ADEQUATE Advanced Diagnostics for Enhanced QUality of Antibiotic prescription in respiratory Tract infections in Emergency rooms

Clinical Protocol WP4b – Adults

Version: 3.0

Effective date: 18-Feb-2022

NCT04547556

VALUE-Dx

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 820755. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and bioMérieux SA, Janssen Pharmaceutica NV, Accelerate Diagnostics S.L., Abbott, Bio-Rad Laboratories, BD Switzerland Sàrl, and The Wellcome Trust Limited.


University
of Antwerp


BIOMÉRIEUX


wellcome


UNIVERSITY OF
OXFORD


UMC Utrecht


Penta
Child Health Research


UNIVERSITÀ
di VERONA


THE UNIVERSITY
of EDINBURGH


University Medical Center Groningen

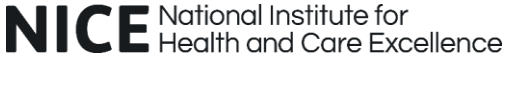

RAMBAM
Health Care Campus


UNIVERSIDAD
DE LA RIOJA


BOSTON
UNIVERSITY


BIOASTER
MICROBIOLOGY TECHNOLOGY INSTITUTE


FIND


NICE National Institute for
Health and Care Excellence


Gesundheit Österreich
GmbH


ESCMID
EUROPEAN SOCIETY
OF CLINICAL MICROBIOLOGY
AND INFECTIOUS DISEASES


ERS
EUROPEAN
RESPIRATORY
SOCIETY
every breath counts


Berry Consultants
Statistical Innovation


IBBL
INTEGRATED BIOBANK OF LUXEMBOURG
FOR NEXT GENERATION HEALTHCARE


ZonMw


janssen


ACCELERATE
DIAGNOSTICS


Abbott


BIO-RAD


BD

Randomised controlled trial of **Rapid Syndromic Diagnostic Testing** (RSDT) for enhancing the quality of antibiotic prescribing for community acquired acute respiratory tract infection (CA-ARTI) in Emergency Rooms in Europe.

Protocol ID	WP4b - adults
Study title	**A**dvanced **D**iagnostics for **E**nhanced **QU**ality of **A**ntibiotic prescription in respiratory **T**ract infections in **E**mergency rooms - **ADEQUATE**
Version	3.0
Date	18-Feb-2022
Principal Investigator	Cristina Prat Aymerich, UMC Utrecht
Sponsor	University Medical Center Utrecht (UMC Utrecht) Heidelberglaan 100 3584 CX Utrecht The Netherlands
Subsidising party	Innovative Medicines Initiative 2 Grant agreement No 820755
Steering Committee	Marc Bonten, UMC Utrecht Cristina Prat, UMC Utrecht Herman Goossens, University of Antwerp Julia Bielicki,Fondazione PENTA Florence Frager, bioMérieux
Trial Methodologist, Statistician	Henri van Werkhoven, UMC Utrecht
Data Safety Monitoring Board (DSMB)	Mark Lyttle, Chair Harry Heijerman, Pulmonologist Michelle Clements, senior Statistician

PROTOCOL SIGNATURE SHEET

Study Title: **A**dvanced **D**iagnostics for **E**nhanced **QU**ality of **A**ntibiotic prescription in respiratory **T**ract infections in **E**mergency rooms – **ADEQUATE**

Version Clinical Protocol: V3.0, 18-Feb-2022

Name	**Signature**	**Date**
Sponsor: Prof. Dr. K.G.M. Moons, Manager Research UMC Utrecht		21st-Feb-2022
Sponsor Principal Investigator: Dr. C. Prat, MD, PhD, Microbiologist UMC Utrecht		18th-Feb-2022
I have read the above named Clinical Protocol and agree to conduct the trial as outlined and in compliance with country, local and internal institutional requirements. Site Principle Investigator: Site name: _____ Name: _____		

Table of contents

1. LIST OF ABBREVIATIONS

AE	Adverse event
ARTI	Acute Respiratory Tract Infections
BAL	Bronchoalveolar lavage
CA-ARTI	Community acquired acute respiratory tract infections
CRP	C Reactive Protein
COVID-19	Coronavirus Infectious Disease 2019
DALY	disability adjusted life years
eCRF	electronic Case Report Form
ETA	Endotracheal Aspirate
Dx	Diagnostics
EQ5D	EuroQoL (Europe Quality of Life) 5 dimensions
ER	Emergency Room
GCP	Good Clinical Practice
GPDR	General Data Protection Regulation
ICD	International Classification of Diseases
ICF	Informed Consent Form
LAR	Legally Accepted Representative
LRTI	Lower Respiratory Tract Infections
NPS	Nasopharyngeal swabs
PCT	Procalcitonin
POCT	Point of Care Testing
PP	Pneumonia Panel (Biofire®Filmarray®)
PPAS	Point Prevalence Audit Study
RP2.1 plus	Respiratory Panel version 2.1 plus (BioFire®FilmArray®)
RSDT	Rapid syndromic diagnostic testing
SAE	Serious adverse event
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOP	Standard Operating Procedures
WP	Work Package

2. SUMMARY

Background and Rationale: Community-acquired acute respiratory tract infections (CA-ARTI) are among the most frequent infectious diseases worldwide. At the same time, uncomplicated acute respiratory infections (ARI) is the most frequent cause of inappropriate antibiotic use. Antibiotic resistance rates are related to antibiotic use in any setting, but opportunities to implement a more judicious antibiotic prescribing are probably most apparent in primary care and emergency departments. Optimal clinical management of CA-ARTI is hampered because of diagnostic delays and suboptimal test sensitivity, leading to incorrect or missing etiologic diagnosis, and over prescription of antibiotics. Highly sensitive molecular assays have increased the detection of respiratory pathogens, but the **impact in clinical decision-making** needs further evaluation. Accurate and rapid identification of infected patients allows for more rational and effective infection control practices and public health responses which will limit morbidity and mortality, economic damage, and can allow low risk/non-infected and recovered people to return to the workforce.

Objective: To assess the impact of rapid diagnostic testing of patients with Acute Respiratory Tract Infection (ARTI) at the emergency department, on (1) hospital admission rates and (2) antibiotic prescriptions (days of treatment) and (3) the non-inferiority in terms of clinical outcome. Geographical and seasonal variation will be assessed on a real time basis including pathogens of public health interest. The impact will be stratified within age groups and risk factors in order to determine the long-term clinical, public health and economic determinants for the integration of diagnostics in a global and sustainable perspective.

Study design: Prospective, multi-center, individually randomised, controlled trial.

Study population: Adults (≥18 years old) consulting in selected participating sites with CA-ARTI.

Study Intervention: The diagnostic intervention is rapid syndromic testing with:

- BioFire FilmArray Pneumonia Panel plus (PP): Sputum (and/or ETA or BAL sample)
- BioFire FilmArray Respiratory Panel 2.1 plus (RP2.1plus): Nasopharyngeal swab

Main study parameters/endpoints:

- Days alive out of hospital (superiority endpoint), within 14 days
- Days on Therapy (DOT) with antibiotics (superiority endpoint), within 14 days
- Adverse outcome (non-inferiority safety endpoint)
    - For initially non-admitted patients: any admission or death within 30 days
    - For initially hospitalised patients: i) any readmission, ii) ICU admission ≥ 24 hours after hospitalisation, or iii) death within 30 days

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Participation in the study involves collection of data that can be obtained from medical charts and follow up questionnaires and interviews. Respiratory samples (e.g. nasopharyngeal swab, sputum) will be obtained as standard of care and diagnostic intervention (Biofire FilmArray) will be used only for participants randomised to the intervention,Based on the results of diagnostic testing (BioFire FilmArray) antibiotics may be withheld when deemed unnecessary, or a different antibiotic class may be selected when certain bacterial pathogens are detected. The risks and benefits of management decisions, complemented with adequate training, are subject to the current investigation.

3. GENERAL CONSIDERATIONS WORK PACKAGE 4 AND VALUE-Dx OVERVIEW

The purpose of VALUE-Dx consortium (www.value-dx.eu) is to facilitate and accelerate the rigorous assessment and implementation of (new) diagnostic technologies into healthcare settings, by establishing the infrastructure, methods, processes and approaches needed to understand, evaluate, assess, and demonstrate the multi-faceted value of diagnostics and overcome the associated barriers to their widespread adoption and use. VALUE-Dx focuses its research on **community acquired acute respiratory tract infections (CA-ARTI)**.

The objectives of VALUE-Dx are:

- To design a health-economic framework to assess and demonstrate the value of diagnostics both for individual patients and for public health impact by reducing antibiotic use and subsequent antibiotic resistance among patients;
- To establish a sustainable European Standardised Care Network adequately trained and resourced to conduct clinical trials evaluating the value of diagnostics;
- To design and implement clinical studies to demonstrate the value of diagnostics in the optimal management of CA-ARTIs;
- To explore, define and attempt to resolve the psychological, ethical and social barriers which prevent the more widespread adoption of diagnostics delivering healthcare to the population.

The clinical studies, to be implemented in WP4, aim to gather evidence on:

i) doses or days of antibiotics prescribed,
ii) proportion of patients not receiving antibiotics;

WP4 studies will be performed separately in primary care and long-term facilities (WP4a)- POCT with Abbott and BD and Emergency Rooms (WP4b)- Rapid syndromic diagnostic testing with bioMérieux.

