Study Title: **P**latform **r**andomised controlled trial of point of care **d**iagnostics for **e**nhancing the quality of antibiotic prescribing for **c**ommunity acquired acute respiratory tract infection in ambulatory care in **E**urope (**PRUDENCE**)

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The Investigators declare that there are no conflicts of interest.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

TABLE OF CONTENTS

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2. LAY SUMMARY

The discovery of antibiotics was a major breakthrough in medical science. Antibiotics are used to treat illness caused by bacteria. Development of these medicines meant that illnesses, like pneumonia, which were once often fatal, could be successfully treated, saving millions of lives. But their effectiveness has decreased because they are misused. Highly resistant 'superbugs' have appeared against which we have no effective treatment. The development of antibiotic resistance is now a major public health concern worldwide.

Bacteria that are exposed to insufficient antibiotic can change and become resistant to their killing effects. Another cause of resistance is when antibiotics are prescribed when there is no evidence to prove which micro-organism is causing a patient's symptoms. Clinical studies have shown that confirming a patient is more likely to be infected with a bacterium (or not) before prescribing an antibiotic by using a point-of-care test in a doctor's surgery could help improve the quality of patient care and aid recovery. This is especially true for community-acquired acute respiratory tract infections (CA-ARTI), the commonest reasons for consulting health services in the community and for antibiotic use.

The aim of PRUDENCE is to determine if there is added value provided by having a CA-ARTI diagnostic (CA-ARTI-Dx) test done in the surgery to give a quick result. Then the result is available when a clinician is considering, or plans to prescribe an antibiotic, which could lead to more appropriate prescribing decisions, without causing harm to patients.

3. SYNOPSIS

Study Title	**P**latform **r**andomised controlled trial of point of care **d**iagnostics for **e**nhancing the quality of antibiotic prescribing for **c**ommunity acquired acute respiratory tract infection in ambulatory care in **E**urope
Internal ref. no. / short title	PRUDENCE
Study registration	
Sponsor	University of Oxford
Funder	Innovative Medicines Initiative 2 Joint Undertaking
Study Design	Pragmatic, platform, randomised controlled trial of point-of-care diagnostics
Study Participants	Patients ≥1 year consulting with CA-ARTI in community care with participating clinicians in participating countries and that meet the eligibility criteria.
Sample Size	Approximately 2500 participants in the main trial. Additionally 32-50 participant (patient) interviews and 28-35 healthcare professional interviews.
Planned Study Period	Trial preparation started in January 2020. All approvals are expected to be in place and recruitment to start 04JAN2021.The end of the study will be at the end of the VALUE-Dx project.

Planned Recruitment period	The first inclusion is planned for January 2021, and the last patient will be recruited at the end of the winter season 2021/22. The recruitment period may start later or be extended if problems arise due to COVID-19 second wave.		
	Objectives	Outcome Measures	Timepoint(s)
Co-Primary	To assess effectiveness of CA-ARTI-Dx in terms of both reductions in antibiotic prescribing, and in terms of patient recovery.	The proportion of participants being prescribed at least one antibiotic course (of any dose or duration) over 28 days from inclusion (estimating possible reduction). Time to return to usual daily activities, where returned to usual daily activity = yes (estimating non-inferiority)	Day 1 – 14 diary or Day 14 telephone call, and Day 28 telephone call
Secondary	To explore whether adding a CA-ARTI-DX to usual primary care has: 1) Additional effects on antibiotic prescribing 2) Effects on antibiotic use 3) Effects on patient recovery and safety, including complications and hospitalisation 4) Effects on use of medications other than antibiotics 5) Effects on clinician's decision making process regarding diagnosis and treatment 6) Effects on patients' perceived ability to understand and cope with their illness	1) Choice of antibiotic class, whether 'delayed' or immediate 2) Antibiotic use over 28 days after inclusion. 3) Patient reported symptoms severities, re-consultations, hospitalization, pneumonia 4) Use of other, non-antibiotic, medication including antiviral medication, and over-the-counter treatments. 5) Responsible clinician's initial working diagnosis and antibiotic prescribing decision before test, if randomised to CA-ARTI-Dx also final decision after test. 6) Patient Enablement Instrument (PEI) score. 7) Costs data: EQ5D at day 1, Day 14 and Day 28; time taken for	1) Day 1 – 14 diary or Day 14 telephone call, and 28 telephone call 2) Day 1 – 14 diary or Day14 telephone call, and 28 telephone call 3) Day 1 – 14 diary or Day 14 telephone call, and 28 telephone call 4) Day 1 – 14 diary or Day 14 telephone call, and 28 telephone call 5) Baseline visit 6) Day 1 -14 diary

	7) Is cost effective	testing; costs associated with subsequent help seeking, complications, work loss and over-the-counter medication use (to be determined in close collaboration with WP5).	7) Baseline visit, Day 1 – 14 diary or Day 14 telephone call, and Day 28 call
Process Evaluation	To identify: 1) Clinicians' views and experiences of using CA-ARTI-Dx, how they fit with/impact on delivery of care and intended future use. 2) Patient/parent views of CA-ARTI-Dx and how they impact consultations including patient satisfaction and intention to consult in future for similar symptoms.	1) Interviews and surveys with healthcare professionals. 2) Interviews and surveys with patients.	
Interventions	All trial interventions are detailed in the Appendices. Further interventions may be added or replaced during the course of the trial, subject to suitable interventions becoming available and all necessary approvals being first obtained.		
Comparator	Usual care of people with CA-ARTI consulting in primary care (general practices, Long Term Care Facility (LTCF), and primary care paediatricians) where an antibiotic is being considered. Clinicians will be referred to local guidelines for managing common infections.		

4. ABBREVIATIONS

AE	Adverse Event
AMR	Antimicrobial resistance
CA-ARTI	Community Acquired Acute Respiratory Tract Infection
CA-ARTI-Dx	Community Acquired Acute Respiratory Tract Infection Diagnostic
CEA	Cost Effectiveness Analysis
CE marked	European conformity marked
CI	Chief Investigator
COVID-19	Coronavirus disease
CRF	Case Report Form
eCRF	Electronic Case Report Form
CTRG	Clinical Trials & Research Governance, University of Oxford
DMEC	Data Monitoring and Ethics Committee

EDC	Electronic Data Capture
GAS	Group A Streptococcus
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GP	General Practitioner
HRA	Health Research Authority
ICF	Informed Consent Form
IMI	Innovative Medicines Initiative
LRTI	Lower Respiratory Tract Infection
LTCF	Long term Care facility
NHS	National Health Service
PC-CTU	Primary Care Clinical Trials Unit
PEI	Patient Enablement Instrument
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
POCT	Point of care test
PRUDENCE	Platform randomised controlled trial of point of care diagnostics for enhancing the quality of antibiotic prescribing for community acquired acute respiratory tract infection in ambulatory care in Europe
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RES	Research Ethics Service
RO	Research Online
RTI	Respiratory Tract Infection
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2 (viral name)
SOP	Standard Operating Procedure
TMG	Trial Management Group
TSC	Trial Steering Committee
UMCU	University Medical Center Utrecht
UOXF	University of Oxford
VAS	Visual Analogue Scale

5. BACKGROUND AND RATIONALE

Introduction

The public health threat posed by antimicrobial resistance (AMR) requires humanity to use existing antibiotics far more wisely and efficiently to preserve the effectiveness of these 'wonder' drugs. A more personalised approach to antibiotic prescribing, one that better targets antibiotics to those individuals who are *likely* to benefit, and directs alternative, non-antibiotic treatments to those who are *unlikely* to benefit, is now a matter of considerable urgency. Unnecessary antibiotic use increases antibiotic resistance, wastes resources, and unnecessarily exposes patients to adverse effects.

Community Acquired Acute Respiratory Tract Infections (CA-ARTI)

Community acquired acute respiratory tract infection (CA-ARTI) is the commonest acute reason for consulting community health care, and is also the commonest syndrome for which antibiotics are prescribed unnecessarily. Point-of-care tests (POCTs) for this condition could transform care.

Community Acquired Acute Respiratory Tract Infections point of care Diagnostics (CA-ARTI-DX)

Diagnostics could transform clinical care, especially in community care settings where the majority of antibiotics are prescribed, by reducing uncertainty about potential benefit antibiotics may offer to individuals. However, diagnostics are hardly ever used to inform prescribing decisions in community care settings in many European countries, largely because of huge gaps in the evidence-base on their potential medical and health-economic value and the multi-faceted barriers that inhibit their uptake into usual care.

Trials of CA-ARTI-DX

Despite the enticing opportunities for health and social gain, diagnostic tests for use in community care settings, in general, have been inadequately evaluated. Clinical studies have almost exclusively focused only on the analytical performance of tests and have largely ignored other important value-determinants such as their contribution to improving patient outcomes, their impact on antibiotic use and AMR, and their cost-effectiveness at broader level (e.g. societal, payers). Some point-of-care diagnostic tests have been taken up into routine care, and have then been found not to be cost-effective when considered in relation to improving patient outcomes. Psychosocial and organisational (e.g. uptake) barriers and facilitators require a far deeper understanding to better inform the development of bespoke solutions.

When clinical trials of the cost-effectiveness of POCTs have been done, trials have generally adopted a traditional two arm approach, which can be inefficient. Innovative trial designs could allow greater efficiency flexibility, for example evaluating more than one intervention and replacing or adding new interventions within the same trial. Critically, CA-ARTI-Dx should better target antibiotic prescribing and use, thus reducing the overall amount of antibiotics used. However, this should not be at the expense of patient recovery. Evaluations should ensure that the introduction of any new CA-ARTI-Dx is based on evidence for reduced antibiotic use/prescribing and evidence that use of the CA-ARTI-Dx does not cause patients harm.

