____/____/____
Consumptive coagulopathy	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy) ____/____/____
Pneumonia by chest X-ray	☐ Yes ☐ No ☐ Unknown If Yes, date started (dd/mm/yyyy) ____/____/____
Other complications	☐ Yes ☐ No ☐ Unknown If Yes, secondary bacterial infection* ☐ Yes ☐ No ☐ Unknown Specify infection: If other complication, specify: *Fill out relevant laboratory information in specimen collection form
Hypotension requiring vasopressors	☐ Yes ☐ No ☐ Unknown
Extracorporeal membrane oxygenation (ECMO) required	☐ Yes ☐ No ☐ Unknown

7. Contact pre-existing condition(s)	
Pregnancy	☐ Yes ☐ No ☐ Unknown If Yes, specify trimester: ☐ First ☐ Second ☐ Third ☐ Unknown

8. Treatment with antivirals	
Did the contact receive an antiviral treatment in the last 14 days (or since baseline)?	☐ Yes ☐ No ☐ Unknown (If No or Unknown, skip to next question) If Yes, which antiviral was received? _____ Date started (dd/mm/yyyy) ____/____/____ Date stopped (dd/mm/yyyy) ____/____/____ Dosage (specify): What were antivirals prescribed for? ☐ Treatment ☐ Prophylaxis

Form B2: Contact follow-up reporting form – for close contacts of Pathogen X index cases (Day 28) (continued)

9. Final contact classification	
Contact was identified as a case during the investigation	☐ Yes, confirmed (tested positive with fully validated IHDT) ☐ No If no, the contact is a non-case
If contact was identified as a case during the investigation, final case classification (select one from each category that apply)	☐ Primary ☐ Co-primary ☐ Secondary ☐ Other, specify: (e.g., tertiary case) & (optional) ☐ Imported & (optional) ☐ Unrelated

10. Status of form completion	
Form completed	☐ Yes ☐ No ☐ Partially If No or partially, reason: ☐ Missed ☐ Not attempted ☐ Not performed ☐ Refusal ☐ Other, specify:

3. For cases and contacts

Form C: Specimen collection forms and laboratory results

Comment: Please note that this table will need to be filled out/updated for each specimen collected according to the mandatory sampling strategy (see Figure 2). **Note that if more than one laboratory is participating in the study, all specimens from an individual participant should be sent to the same laboratory to be run with the same IHDT.**

1. Case and contact details	
Unique ID for participant (use unique case or contact ID)	
Classification at recruitment	☐ Suspected case (protocol Part A) ☐ Confirmed case (protocol Part B) ☐ Close contact

2. Respiratory/other specimen collection (samples to be tested by IHDT)	
Date of sample collection (dd/mm/yyyy)	____/____/____
Time of sample collection	____ : ____
Day of specimen collection per sampling strategy (Figure 2)	(insert day number)
Type of sample collection	☐ AN (Nasal) swab ☐ OP (Throat) swab ☐ NP (Nasopharyngeal) swab ☐ Sputum ☐ Bronchoalveolar lavage (BAL)s ☐ Blood ☐ Urine ☐ Stool ☐ Saliva ☐ Conjunctival swab
Sample transport media (swab samples only)	☐ Viral Transport Media, specify manufacturer _____ ☐ Saline, specify type _____
Transport media volume (swab samples only)	☐ 1mL ☐ 3mL ☐ Other, specify _____
Who collected the respiratory specimen?	☐ Study staff/research nurse ☐ Other professional specimen collection service
Which laboratory was the specimen sent to?	
Was cold chain (4C) maintained throughout shipping?	☐ Yes ☐ No ☐ Unknown
Date sample received by laboratory (dd/mm/yyyy)	____/____/____
Time sample received by laboratory	____ : ____

Form C: Specimen collection forms and laboratory results (continued)

