# Quality control of Antibiotic stewardship

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| Research legislation: | Ordinance on human research with the exception of Clinical trials (HRO). |
| Type of Research Project: | Research project involving human subjects |
| Risk Categorisation: | Risk category A |
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## PROTOCOL SIGNATURE FORM

Study Title *Quality control of antibiotic stewardship*

The project leader has approved the protocol version ***[V1 (dated 06.04.2021]***, and confirms hereby to conduct the project according to the protocol, the Swiss legal requirements, current version of the World Medical Association Declaration of Helsinki and the principles and procedures for integrity in scientific research involving human beings.

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## GLOSSARY OF ABBREVATIONS

| | |
|---|---|
| *BAL* | *Bronchoalveolar lavage* |
| *BASEC* | *Business Administration System for Ethical Committees* |
| *DNA* | *Deoxyribonucleic acid* |
| *CAP* | *Community acquired pneumonia* |
| *CE IVD* | *In-Vitro Diagnostic Medical Device Directive* |
| *CI* | *Confidence interval* |
| *CRF* | *Case report form* |
| *CRP* | *C-reactive protein* |
| *eCRF* | *Electronic Case Report Form* |
| *EDC* | *Electronic data capture* |
| *ETA* | *Endotracheal Aspirate* |
| *US FDA* | *United States Food and Drug Administration* |
| *FOPH* | *Federal Office of Public Health* |
| *GCP* | *Good Clinical Practice* |
| *HAP* | *Hospital acquired pneumonia* |
| *H0* | *Null hypothesis* |
| *H1* | *Alternative hypothesis* |
| *HRA* | *Human Research Act* |
| *HRO* | *Ordinance on Human* |
| *IB* | *Investigator's Brochure* |
| *LOS* | *Length of hospital stay* |
| *LRTI* | *Lower respiratory tract infection* |
| *rDNA* | *ribosomal Deoxyribonucleic acid* |
| *RT-PCR* | *Reverse transcription polymerase chain reaction* |
| *PCR* | *Polymerase chain reaction* |
| *PCT* | *Procalcitonin* |
| *PI* | *Principal investigator* |
| *TAT* | *Turnaround time* |
| *TGA* | *Therapeutic Goods Administration* |

# 1 BACKGROUND AND PROJECT RATIONALE

Pneumonia is a leading cause of morbidity and mortality worldwide. It can be categorized into two main groups as community-acquired (CAP) or hospital-acquired (HAP) with HAP being defined as pneumonia occurring 48 hours or more after hospitalisation.

Clinical presentation cannot distinguish between the wide varieties of causative agents [1]. One of the most important prognostic factors in pneumonia is early and adequate antibiotic therapy [2-5]. Therefore, initial empiric antibiotic therapy is chosen to cover the suspected range of bacteria. Identification of the causative agent is pivotal to adjust empirical antibiotic therapy to targeted therapy, a primary goal of antimicrobial stewardship. Antimicrobial stewardship targets best clinical outcomes with keeping unnecessary or inappropriate antibiotic use to a minimum in order to decrease side effects, reduce the emergence of resistance and for cost-effectiveness purposes [6-8]. Optimal antibiotic therapy includes escalation or de-escalation according to microbiological results, discontinuation and taking allergies into account [9], whereas inappropriate therapy is empirical therapy in discordance with susceptibility testing, without clinical response or with a too broad-spectrum for the identified pathogen.

Routine microbiologic testing methods to identify the causative agent are culture-based and results take 48 to 72 hours. In addition to the time delay, they are not suitable to detect atypical pathogens. The yield of sputum cultures is variable, generally low and influenced by several factors such as specimen collection, transport, time of processing, and prior antibiotic therapy [10-12] with the highest yield in patients before the start of antibiotic therapy. Bronchoalveolar lavage (BAL) has a higher yield than sputum culture, and is of additional value in identifying the causative agent, especially in patients without sputum production and those non-responsive to empirical treatment [13]. However, in patients already receiving antibiotic treatment the diagnostic yield in BAL is also reduced [14]. With multiplex PCR methods, rate of pathogen detection can be improved significantly, especially in antibiotic exposed patients, with the potential to enable targeted therapy [15].