The clinical trial will align – where possible with the other WPs of the project.

- WP1 aims to develop evidence-based clinical algorithms that integrate point of care tests. Initial analysis has provided results on accuracy of the selected groups of items: signs and symptoms, biomarkers, imaging and rapid diagnostic tests, for prediction of influenza and bacterial pneumonia, respectively.
- WP2 will explore analytical performance of some tests in the trials and will collect pathogen and host biomarker data.
- WP3 will provide data management.
- Within WP4a, during the winter season from January to March 2020, a web-based point prevalence audit survey (PPAS) on presentation and management of CA-ARTI in primary care and long-term facilities was performed. The aim is to retrospectively characterize patients who

seek healthcare for CA-ARTI, quantify antibiotic prescriptions and to benchmark patterns of diagnostic in different European countries.

- WP5 is setting-up data collection for health economic modelling, that includes a disease model, diagnostic models and antibiotic resistance predictions. The variables to be included to assess direct and indirect medical costs and quality of life need to be collected in the clinical trials of WP4.
- WP4b, The ADEQUATE study is an individual randomised study that aims to assess the impact of rapid syndromic diagnostic testing (Biofire® bioMérieux) in patients with Acute Respiratory Tract Infection (ARTI) at the emergency department, on (1) hospital admission rates and (2) antimicrobial prescriptions (days of treatment) and (3) the non-inferiority in terms of clinical outcome.
- The ADEQUATE study is composed of an adult and a paediatric part, both with their own protocol, but with a joined sample size calculation and Statistical Analysis Plan (SAP). The study analysis will include both the adult and paediatric populations.
- The current protocol describes the trial of WP4b for the adult population.

4. BACKGROUND AND RATIONALE

4.1. Background

Community-acquired acute respiratory infections (CA-ARTI), including upper and lower respiratory tract infections, are among the most frequent infectious diseases worldwide. Lower respiratory tract infections (LRTI) are among the most lethal communicable diseases and the fourth cause of death globally, responsible for an estimated 3 million deaths in 2016 [1]. LRTI disproportionately affects children younger than 5 years and is the second cause of disability adjusted life years (DALYs). A study published in the Lancet in 2018 estimated the global, regional, and national morbidity, mortality, and aetiologies of LRTI in 195 countries, between the years 1990–2016. In 2016, LRTI caused 652,572 deaths (95% uncertainty interval 586,475–720,612) in children younger than 5 years, 1,080,958 deaths (943,749–1,170,638) in adults older than 70 years, and 2,377,697 deaths (2,145,584–2,512,809) in people of all ages, worldwide *S. pneumoniae* was the leading cause of LRTI morbidity and mortality globally, contributing to more deaths than all other aetiologies combined in 2016 (1,189,937 deaths (690,445–1,770,660)[2], particularly as community acquired pneumonia. Within the European region, geographical variations are present. Over the years, the epidemiology has changed due to changing populations, with increased disease burden in elderly (>70 years) in many regions, varying prevalence of smoking and varying patterns of vaccine usage [3, 4].

On the other hand, uncomplicated ARTI is the most frequent cause of inappropriate antibiotic use [5, 6], and there is a need of more judicious antibiotic prescription to prevent exposure to drug-related adverse events, selection of antibiotic resistance and emergence of opportunistic pathogens that substitute the indigenous microbiota. At the same time, the clinical role of bacteria whose normal ecological niche is in the airways is an unresolved issue because of contamination with oropharyngeal flora. Antibiotic resistance rates are related to antibiotic use in any setting, but opportunities to decrease unnecessary treatments are probably most apparent in primary care and emergency departments. Not only the ecological but also, the economic cost of antimicrobial

resistance per antibiotic consumed is considerable [7-9]. Management is heterogeneous in diverse geographical areas due to non-uniform guidelines, both for diagnostic and antimicrobial stewardship [10].

One of the major challenges in clinical decision-making is the absence of microbiological evidence of the aetiological agent in CA-ARTI at the time the antibiotics must be initiated so rapid diagnostic testing may have an impact. The required sample is not always available, and with conventional testing there may be low sensitivity (40-60% cases without aetiological diagnosis) and an important diagnostic delay before results are available. An accurate and reliable distinction between bacterial and viral causes of CA-ARTI would provide an important opportunity for better antimicrobial stewardship. However, due to substantial overlap in clinical disease presentation and laboratory parameters it is currently impossible to reliably distinguish viral from bacterial aetiology based on available tools.

It has been proposed that implementation of Point-of-Care tests (POCT) with biomarkers or microbiological tests to differentiate viral from bacterial infections may reduce inappropriate antibiotic prescriptions [11]. There is encouraging evidence from randomised trials for biomarkers-guided antimicrobial management. In a multicenter study in adults in primary care the combination of selected clinical symptoms with the addition of C-reactive protein (CRP) measurement improved diagnostic information, but measurement of procalcitonin (PCT) did not add clinically relevant information [12]. In a Cochrane review of controlled trials of biomarkers in patients with CA-ARTI, PCT appeared to be more informative than CRP and other inflammatory markers, as it was associated with an earlier increase upon infection, a better negative predictive value, and was influenced by immunosuppressive medication [13].

The development of highly sensitive molecular assays has increased the detection of respiratory pathogens in patients with CA-ARTI, and increased our understanding of the role of viruses in CA-ARTI [14]. However, diagnostic methods that detect a virus do not always rule-out bacterial infection. Additional diagnostic yield has been demonstrated by using molecular tests but evidence is limited regarding the impact on antibiotic use or costs [15-17].

In this context, a new molecular rapid syndromic testing platform (BioFire; Salt Lake City, UT, USA) might improve clinical decision-making in patients with CA-ARTI. The BioFire FilmArray Pneumonia Panel plus (PP) is a multiplexed nucleic acid amplification test that identifies 34 bacterial and viral targets, including antimicrobial resistance genes from sputum or bronchoalveolar lavage (BAL) specimens. The BioFire FilmArray Respiratory Panel 2.1 plus (RP2.1plus) can simultaneously detect 24 viruses and atypical pathogens from nasopharyngeal swabs. Both panels allow syndromic testing and results can be provided in less than one hour with high sensitivity and specificity [18, 19]. Several single-center studies have reported promising results and ongoing clinical trials are summarized in section 9.2. Additional data is needed to prospectively assess the impact of rapid syndromic testing in daily clinical decision-making as well as to determine its costs and effects.

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [20]has had an unprecedented and dramatic impact on the health care system and on the world economy. One of the most effective tools in the management of this global pandemic is the ability to rapidly and accurately test patients with signs and symptoms of ARTI or with risk factors for exposure. Some countries have used aggressive and widespread testing paired with contact tracing to manage the crisis and have been apparently the most successful at reducing mortality rates, the

strain on the health care system and the spread of the virus. Expanding testing locations could prevent patients from spreading infections due to lack of availability of testing outside hospitals and by reducing the time between sample collection and test result.

4.2. Rationale

Optimal clinical management of CA-ARTI is hampered because of diagnostic delays and suboptimal test sensitivity, leading to incorrect or missing aetiological diagnosis, and over-prescription of antibiotics. Diagnostic and antimicrobial stewardship is important to get the optimal patient management.

There is a need to assess the impact of rapid syndromic diagnostic testing in patients with CA-ARTI presenting to Emergency Rooms on clinical decision making related to:

- Hospitalisation yes or no;
- Start antibiotics yes or no;

At the same time, it must be determined whether the decisions guided by the rapid syndromic diagnostic testing results do not compromise patient safety.

5. OBJECTIVES

5.1. Co-primary objective:

To assess the impact of rapid syndromic testing in patients presenting with CA-ARTI in the ER on:

1. Days in hospital within 14 days after study enrolment
2. Days with antibiotic therapy within 14 days after study enrolment
3. Occurrence of adverse outcome within 30 days after study enrolment

5.2. Secondary objectives:

1. To assess the impact of rapid syndromic testing on healthcare utilization.
2. To assess the impact of rapid syndromic testing on quality of life.
3. To quantify the additional diagnostic yield and sensitivity of rapid syndromic testing because of targets not included in standard of care testing.
4. To quantify the impact of rapid syndromic testing on antimicrobial de-escalation and the choice of antibiotics and prescription of antivirals.
5. To quantify the impact of rapid syndromic testing on detection of antibiotic resistance.
6. To assess the impact of rapid syndromic diagnostic testing on patient bed management and/or isolation measures.

5.3. Exploratory objectives:

- Collect comprehensive data on clinical status and laboratory results for development and validation of clinical algorithms.
- To describe the current routine diagnostic and antimicrobial stewardship policy in Europe and identify good practices
- To estimate the impact of rapid syndromic testing on primary and secondary endpoints for subcategories of hospitals with similar routine diagnostic and antimicrobial stewardship programs.

- To explore clinician views and experiences of the use of rapid syndromic diagnostic testing when patients present to emergency departments (qualitative process evaluation by social sciences).