Laboratory sample identification number	
Diagnostic method	☐ PCR [In-House Developed Test (IHDT), specify name _____] ☐ Viral culture or virus isolation, if applicable
Date of IHDT result	____/____/____
Pathogen X Result	☐ Positive ☐ Negative ☐ Inconclusive, detail _____ (REPEAT TESTING) If positive (and applicable), subtype* ☐ Subtype A ☐ Subtype B ☐ Subtype C ☐ Not able to be typed *Adapt based on known information for Pathogen X
IHDT Ct value	
IHDT pathogen nucleic acid concentration, in genome equivalents/volume, if feasible	
Pathogen X IHDT Result (FROM REPEAT TESTING)	☐ Positive ☐ Negative ☐ Inconclusive, specify _____ If positive (and applicable), subtype* ☐ Subtype A ☐ Subtype B ☐ Subtype C ☐ Not able to be typed *Adapt based on known information on Pathogen X
IHDT Ct value (FROM REPEAT TESTING)	
IHDT pathogen nucleic acid concentration, in genome equivalents/volume, if feasible (FROM REPEAT TESTING)	
# aliquots (250 uL) made of sample after testing	
Date aliquots frozen by laboratory (dd/mm/yyyy)	____/____/____
Time aliquots frozen by laboratory	____ : ____
Freezing temperature	☐ –80°C (preferred) ☐ –20°C
Viral culture results (if applicable)	☐ Positive ☐ Negative ☐ Inconclusive
Additional details from positive viral culture (if applicable)	e.g., viral titer _____
Was Pathogen X sequencing performed? (if applicable)	☐ Yes ☐ No

Form C: Specimen collection forms and laboratory results (continued)

Other laboratory results obtained from specimen	☐ Other pathogens detected in specimen, specify[#]: _____ ☐ Negative (specify pathogens tested: _____) ☐ NA/not tested # Include data suggestive of any secondary (bacterial) infections in this section (sputum/BAL only)
Sample sent to WHO CC (if applicable) (dd/mm/yyyy)	☐ Yes ☐ No If yes, Date: ____/____/____ Name of collaborating centre:

3. Serologic testing methods and results:	
Date of sample collection (dd/mm/yyyy)	____/____/____
Day of specimen collection per sampling strategy (Figure 2)	(insert day number)
Type of sample collection	☐ Whole blood ☐ Serum ☐ Dried blood spot ☐ Other, specify:
Who collected the sample?	☐ Study staff/research nurse ☐ Other professional specimen collection service
Which laboratory was the specimen sent to?	
Date sample received by laboratory (dd/mm/yyyy)	____/____/____
Laboratory identification number	
Diagnostic method/assay used	☐ Total antibody ☐ Microneutralisation ☐ Other, specify:
Antigen used (if applicable)	
Date of result	____/____/____
Pathogen X Result (according to assay thresholds/cut-offs – please report these)	☐ Positive ☐ Negative ☐ Indeterminate/probable Titre (irrespective of result):
Sample sent to WHO CC (if applicable) (dd/mm/yyyy)	☐ Yes ☐ No If yes, Date: ____/____/____ Name of collaborating centre:

First Few X cases and contacts (FFX-Dx) investigation for respiratory pathogens with pandemic potential: template protocol

Form D: Symptom diary for suspected/confirmed cases of Pathogen X and close contacts (Day 1–28)

Note: if a suspected case is determined to NOT be a case after testing with the fully-validated IHDT, the symptom diary no longer needs completion. Symptom diaries will be provided to all participants for recording the presence or absence of various signs or symptoms for 28 days after enrolment (Day 1).

The symptom diary template provided below is generic. This form may be completed either by the participant or by a delegate (if patient is too ill to complete).

Comment: In the context of a novel pathogen with uncertain clinical presentation and spectrum, symptom diaries may be broadened to include vomiting, diarrhoea, abdominal pain, etc., as relevant.