Recently, a diagnostic test system has been introduced that detects a panel of pneumonia-causing pathogens by multiplex PCR, the BIOFIRE® FILMARRAY® Pneumonia plus Panel. The BioFire Pneumonia Panel is a sensitive and specific *in vitro* diagnostic assay for the detection of atypical bacteria, viruses, and antimicrobial resistance genes from BAL and sputum specimens [16, 17]. The comprehensive panel and the fully integrated workflow, enable results from BAL samples in 90 min or a turnaround time (TAT) of 4h [18]. It provides detection of 27 microbes and 7 genomic antibiotic resistance markers. Studies have shown that the time for pathogen identification with the Pneumonia Panel diagnostic system was significantly shorter than with conventional microbiology [17, 18].

The multiplex PCR assay is costly. It has been shown that using the multiplex PCR assay results in a reduction in days of antimicrobial therapy and length of hospital stay (LOS) [19-22]. In Japan, total costs were reduced by $134/case when using the Biofire FilmArray even though no antibiotic stewardship program was implemented.

The purpose of this study is to evaluate whether a rapid multiplex PCR of the BAL can decrease time on inappropriate antimicrobial therapy in patients with LRTI compared to conventional microbiological investigations alone as used in the clinical routine of a tertiary care institution.

# 2 PROJECT OBJECTIVES AND DESIGN

## 2.1 Hypothesis and primary objective

The purpose of this quality control project is to evaluate whether a rapid multiplex PCR of the BAL can decrease time on inappropriate antimicrobial therapy in patients with LRTI compared to conventional microbiological investigations alone as used in the clinical routine of a tertiary care institution.

## 2.2 Primary aim and endpoint

To establish whether a rapid multiplex PCR of the BAL can decrease time on inappropriate antimicrobial therapy in patients with LRTI compared to conventional microbiological investigations alone.

Endpoint being “time on inappropriate therapy” and defined as anti-microbial therapy

1. With no identifiable pathogen or
2. Not active according to in-vitro susceptibility testing of the identified pathogen or
3. With known intrinsic resistance of the identified pathogen to the given therapy or
4. Having a spectrum too broad for the identified pathogen (antimicrobial therapy considered broad when switching to a different antimicrobial therapy with a narrower spectrum continues to show a favourable clinical course) and in the case where there is a lack of evidence suggesting resistance of the microorganism to a narrower antimicrobial therapy or
5. Continuation of the antimicrobial therapy beyond the guideline-suggested duration e.g. for pneumonia 5 to 7 days.

A spectrum too broad for the identified pathogen is considered if the empirical treatment is an antibiotic with a higher spectrum (higher rank number – see list below) than the identified resistance pattern of the identified pathogen. In addition, companion drugs (e.g. to cover atypical organisms, anaerobes or resistant organisms) are considered too broad, if one single agent antibiotic treatment is possible. For the purpose of this study, antibiotic therapy for *S. pneumoniae* will be considered inappropriate if classified as rank>2.
Optimal antimicrobial therapy will be defined by the absence of inappropriate antibiotic therapy. However, antibiotic therapy for pneumococcus will only be considered optimal if classified as rank 1 and adequate if classified as rank 2.

Table 1: Antibiotic ranking

| **Ranking** | **Antibiotics** |
|---|---|
| 5 | Imipenem, Meropenem |
| 4 | Ertapenem |
| 3 | Piperacillin/Tazobactam, Ceftazidime, Cefepime, Aztreonam |
| 2 | Ceftriaxone, Amoxicillin/clavulanic acid |
| 1 | Amoxicillin, Penicillin |

## 2.3 Secondary aims and endpoints

To establish whether a rapid multiplex PCR with its associated quantification of the bacteria in the BAL can improve clinical outcome (time to clinical stability, length of hospital stay, mortality, adverse events), in patients with LRTI compared to conventional microbiological investigations alone.

Other outcomes of interest:
Performance and reliability of resistance markers detected by BioFire Pneumonia Panel compared to in-vitro susceptibility testing by conventional microbiological testing.