6. STUDY PARAMETERS/ENDPOINTS

6.1. Main study parameter/endpoint
Co-primary study endpoints:

1. Days alive out of hospital (superiority endpoint), within 14 days after study enrolment
2. Days on Therapy (DOT) with antibiotics (superiority endpoint), within 14 days after study enrolment
3. Adverse outcome (non-inferiority safety endpoint)
    - For initially non-admitted patients: any admission or death within 30 days after study enrolment
    - For initially hospitalised patients: i) any readmission, ii) ICU admission ≥ 24 hours after hospitalisation, or iii) death within 30 days after study enrolment

6.2. Secondary endpoints

- Direct costs and indirect costs within 30 days after enrolment.
- Change in quality of life as determined by EQ-5D-5L on day 1, 14 and 30 after enrolment. Extended follow up (up to 6 months) in a subgroup of patients that were initially hospitalised.
- Microbiological results obtained as standard of care and with the diagnostic intervention
- Empirical antibiotics, antibiotic type switches, de-escalation based on antimicrobial agent categories [21]. Prescription of antivirals during the main study.
- Detection of antimicrobial resistance (carriage or infection) related to the diagnostic intervention results compared to standard of care and impact on antimicrobial stewardship guidelines and prevention of hospital acquired infections.
- Decisions regarding isolation measures related to test result.

7. STUDY DESIGN AND DURATION

7.1. Study design and justification

This is a prospective, multi-centre, **individually randomised controlled open-label trial.** Approximately 1600 patients will be randomised in the trial in up to 10 investigational sites in the European region. Patients will be followed at day 14 and day 30 after randomisation. In a subset of patients, follow up will be extended to time points 3 and 6 months.

Enrolment will be competitive across sites.

Justification of the design. The aim is to deliver a study outcome that is **valid** (absence of bias), **precise** (sufficiently powered to achieve clinically relevant absence and presence of difference), and **generalizable** (recognizable population). The study needs to be **feasible** in terms of costs

(determined by number of study sites and number of tests to be performed), with an easy patient enrolment and an achievable burden on laboratories in both adult and paediatric sites. Therefore, although an **individually randomised trial** - in contrast to cluster randomisation - requires informed consent from all recruited patients fulfilling inclusion criteria prior to performing the test. This approach has several advantages: 1. Internal validity of the trial is guaranteed by the randomisation, whereas in cluster-randomised trials, patient selection criteria can be difficult to apply in a consistent manner, inducing a risk of selection bias; 2. Required sample size is lower compared to a cluster-randomised trial; 3. Recognizable population can be realized, although generalizability will always be lower compared to enrolling all-comers; 4. Feasibility: compared to a cluster-randomised trial, a lower sample size is needed, reducing costs for study execution; fewer hospitals, fewer patients, and a lesser burden on labs because tests are only performed in 50% of enrolled patients instead of 100% of all-comers. Moreover, although requirement of informed consent to be obtained at the ER could be considered as a drawback of this design, it in fact may help in enrolling the pursued patient population for this specific trial. We aim for patients in which the result of the rapid syndromic testing can guide clinical decision making. This implies that there is also time for obtaining informed consent.

In choosing the primary endpoints the following considerations were made:

- The composite endpoint is designed to capture the relevant outcomes and will be combined for analysis
- It needs to be ensured that the non-inferiority endpoint is not positively impacted by direct effects of the intervention such as decisions to initiate or withhold antibiotic treatment or early discharge from the ER or hospital. E.g. if we would include hospitalisation days in the non-inferiority endpoint, we run the risk of 'compensating' the putative adverse effects.
- **Hierarchical nested design:** Superiority primary endpoints are tested first. Only if superiority to at least one of the two superiority endpoints is confirmed, is the non-inferiority safety endpoint taken into consideration [22]
- Because of the non-blinded nature of the study, outcomes should be defined objectively, so e.g. we cannot use cause-specific re-admission or cause-specific death.
- Reassessment when other microbiological results are available. Discrepant results should be handled by the treating physician based on best practice
- Different time windows have been chosen because i) impact of rapid syndromic testing on hospitalisation / antibiotics is immediate. ii) Shorter follow-up increases power for difference of 1 day. iii) Adverse outcomes may reflect late sequelae.

7.2. Study duration

The study will encompass at least 2 influenza seasons (autumn/winter months) in Europe, however timelines might be extended related to the COVID-19 situation.

Justification. i) most CA-ARTI occur in autumn/winter months. ii) Timeframes allow for training, initiation and patient follow up. iii) To achieve an adequate sample size iv) Fluctuations in microbiological epidemiology of CA-ARTI (seasonal outbreaks *Mycoplasma*, *Bordetella*, variability on virus lineages, pneumococcal serotypes, etc.) are better represented with more than one season.

8. STUDY POPULATION

8.1. Study population

Adults (≥18 years old) presenting at the Emergency Room in selected participating sites with CA-ARTI and able to give informed consent.

8.2. Inclusion criteria

1. **Adults** (≥18 years old) presenting to the **Emergency Room** with an **acute illness** (present for **14 days or less**) with **cough**, **and** with at least 1 other lower respiratory tract **symptom** or **clinical sign** at physical examination:

   - Sputum production,
   - Breathlessness,
   - Chest discomfort or chest pain,
   - Wheeze,
   - Crackles,
   - Self-reported dystermia or documented fever;
   - Documented hypoxemia (adjusting definition for chronic oxygen therapy users, method of measurement) **and no alternative explanation** (infection, such as sinusitis; other, such as asthma).

2. **Managing medical team considers** early in the diagnostic process**:**

   a. to treat patient with antibiotics and/or to hospitalise patient

   AND

   b. that the rapid syndromic diagnostic test result can be awaited up to a maximum of 4 hours before the decision to discharge the patient or to initiate antibiotic therapy.

8.3. Exclusion criteria

1. Development of ARTI more than 48 hours after hospital admission (**hospital acquired**);
2. Patients with **cystic fibrosis;**
3. Less than **14 days** since the last episode of respiratory tract infection;
4. Pregnancy (confirmed by pregnancy test) and breastfeeding;
5. Any clinically significant abnormality identified at the time of screening that in the judgment of the Investigator would preclude safe completion of the study or constrain endpoints assessment such as major systemic diseases or patients with short life expectancy;
6. Inability to obtain informed consent from a competent patient.

Based on standard of care microbiological diagnosis and thoracic imaging (when indicated):

7. Radiologically confirmed acute lobar pneumonia;
8. Known or suspected *Pneumocystis jirovecii* pneumonia or active tuberculosis;

9. Alternative noninfectious diagnosis that explains clinical symptoms (pulmonary embolism, alveolar hemorrhage, acute heart failure, lung cancer).

Patients participating in interventional therapeutic studies related to COVID-19 are not considered *a priori* an exclusion criteria if it does not interfere with randomisation to the diagnostic intervention and the primary outcomes of the ADEQUATE clinical study. Compatibility will be evaluated individually.

8.4. Recommended Study site selection criteria

- Does not currently use equivalent rapid testing routinely in patients with CA-ARTI at the ER (rapid defined as time from sample collection to result interpretation by the physician within 4 hours).
- At least 25% of CA-ARTI patients seen at ER are not hospitalised.
-
- Microbiology lab is capable of performing molecular testing.
- Highly motivated and GCP-trained local Principal Investigator. Clinical research nurse.
- **Geographical balance:** We will prioritize EU Member States and H2020 Associated Countries with **high and medium antibiotic use** and with a range of **country level income** and **antibiotic stewardship programs** (if present, the antibiotic stewardship program will be documented, including the list of participant roles). Variations in **microbiological aetiologies and vaccination policies** will be taken into consideration.

9. DIAGNOSTIC INTERVENTION

9.1. Description of the test and Intended use

Two rapid syndromic diagnostic tests, to be allocated according to clinical syndrome, age and available sample. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

- **BioFire FilmArray Respiratory Panel 2.1 plus** (RP2.1plus): Nasopharyngeal swab
- **BioFire FilmArray Pneumonia Panel plus** (PP): Sputum (and/or ETA or BAL sample)

The **FilmArray Respiratory Panel 2.1 *plus* (RP2.1 *plus*)** is a multiplexed nucleic acid test intended for use with FilmArray® 2.1 or FilmArray® Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The test is FDA approved.
Virus: Adenovirus, Coronavirus (229 E, HKU1, NL63, OC43, SARS-CoV-2), human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, including subtypes H1, H1-2009, and H3, Influenza B, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Parainfluenza Virus (1, 2, 3, 4), Respiratory Syncytial Virus. **Bacteria:** *Bordetella parapertussis* (IS*1001*), *Bordetella pertussis* (*ptxP*), *Chlamydia pneumoniae, Mycoplasma pneumoniae*

The **FilmArray® Pneumonia Panel plus** is a multiplexed nucleic acid test intended for use with FilmArray® 2.0, or FilmArray® Torch systems for the simultaneous detection and identification of

multiple respiratory viral and bacterial nucleic acids, as well as selected antimicrobial resistance genes, in sputum-like specimens (induced or expectorated sputum, or endotracheal aspirates) or Broncho alveolar lavage (BAL)-like specimens (BAL or mini-BAL) obtained from individuals suspected of lower respiratory tract infection.