Symptom diaries

Case or contact study ID number :
Day 1 (enrolment) date _____

Day	Symptoms*						
	No symptoms (check if none experienced)	**Fever ≥38°C**	**Runny nose**	**Cough**	**Sore throat**	**Shortness of breath**	**Other symptoms: specify**
1	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
2	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	

Form D: Symptom diary for suspected/confirmed cases of Pathogen X and close contacts (Day 1–28) (continued)

Day	Symptoms*						
	No symptoms (check if none experienced)	**Fever ≥38°C**	**Runny nose**	**Cough**	**Sore throat**	**Shortness of breath**	**Other symptoms: specify**
3	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
4	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
5	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
6	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
7	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	

Form D: Symptom diary for suspected/confirmed cases of Pathogen X and close contacts (Day 1–28) (continued)

Day	Symptoms*						
	No symptoms (check if none experienced)	**Fever ≥38°C**	**Runny nose**	**Cough**	**Sore throat**	**Shortness of breath**	**Other symptoms: specify**
8	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
9	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
10	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
11	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
12	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	

Form D: Symptom diary for suspected/confirmed cases of Pathogen X and close contacts (Day 1–28) (continued)

Day	Symptoms*						
	No symptoms (check if none experienced)	**Fever ≥38°C**	**Runny nose**	**Cough**	**Sore throat**	**Shortness of breath**	**Other symptoms: specify**
13	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
14	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
15	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
16	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
17	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	

Form D: Symptom diary for suspected/confirmed cases of Pathogen X and close contacts (Day 1–28) (continued)

Day	Symptoms*						
	No symptoms (check if none experienced)	**Fever ≥38°C**	**Runny nose**	**Cough**	**Sore throat**	**Shortness of breath**	**Other symptoms: specify**
18	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
19	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
20	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
21	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
22	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	

Form D: Symptom diary for suspected/confirmed cases of Pathogen X and close contacts (Day 1–28) (continued)

Day	Symptoms*						
	No symptoms (check if none experienced)	**Fever ≥38°C**	**Runny nose**	**Cough**	**Sore throat**	**Shortness of breath**	**Other symptoms: specify**
23	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
24	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
25	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
26	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	
27	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	

Form D: Symptom diary for suspected/confirmed cases of Pathogen X and close contacts (Day 1–28) (continued)

Day	Symptoms*						
	No symptoms (check if none experienced)	**Fever ≥38°C**	**Runny nose**	**Cough**	**Sore throat**	**Shortness of breath**	**Other symptoms: specify**
28	☐ None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No If Yes to cough, productive? ☐ Yes ☐ No ☐ Unknown If Yes to cough, dry? ☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No	☐ Yes ☐ No	

*Please select None for No symptoms. If no symptoms are experienced, then consider the entry complete.

FFX-Dx reporting forms: completion guidance

These notes provide guidance in completing the forms. It is suggested that the investigations could be divided into teams – these could include:

- a **"case reporter" team;**
- a **"contact reporter" team;** and
- a **"go to" team** who would liaise with additional data sources other than the case or contact, such as hospitals, laboratories, etc.

Form A1: Case initial report form – for Pathogen X suspected/confirmed cases (Day 1) and Form A2: Case follow-up form – for Pathogen X confirmed cases (Day 28)

These forms should be completed by the "case reporter" team.

Section	Sources	Verified against
Final case classification	Case reporter/hospital	
Reporter details	Case reporter	
Informant details	Informant	
Exposure information	Informant	
Outcome/status	Informant	Statistical data, mortality, GP/hospital
Illness	Informant	Health-care provider/review of medical records
Clinical course/complications	Informant/interview with health-care provider	Review of medical records
Interaction with national security system	Informant/hospital	National social health information system
Medical history	Informant	Health-care provider/GP/review of medical records
Laboratory results (record in Form C for each sample)	Testing laboratory	Laboratory database

Form B1: Contact initial reporting form – for close contacts of Pathogen X confirmed cases

This form should be completed by the "contacts reporter" team and should be completed after the initial case report form (B1) has been completed by the "case reporter" team, ideally within 24 hours.