A pragmatic trial

The aim of PRUDENCE is to determine the value of the information of having a CA-ARTI-DX result available at the point of making a prescribing decision in addition to usual care clinical assessment.(without a CA-ARTI-Dx), in situations where a CA-ARTI-Dx result could sway the responsible clinician towards not

prescribing an antibiotic which does not result in harm to the patient (increased symptom duration or severity). When there is no doubt in the clinician's mind that antibiotics are definitely not indicated, such as in the case of an obvious viral, self-limiting infection, then performing a test does not influence clinician uncertainty and so should not be performed. Testing everyone with CA-ARTI in primary care is neither feasible nor appropriate. Thus, we wish to include only those patients in whom the responsible clinician is considering or planning to prescribe an antibiotic, and exclude those where they feel an antibiotic is not indicated. Given that the comparison will be to management without a CA-ARTI-Dx result, the trial should be as minimally disruptive to usual care as possible.

Due to uncertainties around COVID-19, the study may be paused in certain networks/regions if it is not feasible to proceed according to this pragmatic protocol.

COVID-19

As of 05 October 2020, 3 569 918 cases of COVID-19 and 191 737 deaths attributed to COVID-19 have been reported in the EU/EEA and the UK and many countries in Europe are reporting an increase in cases. Symptoms of COVID-19 are often the same as symptoms of CA-ARTI, in particular developing a new, continuous cough. Management of COVID-19 varies but generally people with symptoms compatible with COVID-19 should stay at home and contact their healthcare providers. This could be detrimental to the PRUDENCE trial as participants will need to visit their GP or primary healthcare provider to be able to have the CA-ARTI-Dx test performed. To alleviate some of the concerns around inviting participants with CA-ARTI symptoms to the healthcare provider, the PRUDENCE trial will include a SARS-COV-2 POCT in the management algorithm (depending on test availability and the COVID-19 pandemic situation in each country at the time of recruitment). If used, this will essentially exclude any participants that have a positive test result from continuing in the trial.

Platform trial

A platform trial, in contrast to the traditional design, allows multiple arms to be considered simultaneously, and interventions can be dropped and replaced as evidence emerges for effectiveness or lack of it. The intent is to establish an on-going trial infrastructure within a master protocol that uses all the data already accumulated for the assessment of current and subsequently introduced interventions. Two CA-ARTI-Dx have been selected for evaluation in the first study period (see appendix D and E for current CA-ARTI-Dx being evaluated). There will be a pre-specified interim analysis in between study periods. If the study questions for a given CA-ARTI-Dx are answered at the interim per pre-specified criteria, then one or both of the CA-ARTI-Dx will be replaced with a new CA-ARTI-Dx. This study design therefore has the potential to assess multiple CA-ARTI-Dx across the study recruitment period, and provides flexibility for the inclusion of additional CA-ARTI-Dx into the trial should suitable tests become available and resources permit this.

Proposed trial concept

The aim is to conduct a pragmatic and flexible platform clinical trial to evaluate the clinical- and cost-effectiveness of CA-ARTI-Dx to safely reduce antibiotic prescribing in community care settings.

6. OBJECTIVES AND OUTCOME MEASURES

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
Co-primary: To assess effectiveness of CA-ARTI-Dx in terms of both reductions in antibiotic prescribing, and in terms of patient recovery.	The proportion of participants being prescribed at least one antibiotic course (of any dose or duration) over 28 days from inclusion (estimating possible reduction). Time to return to usual daily activities, where returned to usual daily activity = yes (estimating non-inferiority)	Day 1 – 14 diary or Day 14 telephone call, and Day 28 telephone call
Secondary: To explore whether adding a CA-ARTI-DX to usual primary care has: 1) Additional effects on antibiotic prescribing 2) Effects on antibiotic use 3) Effects on patient recovery and safety, including complications and hospitalisation 4) Effects on use of medications other than antibiotics 5) Effects on clinician's decision making process regarding diagnosis and treatment 6) Effects on patients' perceived ability to understand and cope with their illness	1) Choice of antibiotic class, whether 'delayed' or immediate 2) Antibiotic use over 28 days after inclusion. 3) Patient reported symptoms severities, re-consultations, hospitalization, pneumonia 4) Use of other, non-antibiotic, medication including antiviral medication, and over-the-counter treatments. 5) Responsible clinician's initial working diagnosis and antibiotic prescribing decision before test, if randomised to CA-ARTI-Dx also final decision after test. 6) Patient Enablement Instrument (PEI) score.	1) Day 1 – 14 diary or Day 14 telephone call, and 28 telephone call 2) Day 1 – 14 diary or Day14 telephone call, and 28 telephone call 3) Day 1 – 14 diary or Day 14 telephone call, and 28 telephone call 4) Day 1 – 14 diary or Day 14 telephone call, and 28 telephone call 5) Baseline visit 6) Day 1 -14 diary

7) Is cost effective	7) Costs data: EQ5D at day 1, Day 14 and Day 28; time taken for testing; costs associated with subsequent help seeking, complications, work loss and over-the-counter medication use (to be determined in close collaboration with WP5).	7) Baseline visit, Day 1 – 14 diary or Day 14 telephone call, and Day 28 call
To identify: 1) Clinicians' views and experiences of CA-ARTI-Dx and how they fit with/impact on delivery of care and intended future use. 2) Patient/parent views of CA-ARTI-Dx and how they impact consultations including patient satisfaction and intention to consult in future for similar symptoms.	1) Interviews and surveys with healthcare professionals. 2) Interviews and surveys with patients.	

7. STUDY DESIGN

This will be a multi-country, prospective, individually randomised, platform clinical trial in community care with a nested process evaluation. The trial will be a diagnostic strategy intervention study to evaluate the use of clinical algorithms (flow chart) that include a CA-ARTI-Dx, compared to usual clinical care without CA-ARTI-Dx. The trial will initially be three-arm platform trial (see section 5), comparing usual care (with availability of local guidelines for managing infections) without any CA-ARTI-Dx POCT with two different diagnostic strategies using CA-ARTI-Dx POCTs as outlined in the appendices.

The study has the capacity to drop a CA-ARTI-Dx via an interim analysis after the end of the first recruitment period, most likely after the first winter season if recruitment is as expected, based on pre-specified criteria for either success or futility. If a CA-ARTI-Dx is dropped, it may be replaced with a new CA-ARTI-Dx to be evaluated in the second recruitment period. The nested process evaluation will capture data to understand how CA-ARTI-Dx is used in practice and how it influences patient care and experience. These data will inform implementation within the period of the trial and beyond.

8. PARTICIPANT IDENTIFICATION

8.1. Study Participants

Patients aged one year and older, presenting to primary care: with lower respiratory tract infection where cough is the predominant symptom (<28 days); OR, upper respiratory tract infection where acute

sore throat (<14 days) is the dominant symptom; AND for whom the responsible clinician is considering or has decided to prescribe an antibiotic.

8.2. Inclusion Criteria

- Male or Female, aged 1 year or above
- Consulting with symptoms of lower respiratory tract infection where cough is the predominant symptom (<28 days); or, symptoms of an upper respiratory tract infection (<14 days) where sore throat is the dominant symptom; and where the clinician is considering/has decided to prescribe an antibiotic
- Is able and willing to comply with all trial requirements
- Participant or legal guardian(s) of a child is willing and able to give informed consent according to national regulations

8.3. Exclusion Criteria

- Patients with only nasal, ear or rhinosinusitis symptoms
- Patients who have tested positive to SARS-CoV-2 within 28 days of onset of symptoms
- Patients with any serious condition associated with immunocompromised (long term oral steroids or immunosuppressants, terminal cancer)
- Patients for whom the clinician decides on immediate hospital admission
- Patients who will not be able to participate in the study because they do not understand the local language; are terminally ill; have a serious psychiatric disorder; or judgement of the recruiting clinician deems ineligible.

9. PROTOCOL PROCEDURES

9.1. Recruitment

Primary care healthcare workers will recruit participants through recruiting sites which will be the first point of care for that participant (e.g. GP Practice, primary care paediatric centres or LTCF). We will include recruiting sites from up to 12 European Union and/or Horizon 20-20 countries in the trial. Some countries may recruit only from GP practices or only from LTCF. In some countries, where clinicians attend patients in LTCFs, participating general practices will potentially include eligible patients whom they care for and who are resident in a LTCF.

Potential participants will be identified when they present to their recruiting site with symptoms of CA-ARTI. Potential participants will be referred for eligibility assessment and potential trial entry as soon as possible. They will consult with a responsible clinician or appropriately trained delegate, where they will have the trial presented to them and be screened to confirm whether or not they meet the eligibility criteria. If eligible and willing, they will be recruited into the trial.

9.2. Screening and Eligibility Assessment

Participants will be assessed against the eligibility criteria listed in section 8 by the responsible clinician or delegate who will complete the eligibility CRF on paper or online, they will then go on to complete a baseline assessment. If there is a meaningful delay between introduction of the study/initial eligibility assessment and consent/randomisation, then the responsible clinician must confirm that all eligibility criteria are still met.

9.3. Informed Consent

Written versions of the Participant Information Leaflet (PIL) and the Informed Consent Form (ICF) will be presented and explained to the participants in their own language detailing no less than: the exact nature of the study; the implications and constraints of the protocol, and, the known side-effects and risks involved in taking part. Electronic versions of the PIL may be sent to the patient via email to consider participation at home before the baseline visit or be advertised on the GP websites. The study will provide an age appropriate PIL that includes all necessary information in appropriate wording and format for the participant. PIL and other participant-facing study materials will be available in the official national language. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to provide the reason for withdrawal.