The following **bacteria** are reported **semi-quantitatively** with bins representing approximately 10^4, 10^5, 10^6, or $\geq 10^7$ genomic copies of bacterial nucleic acid per milliliter (copies/mL) of specimen, to aid in estimating relative abundance of nucleic acid from these common bacteria within a specimen:
Acinetobacter calcoaceticus-baumannii complex, Klebsiella oxytoca, Serratia marcescens, Enterobacter cloacae complex, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Moraxella catarrhalis, Streptococcus agalactiae, Haemophilus influenzae, Proteus spp., Streptococcus pneumoniae, Klebsiella aerogenes, Pseudomonas aeruginosa, Streptococcus pyogenes
The following atypical bacteria, viruses, and antimicrobial resistance genes are reported **qualitatively**:
Atypical Bacteria *Chlamydia pneumoniae, Legionella pneumophila, Mycoplasma pneumoniae*
Viruses: Adenovirus, Human Rhinovirus/Enterovirus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Coronavirus, Influenza A, Parainfluenza Virus, Human Metapneumovirus, Influenza B, Respiratory Syncytial Virus
Antimicrobial Resistance Genes: CTX-M, NDM, mecA/C and MREJ, IMP OXA-48-like, KPC VIM

9.2. Summary of findings from clinical studies

Both diagnostic tests had a high sensitivity (overall 97%) and specificity (overall 99%) in US FDA product registration trials (available from https://www.biofiredx.com/support/documents/ under Instructions for Use and Manuals) and in independent research [23]. In a performance study that compared the BioFire RP2 Panel to the BioFire RP Panel or PCR and sequencing, the overall percent agreement between the BioFire RP2 Panel and the comparator testing was 99.2% [24].

Biofire respiratory panels:

Several studies have been conducted to examine the clinical impact of the BioFire RP Panel. Findings from such studies included an increase in diagnostic yield, decrease time to diagnosis, lower probabilities of hospital admission, reduced time in isolation, reduced number of supplementary tests such as chest radiographs, reduced hospital length of stay, decreased duration of antimicrobials use, and increase in appropriate antiviral use [25, 26].

As a rapid syndromic diagnostic testing it expedites turnaround time for results, leading to higher rates of early discharge and early discontinuation of antibiotics [27]. In a large retrospective study in paediatric patients with acute viral respiratory tract infections its use was associated with less exposure to antibiotics and chest X-rays and more timely administration of antivirals [28].

BioFire Pneumonia Panel:

Several studies have demonstrated the diagnostic yield and accuracy of the BioFire FilmArray Pneumonia Panel for identification of pathogens in lower respiratory tract specimens and show highly concordant results with optimal sensitivity and specificity for the detection of bacteria and viruses [29-34]. Single-country clinical trials (ClinicalTrials.gov) in community acquired pneumonia are currently active: CAP-NEXT (NCT03360851), PROARRAY (NCT03840603),

RADICAP (NCT04158492) and ventilator associated pneumonia: INHALE https://fundingawards.nihr.ac.uk/award/RP-PG-0514-20018 are currently recruiting patients. Other single-center active projects, not recruiting: NCT02880384 and NCT03756753.

9.3. Summary of known and potential risks and benefits

Based on the BioFire FilmArray antibiotics may be withheld when deemed unnecessary, or a different antibiotic class may be selected when certain bacterial pathogens are detected. These management decisions are made by the patient's treating physician. The risks and benefits of these management decisions are subject to the current investigation.

A risk assessment and monitoring plan will be implemented but based on known intended use of the test (see below). Special effort will be invested on adequate training and monitoring of the sites (both laboratory and clinicians) for an optimal use of the test, and to support in the clinical and therapeutic decisions based on test results.

Safety and warning precautions of the product are summarised in the Instructions for Use as well as the Summary of Results and Limitations, with focus on the following:

- The pneumonia panel detects more pathogens than culture, however, the clinical significance may be unclear regarding their role as commensals, there might be concern for increasing antibiotic usage. Detection of bacterial nucleic acid may be indicative of colonizing or normal respiratory flora and may not indicate the causative agent.
- Asymptomatic carriage of viruses does occur, although less so in adults, or the virus could be a co-pathogen together with a bacterial pathogen, or a recent viral infection could have predisposed to a secondary bacterial pneumonia.
- Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test and pathogens below the limit of detection
- Negative results for antimicrobial resistance gene assays do not indicate susceptibility to corresponding classes of antimicrobials, as multiple mechanisms of antimicrobial resistance exist.
- There is a risk of false positive results due to non-specific amplification and/or cross-reactivity with organisms found in the respiratory tract and they are summarized in the Instructions for use. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible.

Expected **benefits of the study** are, together with the main objective of reducing hospitalisation rates and antimicrobial use without adverse outcome, the opportunity for improved training, harmonised improvement of existing guidelines (local and European), opportunity to grow the different existing networks, opportunity to improve surveillance because of underreporting or not available diagnostic tests.

10. METHODS

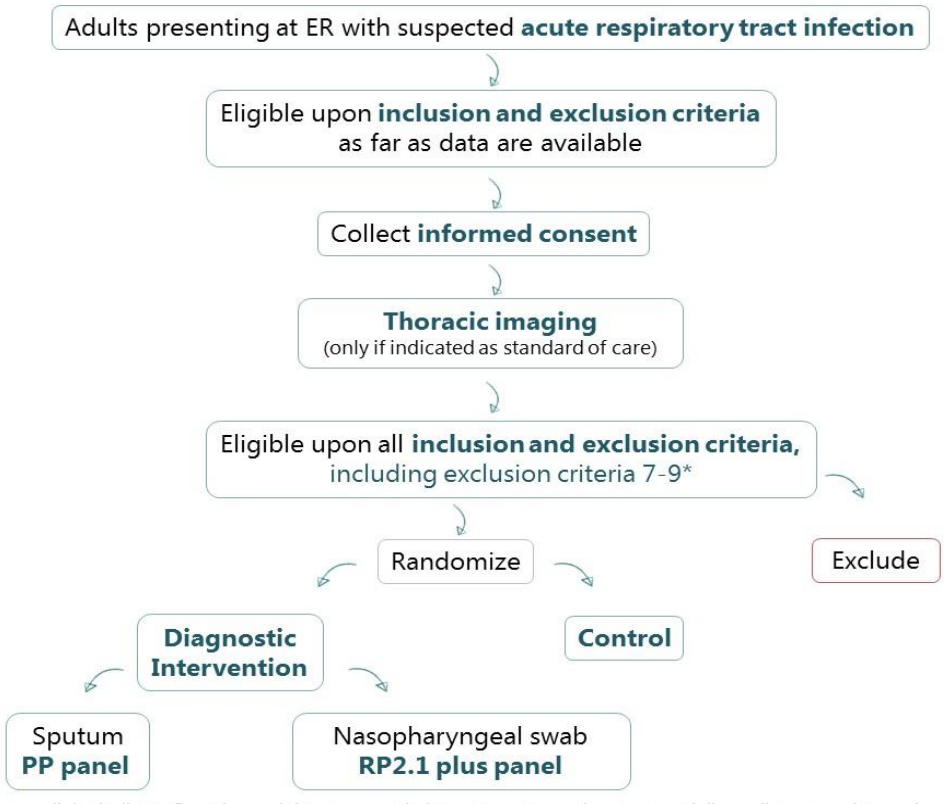
10.1. Screening and enrolment

Patients entering the emergency room will be screened for study. When exclusion criteria 7-9 are unknown at the time of screening, patients will be enrolled nonetheless when they meet these inclusion criteria. After informed consent is obtained, remaining exclusion criteria will be checked.

The health status of patients might rapidly deteriorate between entering the emergency room and randomisation. Therefore, all eligibility criteria need to be re-evaluated and confirmed prior to the decision to randomise the patient.

Screen failures are defined as patients who were found eligible per screening and have given their consent to participate in the trial, but meet exclusion criteria 7 to 9 or did not meet all inclusion criteria anymore, during the evaluation of exclusion criteria 7 to 9. Of note, no diagnostic procedures such as chest radiograph will be performed for the purpose of checking eligibility criteria, only if indicated as standard of care. E.g. “if a chest radiograph is not deemed necessary by the treating physician, a pneumonia (exclusion criterion 7) cannot be excluded, but the patient can still be enrolled as there is no ‘radiologically confirmed acute lobar pneumonia’. When all criteria are met, the patient will be randomised (see next paragraph).

Figure 1. Study flowchart



10.2. Randomisation and blinding

Due to the nature of the intervention, blinding is not possible. After all eligibility criteria have been verified and informed consent has been obtained, randomisation will be performed using the built-in randomisation module of the eCRF system. Allocation will be concealed until the moment of randomisation. To this end, block randomisation will be used with variable blocks of size 2, 4 and 6. Randomisation will be stratified by centre. After the decision to randomise the patient is made, patients will not be excluded from the trial. If the allocated intervention is not applied for any reason, this will be recorded and follow-up for the participant will be completed.

10.3. Data collection

A secured electronic case record form (eCRF) will be specifically designed for this study for data capture.