Section	Sources	Verified against
Reporter details	Contact reporter	
Informant details	Informant	
Contact details	Informant	
Exposure information	Informant	
Illness in contacts	Informant	Health-care provider/review of medical records
Outcome/status	Informant	Statistical data, mortality, GP/hospital
Case classification	Contact reporter	
Laboratory results (record in Form C for each sample)	Testing laboratory	Laboratory database
Medical history	Informant	Health-care provider/GP/review of medical records

Form B2: Contact follow-up reporting form – for close contacts of confirmed Pathogen X index cases (Day 28)

This form should be completed by the "contacts reporter" team.

Section	Sources	Verified against
Reporter details	Contact reporter	
Informant details	Informant	
Final contact classification	Contact reporter	
Exposure information	Informant	
Illness in contacts	Informant	Health-care provider/review of medical records
Clinical course/complications	Informant/interview with health-care provider	Review of medical records
Laboratory results (record in Form C for each sample)	Testing laboratory	Laboratory database

Form C: Specimen collection forms and laboratory results

These forms should be complete by the "go to" team.

Section	Sources	Verified against
Laboratory results	Testing laboratory	Laboratory database

Form D: Symptom diary for confirmed index cases of Pathogen X and close contacts (Day 1-28)

This form should be completed by all participants or delegates (if participant too ill to complete).

Annex 3: Considerations for specimen collection

Please see Figure 2 for the detailed FFX-Dx sample collection plan. Collection and testing of appropriate specimens from cases and contacts must be conducted according to the appropriate sampling strategy.

Table A3.1 provides a list of possible specimens that will be collected, recommendations for swab type (if applicable), transport medium, transport temperature and storage, and a reference for best-practice collection method.

Comment: Investigators need to be familiar with the correct collection techniques (including the appropriate use of PPE for different types of specimens collected and established infection control guidelines), and the safety standards for specimen storage, packaging, and transport.

Table A3.1: Specimen type, transportation (assumes virus Pathogen X), and storage guidelines (assumes molecular testing) for testing for presence of respiratory pathogens. (Adapted from Table 1, WHO protocol to investigate non-seasonal influenza and other emerging acute respiratory diseases *(29)*)

Specimen type	Swab type/ container, if applicable	Transport medium	Transport to laboratory	Storage until testing	Best-practice method for collection (REF)
Nasopharyngeal swab	NP flocked swab	UTM/VTM	4–8°C	≤5 days: 4–8°C >5 days: ≤ –20°C	CDC method *(30)*
Sputum	Sterile container	NA	4–8°C	≤48 hours: 4–8°C >48 hours: ≤ –20°C	CDC method *(30)*
Anterior nasal swab (collected with optimized method)	Large head flocked swab	VTM	4–8°C	≤5 days: 4–8°C >5 days: ≤ –20°C	CDC method *(30)*
Throat swab	Large head flocked swab	VTM	4–8°C	≤5 days: 4–8°C >5 days: ≤ –20°C	CDC method *(30)*
Bronchoalveolar lavage*	Sterile container	NA	4–8°C	≤48 hours: 4–8°C >48 hours: ≤ –20°C	Per institutional routine
Serum	Serum separator tube	NA	4–8°C	≤5 days: 4–8°C >5 days: ≤ –20°C	Per institutional routine, 5 mL optimal
Whole blood	EDTA tube or DBS	NA	4–8°C	≤5 days: 4–8°C >5 days: ≤ –20°C	Per institutional routine, 5 mL optimal. (May be useful antigen detection particularly during the first week of illness; DBS could also be used for serologic testing)
Urine	Sterile container	NA	4–8°C	≤5 days: 4–8°C >5 days: –20°C	Per institutional routine; sterile container; minimum 5–10 mL
Stool	Sterile container	NA	4–8°C	≤5 days: 4–8°C >5 days: –20°C	Per institutional routine; sterile container; minimum 1g
Saliva	Sterile container	NA	4–8°C	≤48 hours: 4–8°C >48 hours: –20°C	*(31)*
Conjunctival swab*	NP flocked swab	UTM/VTM	4–8°C		*(32)*

* More invasive specimen types which may cause patient discomfort and are not performed routinely.