Adequate time will be given to the participant or legal guardian to consider the information given to them and to ask any questions they may have about the trial to decide whether they will participate in the study, however they must still be recruited within the stated number of days of the onset of their symptoms. Participants (or their legal guardian(s)) will be invited to sign and date the latest approved version of the ICF before any study-specific procedures are performed. If the participant is above the legal age of consent for the jurisdiction in which they are being recruited, they must personally sign the consent form. However, if the participant is aged below the legal age of consent in their jurisdiction, then consent will be provided by their legal guardian (either one or both parents will be required to give consent in accordance with the permissions of the jurisdiction where recruitment is taking place). Children will be provided with an assent form at the discretion of the person who is presenting and obtaining the informed consent.

Written Informed Consent will be confirmed by the dated signatures of the participant or their legal guardian and by the person who presented and obtained the informed consent. The person obtaining consent must be suitably qualified and experienced, and be authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participants. The original signed form will be retained at the study site in the patient notes.

An optional consent question will be included on whether they would like to be contacted by the research team to be invited to a telephone interview for the nested process evaluation (see section 9.10).

Participants that lack capacity may be included in the LTCF setting only. Appendix I provides more details on the process for this.

9.4. SARS-CoV-2 POCT

If a participant has had onset of symptoms of CA-ARTI within 5 days of the baseline visit they will be invited to have a SARS-CoV-2 POCT (BD Veritor™ System for Rapid Detection of SARS-CoV-2 – see appendix C for more information on the test). The amount of antigen in a sample may decrease as the duration of illness increases so specimens collected after day 5 of illness are more likely to be negative compared to a RT-

PCR assay. Participants with symptoms for more than 5 days will not have the SARS-CoV-2 test but will continue with the baseline visit and randomisation.

This test is CE marked and will be used within its intended use. Clinicians will be provided with information and training on how to perform the test by the study team or their local co-ordinating centre with the assistance of the test manufacturer (training will be given according to the manufacturers kit instructions for use). Nasal swab specimens will be collected from the participant for analysis. The specimen collection, processing and test procedure will be according the manufacturer's instructions (see appendix C for more information).

If the test result is negative the participant will continue in the trial and be randomised as described in section 9.5. If the test result is positive, the GP will manage the participant according to national guidelines, they will record the test result on the baseline CRF. The participant will have completed participation in the trial and will be excluded from any further trial procedures.

Note/ this procedure has been added into the clinical algorithm in light of the COVID-19 pandemic. The SARS-CoV-2 test may not be included in the trial if test availability is too low or the test is not required due to low prevalence of the disease.

9.5. Randomisation

See section 11.2 for randomisation and stratification details.

If the participant has had symptoms for more than 5 days, or, the participant has had the BD Veritor™ SARS-CoV-2 POCT (section 9.4) and tested negative they will continue with trial randomisation. At the baseline visit the recruiter will enter the participant's baseline data into the online system, which will then enable the randomisation to take place. The randomisation process will take only a few moments via the online system and will not delay trial participation. The Data Monitoring Committee will ensure that the randomisation design will be executed as planned.

9.6. Blinding and code-breaking

PRUDENCE will be an open trial. The participant and the recruiting clinician will know the participant's allocation. Therefore, no unblinding or code breaking is required in the event of a relevant emergency.

The trial team and recruiting clinicians will be blind to emerging results. During the course of the trial, only those on the Data Monitoring Committee will have access to the unblinded data as part of the interim analysis.

9.7. Description of study intervention(s), comparators and study procedures (clinical)

9.7.1. Description of study intervention(s)

The interventions will be usual care (see section 9.6.2) with the addition of clinical algorithms (flow chart) including a CA-ARTI-Dx, details of the current clinical algorithm (flow chart) and diagnostic tests under evaluation can be found in the appendices.

9.7.2. Description of comparator(s)

Usual care of eligible patients with CA-ARTI consulting in primary care (e.g. general practices, LTCF, and primary care paediatricians) where the clinician is considering or going to prescribe an antibiotic. Clinicians will be referred to local guidelines for managing common infections where available.

9.7.3. Description of study procedure(s)

Clinicians will be provided with information and training on how to perform the CA-ARTI-Dx tests by the study team or their local co-ordinating centre with the assistance of the test manufacturer (training will be given according to the manufacturers kit instructions for use). Guidance for clinicians on how to respond to the test result are included in the clinical algorithm (study flow chart) for the diagnostics under evaluation in appendix B.

Depending on the outcome of the independent Data Monitoring and Ethics Committee (DMEC) review after period 1, additional CA-ARTI-Dx algorithms may be evaluated in period 2.

All interventions are CE marked and will be used within the scope of their intended use.

9.8. Baseline Assessments

After obtaining consent, the recruiter will complete the web-based baseline Case Report Form (CRF). The first part of this CRF will include: baseline demographic variables; comorbidities and relevant medical history; clinical severity and duration of symptoms. They will follow the clinical algorithm (study flow chart) outlined in appendix B, explaining and performing the SARS-CoV-2 test procedure if applicable If the participant is eligible to continue to randomisation, the responsible clinician's initial working diagnosis (before CA-ARTI-Dx if applicable); and responsible clinician's initial prescribing decision (before CA-ARTI-Dx if applicable) will be recorded on the baseline CRF. The recruiter will create equipoise for the participant about the randomisation process and allocation to different arms. All recruiters will be trained by the local PRUDENCE co-ordinating centre teams in how to do this as part of the trial training. They will continue to follow the clinical algorithm (study flow chart) outlined in appendix B, either their usual care process or one of the CA-ARTI-Dx strategies with usual care according to the participant's group allocation. Clinicians will be asked to refer to local guidelines for managing common infections for usual care, and be provided with the CA-ARTI-Dx strategies (clinical algorithm/flow chart outlined in Appendix B) as well as kit instructions/manuals on how to perform the CA-ARTI Dx for the intervention arms. The biological sample (e.g. finger prick blood test, nasal swab) required for the CA-ARTI-Dx will be taken in patients randomised to care with the addition of that CA-ARTI-Dx. Once the CA-ARTI-Dx test is complete (or the participant tested positive for SARS-CoV-2 so did not complete randomisation) the clinician will complete the second part of the baseline CRF.

Baseline CRF data items to be collected will include:

Part 1:

- Baseline demographic variables

- Comorbidities and relevant medical history
- Clinical severity and duration of symptoms
- BD Veritor™ SARS-CoV-2 POCT result (if applicable)
- Responsible clinician's initial working diagnosis (before CA-ARTI-Dx if applicable)
- Responsible clinician's initial prescribing decision (before CA-ARTI-Dx if applicable)

RANDOMISATION

Part 2:

- CA-ARTI-DX result (if applicable)
- Responsible clinician's final working diagnosis (after CA-ARTI-Dx if applicable)
- Responsible clinician's final prescribing decision (after CA-ARTI-Dx if applicable)
- Antibiotic prescription details: class, whether 'delayed' or for immediate consumption (if applicable)
- Any other medication prescribed and advised
- Information about any additional usual care advice given to the participant
- Information about any additional diagnostic/laboratory tests requested

With the exception of participants that tested positive to SARS-CoV-2, the recruiters will provide all other participants with instructions on completing a simple, user-friendly diary to be completed over 14 days from the baseline visit (day 1). Contact details will be collected from these participants including mobile/home telephone numbers and email addresses to enable the collection of the follow-up data.

9.9. Subsequent Visits

There is no requirement for participants to have a research-specific face-to-face follow-up visit as part of their study participation, as all subsequent measurements consist of self-completed questionnaires or through telephone calls by the trial team (including process evaluation interviews for some of the participants).

Participants (or their legal guardian or their carer) will be asked to complete a simple, user-friendly diary each day until they have returned to usual activity for two consecutive days, or up to day 14 (whichever is sooner), the diary will request the following information:

- Employment status
- Return to usual activities each day
- Antibiotic use
- Use of other prescription medication
- Individual symptom severity
- Health care resource use, including hospitalisation
- Use of over-the-counter and non-prescription medication or remedies
- Out-of-pocket expenditure
- Number of work/school/nursery days lost
- EQ-5D-5L (or alternative as recommended for age) and Visual Analogue Scale (VAS) (Day 1, Day 14 and Day 28)
- Patient Enablement Instrument (Day 1)

- Process evaluation patient survey (see section 9.10) (Day 1)

The diary will be available on-line (study team preferred option) and in a paper version for posting back to the national network coordinator when specifically requested or where the participant does not have daily access to the internet.

Participants who have not completed/returned their diary by day 14 will be telephoned on or after day 14 (+ 3 days) by the study team and asked a brief set of questions to establish a minimal data set including antibiotic use, day returned to usual activity, hospitalisation, COVID-19 testing and EuroQoL EQ-5D. All participants will be telephoned again on or after day 28 (+ 3 days) regarding antibiotic use, day returned to usual activity if not already stated by day 14, hospitalisation, COVID-19 testing and EuroQoL EQ-5D in the preceding 14 days. We will also repeat the brief process evaluation patient survey (see section 9.10). The study team will attempt to call the participant no more than 3 times on each occasion.

Participants may receive reminder text messages about these telephone calls and to return the diary if they give the trial team their mobile telephone number.

Participants who have visited hospital with complications related to CA-ARTI and who have had a chest X-ray may have their clinical record in primary care examined by the study team for confirmation of diagnoses of complications including pneumonia.

9.10. Sample Handling

The biological sample (e.g. finger prick blood test, nasal swab) required for the CA-ARTI-Dx will be taken in patients randomised to care with the addition of that CA-ARTI-Dx. The samples will be handled and processed according to the kit instructions for the CA-ARTI-DX and any leftover sample will be disposed of as per local procedures for clinical waste. No other samples will be taken.

If the responsible clinician considers that a microbiological sample is necessary for the purposes of routine care, they will need to obtain an additional sample according to their routine processes.

