Statistical Analysis Plan

Study Protocol Title: Advanced Diagnostics for Enhanced QUality of Antibiotic prescription in respiratory Tract infections in Emergency rooms

The ADEQUATE Study

Version 1.0; 4th January 2024

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

Approval Signatures:

By signing below, I indicate that I have reviewed this document in its entirety and approve its contents.

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HISTORY OF CHANGES

Date	Version	Rationale	Authors
4th Jan 2024	1.0	First version	Julia Bielicki (SGUL) Andrew Atkinson (UKBB) Malte Kohns (UKBB)

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1. General considerations

The ADEQUATE study is a prospective, multi-center individually randomised controlled open-label trial.

This document describes the statistical analysis for the paediatric study to determine the impact of rapid syndromic diagnostic testing of patients with community acquired Acute Respiratory Tract Infection (CA-ARTI) at the emergency department compared to the usual standard of care diagnosis approach.
The study population is children (<18 years) consulting in selected participating sites with CA-ARTI, randomised after informed consent is given by the patient, legal representative or by parents or legal guardians and participant's assent where age-appropriate.
The study intervention consists of rapid syndromic testing using BioFire Filmarray test devices on respiratory samples (intervention arm; BioFire FilmArray Respiratory Panel 2.1 plus (RP2.1plus) with nasopharyngeal swabs).
The study control arm consists of the standard or care diagnosis approach used in the respective hospital emergency department.
Visit schedule and study procedures are displayed in "clinical protocol WP4b – pediatric version" version 3.0 (11.11.22).

2. Main study endpoints

Co-primary endpoints:

- EP1: Days on antibiotic treatment (superiority endpoint) within 14 days after study enrolment
- EP2: Days alive out of hospital (superiority endpoint) within 14 days after study enrolment

EP1 and EP2 are tested separately, and superiority is confirmed if either EP1 or EP2 (or both) of the endpoints are superior in terms of the primary analysis.

There is no hierarchy in terms of these endpoints. Therefore, we note that the following text in the protocol amendment is incorrect: "The superiority comparison on EP1 will be considered the primary outcome comparison for the trial."

To recap, and adopting the framework of the ICH E9 (R1) addendum [1], the *primary estimand* for the trial is defined by the following components:

- Treatment regimens to be compared: standard of care in the emergency room versus rapid syndromic diagnostic testing (intervention)
- Patient population: Children (<18 years) consulting in selected participating sites with CA-ARTI
- Outcome definition: EP1 or EP2.
- Population-level summary: Difference in log transformed times for EP1 or EP2 between the two arms.
- Intercurrent event (ICE) strategies: For the primary estimand, we will pursue a treatment policy strategy (ie "intention to treat"). For children lost to follow-up, we censor their data at the date/time of their being lost to follow-up, and include the censored time in the primary analysis (refer to section 6.4).

2.1. Secondary endpoints

1. Adverse outcome
    a. For initially hospitalised patients: i) any readmission, ii) ICU admission ≥ 24 hours after hospitalisation, or iii) death, within 30 days after study randomization
    NOTE: adverse outcomes no longer part of the primary outcome
    b. For initially non-admitted patients: any admission or death within 30 days after study randomisation.
    NOTE: originally enrollment, not randomisation.
2. Antibiotic treatment (yes/no)
3. Time from randomisation to discharge (for those hospitalised).
4. Time from randomisation to management decision (for those admitted or treated with antibiotics)
5. Direct costs and indirect costs within 30 days after enrolment.
6. 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.
7. Microbiological results obtained as standard of care and with the diagnostic intervention.
8. Proportion of hospitalised participants (by arm) with detection of cephalosporin-, carbapenem- or chinolone-resistant Enterobacteriaceae on any standard of care samples >7 days after randomisation.
9. Hours in individual or cohort isolation in hospitalised participants (by arm).
10. Descriptive statistics of empirical antibiotics, antibiotic type switches, and time to de-escalation based on antimicrobial agent categories.

3. Sample size calculation

The sample size calculation and its rationale is defined in the amendment to the protocol [2].

4. 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 is performed using the build-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 is made, patients will not be excluded from the trial for the primary estimand (i.e. “intention to treat” or “treatment policy” type strategy for intercurrent events).