### 2.4 Project design

Patients hospitalized with LRTI and with a clinical indication for bronchoscopy with BAL will be screened for inclusion into the project. A total of 740 patients will be included. Samples will be allocated according to a one week off and two weeks on schedule. During the one week off, the samples will only be analysed with the conventional microbiological diagnostics. During the two weeks on, samples will be analysed with both the conventional microbiological diagnostics and Biofire Pneumonia Panel. In addition to the results obtained by the Biofire Pneumonia Panel, an antimicrobial recommendation based on the identification of the causative pathogen (please see Table 2 and Table 3 in section 4.3) will be available electronically to the treating physician as soon as possible after the bronchoscopy. Similarly, patients in the standard care group will be treated according to the current guidelines, which may include consultation with an infectious disease specialist or pneumologist.

Time on inappropriate antibiotic treatment will be assessed as well as time to clinical stability on a daily basis, length of hospital stay, 30-day mortality and adverse events until discharge.

### 2.5 Study intervention

The BIOFIRE® FILMARRAY® Pneumonia *plus* Panel tests for 18 bacteria (11 Gram negative, 4 Gram positive and 3 atypical), 7 antibiotic resistance markers, and 9 viruses that cause pneumonia and other lower respiratory tract infections. It offers an overall sensitivity and specificity for BAL-like samples of 96,2% and 98.3%, respectively. The Biofire Pneumonia Panel is run on the BIOFIRE® FILMARRAY® System, a US FDA, CE-IVD, and TGA certified multiplex PCR system. The system integrates sample preparation, nucleic acid extraction and purification, amplification, detection and analysis into one simple system that requires just 2 minutes of hands-on time, with a total run time of about 75 to 90 minutes [16, 18].

The new panel complements the existing BIOFIRE® FILMARRAY® Respiratory Panel 2+ to provide a comprehensive diagnostic tool for pneumonia and other lower respiratory tract infections. A rapid and accurate identification of the causative agent of both community and health-care associated respiratory tract infections can help improve patient management by timely and effective antimicrobial therapy. A rapid diagnosis can assist with directing appropriate infection control practices thereby aiding in the prevention of secondary spread of infection, shorten hospital stays, reduce ancillary testing, and reduce overall health care costs. The disposable cartridge is compatible with standard waste disposal procedures of hospitals and laboratories.

## 3 PROJECT POPULATION AND STUDY PROCEDURES

### 3.1 Project population, inclusion and exclusion criteria

Inclusion criteria:

- Age ≥ 18 years
- Clinical indication for diagnostic bronchoscopy with bronchoalveolar lavage
- Suspicion of lower respiratory tract infection – In immunocompetent patients infiltrate must be confirmed; in immunocompromised patients an infiltrate is not required

- Evidence of systemic inflammation (such as abnormal white blood cell count – either leukocytosis (>10.0x$10^9$/l) or leukopenia (<4.0x$10^9$/l) – or C-reactive protein (CRP) or procalcitonin (PCT) values above the local upper limit.

Exclusion criteria:

- Ambulatory patients
- Patients intubated at the time of inclusion
- Neutropenic patients as defined by neutrophils <0.5x$10^9$/l
- Haemodynamic instability or signs of life-threatening infection precluding a narrowing of antimicrobial therapy
- prior enrolment in an intervention study within the last 30 days
- Women who are pregnant or breast feeding

### 3.2 Recruitment, screening and informed consent procedure

Data from patients that present to the hospital or are already hospitalized with suspicion of a lower respiratory tract infection, and in whom bronchoscopy with BAL has been indicated, will be analysed. Patients for whom the general informed consent is missing, will be approached by the pneumology team and the consent form will be obtained.

### 3.3 Study procedures

Data from patients that present to the hospital or are already hospitalized with suspicion of a lower respiratory tract infection, and in whom bronchoscopy with BAL has been indicated, will be analysed. BAL samples will be analysed either using the Biofire Pneumonia Panel together with the conventional microbiologic investigation or using the conventional method alone.

Time on inappropriate antibiotic therapy will be recorded in hours daily until discharge or 30 days of follow-up. Time to clinical stability will be assessed on a daily basis, length of hospital stay, 30-day mortality and adverse events daily until discharge.