- **Main study** (Follow up until 30 days) – Standard of care clinical and microbiological data will be collected but not study specific biological samples will be obtained for research purposes. The **clinical data set** will summarize the illness episode and outcome, microbiological testing and antimicrobial use including the total hospitalisation or, in case of discharge, the time window defined on the primary endpoints. Information including data for health economic analysis will be collected on day of enrolment, day 14 (visit window: day 14 – 16) and on day 30 (visit window: day 30 – 32), after randomisation. Participants/LAR will be asked to consent to being contacted by study staff for the follow-up visits to minimise loss to follow-up. In case of failure to successfully contact participant/LAR at the end of trial participation, the participant's general practitioner/family doctor will be contacted to complete information on the primary endpoints.

- **Extension study** – in a subset of patients (around 300 patients per arm), patients that were initially hospitalised will be followed post-discharge at month 3 (visit window: +7 days) and month 6 (visit window: +7 days) to study health economic and social impact.

- Qualitative Evaluation: Only clinician (semi-structured) qualitative interviews

10.4. Clinical data set

Inclusion. Check for inclusion and exclusion criteria and randomisation. Informed Consent Form.**Baseline registration and investigations.**
Signs and symptoms at ER and management plan
Participant background
Vaccination
Co-morbidities and chronic medication
Standard of care haematology and biochemistry
EQ-5D-5L questionnaire
Management: Clinical decision after randomisation and initial results
Investigations: respiratory, urine, faeces, blood, SARS-CoV-2, radiology (only when standard of care)
Day 14: symptoms, EQ-5D-5L questionnaire
Day 30: symptoms, EQ-5D-5L questionnaire
Month 3 (if applicable): symptoms, EQ-5D-5L questionnaire
Month 6 (if applicable): symptoms, EQ-5D-5L questionnaire
Outcomes and safety:
Antibiotic treatment
Antiviral and antifungal agents
Hospital course
Device deficiency
(Severe) Adverse Event
Deviation
End of study

Table 1. Visit Schedule

Procedures	Screening	Enrollment	Hospitalization Period[e]	Face to face, on line questionnaire or phone call		Phone call Extended FU	Phone call Extended FU
	Pre- study procedures Day 1	Day 1	> Day 1	Day 14	Day 30	3 months[f]	6 months[f]
Informed consent	x						
Eligibility criteria	x						
Demographics[a]	x						
Baseline assessment[b]	x						
Vaccination status	x						
Comorbidities and chronic medications	x						
Hospital course[c]		x	x[e]				
Microbiological testing[d]		x	x				
Concomitant therapy review	x	x	x[e]	x	x	x[f]	x[f]
Symptoms review and hospitalizations	x	x	x[e]	x	x	x[f]	x[f]
Quality of life questionnaire EQ5D		x		x	x	x[f]	x[f]

10.5. Baseline and follow-up data for health-economic analysis

Follow up Assessments: Timepoints: Day 1, Day 14 and Day 30. · 3 months and 6 months for patients included in Extended Follow up

Individual patient data will be clustered in four main categories:

patient status prior to consultation (including quality of life assessment), diagnostics (including microbiological diagnostic tests and thoracic imaging), medicines (antibiotics and any other prescribed medication at the ER/admission including several parameters, antibiotic prescription in the previous 14 days) and patient follow-up (quality of life, adherence to treatment, duration of complaints).

Quality of life- (see eCRF completion guidelines on guidance)The EQ-5D (https://euroqol.org) contains 5-dimensions ("5D") related to everyday living: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Patients rate the problems they have with each of these dimensions on a 5-level scale ("5-L") from 1 (no problems) to 5 (extreme problems). The second part of the EQ-5D-5L asks patients to grade their current global health status from 0 (worst health you can imagine) to 100 (best health you can image). The questionnaire form takes ± 5-10 min to administer. It needs to be completed by patient or person who has known the patient for > 5 years. (Order of preference: caregiver > spouse > children 18+ > sibling > other acquaintance). For patient questionnaires, patients will receive a link per email to complete the questionnaires online, which will directly be incorporated into the eCRF and be regarded as source data once digitally completed by the patients. If this is not possible or if patients are seen face to face, questionnaires will be completed per paper (source data) and entered in the eCRF by study staff at the site. In this case paper versions will be stored as source data. The third option to have the questionnaires completed is the use of the Interviewer version in which the study staff will interview the patient and complete the data on the paper form (source data) after which data will be entered in the eCRF.

10.6. Microbiological testing

Results from standard of care testing. Microbiological tests according to standard of care from site.
Known previous colonization with multidrug resistant microorganism.
Known previous SARS-CoV-2 infection if required hospitalisation and data is available

- Type of samples collected: blood, ETA, BAL, pleural fluid, urine
- Type of tests: cultures, recording quality sputum (<10 epithelial cells and >25 leukocytes per field magnification x 100), antigen detection, antibody detection, molecular testing
- Test results: list of microorganisms. Selected susceptibility patterns

Susceptibility testing will be grouped based on antimicrobial resistant phenotypes definitions: methicillin resistant *Staphylococcus aureus* –MRSA-, carbapenem resistant Enterobacteriaceae, Extended Spectrum Beta Lactamases Enterobacteriaceae –ESBL-, carbapenem non susceptible *Pseudomonas aeruginosa*, multidrug resistant *P.aeruginosa*. (Based on https://www.cdc.gov/nhsn/PDFs/pscManual/11pscAURcurrent.pdf)

The data collected will allow also reporting pathogens of public health interest.
Within VALUE-Dx WP3 there is current work on inter-operability with lab devices, with the objective to achieve direct connectivity with these systems to have antimicrobial resistance information from lab results easily available.

Study samples. For diagnostic intervention: nasopharyngeal swab; endotracheal aspirate, spontaneously produced sputum collected as standard of care. Specific procedures for collection and processing will be provided. Data to be recorded:

- Test result (including data on genomic copies provided by the test)
- Time at which Test Result was Generated
- Time at which Test Result was received by care team and how it is communicated
- Time at which antibiotics were prescribed
- Results from standard of care diagnostic tests (e.g. cultures) with comparable targets and assessment of discrepant results

10.7. Qualitative evaluation

A qualitative process evaluation will be nested within the trial. The process evaluation will capture data to understand how clinicians adopt and experience RSDTs when delivering care in ERs. Related documents will be also be sent for ethical approval.

Recruitment: We will select 3-6 trial sites, sampling where possible to get sites from a range of countries. Once a site has completed recruitment for the trial, or once a site has had the RSDTs for at least 2 months, we will ask the PI at each site to identify 2-8 healthcare professionals who would be willing to share their experiences of taking part in the trial. Healthcare professionals will include clinicians working in the emergency department with the main criteria for inclusion in interviews being that clinicians should have used the RSDTs at their site. Potential clinician participants will be contacted in person or by email by the PI or another member of the research team. Emails will include further study information.

Sampling for clinician interviews will likely be by convenience and snowball sampling, through the site PIs, with colleagues recommending others who may be interested in participating in an interview. If there is a good response rate the research team will purposively select participants

based on site, site patient recruitment and use of RSDTs and job role. We will aim to complete 20-25 interviews with clinicians. All participants will only be required to take part in a single interview and a 15 euro voucher will be provided for each participant as reimbursement for their time.

Interviews: Interviews will be conducted by telephone (or alternative means such as Skype teleconferencing) and all participants will be asked to provide verbal consent prior to the interview starting. Interviews may be carried out in person if suitable and, if so, written informed consent will be sought. The researcher will make a written record of this consent. Interviews will be audio-recorded with participants permission. All interviews will be expected to last approximately 30-45 minutes. Clinician interviews will follow a clinician topic guide and will ask about experiences of using RSDTs in usual practice, interpreting results and the impact of results on patient management and clinical decision making.

11. TRAINING

11.1. Good Clinical practice training

The Principal Investigator of a study site needs to provide a valid ICH-GCP certificate to timely identify and enrol eligible patients, collect informed consent forms, collect source data, enter data into clinical database. See the ADEQUATE monitoring plan for more details. Guidelines will preferably follow ISO20916:2019 because of the specific differences in device related events and deficiencies reporting.

11.2. Medical Device training, being part of the Site Initiation Visit.

Training of the hospital staff involved in the study will be implemented, with an emphasis on sample collection, sample processing, correctly identifying causative organisms and performance of susceptibility testing in coordination with bioMérieux. Where required, guidance will be provided to improve procedures and align them with standardized uniform manners following the principles of Good Clinical Laboratory Practice (GCLP). Quality Control of the device will be performed as described in the ADEQUATE monitoring plan.

11.3. Data management training

All sites will be trained to use the data management system (Research Online) as part of the site initiation visit. To assure high quality, the Data Management Department of the Julius Centre (JC) of UMCU works according to a Quality Management System. All work is carried out in accordance with UMCU written Standard Operating Procedures (SOP) and Work instructions.

12. SAFETY REPORTING

12.1. Adverse events

Definitions

Adverse Event (AE)

For this study, an adverse event is defined as any untoward medical occurrence, inappropriate patient management decision, unintended disease or injury, or untoward clinical signs in patients, users, or other persons, with any connection to study related activities, whether or not related to the IVD medical device.

Adverse device effect (ADE)

An adverse event related to the use of the BioFire®.