Participants included in the LTCF setting may be invited to participate in a sampling sub-study as described in appendix J.

9.11. Nested Process Evaluation

A mixed-methods process evaluation will be nested within the trial. The process evaluation will capture data to understand how the intervention is used and viewed by clinicians and patients in order to explain how clinicians and patients adopt CA-ARTI-Dx in community care. Not all countries included in the PRUDENCE study will participate in the Nested Process Evaluation Interviews. See appendix G for the current list of participating countries.

<u>Interviews:</u>

4-5 countries from the 12 that participate in the trial will be used as case studies that reflect diversity in relevant factors such as healthcare system, community care settings, income, geographic location in Europe and antibiotic use.

We will carry out interviews (telephone, online (e.g. using Microsoft Teams or face to face as appropriate) with patients participating in the trial throughout the recruiting period. All patients will be eligible to take part in an interview unless they do not have capacity. Interviews will capture experiences of and satisfaction with care when a CA-ARTI-Dx is used and subsequent views and experiences of management of symptoms (including antibiotic use). Some patients recruited to intervention arms in the trial (approximately 8-10 per country) will be purposively sampled across recruiting periods to give a total of 32-50 interviews. We will seek to obtain variation in age, symptom presentation, type of CA-ARTI-Dx carried out and whether an antibiotic is prescribed. Parents/carers of child patients under 12 will be invited to interviews. Patients aged 12 years and older will be invited to interviews, with parents able to sit in on interviews with children under 16 where desired. Patients will be provided with Patient Invitation Letter and Patient PIL. We will obtain written or oral consent prior to an interview. A password protected copy of the consent will be emailed to participants.

In addition, we will conduct (telephone, online (e.g. using Ms Teams) or face to face as appropriate) interviews with healthcare professionals to capture experiences using CA-ARTI-Dx in daily clinical practice, and identify any additions and improvements required when rolling out CA-ARTI-Dx and any training materials during the trial in order to meet clinicians' and patients' needs and support use. Any health professionals supporting PRUDENCE will be eligible to take part and CIs at each site will identify and invite professionals. Different health professionals (approximately 7 per country, each representing a participating practice/faculty) will be purposively sampled across each of the recruiting periods to give a total of 28-35 interviews. We will seek to obtain variation in professional role, practice setting, and experience. Healthcare professionals will be provided with Clinician Invitation Letter and Clinician PIL. We will obtain written or oral consent prior to an interview. A password protected copy of the consent will be emailed to participants.

Semi-structured topic guides will be informed by existing literature and theory, including the Theoretical Domains Framework and the Common Sense Model, to ensure that questions elicit likely key determinants of behaviour, and Normalization Process Theory to identify how POCT diagnostics could be embedded in routine practice. Data will be analysed using thematic and framework analysis (1, 2).

Surveys:

Surveys will assess the views of clinicians and patients on the use of CA-ARTI-Dx in ARTI consultations, examine how CA-ARTI-Dx are implemented and experienced.

Patient surveys will be included in the participant diary on Day 1 and in the follow-up telephone call at Day 28 (as described above). Surveys will include brief statements measuring patient satisfaction with the consultation, beliefs about the necessity of antibiotics for respiratory symptoms and future behavioural intentions to manage symptoms and (re)consult for future illness. Patient surveys will be included for all participants in all trial sites.

Clinician surveys will be sent electronically to all participating clinicians at each site as sites are recruited, prior to patient recruitment starting at that site. Clinicians will be asked to complete the survey at two time points: prior to patient recruitment at their site and on completion of patient recruitment at their site. Additional timepoints may be added if sites adopt a new CA-ARTI-Dx during the trial. Surveys will

include brief statements measuring attitudes about use of CA-ARTI-Dx, implementation of CA-ARTI-Dx and future behavioural intentions to use CA-ARTI-Dx. Clinician surveys will be conducted in all participating countries in the trial.

9.12. Early Discontinuation/Withdrawal of Participants

Each participant has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- BD Veritor SARS-CoV-2 positive test result (discontinued after baseline visit prior to randomisation)
- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Withdrawal of consent

The reason for withdrawal will be recorded in the CRF. Data that has already been collected about the participant will be kept.

9.13. Definition of End of Study

The trial will end when all patients enrolled into the study have completed the 28 day follow-up and all subsequent data collection is complete.

10. SAFETY REPORTING

All CA-ARTI-Dx tests being evaluated in this study are CE-marked and being used within the scope of their intended use. As a result of this no adverse events will be recorded in this study. All Serious Adverse Events (SAEs) occurring during the 28 days participants are enrolled on the trial will be recorded as detailed in Section 10.2 Reporting Procedures for Serious Adverse Events.

10.1. Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other 'important medical events' may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

10.2. Reporting Procedures for Serious Adverse Events

All SAEs must be reported on the study SAE Report Form by the person who has discovered the SAE or nominated delegate, either in paper form or through the study database to the local network coordinator and the University of Oxford PC-CTU within 24 hours of the site study team becoming aware of the event. The University of Oxford PC-CTU Project Manager or delegate will perform an initial check of the report, request any additional information, and ensure it is reviewed by the CI or other delegated personnel for relatedness and expectedness. Additional and further requested information (follow-up or corrections to the original case) will be detailed on a new SAE Report Form and emailed to the sponsor or delegate. If the event has not resolved, at the 28 day time point the SAE will be reviewed again to see if resolution has occurred. If the event is considered 'resolved' or 'resolving' no further follow up is required. If not the event must be followed up until such a time point.

SAEs will be reviewed at each Data Monitoring Committee meeting.

A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related' (resulted from administration of any of the research procedures) and 'unexpected' in relation to those procedures. Reports of related and unexpected SAEs should be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the HRA report of serious adverse event form (in the UK).

11. STATISTICS AND ANALYSES

11.1. Statistical Analysis Plan (SAP)

The statistical aspects of the study are summarised here with details fully described in a statistical analysis plan (SAP) that will be available from the time that the first participant is recruited. The SAP will be finalised before the interim analysis scheduled upon completion of the first study period evaluating the initial CA-ARTI-DX tests. This will allow the possibility of additional CA-ARTI-Dx to be evaluated in the second study period (replacing either one or all of the initial CA-ARTI-Dx), pending results of the scheduled interim analysis.

11.2. Randomization

Participants will be randomized to one of the CA-ART-Dx tests with usual care or usual care (see appendix B for a flow chart/algorithm of the current process). Randomisation stratification for the current CA-ARTI-Dx being evaluated are outlined in appendix F.

11.3. Co-Primary Endpoints

The study has two co-primary endpoints:

1) Whether a participant is prescribed at least one antibiotic course (of any dose or duration) over 28 days from inclusion (yes/no)
2) Time to return to usual daily activities (in days)

11.4. Primary Hypotheses

The study will evaluate the following hypotheses for each CA-ARTI-Dx evaluated:

1) CA-ARTI-Dx with usual care use is superior to usual care, as demonstrated by the following two criteria:
    a. The proportion of participants prescribed at least one antibiotic course is lower for the CA_ARTI-Dx arm relative to the usual care arm
    b. The mean time to return to usual daily activities is no greater than 1 additional day for the CA-ARTI-DX arm relative to the usual care arm

11.5. Primary Analyses

Analyses will be conducted separately for comparing each CA-ARTI-Dx test versus usual care.

For each CA-ARTI-Dx, the first co-primary endpoint (prescribing of antibiotics) will be analysed with a logistic regression model on Treatment (CA-ARTI-Dx vs. usual care), adjusting for primary symptom (cough, sore throat) and flu season. If the one-sided p-value for the odds ratio of the CA-ARTI-Dx versus usual care is less than or equal to a pre-specified nominal alpha value (e.g. 0.025), the CA-ARTI-Dx will be deemed superior to the control with respect to prescribing of antibiotics. The exact nominal alpha value will be chosen to control one-sided Type I error at approximately 0.025 while accounting for interim analyses.

The second co-primary endpoint (time to return to usual activities) will be analysed with a piecewise exponential time-to-event regression model on treatment (CA-ARTI-Dx vs. usual care), adjusting for primary symptom (cough, sore throat) and flu season. If the upper boundary of the pre-specified 95% (or pre-specified alternative %) confidence interval for the mean difference between treatment arms (CA-ARTI-Dx minus usual care) is less than 1.0 days, the CA-ARTI-Dx will be deemed non-inferior to the control with respect to time to return to usual activities. The percent confidence for the specified confidence interval will be chosen to control one-sided Type I error at approximately 0.025 while accounting for interim analyses.

If a given CA-ARTI-Dx is superior to control on the first co-primary endpoint (antibiotic prescriptions), and non-inferior relative to control on the second co-primary endpoint (time to return to usual activities), the implementation of the CA-ARTI-Dx will be deemed superior to usual care.

11.6. Sample Size Justification

For the first co-primary endpoint, it is expected that approximately 90% of participants receiving usual care will be prescribed an antibiotic during the 28 days of primary outcome follow-up. A clinically meaningful and feasible reduction in the proportion of those prescribed antibiotics is considered to be 15%. Therefore, 100 participants per arm will be required for 80% power (and 133 subjects for 90% power)

to detect an improvement of 90% to 75% in antibiotic prescribing for each CA-ARTI-Dx hypothesis vs. usual care, respectively, using a traditional two-sided 0.05 Type I error.

For the second co-primary outcome, we assume an exponential time to recovery with a mean of 4 days (based on ALICE data). Under the exponential distribution, a mean increase of 1 day is equivalent to a hazard ratio of 0.80. Hence, to demonstrate no worse than a mean 1 day increase in time to recovery (or equivalently a hazard ratio no less than 0.80) within 28 days of follow-up, 318 patients per group are required to provide 80% power using a traditional two-sided 0.05 Type I error for each CA-ARTI-Dx hypothesis vs. usual care.