Annex 4: Considerations for the Development and Early Validation of an IHDT for molecular detection of Pathogen X in clinical samples

Summary: Phases I (Early Development) and II (Early Validation), below, are expected to be done by the Developer(s) of the IHDT, specifically an expert laboratory or laboratory consortium with substantial experience in development and validation of IHDTs and potentially with expertise in pathogens similar to Pathogen X (optimally, candidates for IHDT development could be identified in advance). The IHDT Developer laboratory may or may not be located near the site of the outbreak, and may not have access to clinical specimens from patients infected with Pathogen X. Following a successful Early Validation of the IHDT by the IHDT Developer (Phase II, below), it is expected that an End User laboratory wishing to implement the IHDT for testing at the site of the outbreak would perform its own validation of the IHDT (End User Validation; **Section 2.2**). This End User Validation includes a focused analytical validation in the End User laboratory and a limited clinical validation involving testing of patients suspected of infection with Pathogen X, including confirmation of the first 10+/10- results by an independent laboratory. **The End User Validation is performed in Part A of the FFX-Dx protocol (Section 2.2); Early Development and Early Validation phases are assumed to have been completed by the IHDT Developer prior to FFX-Dx protocol initiation.**

I. IHDT Early Development

Work by the IHDT Developer to **design and develop** the molecular test (IHDT) for Pathogen X prior to analytical and clinical validation in Phase II. Includes development of primers, probes, and an initial RT-PCR/PCR assay. This work could be done by the same laboratory involved in Pathogen X discovery/early characterization or by another laboratory or laboratory consortium.

Note: the plan below assumes that the IHDT is a real-time RT-PCR/PCR assay, but can be adapted to accommodate other types of molecular assays. [Note that the FFX-Dx protocol assumes the ability to generate Ct values and/or target nucleic acid concentrations (in genome equivalents) that can be used to assess Pathogen X kinetics and the relative yield of different sample types.]

Information and materials to be generated:

Sequence of Pathogen X

Primer and probe sequences/targets/concentrations

Recommendations:

- The developed assay should ideally be a dual target assay.
- Consider strategy to choose targeted amplification regions and the specific primer and probe regions, including impact of known or expected variation in the Pathogen X genome and goal of avoiding cross-reactivity.

Materials to use a) as a quantitative reagent for LOD determination, b) to create contrived samples, and c) as positive control material

- Inactivated/quantified target nucleic acid (native genomic RNA or in-vitro transcribed (IVT) RNA, or DNA)
- Inactivated Pathogen X stock (ideally, quantified)
- Other reagents (e.g., plasmid, if relevant)

Assay details*

- Instruments used for extraction, PCR detection:
  - Recommendation: Use readily available instruments, and preferably those that have already been regulated for IVD use.
- Extraction details (sample input volume, nucleic acid elution volume, extraction protocol details).
- Amplification details:
  - Input volume of purified nucleic acid added to RT-PCR reaction mix.
  - Amplification assay details (instrument, master mix, assay conditions).
- Preliminary LOD using genomic or IVT RNA (or plasmid, if relevant).
- Preliminary specificity using control samples:
  - Recommendation: UTM/VTM, water, mucin, leukocyte DNA or DNA from other organisms; n = 50–60 each.
- Suitable controls should be carefully considered (including internal controls and process controls), balancing the goals of rapid assay development, intention to ultimately transfer assay to End User laboratories, and the goal of avoiding future re-design (particularly if a version of the assay ultimately may be validated for emergency use authorization by any relevant regulatory authority).
  Consider:
  - External positive controls (including concentration ideally near LoD)
  - External calibration/quantification controls (if applicable)
  - External negative control
  - Extraction control (can be the external positive control)
  - Other controls (e.g., internal control, sample adequacy control with a defined reference range)
- Preliminary cutoff for positive versus negative result.
- Preliminary definition of indeterminate/inconclusive/equivocal.