5. Variables

Clinical data set is described in detail in electronic Case Report Form (eCRF) casebook and eCRF specifications (current version 3.0, 22-Dec-2021) includes the following forms:

Inclusion.
Eligibility. 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
Study samples (only for intervention group)
Investigations:
Standard of care microbiological results: respiratory, urine, faeces, blood, SARS-CoV-2 detection, radiology (only when standard of care)
Follow up:
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
(Serious) Adverse Event
Deviation
End of study

Primary variables are extracted from the following forms: Clinical decision form, day 14 and day 30 forms, antibiotic form and hospital course form.

Safety variables: Extracted from Follow up and Outcomes and Safety Forms. As described in protocol and in safety management plan, the form includes causality assessment and severity of events reported as having a possible, probable or causal relationship to study device, study procedure and disease under investigation (if applicable)

Secondary variables are extracted from the following forms: baseline, day 14 and day 30, investigations, Antibiotic treatment, Antiviral and antifungal and Hospital course.

5.1. Definition of variables

1. Comorbidities

The original Charlson comorbidity index score (Charlson ME et al. Journal of chronic diseases. 1987;40(5):373-83) assigns four categories (0, 1-2, 3-4, and ≥ 5) according to the points assigned to a list of comorbidities. For this study, modified Charlson comorbidity index is based on the ISARIC WHO requirements Clinical Characterisation Protocol and includes conditions selected a priori by a global consortium to provide rapid, coordinated clinical investigation of patients presenting with any severe or potentially severe acute infection of public interest and enabled standardisation as described in "Risk stratification of patients admitted to hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: development and validation of the 4C Mortality Score" [3]. Comorbidities collected: chronic cardiac disease (including congenital heart disease, not hypertension), chronic respiratory disease (excluding asthma), chronic renal disease (estimated glomerular filtration rate ≤30), mild to severe liver disease, dementia, chronic neurological conditions, connective tissue disease,

diabetes mellitus (diet, tablet, or insulin controlled), HIV or AIDS, and malignancy. The grading scale assigns 3 categories according to number of comorbidities: 0, 1 +1

2. Antibiotic treatment

Days on antibiotic treatment is calculated from the date and time of first antibiotic prescription after enrolment (recorded in clinical decision form Yes/No, Time) until day 14. The duration during the first 14 days is calculated from the antibiotic prescription form. Additional antibiotic courses are requested in Follow up forms day 14 and day 30.
Antibiotic switches will be described as (1) switches from broad-spectrum to narrow-spectrum antibiotics, (2) switches from narrow-spectrum to broad-spectrum antibiotics, (3) switches from intravenous to oral antibiotics, (4) switches from oral to intravenous to oral antibiotics. All analyses will be performed to describe both the proportion of patients switches as well as the time until the respective switch (ie multiple switches per patient are possible). Broad and narrow spectrum are defined in protocol as in reference https://www.cdc.gov/nhsn/PDFs/pscManual/11pscAURcurrent.pdf

3. Days alive out of hospital

Calculation of days alive out of hospital is based on time and date collected on the clinical decision form and in hospital course forms, completed both if initial hospitalization or in follow up at day 14 and day 30. The form includes also intensive Care Unit (ICU) admission (if that is the case), and ICU length of stay, as well as severity parameters to monitor the safety variables. Days alive out of hospital is calculated as the maximum of 0 and (14 days minus hospital length of stay in hours), whereby hospital length of stay is calculated from admission to discharge.

6. Planned analysis

6.1. Descriptive statistics

Descriptive statistics will be produced and tabular summaries will be presented, stratified according to the allocated group (rapid syndromic testing (intervention) vs. control (standard of care)). Categorical data will be summarized by the number and percentage of subjects in each category. The median and inter-quartile range will be used to summarise continuous variables. Differences in baseline characteristics between groups will be statistically tested using the chi-square test or Fisher's exact test (categorical) or the Wilcoxon test (continuous), and commented on if significant at the 5% level (refer to the supplementary tables S1-S5) .
For the study samples (diagnostic intervention) data collected includes:
Type of sample: nasopharyngeal swab
Test result
Time between randomization and generation of Test Result
Time between randomization and receipt of Test Result by care team
Time between randomization and prescription of antibiotics

6.2. Analysis populations

The primary estimand and associated primary analysis (see below) will follow the "treatment policy" strategy for the ICE "treatment change at baseline" (akin to "intention-to-treat" principle) in which groups are compared based on the allocated regimen.
Further, we define the following analysis populations in the context of the intercurrent event (ICE) "Treatment change at baseline".