Given the very high power for the first co-primary endpoint (reduction in prescribing of antibiotics), the power for the joint co-primary hypothesis is essentially equal to the statistical power of the second co-primary endpoint (time to recovery), which is approximately 93% or greater power across the two seasons for each CA-ARTI-Dx, and approximately 69% or greater probability of obtaining superiority after one season (see appendix F).

Given the wide range of uncertainty in the above assumptions underlying these calculations, we propose to conduct a simulation study to further characterize the statistical power of the design and to refine the decision criteria. The virtual trial simulations will explore the impact of the assumed control group distributions of antibiotic prescriptions and time to recovery, distribution of participants across strata (primary symptom, flu season), the expected CA-ARTI-Dx effects, the extent of missing data, differing accrual rates between winter seasons, and varying correlation between endpoints. In addition, the simulations will incorporate a single interim analysis to evaluate the primary hypotheses in between winter seasons, and explore the possibility/likelihood of evaluating additional CA-ARTI-Dx in the second winter season.

Further sample size justification is given in appendix F for the specific CA-ARTI-Dx tests under evaluation.

11.7. Adaptive Arm Stopping and Addition of New Arms

The trial will continue through two winter seasons of CA-ARTI (typically October to May), and each winter season will encompass a shorter influenza season (length varies by year). A single interim analysis will be conducted in between winter seasons, in which both of the co-primary endpoints are evaluated for each of the CA-ARTI-Dx. If early success or futility is determined for either CA-ARTI-Dx relative to usual care, a new CA-ARTI-Dx will replace the given CA-ARTI-Dx in the second winter season. Otherwise the given CA-ARTI-Dx will be continued in the second CA-ARTI season. The co-primary endpoints and primary analyses will remain the same for any newly introduced CA-ARTI-Dx arms. Decision rules for claiming superiority of a given CA-ARTI-Dx upon conclusion of either winter season will be calibrated to control the overall Type I error for each CA-ARTI-Dx at the approximate one-sided 0.025 level. These thresholds will be pre-specified. Any additional CA-ARTI-Dx introduced in the second winter season will require a statistical Appendix/Addendum to update the SAP.

11.8. Analysis populations

The primary analyses will include all participants in the intention to treat (ITT) population, i.e. all participants randomized and analysed in their respective randomized assignment. Additional populations

will be described in the SAP; this includes all participants who underwent the intervention (a safety population); all eligible participants (a per protocol analysis); and an "as treated" population.

11.9. Procedure for Accounting for Missing, Unused, and Spurious Data.

For a given endpoint, the primary analyses will be conducted using complete cases, i.e. ignoring patients with missing endpoints. Sensitivity analyses will be conducted exploring the impact of missing data and any biases that may be present.

11.10. Secondary Endpoints & Analyses

The secondary endpoints and analyses of the study are fully described in a statistical analysis plan (SAP) and will be available prior to any interim analyses.

11.11. Cost Effectiveness Analysis

Cost-effectiveness analysis (CEA) will compare the direct medical costs, productivity costs, and health outcomes (in terms of days on antibiotic regime, doses of antibiotics prescribed, number of days where CA-ARTI limits usual activities and Quality Adjusted Life Years gained) between the CA-ART-Dx arms and the routine care arm. The analysis will source data from the trial (resource use, EQ-5D-5L and VAS scores), and use other relevant data from the countries in which the trial is set (e.g., unit costs, and type of health care provided within each country if available information is obtained from each country, otherwise unit costs would be estimated as a range for all participant countries).

Uncertainty will be explored using sensitivity and subgroup analyses. Value of perfect information analysis may also be performed to identify which sources of uncertainty should be reduced through additional research to efficiently improve decision making.

These analyses will be conducted after each season, and depending on the evolution of the trials' arms, the comparators may change accordingly.

11.12. Process Evaluation Analysis

Qualitative data collection and analysis will be done concurrently. Interviews with clinicians and patients, will be analysed using thematic and framework analysis taking an inductive approach (1-3). NVivo software will be used to assist with the organisation of data. A thematic framework will be used to chart data across all interviews and will aid comparisons between participants.

Quantitative data from surveys with clinicians and patients will be analysed using descriptive statistics.

12. DATA MANAGEMENT

The data management aspects of the study are summarised here with details fully described in the Data Management Plan.

12.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name.

12.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

12.3. Data Recording and Record Keeping

Research Online (RO) is an electronic data capture (EDC) system that will be used for data collection. Web-based case report forms (eCRF) are implemented into the system to facilitate the study-specific data collection. These forms can easily be accessed by all standard web browsers. The baseline CRF, Symptom Diaries, Days 14 and 28 phone questionnaires and SAE Forms will be entered onto this system.

Multiple validation and range checks will be programmed in the eCRF to assure complete and high-quality data. Data that does not comply with these rules or ranges will generate a query that must be resolved immediately or at a later stage. Electronic workflows will employ multiple skip and jump rules to ensure that only information that is applicable to the patient will appear. After the data of the last subject is entered, the database can rapidly be closed and data made available for cleaning, further analysis and publication purposes.

RO meets all requirements according to GCP standards for electronic data entry with respect to safeguarding data integrity and data security regulations. Users will have role-based access to the system by logging in using their personal username and password. The system will log all data entry steps with timestamps and user information. The role-based access to the system will avoid unauthorised data access and prevents users from performing actions that they do not have authorisation for.

Project management and monitoring of the study is facilitated by the integrated real live study progress reports. RO data traffic over the Internet is encrypted using secured data communication protocols. Dedicated databases and web servers are hosted in a secure data centre, the database (PostgreSQL) is backed up on a daily basis.

For process evaluation interviews:

Each interview will be audio-recorded with the participant's permission. Recordings will allow verbatim transcription of interviews in Microsoft Word. Transcription will be completed by an independent transcriptionist who holds a contract with a partner university. If any interview is conducted in a language other than English recordings will be transcribed and translated by a member of the research team or by an independent transcriptionist and translator who holds a contract with a partner university. Once transcribed and transcripts are checked, recordings will be deleted. Transcripts will be labelled with a

unique participant number and will omit any identifiable data either identifying the participant or their general practice.

13. QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, GCP, relevant regulations and PC-CTU Standard Operating Procedures. All PIs, coordinating centre staff and site staff will receive training in trial procedures according to GCP where required.

13.1. Risk assessment

A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the study to reflect significant changes to the protocol or outcomes of monitoring activities.

13.2. Study monitoring

Prior to starting the study, each countries coordinating centre teams will be trained by the core trial team based at the UOXF and UMCU, and will cascade training, delegation of responsibilities, and set-up of their sites in their networks. The University of Oxford Primary Care and Vaccines Clinical Trials Unit will take overall management of regulatory aspects, and the Julius Center of UMCU will cover training, site set-up, initiations and logistics. The industry partners whose CA-ARTI-Dx are being evaluated in the study will be responsible for logistics and training for their respective tests.

Regular monitoring will be performed according to the study specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the study specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

The monitoring will be performed by the trial management team (or delegate) according to the risk assessment and monitoring plan. Additionally, routine audits will be performed by the CTU Quality Assurance manager (or delegate).

13.3. Study Committees

A Data Monitoring and Ethics Committee (DMEC) and Trial Management Group (TMG) will be appointed in line with standard CTU procedures. The responsibilities of each group are as follows:

- DMEC- to review the data at each interim analysis, as the updates to the randomisation scheme occur in order to ensure that the process is working correctly and to review and monitor the accruing data to ensure the rights, safety and wellbeing of the trial participants. As there will be no TSC appointed, the DMEC will provide overall supervision of the trial on behalf of the Sponsor and the Funder to ensure that it is being conducted in accordance with GCP and provide advice on all aspects of the trial.

- TMG- is responsible for the day-to-day running of the trial, including monitoring all aspects of the trial and ensuring that the protocol is being adhered to.

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14. PROTOCOL DEVIATIONS

A study related deviation is a departure from the ethically approved study protocol or other study document or process (e.g. consent process or administration of study intervention) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the study master file.

A PC-CTU SOP is in place describing the procedure for identifying non-compliances, escalation to the central team and assessment of whether a non-compliance/deviation may be a potential Serious Breach.

15. SERIOUS BREACHES

A "serious breach" is a breach of the protocol or of the conditions or principles of Good Clinical Practice which is likely to affect to a significant degree:

(a) the safety or physical or mental integrity of the trial subjects; or

(b) the scientific value of the research.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

16.3. Approvals

Following Sponsor approval the protocol, informed consent form, participant information leaflets and any proposed informing material will be submitted to an appropriate Research Ethics Committee (REC), regulatory authorities, and host institution(s) for written approval. The PI and coordinating centres for each country will ensure and confirm correct regulatory approvals are gained prior to recruitment.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents. The PI and coordinating centres for each country will ensure and confirm any substantial amendments are approved for their networks and region.

16.4. Other Ethical Considerations

The study will include children and pregnant women.

16.5. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation, Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties. The PI and coordinating centres for each country will be responsible for submitting any required progress reports and final report for their networks and regions.

16.6. Transparency in Research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database.

Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration.

16.7. Participant Confidentiality

The study will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data. All study records (which includes some personal data such as names on consent forms) will be retained up to 3 years after the youngest subject reaches 18 years old.

16.8. Expenses and Benefits

Not applicable.

17. FINANCE AND INSURANCE

17.1. Funding

Innovative Medicines Initiative 2 Joint Undertaking.

17.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

Where local insurance is required in other participating countries this will be obtained by the University of Oxford.