Serious Adverse Event (SAE)

All SAEs, regardless of relationship to the study device or procedure will be documented in the source. It is the Investigator's responsibility to determine the "seriousness" of an AE using the protocol defined terms, listed below. A SAE is an AE that results in one or more of the following for this study:

- Resulted in death: An AE that resulted in the patient's death.
- Life-threatening illness or injury: The patient was at imminent risk of dying at the time of the adverse event.
- Permanent impairment: An AE that resulted in permanent impairment of a body function, including chronic diseases or permanent damage to a body structure.
- Required in-patient or prolonged hospitalisation.
- Resulted in medical or surgical intervention to prevent life threatening illness or injury or permanent impairment to a body structure or body function.
- Led to fetal distress, fetal death or congenital abnormality or birth defects, including physical or mental impairment.

Notes:

1. Hospitalisation on Day-1 should not be reported as an AE or SAE if this is a direct consequence of the initial referral to the hospital.
2. SAEs resulting in death should be reported using the primary cause of death as the event term. The only exception is "Sudden Death" when the cause is unknown.
3. Planned hospitalisation for a pre-existing condition is not considered a SAE.

Serious adverse device effect (SADE)

Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

Categories of adverse events

ADVERSE EVENTS	**Non-device-related**	**Device-related**
		Applies to: - Inaccurate test result leads to indirect harm to the patient - Device causes direct harm to user or another person
Non-serious	Adverse event	Adverse device effect
Serious	Serious adverse event	Serious adverse device effect

SEVERITY OF ADVERSE EVENTS

It is the Investigator's responsibility to assess the severity of an AE. A change in severity may constitute a new reportable AE.

Also, the following guideline should be used to determine the severity of each adverse event:

- MILD: Awareness of signs or symptoms, but does not interfere with the patient's usual activity, or is a transient event that resolves without treatment and with no sequelae.
- MODERATE: A sign or symptom, which interferes with the patient's usual activity.
- SEVERE: Incapacity with inability to do work or usual activities.

RELATIONSHIP OF ADVERSE EVENTS

It is the Investigator's responsibility to assess the relationship between all AEs to the study device and procedure. The following guidelines should be used in determining the relationship of an AE to a device, procedure, or other causality

Not related	Relationship to the procedures or device can be excluded when: • The event is not a known side effect of the product category the device belongs to or of similar device and procedures; • The event has no temporal relationship with the use of the device or the procedures; • The event involves a body-site or an organ not expected to be affected by the device or the procedure or the disease under investigation; • The event can be attributed to another cause (e.g. an underlying or concurrent illness/clinical condition, an effect of another device, drug, treatment, or other risk factors); • Harms to the patient are not clearly due to use error; or • To establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedure and the event.
Unlikely	The relationship with the use of the device seems not relevant and/or the event can be reasonably explained by another cause, but additional information may be obtained.
Possible*	The relationship with the use of the device is weak but cannot be ruled out completely. Alternative causes are also possible (e.g. an underlying or concurrent illness/condition and/or an effect of another device, drug, or treatment). Cases where relatedness cannot be assessed, or no information has been obtained should also be classified as possible.

Probable*	The relationship with the use of the device seems relevant and/or the event cannot reasonably be explained by another cause, but additional information may be obtained.

Causal Relationship*	The event is associated with the device or with procedures beyond reasonable doubt when:
	• The event is a known side effect of the product category the device belongs to or of similar device and procedure
	• The event has a temporal relationship with the device uses/application or procedures
	• The event involves a body-site or organ that
	  ○ The device or procedures are applied to
	  ○ The device or procedures have an effect on
	• The event follows a known response pattern to the medical device (if the response pattern is previously known)
	• Other possible causes (e.g. an underlying or concurrent illness/clinical condition and/or an effect of another device, drug, or treatment) have been adequately ruled out
	• Harm to the patient is due to error in use
	• To establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedure and the event.

*Denotes “related” to the study procedure or device and should be reported (AE) as part of the study.

12.2. Reporting Adverse Events

The principal investigator shall:

a) record all AEs and device deficiencies, regardless of relationship to the study device or study procedure in the source, together with an assessment as to whether the device or study procedure were a cause of the event,

b) report to the Sponsor, without unjustified delay (max. 48 hours after becoming aware of the event), all SAEs, that deemed to have a possible, probable, or causal relationship to the study procedure, disease under investigation or device, from the time of signing the informed consent through study completion (day-30 for the main study or 6 months for the extension study) and device deficiencies that could have led to a SADE; this information shall be promptly followed by detailed written reports, as specified in the Safety Management Plan,

c) report to the Sponsor, without unjustified delay (max. 7 calendar days after becoming aware of the event), all AEs, that deemed to have a possible, probable, or causal relationship to the study procedure or device from the time of signing the informed consent through study completion (day-30 for the main study or 6 months for the extension study); this information shall be promptly followed by detailed written reports, as specified in the Safety Management Plan,

d) ensure expedited reporting to the ethics committee, or per the timelines of the national regulations, SAEs that deemed to have a possible, probable, or causal relationship to the study

procedure, disease under investigation or device, from the time of signing the informed consent through study completion (day-30 for the main study or 6 months for the extension study) and device deficiencies that could have led to a SADE, when required by the national regulations or Safety Management Plan or by the ethics committee,

e) ensure annually reporting to the ethics committee, or per the timelines of the national regulations, AEs that deemed to have a possible, probable, or causal relationship to the study procedure or device, from the time of signing the informed consent through study completion (day-30 for the main study or 6 months for the extension study), when required by the national regulations or Safety Management Plan and

f) ensure to report to regulatory authorities, SADEs and device deficiencies that could have led to serious adverse device effect, as required by national regulations, and supply the Sponsor, upon Sponsor's request, with any additional information related to the safety reporting of a particular event. Sponsor remains responsible for adequate reporting to regulatory authorities.

The study site will report applicable safety events to the Sponsor by entering the event into the AE form in the eCRF, which will trigger an automated email to the Sponsor and manufacturer. Refer to the ADEQUATE Satefy Management Plan for more extensive guidance on reporting.

12.3. Device Deficiency

Device Deficiency Definition

A device deficiency is defined as inadequacy of the BioFire® and/or kits with respect to its identity, quality, durability, reliability, usability, safety or performance.

Device deficiencies include, but are not limited to, malfunctions, use errors, and inadequacy in the information supplied by the manufacturer including labelling.

A device deficiency may or may not be associated with an AE/SAE.

Reporting Device Deficiency

All device deficiencies related to devices in the procedure shall be documented throughout the study. The study site must report device deficiencies related to the BioFire and/or kits, within 48 hours after becoming aware of the event through the Device Deficiency form in the eCRF.

Manufacturer representatives will organize collection of the device for evaluation, as needed.

12.4. Deviations from the Clinical Study Protocol

A protocol deviation is any non-compliance with the study protocol, Good Clinical Practice.A deviation can be identified from a number of sources. Potential sources include, but are not limited to, a member of the Investigator's staff, a Sponsor representative during monitoring visits, or a member of the data management or statistical groups when entering or analysing data. Regardless of the source, it is crucial to document the deviation in the protocol deviation eCRF. The Investigator will report protocol deviations to the IRB/EC as required by the IRB/EC procedures.

Any deviation from the protocol or procedures should be recorded on the Deviation Form in the eCRF. Standard of care assessments not completed at a site should not be considered protocol deviations.

Steps to be taken to assure the accuracy and reliability of data include the selection of qualified investigators and appropriate sites, the review of protocol procedures with the Investigator and associated personnel prior to the study, and periodic monitoring visits by the Sponsor. The Sponsor will review eCRFs for accuracy and completeness during on-site and/or remote monitoring visits; any discrepancies will be resolved with the Investigator or designees, as appropriate.

12.5. Data Safety monitoring board (DSMB)

A Data Safety Monitoring Board has been established with the aim to safeguard the interests of trial participants, assess the safety of the interventions during the trial, and monitor the overall conduct of the trial. A specific charter document describes the roles and responsibilities of the independent DSMB for the ADEQUATE trial, including the timing of meetings, methods of providing information to and from the DSMB, frequency and format of meetings, statistical issues and relationships with other committees.

13. STATISTICAL CONSIDERATIONS

13.1. Sample size calculation

Co-primary endpoints used in the study:

1. Days on antibiotic treatment within 14 days (superiority endpoint)
2. Days alive out of the hospital within 14 days (superiority endpoint)
3. Adverse outcome within 30 days (non-inferiority safety endpoint)

Required sample sizes are presented for endpoint 2 and 3. Endpoint 1 is non-limiting due to having an expected smaller standard deviation compared to endpoint 2 and having the same clinically relevant effect size.

The intervention is considered successful if superiority is demonstrated for either or both endpoints 1 and 2 AND non-inferiority is demonstrated for endpoint 3. We use a hierarchical nested design: superiority primary endpoints are tested first. Only if superiority to at least one of the two superiority endpoints is confirmed, the non-inferiority safety endpoint is taken into consideration. To maintain an overall alpha < 0.05, the two superiority endpoints will be tested using a two-sided alpha of 0.025, while the non-inferiority endpoint will be tested using a one-sided alpha of 0.05. The minimal power for the superiority endpoints is set to 0.9 and for the non-inferiority endpoint to 0.95. This is to maintain overall power of the trial, given the hierarchical testing. For ‘Adverse outcome’ we considered different non-inferiority margins. For ‘Days on antibiotic treatment’ and ‘Days alive out of the hospital’ we defined 1 day as a clinically relevant effect size.