- "on treatment" strategy
    - Scenario 1 ("strict"): We exclude those patients not adhering to their randomised treatment allocation, and repeat the primary analysis.
    - Scenario 2 ("treatment taken"): We analyse the patient groups according to the treatment they received (not as they were randomised), and repeat the primary analysis.
    - Scenario 3: An endpoint review board (ERB) will determine the appropriateness (or otherwise) of antibiotic prescribing (EP1) or admission (EP2) at baseline. These adjudications will result in 4 patient groups for both EP1 and EP2:

        A. Randomised arm 1 (standard of care) appropriate treatment/no treatment, respectively admission/non-admission.

        B. Randomised arm 1 (standard of care) not appropriate treatment/no treatment, respectively admission/non-admission.

        C. Randomised arm 2 (rapid testing) appropriate treatment/no treatment, respectively admission/non-admission.

        D. Randomised arm 2 (rapid testing) not appropriate treatment/no treatment, respectively admission/non-admission.

      The primary analysis will be repeated including only patients in groups A and C. Potential covariate imbalance between the arms will be considered by fitting adjusted models and/or using re-weighting approached.

- "hypothetical" strategy

    Based on the adjudications of the ERB, we perform comparisons of the treatment groups by multiply imputing fictitious outcomes for those "not appropriate" (groups B and D, if counter-factual) with reference to those adjudicated as appropriate in terms of EP1 and EP2 (groups A and C); the primary analysis is repeated with the respective hypothetical patient groups (details of reference based multiple imputation in e.g. Chapter 10 of [4]. Potential covariate imbalance between the arms will be considered by fitting adjusted models and/or using re-weighting approached.

6.3. Hypothesis framework

For the primary and secondary, the null hypothesis will be that there is no true difference in effect between any of the treatment arms.

6.4. Primary analysis

To investigate differences between the two arms for endpoint EP1, a two-sample t-test of the log transformed mean time (in hours) on antibiotic treatment comparing those on the standard of care arm (control) and the intervention arm will be performed, assuming a pooled variance estimate. Results will be summarised as in **Table 2** below.

This analysis will be repeated for endpoint EP2.

Table 2: Descriptive statistics for the differences between the arms

Primary endpoint	Control Group	Intervention Group	Mean difference in log days on antibiotic therapy (95% CI)	p-value from the t-test of the difference between log days of antibiotic therapy
EP1: Days on antibiotic treatment (median, IQR))				

EP2: Days alive out of hospital (median, IQR))				

6.5. Supplementary analyses of the co-primary endpoints

An adjusted linear mixed effects model will be fitted with log transformed days on antibiotic treatment (EP1) as dependent variable, and an indicator variable for the randomized arm, age groups (<5y, 5-17y) and comorbidities (stratified according to modified Charlson comorbidity index: 0, 1, +1) as independent variables. Further independent variables will be considered in posthoc analyses. The model will include a random intercept for each country (and potentially, emergency department in country if cluster sizes allow).
Zero-inflated or similar models will be considered if data are heavily skewed.
An analogous analysis will be repeated with days alive out of hospital (EP2) as dependent variable. Again, we anticipate EP2 data to be heavily right skewed in the full analysis set, and therefore suitable transformations and/or modelling approaches will be considered (as appropriate).

6.6. Subgroup analyses of the primary endpoint

- By age groups (<5, ≥5)
- By admission at baseline (yes/no)
- By receipt of antibiotics at baseline (yes/no)
    - For those on antibiotic therapy at baseline, we will dichotomise days on treatment into two groups (0="1-5 days", 1=">5 days"), and fit a (mixed effects) logistic model with this grouping as dependent variable, adjusting as above.
- By country
- By emergency department (if the number of patients allows).

6.7. Analysis of secondary endpoints

1. Adverse outcomes: Descriptive statistics and appropriate tests by randomized arm, based on:
i. *any* of the adverse outcomes occurring in definitions (a.) and b.) of section 2.1):
ii. the individual constituent endpoints defined in section 2.1.