17.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

18. PUBLICATION POLICY

The Investigators (those listed on the protocol and one or two investigators from each network, to be decided at publication) will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the **IMI2 JU**. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Not applicable

20. ARCHIVING

Each country specific co-ordinating centre will store and archive de-identified research data, such as paper diaries or research documents with personal information, such as consent forms, securely up to 3 years after the youngest subject reaches 18 years old according to the University of Oxford Primary Care Clinical Trial Units SOP.

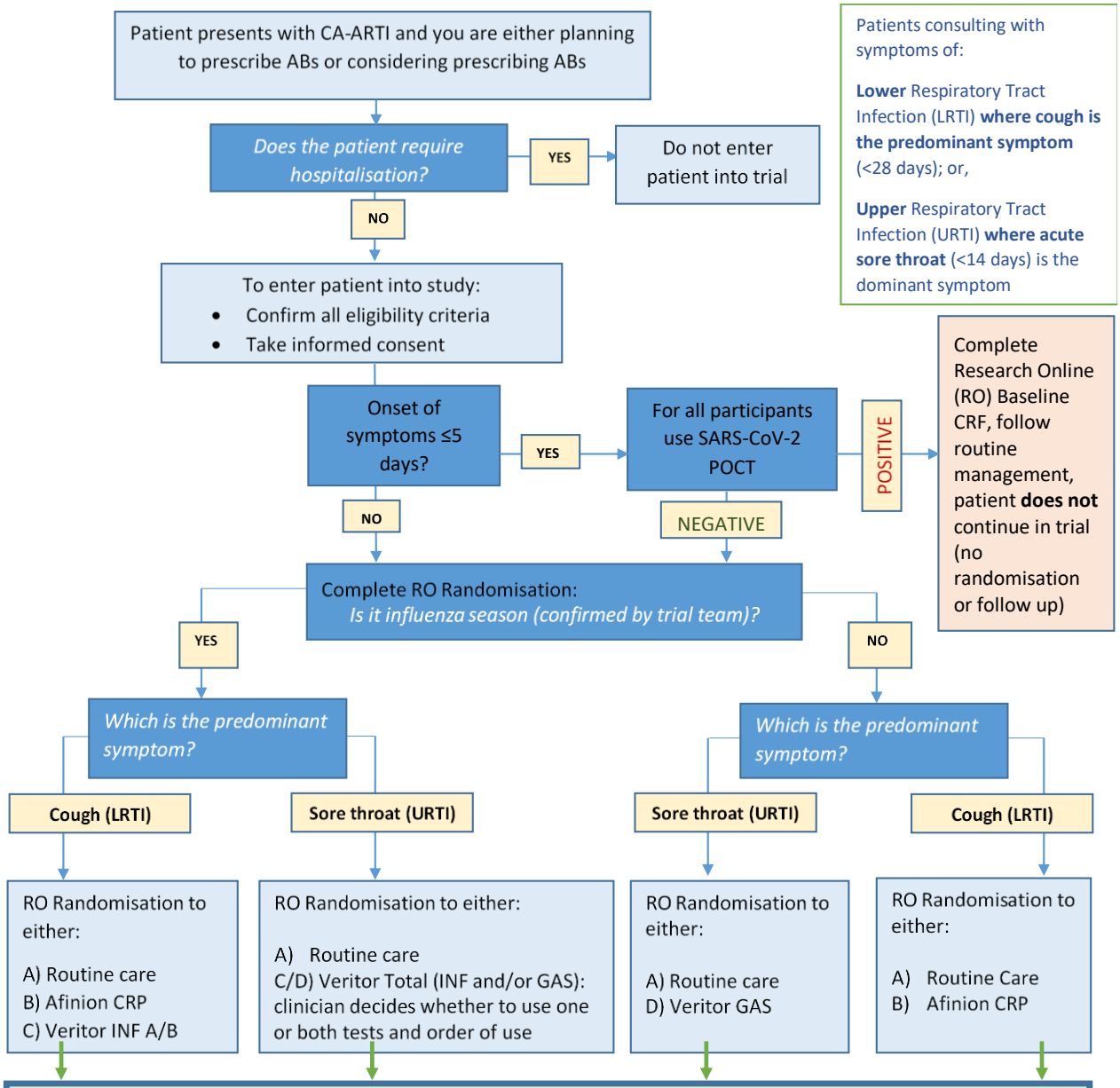
21. REFERENCES

1. Braun V, Clarke V. Using thematic analysis in psychology. Qualitative research in psychology. 2006;3(2):77-101.
2. Gale NK, Heath G, Cameron E, Rashid S, Redwood S. Using the framework method for the analysis of qualitative data in multi-disciplinary health research. BMC Medical Research Methodology. 2013;13(1):117.
3. Fereday J, Muir-Cochrane EJIjoqm. Demonstrating rigor using thematic analysis: A hybrid approach of inductive and deductive coding and theme development. 2006;5(1):80-92.

22. APPENDIX A: Schedule of Procedures

Procedures and data capture	Visits					
	Prior to Day 1	Day 1	Day 1 - 14	Day 14 (+ 3 days)	Day 28 (+ 3 days)	Day 28+
	Before patient recruitment	Baseline Visit	Daily Diary	Telephone call by study team	Telephone call by study team	Retrospective data collection by study team
Eligibility assessment		X				
Informed consent		X				
Demographics		X	X			
Comorbidities and medical history		X				
Symptom severity		X	X	X	X	
Clinicians initial diagnosis and prescribing decision		X				
SARS-CoV-2 Test (if applicable)		X				
Randomisation		X				
CA-ARTI-Dx (if applicable)		X				
Clinicians working diagnosis and prescribing decision		X				
Antibiotic prescription details		X				
Any other medication prescribed/advised		X				
Additional advice given		X				
Additional tests requested		X				
Return to usual activities			X	X	X	
Antibiotic use			X	X	X	
Healthcare resource use			X	X	X	
Use of over-the-counter and non-prescription medication or remedies			X			
EQ-5D-5L			X	X	X	
Patient enablement instrument			X			
Details of hospital admissions			X	X	X	X
Process evaluation survey: clinicians	X					X
Process evaluation survey: participants			X		X	
Qualitative interviews: clinicians and participants		Through-out the recruiting period				

23. Appendix B: Clinical CA-ARTI-Dx Algorithm (Flow Chart)



A) ROUTINE CARE: Follow best routine care based on your clinical judgement and any corresponding national guidelines.

B) AFINION CRP: Depending on CRP level AND type/severity of symptoms treat according to national guidelines:
For adults (≥17 years old):
< 20 mg/L: Antibiotics are not recommended and should not be prescribed unless clinical judgement indicates otherwise
20 – 100 mg/L: Benefit from antibiotics is more likely the higher the CRP level, consider clinical signs, symptoms, risk factors (age, COPD) before prescribing antibiotics, consider a delayed prescription where feasible
>100 mg/L: Immediate antibiotic treatment should be offered
For children (≥1 to 16 years old):
< 5 mg/L: do not routinely offer antibiotics / antibiotics unlikely to be beneficial and usually should not be prescribed
≥5 mg/L: consider clinical signs, symptoms, risk factors and CRP level before prescribing antibiotics

C) VERITOR INF A/B: if positive follow national guidelines for treatment of influenza. If negative – infection due to influenza cannot be ruled out because the antigen present in the sample may be below the detection limit of the test, follow clinical judgement and any corresponding national guidelines.

D) VERITOR GAS test: if positive follow national guidelines for treatment of GAS; if negative - infection due to Strep A cannot be ruled-out because the antigen present in the sample may be below the detection limit of the test, follow clinical judgement and any corresponding national guidelines.

24. Appendix C: BD Veritor™ System for Rapid Detection of SARS-CoV-2

BD Veritor™ SARS-CoV-2

Summary of the test:

A novel coronavirus (2019-nCoV) was identified in December 2019, which has resulted in hundreds of thousands of confirmed human infections worldwide. Cases of severe illness and deaths have been reported. On February 11, 2020 the International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2. The median incubation time is estimated to be approximately 5 days with symptoms estimated to be present within 12 days of infection. The symptoms of COVID-19 are similar to other viral respiratory diseases and include fever, cough, shortness of breath.

The BD Veritor System for Rapid Detection of SARS-CoV-2 is a rapid (approximately 15 minutes) chromatographic digital immunoassay for the direct detection of the presence or absence SARS-CoV-2 antigens in respiratory specimens taken from patients with signs and symptoms who are suspected of COVD-19. The test is intended for interpretation in both laboratory and near patient testing environments only with the BD Veritor Plus Analyzer Instrument. The test is not intended to be interpreted visually.

Rationale for use in PRUDENCE:

Recruitment to the PRUDENCE trial is due to start in January 2021. The worldwide COVID-19 pandemic is ongoing with many countries, including some of those countries participating in PRUDENCE, experiencing a second wave or increase in COVID-19 cases as the winter of 2020/21 starts. To encourage recruitment and alleviate concerns over recruiting participants with COVID-19, a SARS-CoV-2 POCT will be used to exclude positive patients from continuing into the interventional and follow-up part of the trial. These patients will be managed following national guidelines. Only patients with onset of symptoms within five days of the baseline visit will be tested for SARS-CoV-2 as the reliability of the test after this time is reduced.

Intended Use:

The BD Veritor™ System for Rapid Detection of SARS-CoV-2 is a chromatographic digital immunoassay intended for the direct and qualitative detection of SARS-CoV-2 nucleocapsid antigens in nasal swabs from individuals who are suspected of COVID-19 by their healthcare provider within the first five days of the onset of symptoms.

Results are for the identification of SARS-CoV-2 nucleocapsid antigen. This antigen is generally detectable in upper respiratory samples during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results should be treated as presumptive, do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions.

Patient Sample:

Acceptable specimens for testing with this kit include nasal swab specimens obtained by the dual nares collection method. It is essential that correct specimen collection and preparation methods be followed.