The distribution of endpoint 2 is yet unknown for the ARI population. We supported our sample size calculation using data of patients presenting with CAP to Dutch ERs and hospitalised to a non-ICU ward (ClinicalTrials.gov: NCT02604628). The standard deviation (SD) of ‘Days alive out of the hospital within 14 days’ in this population was 4 days. The theoretical maximum SD with a 14 days follow-up period is 7 (which would be the case if 50% had zero days and 50% had 14 days alive out of the hospital). However, we expect that in the target population the variability for this endpoint will be less than in the CAP population. We varied the SD to assess the impact on

the sample size. We used a standard correction factor of 1.15 for non-normality of the data. (Table 1) Hence for the superiority endpoints a sample size of 457 per arm would be adequate.

Table 1: Sample sizes for Days alive out of the hospital using different assumptions

SD	Relevant effect size	Alpha	Beta	Correction	Sample size per arm
3	1	0.025	0.1	1.15	257
4	1	0.025	0.1	1.15	457
5	1	0.025	0.1	1.15	714
6	1	0.025	0.1	1.15	1,028
7	1	0.025	0.1	1.15	1,399

For adverse outcome, if we assume an incidence of 10% the sample size would be 780 per arm, and if we assume 5% it would be 412, in both cases using a non-inferiority margin of 5% (Table 2). A high power for adverse outcome is considered important because both the superiority and the non-inferiority hypothesis need to be confirmed in order to declare the intervention a success so a total of 780 adults per study arm would be required to reach 95% power

Table 2: Sample sizes for adverse outcome using different assumptions.

Incidence *	Non-inferiority margin	Alpha**	Beta	Sample size per arm
0.05	0.05	0.05	0.05	412
0.10	0.05	0.05	0.05	780

* same for intervention and control arm; ** one-sided alpha

To account for potential drop-outs we will aim for a **total of 1600 adults** in the study. The sample size includes participants that are randomised and does not include enrolled but non-randomised patients.

13.2. Analysis populations

A statistical analysis plan will be prepared after protocol approval and once site selection process has been initiated.

The main analyses will be performed for the total population

Analyses will be stratified by:

- age groups (i.e. 18-49, 50-65, >65)
- clinical syndrome: exacerbation of chronic pulmonary disease, influenza-like illness, laryngitis/laryngotracheitis (croup), acute bronchitis
- risk factors. Patient groups at risk for developing severe disease are well known, such as the elderly, patients with chronic pulmonary, cardiovascular or metabolic disease or immunocompromised patients. Clinical predictors of severity are also well known. Still,

within risk groups may exist the uncertainty whether to hospitalise and/or to treat and the impact of testing may be assessed.
- Vaccination status. Influenza. Pneumococcal.

The primary analysis will follow the intention-to-treat principle in which groups are compared based on the allocated regimen. In the per protocol analysis we will exclude participants not receiving the test according to the randomised regimen (e.g. randomised to rapid syndromic testing but not receiving the test).

13.3. Primary study parameter(s)

Descriptive statistics will be produced and tabular summaries will be presented, stratified according to the allocated group (rapid syndromic testing vs. control). Categorical data will be summarized by the number and percentage of patients in each category. Appropriate summary statistics will be used for continuous variables depending on the distributional assumptions. These include measures of central tendency (mean or median) and dispersion (standard deviation or inter-quartile range).

'Days on antibiotic treatment' and 'Days alive out of the hospital' will be analysed using a linear regression analysis, including as covariates stratification by age groups and predefined risk factors. Upfront we may expect that the assumption of normally distributed residuals is violated, in which case we will determine the confidence interval by bootstrapping.

Adverse outcome will be analysed using Cox proportional hazards regression adjusted for age groups and predefined risk factors. Risk differences and 90% CI will be inferred by comparing the cumulative incidence of failure at day 30 from the Cox model and bootstrapping for the confidence interval. A 90% CI will be used to be compatible with a one-sided alpha of 0.05.

13.4. Secondary study parameter(s)

Economically relevant parameters will be gathered within the trial context from individual participants but also the following data from the participant sites/countries will be extracted when possible:

- Patient numbers relative to population and hospital catchment area
- Estimations on the number of emergency department visits associated with acute respiratory infections based on a list of ICD codes.
- For the long-term economic model, the probability of susceptibility to received antibiotics and the resulting illness duration, additional outpatient visits and a second antibiotic course will be estimated based on surveillance data on every site. When needed, assumptions regarding the incidence and hospitalisation rates will be derived from literature and expert opinion

Types of costs: Direct medical costs, direct and indirect non-medical costs.
Hospital costs will be determined using bottom up calculation based on representative sites that have the information available on electronic databases that allow **unit cost prices** assignment to the variables recorded. These descriptive data will include health care utilization for the entire hospitalisation, for ICU stay (unit cost price per hospitalisation day, unit cost price for ICU hospitalisation day). Unit cost price for recorded diagnostic tests and thoracic imaging (referred to the procedure of testing as coded/reimbursed by official nomenclature).

When not available, resource use will be measured as volume of hospital admission days, tests performed, etc).
Analysis of the antibiotic domain based on antibiotic type switches and de-escalation. For the health economic analysis, a decision-tree **deterministic** approach with specification of the base case and alternative scenarios, together with threshold analyses to determine efficient ranges for the values of some parameters. Analysis of the antibiotic domain based on antibiotic type switches and de-escalation.

Regarding qualitative interviews, 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. 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 and sites.

13.5. Mid-term data analysis

A data snapshot will be taken, after the first season, in terms of number of recruited cases per category, impact of seasonal outbreaks or emerging pathogens. Sample size assumptions may also need to be validated.

14. ETHICAL CONSIDERATIONS

14.1. Regulation statement

The study will be performed in accordance with all applicable laws and regulations including the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP) the ethical principles that have their origins in the Declaration of Helsinki (current official version: Fortaleza, 2013; https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-researchinvolving-human-subjects/), the updated version of the General Data Protection Regulation (EU) 2016/679 (GDPR), ISO 20916 and other applicable privacy laws.

14.2. Recruitment and consent

The investigator or authorised delegate must obtain written informed consent before any clinical study related procedure/activity takes place. Patients (or, if applicable, their legally accepted representative (LAR) will be approached for the study by the investigator or authorised delegate. Written versions of the Participant Information Sheet and Informed Consent will detail the exact nature of the study; the implications and constraints of the protocol; and any risks involved in taking part. It will be stated clearly in the patient information letter that participation in this study is completely voluntary and that withdrawal is possible at any time and without any consequences. Staff will explain the details of the study to the participant and/or legal representative (if applicable) and allow them time to discuss and ask questions. The consenting party will be asked to sign and date an informed consent form. Obtaining a patient's consent to participate in medical research may be complicated by COVID-19 measures. In the case of the patient being in in isolation and unable to sign, he/she will give his/her oral consent, the researcher will sign directly, and the patient will sign as soon as possible after completion of the isolation period. Deviations in consent procedures might occur per participating country, following national law and locally accepted procedures with regards to challenging COVID-measures.

Participant Information Sheets will be available in the common local language. Written Informed Consent will be confirmed by the dated signatures of the participant 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 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.

If a patient is illiterate, an impartial witness must be present during the entire informed consent discussion. All items addressed in the Patient Informed Consent Form must be explained. The language used shall be as non-technical as possible and must be understandable to the patient in the impartial witness, where applicable.

For clinicians taking part in interviews we will provide the Clinician Participant Information Sheet, the Clinician Interview Informed Consent Form, the Clinician Interview Invitation and the Clinician Interview Topic Guide.

14.3. Withdrawal of individual patients

Patients are free to withdraw consent at any time without providing a reason. Patients who wish to withdraw consent for the study will have anonymized data collected up to the point of that withdrawal of consent included in the analyses. The patient will not contribute further data to the study. Data up to the time of withdrawal will be included in the analyses unless the patient appeals to the 'right to be forgotten' according to the national GDPR regulations. The investigator can decide to withdraw a patient from the study for urgent medical reasons. Enrolled patients meeting one or more of the exclusion criteria *prior* to randomisation, will be withdrawn by the investigator. Patients withdrawn prior to randomisation will be replaced (i.e. they will not count towards the sample size). Patients withdrawn after randomisation will not be replaced.

The participants cannot be enrolled at the same time into any interventional clinical study unless it is a COVID-19 study that does not interfere with this study. Patients may be co-enrolled in another observational study if the local study coordinators have been informed and have given their approval, to ensure the other study would not interfere with the results of this study or compromise patient welfare.

15. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

15.1. Steering Committee

The missions of the steering committee are to define the objectives of the research, to propose protocol modifications during the research, to organize the research, to determine the methodology, to coordinate information and to monitor the conduct of research. The steering committee will decide during ongoing research what to do in unexpected situation. The steering committee will meet regularly until the end of inclusions.

15.2. Handling and storage of data and documents

Data will be recorded in a secured electronic case record from (eCRF) specifically designed for this study and validated for authenticity, accuracy, reliability and consistent intended performance. An eCRF should be completed for each participant.