The following recommendations will be made to the treating physician depending on the pathogen detected by the Biofire Pneumonia Panel:

Table 2: Antibiotic recommendations

| **Test result** | **Antibiotic choice** | **Penicillin Allergy Type IV** | **Penicillin Allergy Type I (anaphylaxis)** |
|---|---|---|---|
| No pathogen detected | | | |
| Acinetobacter calcoaceticus-baumannii complex | Meropenem (Meronem) | Meropenem (Meronem) | Meropenem (Meronem) |
| Enterobacter cloacae | Cefepim (Cefepim) or Ertapenem (Invanz) | Cefepim (Cefepim) or Ertapenem (Invanz) | Ertapenem (Invanz) |
| Escherichia coli | Ceftriaxone (Rocephin) | Ceftriaxone (Rocephin) | Ertapenem (Invanz) |
| Haemophilus influenzae | Amoxicillin & clavulanic acid (Augmentin)<br>Ceftriaxon (Rocephin) | Ceftriaxone (Rocephin) | Levofloxacin (Tavanic) |

| | | | |
|---|---|---|---|
| Klebsiella aerogenes | Cefepim (Cefepim) or Ertapenem (Invanz) | Cefepim (Cefepim) or Ertapenem (Invanz) | Ertapenem (Invanz) |
| Klebsiella oxytoca | Ceftriaxone (Rocephin) | Ceftriaxone (Rocephin) | Ertapenem (Invanz) |
| Klebsiella pneumoniae group | Ceftriaxone (Rocephin) | Ceftriaxone (Rocephin) | Ertapenem (Invanz) |
| Moraxella catarrhalis | Amoxicillin & clavulanic acid (Augmentin) | Ceftriaxone (Rocephin) | Bactrim, Azithromycin, Doxycycline |
| Proteus spp. | Rocephin | Ceftriaxone (Rocephin) | Ertapenem (Invanz) |
| Pseudomonas aeruginosa | Ceftazidim (Fortam)<br>Piperacillin & Tazobactam (Tazobac) high dose | Cefepim (Cefepim) or Ceftazidim (Fortam) | Meropenem (Meronem) |
| Serratia marcescens | Cefepim (Cefepim) or Ertapenem (Invanz) | Cefepim (Cefepim) or Ertapenem (Invanz) | Ertapenem (Invanz) |
| Staphylococcus aureus | Floxapen, Cefazolin or Penicillin if susceptible | Cefazoline | Clindamycin,<br>Linezolid |
| Streptococcus agalactiae | Penicillin or<br>Ceftriaxone (Rocephin) | Ceftriaxone (Rocephin) | Clindamycin (Dalacin)<br>Levofloxacin (Tavanic) |
| Streptococcus pneumoniae | Penicillin<br>Benzylpenicillin or Amoxicillin | Ceftriaxone (Rocephin) | Clindamycin (Dalacin)<br>Levofloxacin (Tavanic)<br>Azithromycin |
| Streptococcus pyogenes | Penicillin<br>Benzylpenicillin or Amoxicillin | Ceftriaxone (Rocephin) | Clindamycin (Dalacin)<br>Levofloxacin (Tavanic) |
| Legionella pneumophila | Levofloxacin (Tavanic)<br>Azithromycin (Zithromax) | Levofloxacin (Tavanic)<br>Azithromycin (Zithromax)<br>Klacid<br>Doxycyclin | Levofloxacin (Tavanic)<br>Azithromycin (Zithromax)<br>Klacid<br>Doxycyclin |
| Mycoplasma pneumoniae | Levofloxacin (Tavanic)<br>Azithromycin (Zithromax)<br>Klacid<br>Doxycyclin | Levofloxacin (Tavanic)<br>Azithromycin (Zithromax)<br>Klacid | Levofloxacin (Tavanic)<br>Azithromycin (Zithromax)<br>Klacid<br>Doxycyclin |

| | | | |
|---|---|---|---|
| | | Doxycyclin | |
| Chlamydia pneumoniae | Levofloxacin (Tavanic)<br>Azithromycin (Zithromax)<br>Klacid<br>Doxycyclin | Levofloxacin (Tavanic)<br>Azithromycin (Zithromax)<br>Klacid<br>Doxycyclin | Levofloxacin (Tavanic)<br>Azithromycin (Zithromax)<br>Klacid<br>Doxycyclin |