Specimens obtained early during symptom onset will contain the highest viral titers; specimens obtained after five days of symptoms are more likely to produce negative results when compared to an RT-PCR assay. Inadequate specimen collection, improper specimen handling and/or transport may yield a falsely negative result; therefore, training in specimen collection is highly recommended due to the importance of specimen quality for generating accurate test results.

Freshly collected specimens should be processed as soon as possible, but no later than one hour after specimen collection.

It is essential that correct specimen collection and preparation methods be followed.

Kit Storage:

Kits may be stored at 2–30 °C. DO NOT FREEZE. Reagents and devices must be at room temperature (15–30 °C) when used for testing.

BD Veritor™ SARS-CoV-2 Reference Information and User Manual:

https://www.bd.com/en-uk/products/diagnostics-systems/point-of-care-testing/bd-veritor-system-for-rapid-detection-of-sars-cov-2

Consult the BD Veritor™ User Instructions for complete information:

- BD Veritor™ Plus SARS-CoV-2 Assay instructions for use (IFU)
- Rerence guide - BD Veritor™
- Quick reference guide - Analyze Now mode for BD Veritor™ System for rapid COVID-19 (SARS-CoV-2) testing
- Nasal Swab Quick reference guide - BD Veritor™

25. Appendix D: CA-ARTI-Dx 1 - Abbott Afinion™ CRP (study recruitment period 1)

Abbott Afinion™ CRP

Summary of the test:

The Abbott Afinion™ CRP test is a rapid in vitro diagnostic test for quantitative determination of C-reactive protein (CRP) in blood. C-reactive protein (CRP) is one of the cytokine induced acute-phase proteins, the levels of which rise during a general, unspecific response to infections and non-infectious inflammatory processes.

Afinion CRP is a solid phase immunochemical assay. The Afinion CRP Test Cartridge contains all the reagents necessary for measuring the CRP concentration in whole blood, serum or plasma. The sample material is collected using the integrated sampling device and the test cartridge is placed in the Afinion Analyzer. The sample is then automatically diluted with a liquid that also lyses the blood cells. The sample mixture is passed through a membrane coated with anti-CRP antibodies; the CRP in the sample is concentrated onto this membrane. A solution containing anti-CRP antibodies conjugated with ultra-small gold particles is passed through the membrane. The gold-antibody conjugate binds to the immobilized CRP on the membrane, which turns red-brown. Excess goldantibody conjugate is removed by a washing solution. The Afinion Analyzer measures the colour intensity of the membrane, and this is proportional to the amount of CRP in the sample. The CRP concentration is displayed on the analyzer screen.

Rationale for use in PRUDENCE:

C-reactive protein (CRP) is a major acute phase biomarker, which can be used to differentiate between self-limiting or viral and severe bacterial infections, and may help healthcare professionals to identify patients with RTIs who would benefit from antibiotics, and those who would not. Used alongside clinical observations of signs and symptoms, and the patient's history, CRP may help to resolve diagnostics uncertainty in patients with CA-ARTI without affecting recovery rates or duration of illness.[1-7]

The Afinion CRP assay is a simple fingerstick test, minimally invasive for the patient, from sample to decision in just 3 minutes, with an easy-to-use all-in-one cartridge and no analytical steps or user calibration necessary.

Intended Use:

Afinion 2 System, consisting of the Afinion 2 Analyzer and the Afinion Test Cartridges, is for in vitro diagnostic use only. Afinion 2 Analyzer is a compact multi-assay analyzer for point-of-care testing and is designed to analyse the Afinion Test Cartridges.

Afinion™ CRP is an in vitro diagnostic test for the quantitative determination of C-reactive protein (CRP) in human whole blood and in human serum and plasma. The measurement of CRP provides information for the detection and evaluation of infection, tissue injury, inflammatory disorders and associated diseases.

Patient sample:

Use capillary blood directly from finger, venous whole blood (with EDTA or heparin), serum or plasma. Venous whole blood should be analysed within 3 days. Serum or plasma can be stored refrigerated (2-8°C)

for 10 days. Capillary samples cannot be stored. Mix the sample by inverting the tube 8-10 times before use.

Kit Storage:

Store the test kit refrigerated (2-8°C) until the expiry date or at room temperature (15-25°C) for maximum 4 weeks. The test cartridge must reach a temperature of 15-30°C before use; leave the unopened foil pouch on the bench for at least 15 minutes. Use the test cartridge within 10 minutes after opening the foil pouch. Hold the test cartridge by the handle.

Store the controls refrigerated (2-8°C). Opened controls are stable for 4 weeks. The control can be used directly from the refrigerator. Mix the control by inverting the vial 8-10 times before use. The measured value should be within the acceptable range stated in the Afinion™ CRP Control Package Insert.

Abbott Afinion™ CRP Reference Information and User Manual:

https://www.alere.com/en/home/product-details/afinion-crp.html

Consult the AFINION™ User Instructions for complete information:

- Afinion CRP Package Insert.
- Afinion CRP Control Package Insert.
- AFINION 2 Analyzer User Manual.

References:

1. Cals JWL et al. BMJ 2009;338(51):1374
2. Little P et al. The Lancet 2013;382(9899):1175-1182
3. Andreeva E, Melbye H. BMC Family Practice 2014;15(1):80
4. Cals JWL et al. The Annals of Family Medicine 2010;8(2):124-133
5. Butler CC, Gillespie D, White P et al. New England Journal of Medicine 2019;381(2):111-120. doi:10.1056/nejmoa1803185
6. Verbakel JY et al. BMJ Open 2019;9:e025036
7. O'Brien K et al. C-reactive protein point-of-care testing (CRP POCT) to guide antibiotic prescribing in primary care settings for acute respiratory tract infections (RTIs). Rapid assessment on other health technologies using the HTA Core Model® or Rapid Relative Effectiveness Assessment. EUnetHTA Project ID:OTCA012. 2019; https://www.eunethta.eu/wp-content/uploads/2019/02/EUnetHTA_OTCA012_CRP-POCT_31012019.pdf

26. Appendix E: CA-ARTI-Dx 2 - BD Veritor™ System (study recruitment period 1)

BD Veritor™ System

Summary:

This CA-ARTI-DX has the capability of testing for Respiratory Syncytial Virus (RSV), Influenza A+B (Flu) and for Group A Streptococcus (GAS). **For this study we will only be including Flu and GAS**. The BD Veritor™ System for Rapid Detection of Flu A+B is a rapid chromatographic immunoassay for the direct and qualitative detection of influenza A and B viral nucleoprotein antigens from nasopharyngeal wash, aspirate and swab in transport media samples from symptomatic patients. The BD Veritor System for Rapid Detection of Group A Strep is a rapid chromatographic immunoassay for the direct and qualitative detection of Group A Streptococcus antigen from throat swabs of symptomatic patients.

Rationale for use in PRUDENCE:

Clinical diagnosis of influenza alone is unreliable, using a POCT for influenza would increase appropriate use of antivirals and antimicrobials in patients consulting with influenza like illness during the influenza season. Group A Streptococcus is a common bacterial cause of upper respiratory tract infection, but clinical diagnosis alone is unreliable. Signs and symptoms of GAS and non-streptococcal pharyngitis often overlap making accurate diagnosis on clinical grounds alone difficult.

The BD Veritor System device can be used for both influenza and GAS diagnosis independently or collectively depending on the clinical signs and symptoms of the patient. The test has a streamlined workflow, with minimal hands-on time and an objective, digitally displayed result delivered in approximately 10 minutes.

Intended Use:

The BD Veritor System for Rapid Detection of Flu A+B is a rapid chromatographic immunoassay for the direct and qualitative detection of influenza A and B viral nucleoprotein antigens from nasal and nasopharyngeal swabs of symptomatic patients. The BD Veritor System for Rapid Detection of Flu A+B (also referred to as the BD Veritor System and BD Veritor System Flu A+B) is a differentiated test, such that influenza A viral antigens can be distinguished from influenza B viral antigens from a single processed sample using a single device. The test is to be used as an aid in the diagnosis of influenza A and B viral infections. A negative test is presumptive and it is recommended that these results be confirmed by viral culture or a molecular assay cleared for diagnostic use in the country of use. Negative test results do not preclude influenza viral infection and should not be used as the sole basis for treatment or other patient management decisions. The test is not intended to detect influenza C antigens.

The BD Veritor System for Rapid Detection of Group A Strep is a rapid chromatographic immunoassay for the direct and qualitative detection of Group A Streptococcus antigen from throat swabs of symptomatic patients. It is intended to be used in conjunction with the BD Veritor System Reader as an aid in the diagnosis of Group A Strep. All negative test results should be confirmed by bacterial culture because negative results do not preclude Group A Strep infection and should not be used as the sole basis for treatment. The BD Veritor System for Rapid Detection of Group A Strep test is intended for use in point-of-care or laboratory settings.

Patient sample:

Flu A + B: Acceptable specimens for testing with the BD Veritor System Flu A+B test include nasal swabs and nasopharyngeal (NP) swabs. Freshly collected specimens should be processed within 1 hour. It is essential that correct specimen collection and preparation methods be followed. Specimens obtained early in the course of the illness will contain the highest viral titers. Inadequate specimen collection, improper specimen handling and/or transport may yield a false negative result; therefore, specimen collection requires specific training and guidance due to the importance of specimen quality to accurate test results.

GAS: For optimal performance, collect the throat swab with the swab that is provided in the kit. Swab the posterior pharynx, tonsils and other inflamed areas. Excess blood or mucus on the swab specimen may interfere with test performance. Avoid touching the tongue, cheeks and teeth and any bleeding areas of the mouth with the swab when collecting specimens. Testing should ideally be performed immediately after the specimens have been collected. Swab specimens may be stored in clean, dry plastic tubes for up to 8 hours at room temperature or 48 hours at 2–8 °C.