*may be modified by the laboratory performing IHDT early validation, below

Note: depending on feasibility and local regulations, consider ability to produce and distribute actual primers/probes/control materials to other laboratories for validation.

II. IHDT Early Validation

Rigorous and focused analytical studies performed **by the IHDT Developer** (expected to be an **expert laboratory or laboratory consortium**) to **validate** the IHDT in preparation for use for clinical (and research) testing prior to EUA by any relevant regulatory authority. This work can be done prior to availability of clinical samples from patients infected with Pathogen X and can be done by the same laboratory involved in IHDT Early Development or by another laboratory or laboratory consortium.

Note that any potential requirements for IRB/ethics approval for use of discarded clinical specimens for early validation work should be considered in advance.

A. Analytical sensitivity (Limit of Detection)

- Must utilize the entire test system from sample preparation and extraction to detection.
- Spike inactivated/quantified target nucleic acid (native or IVT RNA, or DNA if appropriate; or, optimally, quantified inactivated organism) into pooled target clinical matrix (e.g., NP swab, sputum, BAL) from individuals who do not have Pathogen X infection. Note: if IVT RNA is used for LOD determination, the LOD should be re-determined once inactivated organism/genomic RNA is available.
- The standard used for LOD determination should ideally be quantified in genomic equivalents (copy numbers). (Quantification in PFU/mL should ideally be avoided for LOD determination).
- Mitigation of RNA degradation should be considered (e.g., RNAse inhibitor, spiking RNA into lysis buffer rather than into clinical sample, generating armored RNA).

Minimum testing to establish LOD:

Identify the preliminary Limit of Detection (LoD) by testing a 2-3-fold dilution series (with 3-6 replicates per concentration) in pooled clinical matrix, and then confirm with 20 replicates of the concentration determined to be the preliminary LoD (optimally, should also test 20 replicates at one dilution above and one dilution below the preliminary LOD). For purposes of this document, the preliminary LoD is the lowest concentration that gives positive results 100% of the time and the final LoD is the lowest concentration at which at least 19 of 20 replicates (95%) are positive. The preliminary LoD studies should include at least one concentration that does not yield 100% positive results.

Alternatively, statistical methods like probit regression analysis can be used to define the LOD, and may require fewer replicates.

LOD should be reported in genome equivalents/volume unit.

Note: there is no target LOD at this stage because the relevance of that target LOD is not yet known.

Minimum sample type evaluation:

- For LOD determination, testing of NP swab samples is sufficient to represent upper respiratory tract samples [oropharyngeal (OP) and anterior nares (AN) swabs]; NP swab is considered the most challenging upper respiratory matrix.
- For lower respiratory tract samples (sputum and BAL), sputum is considered to be the most challenging (and sputum testing can suffice to support both upper and lower respiratory matrices).
- **Optimally, validate NP swab first, and subsequently, validate sputum.**
- Stool, urine, saliva, and blood would each need to be tested separately.

B. Inclusivity (analytical reactivity)

- Perform an *in silico* inclusivity analysis that establishes the extent to which variation in the Pathogen X genome may impact sensitivity of test performance.
- Utilize control material that covers the known or expected diversity of the target pathogen.

C. Cross-reactivity (Analytical specificity)

Note: per the International Medical Device Regulators Forum (IMDRF), the term "analytical specificity" includes cross-reactivity and interference.

1. Conduct an *in silico* analysis of published genome sequences using the assay's primers and probes. If *in silico* analyses of the target primers and probes indicate ≥ 80% homology between the cross-reactivity microorganism(s) and your test primers/ probe(s), conduct wet testing with that organism(s). We recommend using high concentrations (e.g., 10^6 CFU/ml or higher for bacteria and 10^5 pfu/ml or higher for viruses). *In silico* analyses alone may be appropriate for organisms that are difficult to obtain.
2. Test original (deidentified) clinical specimens containing the most common respiratory viruses/bacteria or negative clinical specimens spiked with virus/bacterial isolates. For each organism, 3–5 separate clinical specimens should be tested. Optimally, specimen and/or organism banks can be created in advance for this purpose.