If **no bacterial pathogen is detected by the PCR**, antibiotic therapy aimed solely for respiratory infection, should be stopped within 48 hours after the PCR results are made available. If antibiotics are continued longer than 48 hours, the attending physician is required to provide justification for the continued therapy. The justification includes, but is not limited to, clinical instability, infection parameters, vital signs, etc.

If a **bacterial pathogen is identified by PCR**, physicians will be recommended to eventually narrow antibiotic therapy within 48 hours after the PCR results are made available. If antibiotics are not narrowed according to the recommendation provided and/or within 48 hours after the PCR results are made available, the attending physician will be required to provide justification for their course of action.

If a **bacterial pathogen is detected by PCR**, but is deemed a coloniser or another cause is determined for the status of the patient during the bronchoscopy (e.g. cancer), antibiotic therapy should be stopped 48 hours after the PCR results are made available.

If **more than one bacterial pathogen is detected by the PCR**, antibiotic therapy with the narrowest scope that covers all the bacteria detected, will be administered.

Table 3: Antiviral recommendations

| **Test result** | **Antiviral Therapy immunocompetent host** | **Antiviral Therapy immunocompromised host or Severe Pneumonia** | |
|---|---|---|---|
| Influenza A & B | Oseltamivir (Tamiflu)<br><br>Within 5 days if hospitalized. Earlier better. | Oseltamivir (Tamiflu) | empirically treat bacterial co-infection if severe disease (extensive pneumonia, respiratory failure, hypotension, and fever):<br><br>Amoxicillin & clavulanic acid (Augmentin) or<br>Ceftriaxone (Rocephin) |
| Adenovirus | Supportive therapy | | |
| Coronavirus | Supportive therapy | Supportive therapy | |
| Parainfluenza virus | Supportive therapy | Seek expert advice (infectiology consult<br><br>Trial of:<br>Intravenous immune globulin<br>and/or<br>Intravenous or inhaled Ribavirin | |
| Respiratory Syncytial virus | Supportive therapy | Seek expert advice (Infectious Disease Consultation)<br><br>Ribavirin in HSCT patients | |
| Human Rhinovirus/<br>Enterovirus | Supportive therapy | Supportive therapy | |
| Human Meta-pneumovirus | Supportive therapy | Supportive therapy | |
| MERS | Supportive therapy | Supportive therapy | |

The **Data Evaluation Monitoring Committee** will be responsible for the individual evaluation of each patient case, including the assessments about the inappropriateness of the antibiotic therapy and clinical improvement. The committee will consist of the following members: Dr. Andrei Darie, Dr. Kathleen Jahn, Dr. Veronika Bättig, Dr. Elisabeth Wehrle, Prof. Dr. Nina Khanna, Prof. Adrian Egli, and Prof. Dr. Daiana Stolz.

### 3.4 Withdrawal and discontinuation

Patients who do not undergo bronchoscopy after allocation will be withdrawn from the study.

## 4 STATISTICS AND METHODOLOGY

### 4.1. Statistical analysis plan and sample size calculation

The primary hypothesis is that the time a patient is under inappropriate antibiotic therapy would be reduced when a 'test' method for the microbial detection (for Flagship II was a PCR test) is used than when the gold standard method (culture) is used. As explained in the methodology a patient will be diagnosed using either multiplex PCR or no PCR, in addition to the conventional methods.

The null and alternative hypotheses can be stated as follows:

H0: μtest - μcontrol = 0

H1: μtest - μcontrol < -x

Where the difference 'x' between groups (decrease in the average time on in inappropriate therapy) could be estimated using the results obtained in the study Flagship II.