Storage:

Kits may be stored at 2–30 °C. DO NOT FREEZE.

Reagents, specimens and devices must be at room temperature (15–30 °C) for testing.

BD Veritor™ System Reference information and User Manuals:

https://www.bd.com/en-us/offerings/capabilities/microbiology-solutions/point-of-care-testing/veritor-system

Consult the **BD Veritor™** User Instructions for complete information:

- BD Veritor™ reader system brochure
- BD Veritor™ Flu A+B CLIA-waived kit instructions for use
- BD Veritor™ Group A Strep CLIA-waived kit instructions for use

27. Appendix F: CA-ARTI-Dx specific Randomisation, Stratification and Sample Size Justification

Randomisation and stratification Outline:

Randomization will be stratified by predominant cough or sore throat of a participant and the status of influenza season (yes/no), resulting in 2 or 3 trial arms depending on in/out of flu season. More specifically, randomization within each stratum will be the following with respect to CRP vs. Veritor vs. Usual care:

1) Flu season, subjects with predominantly cough: 1:1:1 (CRP, Veritor-Flu, Usual care)

2) Flu season, subjects with predominantly sore throat: 1:1 (Veritor-Total, Usual care)

3) Not Flu season, subjects with predominantly cough: 1:1 (CRP, Usual care)

4) Not Flu season, subjects with predominantly sore throat: 1:1 (Veritor-GAS, Usual care)

The Veritor test is classified as either Veritor-Flu only, Veritor-GAS only, or Veritor-Total, depending on the season. Veritor –Total allows the GP to use one or both tests (Flu and/or GAS) and in what order. The reason for different tests depending on primary symptom and flu season is because certain tests are only expected to be helpful for specific combinations.

Sample size justification:

For the first co-primary outcome, under assumptions of 50% of participants presenting with predominantly cough, and 50% of participants being enrolled during the influenza season, and 5% of participants missing the primary endpoint, 1250 participants per winter season (2500 participants total) will provide approximately 247 subjects per arm per winter season for the CRP vs. usual care analysis (>99% power for a single winter season) and 395 subjects per arm per winter season for the Veritor vs. usual care analysis (>99% power for a single winter season). For the secondary co-primary outcome, under the same assumptions as above, 1250 participants per winter season (2500 participants total) will provide approximately 247 subjects per arm per winter season for the CRP vs. usual care analysis. This corresponds to approximately 69% power for a single winter season, and 93% power for both winter seasons combined. In addition, we expect 395 subjects per arm for the Veritor vs. usual care analysis, providing approximately 87% power for a single winter season, and 99% power for both winter seasons combined.

28. Appendix G: Network List

Country	Primary Care Network (✓)	Long Term Care Network (✓)	Countries participating in Process Evaluation Interviews (✓)	Appendix H and I applicable (✓)
UK	✓		✓	
Greece	✓		✓	
Georgia	✓		✓	
Ireland	✓			
Poland	✓			
Germany	✓		✓	
Belgium	✓			
Hungary	✓			
Spain	✓	✓		✓
France	✓	✓		✓
Italy		✓		✓
Switzerland		✓		✓
Israel		✓		✓

29. Appendix H: Amendment History

Amendment No.	**Protocol Version No.**	**Date issued**	**Author(s) of changes**	**Details of Changes made**

List details of all protocol amendments here whenever a new version of the protocol is produced. This is not necessary prior to initial REC / HRA submission.

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee and HRA (where required).

30. APPENDIX I: Update to section 9.3 Informed Consent for Long Term Care Facility Network only – Participants lacking capacity

Rationale to include patients who lack capacity:

The increase in the aged population and the need for cost optimization of healthcare systems have led to a rapid rise in the demand for elderly healthcare facilities that deliver long-term care. Owing to immunosenescence, comorbidities, and malnutrition, the incidence of Respiratory Tract Infection (RTI) in LTCF residents is higher than in the general population. Nevertheless, previous studies have reported an underrepresentation of elderly adults in clinical trials assessing the management of RTI, particularly in the LTCF setting, where most residents are frail subjects who lack capacity. To ensure the generalizability of clinical trial findings and the fairness and equity in research participation, the PRUDENCE trial will also include LTCF residents with legal guardian(s) willing and able to give informed consent according to national regulations.

Informed consent process:

Participants lacking capacity to consent for themselves (for example, patients in LTCF with dementia) will be identified and the study team will seek advice from a consultee/legal guardian on what the wishes and feelings of the person might be and whether or not they should take part. A consultee/legal guardian will be a person engaged in caring for the participant (not professionally or for payment) or is interested in his/her welfare, and is prepared to be consulted. The study team will provide information leaflets for the consultees/legal guardians, including the same level of information that the participant would receive if they had capacity. The consultees/legal guardians will be asked to sign either a “record of consultation” form or an Informed Consent Form on behalf of the participant to confirm that they have received this information and had the opportunity to ask any questions and give advice. The networks will follow their national regulatory requirements for recruiting participants lacking capacity, this will be the responsibility of the PI for that network.

31. APPENDIX J: Update to section 9.9 Sample Handling for Long Term Care Facility Network only – Sampling sub-study

Microbiology-Biomarker study in the LTCF networks

The microbiology-biomarker sub-study in PRUDENCE will be carried out in LTCFs only and will be led by Work package 2 of the ValueDx project, based at the University Of Antwerp central laboratory. The PI of each LTCF network/site will be responsible for adhering to the regulatory requirements for sample collection, storage and shipment in their country.

Study objectives:

- To assess selection of resistant pathogens under antibiotic pressure
- To study the evolution of the respiratory and gut microbiome as a function of the infection and of the antibiotics used
- To describe the epidemiology of the infections (as also detected by the tests)

- To develop an immune and biochemical profile that will serve to distinguish infection from non-infectious causes, differentiate different aetiologies such as viral infection from bacterial causes, and for prognosis prediction

- To define secondary outcomes specific for the elderly population

Study processes:

Informed consent:

All LTCF participants recruited into the main PRUDENCE trial will be invited to participate in the sampling sub-study. LTCF participants will be provided with the PRUDENCE trial PIL which includes additional information on the sampling sub-study processes. The LTCF consent form will include optional consent questions regarding the additional sample collection, analysis and storage with the purpose of further research. This will be optional and participants can choose not to participate in the sampling sub-study and still participate in the main PRUDENCE trial.

Sample Collection and Handling:

Approximately 200 participants participating in the main trial will have the following samplings taken:

- Blood: each patient will be sampled at four distinct time-points (day of inclusion, 2 days after inclusion, 7 days after inclusion, and 28 days after inclusion). At each time-point EDTA and heparin blood (cytokines, protein markers) as well as Pax tube blood (transcripts) will be collected.

- Nasopharyngeal swab: each patient will be sampled twice on day of inclusion, after antibiotic use and 28 days after inclusion. Two samples at each of the three time points will be collected in STGG medium (skim milk, tryptone, glucose, and glycerin medium to sustain bacteria and DNA) and UTM (universal transport medium).

- Stool: four samples will be obtained per patient in order to perform a longitudinal analysis of resistance development/loss, as well as microbial community shifts that might be observed as an effect of the antimicrobial therapy. The time-points that will be considered are: day of inclusion, day 7, day 28-30 and day 90. Two stool aliquots at each time point will be dispensed in a STGG and UTM medium.

Sample storage and shipment:

Once collected samples need to ideally be stored at -80°C. If the LTCF does not have direct access to a -80°C freezer, all samples should be processed within 1 -2 hours and stored at -20°C until transported on dry ice to a local laboratory. Specifically, this entails centrifugation of EDTA and heparin blood, and freezing plasma according to the laboratory manual. PAX tubes, NP samples and stool samples should be frozen immediately in the provided container/tube. Once at the local laboratory the samples will be frozen and stored at -80°C, and then transported on dry ice to a central laboratory in Antwerp, Belgium. Transportation should be performed ensuring adherence to country and international specific requirements for the shipment of samples. .

The participant's date of recruitment and participant trial ID number will be used as identifiers for these samples, additionally all samples will have a study specific bar code label attached. Only the laboratory and trial team will have access to this information for the purposes of sample identification and tracking.

Laboratory Analysis:

Analysis may be sometime after the samples have been taken and once the samples are received in the central laboratory in Belgium. The respiratory and gut samples will undergo shotgun metagenomics and metatranscriptomics sequencing in order to characterize the microbial community and the resistance burden. Any human genetic material will be removed prior to data analysis.

Blood samples analysis:

Pax blood will be used to study gene expression profiles of cytokine receptors. EDTA and heparin blood will be utilized to study different cytokines for instance IFNy, TNF-α, IL-1β, IL-2, IL-6, IL-8, TGFβ-1, IL-4, IL-5, IL-13, IL-33, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-12p70, TSLP, G-CSF , VCAM, ICAM-1, SAA, CRP, KC-GRO, GM-CSF, IP-10, IL-1RA, IL-2Ra, IL-2R, IL-7, IL-9, IL-3, IL-15, IL-16, IL-18, PDGF-B, BDNF, M-CSF, Eotaxin, IFN-β, IL-12/IL-23p40, MCP-1, MCP-2, MCP-3, MIP-1α, MIP-1β, MIP-3α, RANTES, Fractalkine, MIG, CTACK, EPO, HGF, FGF-B, VEGF-A, VAP-1.

Laboratory Manual:

Before the start of the study, WP2 of the ValueDx project will collate a sample collection manual that will be shared with the LTCF networks and sites.

Biobanking of Samples:
Following analysis, the remaining samples will be stored in a bio bank at the University of Antwerp central laboratory as part of the ValueDx project. The samples will be pseudonymized and prior consent from the participant will be sought for long-term storage with the purpose of further research into infectious diseases..