The proportion of such adverse outcomes on each arm, and risk factors for them, will be analysed by fitting adjusted and unadjusted (exact) logistic regression models (with robust standard errors to adjust for cluster effects).
Time to adverse outcome from randomisation will be analysed by fitting Cox Proportional Hazards regression models, adjusted for age (also categorical, <3 months and ≥3 months) and predefined risk factors (very low birthweight, (i.e. birthweight <1.5kg), and comorbidity score). Risk differences and 95% CI will be inferred by comparing the cumulative incidence of failure at day 30 from the Cox model.

2. Dichotomise by antibiotic treatment (no/yes) and analyse as a categorical endpoint by fitting univariable and multivariable logistic regression models with "treated" as dependent variable and randomized arm as independent variable. The analysis approach will be analogous to that defined for the linear models defined in section 6.5.

3. Time from randomisaton to discharge (only for those admitted): A time to event analysis will be performed with randomisation as "time 0". The Kaplan-Meier product limit will be calculated to

visually present differences between the arms, along with unadjusted and adjusted Cox proportional hazards models (or equivalents) to investigate risk factors. Potential intra-centre correlation will be taken into account by calculating sandwich type standard errors. The analysis will take into account competing risks (e.g. death) if deemed methodologically appropriate.

4. Time from randomization to management decision

Collected data:

- Time from randomization to treat with ATB (in minutes)
- Time from randomization to admit in mins(in minutes)

Analysis: Time to event analysis with randomization as time 0, stratified by randomized arm. An analogous approach to point 3 above.

5. The health economics analysis will be defined in a separate SAP.
6. The analysis of quality of life endpoint(s) will be defined in a separate SAP.

7. Proportion of hospitalised participants (by arm) with detection of cephalosporin-, carbapenem- or chinolone-resistant Enterobacteriaceae on any standard of care samples >7 days after randomisation.

Analysis: chi-square test (or equivalent).

8. Hours in individual or cohort isolation in hospitalised participants (by arm).

Analysis: t-test of the log transformed mean time (for those isolated).

9. Proportion of participants with an identified respiratory pathogen in both study groups on randomisation day samples.

Collected data: bioFire results, standard of care results with sample type and date
Analysis: Test of proportions (z-test) between the two groups.

10. Time to de-escalation in the subgroup of those treated

Collected data: standard of care microbiology results, microbiology sub study results
Analysis: Unadjusted time to event analysis (Kaplan-Meier curve and associated log rank test to test differences between arms); adjusted analyses will be considered if the number of events on both arms allows.

7. 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. Among the sample size assumptions for the co-primary endpoints we will validate the distribution of days alive out of the hospital and the incidence of adverse outcome and re-calculate the sample size if needed. A putative sample size re-estimation will not take into consideration the intervention effect observed at the time of the interim analysis.

8. Missing data

We do not envisage there being a large (>5%) proportion of missing baseline and primary endpoint data. Analyses will be based on the complete case data only ie excluding those patients with one or more missing records *relevant for the specific analysis to be performed*.
If deemed appropriate, analysis of suitably multiply imputed data will be performed making the missing at random (MAR) assumption. In line with common practice, the imputation model will include all covariates from the respective substantive analysis, and any other auxiliary variables deemed to be related to the missingness process. The estimates from the multiply impute data sets will be presented in comparison with those from the respective complete case analysis, and any discrepancies discussed. If the MAR assumption does not seem plausible then suitable sensitivity analyses will be considered.

9. Opt-out studies

The opt out studies statistical analysis is lead by WP5 and WP2 respectively and statistical analysis will be described separately.

9.1. Microbiology study and biobanking pediatric protocol

In a subset of study sites and participants (up to 150 participants at 3 study sites), additional oropharyngeal samples will be obtained. The aim of the microbiology study is to use suitable methods, including metagenomic sequencing, to characterise changes in microbiological colonisation and antimicrobial resistance patterns dependent on treatment with antibiotics. The SAP for this study is not addressed in this document.

10. Statistical software employed

The statistical software R (version 3.6.1 or later) with RStudio will be used for the analyses.

11. Multiple testing and p-value considerations

Allowance for multiple treatment comparisons will be made, as appropriate. For frequentist statistics, a p-value of less than 0.05 will be considered statistically significant throughout (unless stated otherwise).

12. References

[1] CHMP, “ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles in clinical trials," 17 February 2020. [Online]. Available: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e9-r1-addendum-estimands-sensitivity-analysis-clinical-trials-guideline-statistical-principles_en.pdf.