Assuming that the means would fall on the extremes of the 95% CIs

Mean for control: 75.01 h
SD for control: 74.14 h
Mean for test: 58.51 h
SD for test: 58.89 h
Within-group standard deviation: 67.39 h

An alpha of 0.05 (two tailed), equivalent to an alpha of 0.025 (one tailed) if looking at the decrease in time under inappropriate therapy.

The probable random effect due to the patients is assumed to be small compared to the variation of the measurements, but it was nevertheless taken into account when calculating the 95%CI for the means.

The samples are independent and of equal size.

Table 4. Power calculation for different sample sizes for scenario 2, assuming an alpha=0.05 two-tailed, mean for control=75.01 h and a within-groups standard deviation of 67.39

| Test group sample size | Control group sample size | **Power** if mean for test = 58.5h (Difference =-16.51h) |
|---|---|---|
| 20 | 20 | 0.117 |
| 40 | 40 | 0.191 |
| 60 | 60 | 0.265 |
| 80 | 80 | 0.337 |
| 100 | 100 | 0.407 |
| 120 | 120 | 0.472 |
| 140 | 140 | 0.533 |
| 160 | 160 | 0.589 |

| | | |
|---|---|---|
| 180 | 180 | 0.64 |
| 200 | 200 | 0.686 |
| 220 | 220 | 0.727 |
| 240 | 240 | 0.764 |
| 260 | 260 | 0.796 |
| 280 | 280 | 0.825 |
| 300 | 300 | 0.85 |
| 320 | 320 | 0.872 |
| 340 | 340 | 0.891 |
| 360 | 360 | 0.907 |
| 380 | 380 | 0.921 |
| 400 | 400 | 0.933 |
| 420 | 420 | 0.944 |
| 440 | 440 | 0.953 |
| 460 | 460 | 0.96 |
| 480 | 480 | 0.966 |
| 500 | 500 | 0.972 |
| 520 | 520 | 0.977 |
| 540 | 540 | 0.98 |
| 560 | 560 | 0.984 |
| 580 | 580 | 0.986 |
| 600 | 600 | 0.989 |
| 620 | 620 | 0.991 |
| 640 | 640 | 0.992 |
| 660 | 660 | 0.994 |
| 680 | 680 | 0.995 |
| 700 | 700 | 0.996 |

The mean time for the test would fall close to the upper limit of its 95%CI and that for the control would fall close to the lower limit of its 95%CI. In this case, a study with sample size of 300 per group (a total of 600 patients) would have a power of 85% to yield a statistically significant result. The computation assumes that the mean difference is -16.51 h. In this case, the precision would be (95.0% confidence level) of plus/minus 9.97 points. An observed difference of -16.51 would be reported with a 95.0% confidence interval of -26.48 to -6.54. To account for a 10% lost to follow-up, 740 patients should be included.

A per-protocol-analysis will include all patients that underwent bronchoscopy with BAL and had antibiotic or antiviral treatment recommended in the intervention group according to Pneumonia Panel test results. Patients without a treatment recommendation will not be considered in the per-protocol-analysis.

Primary analysis - For the primary analysis the intention-to-treat population will be considered. For the time of inappropriate antibiotic or antiviral therapy as primary endpoint a t-test for independent samples will be performed. It is expected that very few patients will have an unusually long time on inappropriate therapy, therefore the t-test is assumed to be stable under outlying times.

Results are reported as differences of mean times with corresponding 95% confidence intervals and p-values.

A p-value <0.05 is considered as significant. All evaluations will be done with the current version of the statistical software R. For sensitivity analysis, the primary analysis will be repeated on the per-protocol population.

Secondary analysis - For all secondary endpoints complete case analyses will be used.

Study groups will be compared using t-tests, Mann Whitney U-tests or Fisher's exact tests as appropriate. Unadjusted and adjusted estimates of the effect size and corresponding 95% confidence intervals using linear, logistic, or Cox proportional hazards regression will be calculated as appropriate.

### 4.2. Handling of missing data

Should missing data occur, data will be used to the fullest degree possible by choosing the proper statistical methods.
Sensitivity analyses will include a per-protocol analysis, analyses with and without adjustment for baseline characteristics and analyses with and without imputation methods for missing data.