Note: it is not necessary to separately evaluate interference if well-established nucleic acid extraction methods are used.

D. Performance evaluation

A performance evaluation utilizing individual positive contrived specimens should be performed [i.e., 10 clinical specimens (target matrix) from unique individuals, spiked with control material (inactivated/quantified target nucleic acid, or isolate), ideally at no more than 3x LOD]. It is not necessary to test additional clinical specimens negative for Pathogen X because this has been accomplished with the cross-reactivity experiment (C, above). However, if a different matrix is being evaluated, testing of 10 separate negative clinical specimens (target matrix) would be prudent.

E. Sample Stability (Note: data collection should not delay transfer of assay to End User)

Testing depends on the availability of inactivated Pathogen X (or clinical samples) at the time of validation. If available, testing should be conducted to demonstrate sample stability throughout the real-world conditions in which samples are expected to be collected and tested. When the test is intended to be performed on the sample immediately or shortly after obtaining the sample, sample stability testing could be relatively short (i.e., 2 hours at room temperature) and conducted with contrived samples at 3x LoD using inactivated (virus) spiked into negative clinical matrix. Fresh versus frozen studies should be performed.

Conditions to consider: e.g.,
At least one of the following: Saline, VTM or UTM
RT (define), 4°C, –20°C, –80°C
Time (consider transport time to laboratory, and consider extended delays)
Minimum # of replicates at each condition: n = 3
Expectation: </= 3 Ct change between baseline and other condition (e.g., time 0 vs time X, fresh vs frozen).
Note: if initial stability studies are performed with contrived samples, they would optimally be repeated with actual clinical samples when available.

Note: additional validation data may or may not be required for receipt of EUA; this should be determined in consultation with the relevant regulatory authority. For example, some authorities might require a slightly larger performance evaluation (D) for EUA.

Additional recommendations:

- It is not necessary to separately test precision (in part because testing has already been accomplished by replicate testing during LOD determination and confirmation).
- It is not necessary to test a panel of interfering substances at this stage.

Annex 5: Requirements of executing group

Requirements of executing group

Part A investigation:

The End User laboratory may be selected by a national authority (e.g., ministry of health or similar) to perform clinical testing at the site of the outbreak. Requirements for an End User laboratory are outlined in the table below. **Each laboratory wishing to perform clinical testing with the IHDT must complete the End User Validation (focused analytical and limited clinical validation) described in Section 2.2**.

Part B investigation:

Part B testing using the IHDT can be performed by any End User laboratory that has validated the IHDT as outlined in Part A (Section 2.2). Other requirements for the clinical study management and the communication with the public health authorities that apply are outlined in the table below. The executing group/laboratory is expected to be selected by a national authority (e.g., ministry of health or similar). Conceivably, multiple sites can contribute to the enrolment.

While the End User Laboratory and clinical team implementing the FFX-Dx protocol are not expected to be funded in an interpandemic phase, both need to be established and operational with minimal requirements in place as outlined in the table below, including data and sample sharing agreements.

Note: it is expected that Part A and Part B are executed at the very first location of the outbreak.

Table A5.1 articulates the essential/acceptable and desirable features of the End User laboratory and clinical team implementing the FFX-Dx protocol.