[2] J. A. Bielicki und et al., "ADEQUATE Advanced Diagnostics for Enhanced QUality of Antibiotic prescription in respiratory Tract infections in Emergency rooms (Adequate) - Clinical protocol WP4b - Paediatric," Version 3.0, 11th November 2022.

[3] S. R. Knight und et al., "Risk stratification of patients admitted to hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: development and validation of the 4C Mortality Score," BMJ, 370, doi: 10.1136/bmj.m3339, m3339, 2020.

[4] J. R. Carpenter und M. G. Kenward, Multiple Imputation and Its Application, New Jersey: Wiley, 2013.

13. Supplementary material: Table shells

Table S1: baseline characteristics

Baseline characteristics (n (%), median (IQR))	**Control group**	**Intervention group**
Age		
Sex		
Body mass index		
Standard home living status		
Previous confirmed COVID episode Hospitalization Pulmonary involvement ICU admission		
Comorbidities as defined in modified Charlson comorbidity index		
Chronic cardiac disease, including congenital heart disease (not hypertension)		
Hypertension		
Chronic Pulmonary disease (and type)		
Chronic neurological disease		
Chronic renal disease		
Liver disease and severity		
Diabetes (with and without complications)		
Connective tissue disease		
Malignancy		
HIV or AIDS		
Chronic/Repeat medication Inhaled corticosteroids Oral low dose steroids Oral high dose steroids List of other medications, including biological response modifiers		
Vaccine history - pneumococcal polysaccharide - pneumococcal conjugate - influenza - COVID specific vaccine		

Table S2: Acute respiratory tract infection episode

Clinical presentation (n (%), median (IQR))	Control group	Intervention group
Clinical symptoms at admission, N,% (and rating of severity and duration of complaints)		
Previous medication for the current episode - Systemic steroids - Oral steroids - Beta agonist - Pain or fever medication Previous antibiotic treatment		
Patient attending ER spontaneously or referred by general practitioner.		
Selected list of signs at clinical examination.		
Lung parenchyma lesions at admission Yes-No		
Diagnosis at the time of clinical decision (N, %)		
Pediatric patients Pneumonia Bronchiolitis Bronchitis/viral induced wheeze Laryngotracheitis Influenza like illness		
Clinical decision		
Antibiotic treatment Yes –No % Interval time between admission and prescription		
Hospitalization Yes-N %		
Suspected etiology at time of clinical decision Bacterial Viral Undecided		
Final diagnosis at end of study ICD10 codes		

Table S3: Antibiotic treatment

Antibiotic treatment			**Control group N, %**	**Intervention group N, %**
Data for calculation of Days of Antibiotic Treatment and Antibiotic Switches				
Name of antibiotic Registered in alphabetical order	Descriptives grouped per antibiotic class	Wide spectrum Narrow spectrum		
Route of administration (when applicable to antibiotic type)	Intravenous Intramuscular Enteral			
Initial prescription Antibiotic Switch Addition to ongoing therapy				
Data for calculation of Days of Antibiotic Treatment (DOTs) and Defined Daily Dose (DDD)				
Dosing interval	Single dose Once daily 12 hourly 8 hourly 4 hourly Continuous infusion			
Regular dose	Yes, no			
Loading dose given	Yes, no			
Number of days	Calculation from start date and stop date			

Table S4: Descriptives on microbiological results obtained as standard of care.

Sample collected as standard of care– N-%		Name of microorganism	Result
Nasopharyngeal swab-aspirate or lower respiratory tract sample for molecular testing	Antigen Testing	(Influenza RSV SARS-CoV-2)	Positive Negative
Nasopharyngeal swab-aspirate or lower respiratory tract sample for molecular testing	Molecular test	List of targets	Positive Negative Ct if available
Endotracheal aspirate	Culture	List of microorganisms	Susceptibility testing[1]
Spontaneously produced sputum			
Blood culture			
Urine for antigen detection Positive Negative	Antigen detection	(*Legionella, S.pneumoniae*)	Positive Negative

[1]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*.

Table S5: Safety variables

Adverse outcome	All	Control group	Intervention group
In hospital mortality Yes No			
ICU admission Yes No			
Hospital admission after initial discharge			
Requirement during hospitalization Yes-No Supplemental oxygen Non invasive mechanical ventilation invasive mechanical ventilation Extracorporeal life support			
Hospital length of stay ICU length of stay Duration of mechanical ventilation			