All information obtained during the study (except the ICF data) will be entered digitally in conformity with the applicable laws and regulations. All data (except the ICF data) will be coded using a unique study number. Data will be handled in accordance with local privacy regulations and the European General Data Protection Regulation (GPDR, 2016/679, effective as of May 25th, 2018)

15.3. Storage of samples.

A sample required for the CA-ARTI diagnostics will be taken in patients randomised to care with the addition of that diagnostic, according to local laboratory requirements.

15.4. Monitoring and Quality Assurance

Remote/centralized and/or on-site study monitoring will be carried out by the Sponsor. A monitoring plan will be scheduled and will define the monitoring frequency and procedures. Monitoring will start before recruitment begins, throughout the trial (data monitoring including but not limited to recruitment rates, consent procedures, access/storage of patient identifiers, sample handling for performing the diagnostic intervention test, data entry, data queries, unusual data patterns), between recruiting season and at the end of the trial (source document verification, collect trial supplies, close-out meetings/visits). We will use a risk adapted approach within the protocol design to enable safety reporting requirements to reflect the amount of safety data available and the level of risk for this non-interventional study. We refer to the monitoring plan for details.

This study does not involve any experimental treatment, but the diagnostic intervention may interfere with the standard of care and it involves data collection. The need for additional insurance for study patients may differ per country local regulations.

15.5. Public disclosure and publication policy

All information disclosed or provided by the Sponsor (or any company/institution acting on their behalf) or produced during the study, including, but not limited to, the protocol, the eCRF, and the results obtained during the course of the study, is confidential prior to the publication of results and in accordance to consortium agreement and open access regulations.

The detailed procedures for the review of publications are set out in the clinical trial agreement entered into with the Sponsor in connection with this study. These procedures are in place to ensure coordination of study data publication and adequate review of data for publication against the validated study database for accuracy. UMCU will adhere to all applicable local laws governing transparency in clinical trials including the trial posting on clinicaltrials.gov and all other applicable registrations.

16. REFERENCES

1. WHO. *World Health Organization. The top 10 causes of death*. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. .
2. Collaborators, G.B.D.L.R.I., *Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016.* Lancet Infect Dis, 2018. **18**(11): p. 1191-1210.
3. Collaborators, G.L., *Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015.* Lancet Infect Dis, 2017. **17**(11): p. 1133-1161.
4. Feldman, C. and E. Shaddock, *Epidemiology of lower respiratory tract infections in adults.* Expert Rev Respir Med, 2019. **13**(1): p. 63-77.
5. O'Connor, R., et al., *Antibiotic use for acute respiratory tract infections (ARTI) in primary care; what factors affect prescribing and why is it important? A narrative review.* Ir J Med Sci, 2018. **187**(4): p. 969-986.
6. Prat, C. and A. Lacoma, *Bacteria in the respiratory tract-how to treat? Or do not treat?* Int J Infect Dis, 2016. **51**: p. 113-122.
7. Cassini, A., et al., *Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis.* Lancet Infect Dis, 2019. **19**(1): p. 56-66.
8. Naylor, N.R., et al., *Estimating the burden of antimicrobial resistance: a systematic literature review.* Antimicrob Resist Infect Control, 2018. **7**: p. 58.
9. Shrestha, P., et al., *Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use.* Antimicrob Resist Infect Control, 2018. **7**: p. 98.
10. *National Institute for Health and Clinical Excellence. Respiratory Tract Infections - Antibiotic Prescribing: Prescribing of Antibiotics for Self-Limiting Respiratory Tract Infections in Adults and Children in Primary Care*, in *Respiratory Tract Infections - Antibiotic Prescribing: Prescribing of Antibiotics for Self-Limiting Respiratory Tract Infections in Adults and Children in Primary Care*. 2008: London.
11. Esposito, S. and N. Principi, *Defining the aetiology of paediatric community-acquired pneumonia: an unsolved problem.* Expert Rev Respir Med, 2019. **13**(2): p. 153-161.
12. van Vugt, S.F., et al., *Use of serum C reactive protein and procalcitonin concentrations in addition to symptoms and signs to predict pneumonia in patients presenting to primary care with acute cough: diagnostic study.* BMJ, 2013. **346**: p. f2450.
13. Schuetz, P., et al., *Procalcitonin to initiate or discontinue antibiotics in acute respiratory tract infections.* Cochrane Database Syst Rev, 2017. **10**: p. CD007498.
14. Ruuskanen, O., et al., *Viral pneumonia.* Lancet, 2011. **377**(9773): p. 1264-75.
15. Oosterheert, J.J., et al., *Impact of rapid detection of viral and atypical bacterial pathogens by real-time polymerase chain reaction for patients with lower respiratory tract infection.* Clin Infect Dis, 2005. **41**(10): p. 1438-44.
16. Rogers, B.B., et al., *Impact of a rapid respiratory panel test on patient outcomes.* Arch Pathol Lab Med, 2015. **139**(5): p. 636-41.
17. Vos, L.M., et al., *More targeted use of oseltamivir and in-hospital isolation facilities after implementation of a multifaceted strategy including a rapid molecular diagnostic panel for respiratory viruses in immunocompromised adult patients.* J Clin Virol, 2019. **116**: p. 11-17.
18. Poritz, M.A., et al., *FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection.* PLoS One, 2011. **6**(10): p. e26047.
19. Chan, M., et al., *Comparison of the Biofire FilmArray Respiratory Panel, Seegene AnyplexII RV16, and Argene for the detection of respiratory viruses.* J Clin Virol, 2018. **106**: p. 13-17.
20. Zhu, N., et al., *A Novel Coronavirus from Patients with Pneumonia in China, 2019.* N Engl J Med, 2020. **382**(8): p. 727-733.
21. **CDC**. *Centers for Disease Control and Prevention. National Health Care Safety Network. Antimicrobial use and resistance manual.*

]; Available from: https://www.cdc.gov/nhsn.

22. Timsit, J.F., et al., *Appropriate endpoints for evaluation of new antibiotic therapies for severe infections: a perspective from COMBACTE's STAT-Net.* Intensive Care Med, 2017. **43**(7): p. 1002-1012.
23. Leber, A.L., et al., *Multicenter Evaluation of BioFire FilmArray Respiratory Panel 2 for Detection of Viruses and Bacteria in Nasopharyngeal Swab Samples.* J Clin Microbiol, 2018. **56**(6).
24. Shu, Y.L., et al., *Dual seasonal patterns for influenza, China.* Emerg Infect Dis, 2010. **16**(4): p. 725-6.
25. Brendish, N.J., et al., *Routine molecular point-of-care testing for respiratory viruses in adults presenting to hospital with acute respiratory illness (ResPOC): a pragmatic, open-label, randomised controlled trial.* Lancet Respir Med, 2017. **5**(5): p. 401-411.
26. Shengchen, D., et al., *Evaluation of a molecular point-of-care testing for viral and atypical pathogens on intravenous antibiotic duration in hospitalised adults with lower respiratory tract infection: a randomized clinical trial.* Clin Microbiol Infect, 2019. **25**(11): p. 1415-1421.
27. Brendish, N.J., et al., *Impact of turnaround time on outcome with point-of-care testing for respiratory viruses: a post hoc analysis from a randomised controlled trial.* Eur Respir J, 2018. **52**(2).
28. Lee, B.R., et al., *Impact of multiplex molecular assay turn-around-time on antibiotic utilization and clinical management of hospitalised children with acute respiratory tract infections.* J Clin Virol, 2019. **110**: p. 11-16.
29. Webber, D.M., et al., *Evaluation of the BioFire(R) FilmArray(R) Pneumonia Panel for Detection of Viral and Bacterial Pathogens in Lower Respiratory Tract Specimens in the Setting of a Tertiary Care Academic Medical Center.* J Clin Microbiol, 2020.
30. Edin, A., H. Eilers, and A. Allard, *Evaluation of the Biofire Filmarray Pneumonia panel plus for lower respiratory tract infections.* Infect Dis (Lond), 2020: p. 1-10.
31. Hughes, A.E.O., et al., *Comparable Detection of Viral Pathogens in Lower Respiratory Tract Specimens With the BioFire Respiratory Panel 2 and BioFire Pneumonia Panel.* J Clin Microbiol, 2020.
32. Yoo, I.Y., et al., *Evaluation of the BioFire(R) FilmArray(R) Pneumonia Panel for rapid detection of respiratory bacterial pathogens and antibiotic resistance genes in sputum and endotracheal aspirate specimens.* Int J Infect Dis, 2020.
33. Lee, S.H., et al., *Performance of a multiplex PCR pneumonia panel for the identification of respiratory pathogens and the main determinants of resistance from the lower respiratory tract specimens of adult patients in intensive care units.* J Microbiol Immunol Infect, 2019. **52**(6): p. 920-928.
34. Yugueros-Marcos, J., et al., *New molecular semi-quantification tool provides reliable microbiological evidence for pulmonary infection.* Intensive Care Med, 2018. **44**(12): p. 2302-2304.
35. Hansell, D.M., et al., *Fleischner Society: glossary of terms for thoracic imaging.* Radiology, 2008. **246**(3): p. 697-722.
36. Charlson, M.E., et al., *A new method of classifying prognostic comorbidity in longitudinal studies: development and validation.* J Chronic Dis, 1987. **40**(5): p. 373-83.

www.imi.europa.eu www.value-dx.eu