Table A5.1: Requirements for End User Laboratory in Part A/B and clinical team implementing FFX-Dx protocol

Feature	Essential/Acceptable	Desirable	Comment
General requirements			
Study coordination	Designated, active Focal Point/Principal investigator/Support Center		Designation by national authority
Data Management	Data management aligned to standards necessary for validation and following best practice guidelines for security	In-house health informatics expertise	
Data Analysis	An active collaboration with external group for data analysis	In-house medical statistics, epidemiology, or clinical trial unit with data analytics expertise	
Pre-approved ethics and governance processes	If required, pre-approved ethical, data governance and/or regulatory approvals to facilitate the study or access to rapid approval mechanisms Data sharing agreements in place	Sample sharing agreements in place. Linked to pre-approvals of other Unity Studies FFX/HHT investigations to facilitate participant involvement in these studies	Linkage to FFX
Engagement with Unity studies network of sites	Commitment from the site to participate in the Unity Studies network according to the duties described through the acceptance and signature of the terms of reference	**Pre-selection of Unity Studies sites as part of preparedness framework. Ideal is overlap or linkages with other clinical platforms**	
GCP/GLP	Principal investigator and clinical study team for the site to provide CV, conflict of interest form, and Good Clinical Practice certificate (complete within 2 years)		Good clinical practice, good laboratory practice
Clinical requirements			
Routine Clinical Sample Handling	Active clinical and research group with experience in clinical studies with routine access to medical laboratory <60 minutes from site	Active in-house or mobile clinical biomedical laboratory	
Cold chain	Available for clinical and study sample transport		

Feature	Essential/Acceptable	Desirable	Comment
Occupational Health	Access to medical opinion within 20 minutes from site	On site medical practitioner	To assess nosocomial transmission or suspected cases among laboratory staff
Clinical Waste Handling	Clinical waste policy and code of practice to safely handle waste associated with a potential new or dangerous pathogen		
End User Laboratory requirements			
Laboratory Accreditation	ISO 9001 and Certificates of the equipment required to perform the validation and clinical testing	ISO 15189	International standard for quality and competence in medical laboratory activities and quality management systems
Biosafety	BSL2 with BSL3 practices; able to unpack samples possibly containing BSL-3 organisms	BSL3 with virus isolation expertise in house	BSL4 facility not required to be selected as a site
Technical personnel	Commitment to dedicate sufficient time and resources to deliver End User Validation and Part B testing as a priority	Dedicated team comprising at least 1 technician and senior laboratory expert	
Equipment	1 PCR cycler, manual extraction of RNA/ DNA (note that manual extraction increases risk of contamination, but for some pathogens, manual extraction may be optimal)	In-house sequencing platform and bioinformatics unit, 2 PCR cyclers and automated extraction of RNA/DNA	
Laboratory Data Management	Laboratory information system to handle samples	Resilient IT infrastructure that enables real-time data sharing for monitoring and patient/physician information	
Laboratory internal processes	Willing to participate in QC program and share results/ data	Data sharing agreement(s) in place Willing and able to share materials with other laboratories	
Sample sharing	Willing to share samples for confirmatory testing (by another laboratory, in Part A)	Sample sharing agreement(s) in place Willing to perform confirmatory testing for other End User labs when needed	

Feature	Essential/Acceptable	Desirable	Comment
Total turnaround time, per sample	<48hours	<8h (max 24h)	Total turnaround time is time from **sample collection** to result reporting to healthcare provider (and thus also involves clinical team)
Operational capacity	100 samples per day with lab turnaround time < 24 hours and daily service	1,000 samples per day with lab turnaround time < 8 hours and 24/7 service	Lab turnaround time here is time from **sample receipt** in lab to results reporting
Sample Biobanking	Storage of samples at –20° Celsius and 4–8° Celsius with sufficient capacity with aliquoting capability	Comprehensive biobanking facility (4–8° Celsius, –20° Celsius, –80° Celsius, liquid nitrogen) with ability to dedicate on site storage to the samples and a range of storage options and electricity backup and permanent temperature monitoring	
Test portfolio	Ability to test for common causes of respiratory infections (see exclusion criteria 2.5)		
Specialist expertise	Routine molecular diagnostics with robust controls	Multidisciplinary expertise across Virology, Microbiology, Immunology, and/or Entomology	

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