## 5 REGULATORY ASPECTS AND SAFETY

### 5.1 Local regulations / Declaration of Helsinki

This research project will be conducted in accordance with the protocol, the Declaration of Helsinki, the principles of Good Clinical Practice, the Human Research Act (HRA) and the Human Research Ordinance (HRO) as well as other locally relevant regulations. The Project Leader acknowledges his responsibilities as both the Project Leader and the Sponsor.

### 5.2 Notification of safety and protective measures (HRO Art. 20)

The project leader is promptly notified (within 24 hours) if immediate safety and protective measures have to be taken during the conduct of the research project. The Ethics Committee will be notified via BASEC of these measures and of the circumstances necessitating them within 7 days.

### 5.3 Serious events (HRO Art. 21)

If a serious event occurs, the research project will be interrupted and the Ethics Committee notified on the circumstances via BASEC within 7 days according to HRO Art. 21[1].

### 5.4 Procedure for investigations involving radiation sources

n/a

### 5.5 Amendments

Substantial changes to the project set-up, the protocol and relevant project documents will be submitted to the Ethics Committee for approval according to HRO Art. 18 before implementation. Exceptions are measures that have to be taken immediately in order to protect the participants.

### 5.6 End of project

[1] A serious event is defined as any adverse event where it cannot be excluded, that the event is attributable to the sampling of biological material or the collection of health-related personal data, and which:
a. requires inpatient treatment not envisaged in the protocol or extends a current hospital stay;
b. results in permanent or significant incapacity or disability; or
c. is life-threatening or results in death.

Upon project completion or discontinuation, the Ethics Committee is notified within 90 days.

### 5.7 Insurance

n/a

## 6 FURTHER ASPECTS

### 6.1 Overall ethical considerations

This project is a quality control of antibiotic stewardship with no risks to the patients.

### 6.2 Risk-Benefit Assessment

There is no risk associated with the project as all treatments are standard of care in the hospital. The benefit is that the patient will take antibiotics for a shorter time period, spend less time in the hospital and have less associated costs. A shorter treatment could theoretically result in a lack of response or a recurrence of symptoms.

## 7 QUALITY CONTROL AND DATA PROTECTION

### 7.1 Quality measures

The PI is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs and Working Instructions. The PI is responsible for proper training of all involved study personnel.

For quality assurance the sponsor, the Ethics Committee or an independent trial monitor may visit the research sites. Direct access to the source data and all study related files is granted on such occasions. All involved parties keep the participant data strictly confidential.

### 7.2 Data recording and source data

Study data will be recorded in an electronic data capture (EDC) system via an electronic Case Report Form (eCRF) which will be designed specifically for the project using OpenClinica. A unique study code will be used for identification of participants in the eCRF. Subjects will not be identified on the eCRF by name or date of birth.
Only authorized personnel will be able to make eCRF entries via a personalized login to the eCRF and are responsible for entering complete data.
If source data is available on a print-out (e.g. Laboratory values, bronchoscopy records), this print-out will be kept on file in a source data folder, and the data necessary for the study is to be transferred to the eCRF immediately.
Source data include the paper and electronic records of the institution and all study documents (AE/SAE form, informed consent forms, laboratory reports, bronchoscopy reports, etc.).

### 7.3 Confidentiality and coding

Project and participant data will be handled with the utmost discretion and is only accessible to authorised personnel who require the data to fulfil their duties within the scope of the study. On the CRFs and other study specific documents, participants are only identified by a unique participant number.
Only the study (co-investigator, study physician, study nurse) team and the data evaluation monitoring committee will have access to the data. Protected health care information will be kept accessible only to the local study team, study monitors and regulatory authorities.

### 7.4 Retention and destruction of study data and biological material

All study data must be archived for a minimum of 10 years after study termination or premature termination of the clinical trial. Study data will be archived in a password protected database on a protected server at the host institution.

## 8 FUNDING / PUBLICATION / DECLARATION OF INTEREST

Data derived from this trial are considered the property of the investigators of this trial.

Study results will be presented at national and international conferences and published in peer reviewed medical journals.

Publications will be prepared without the use of professional writers. Intended publications will be discussed among the investigators with the principal investigator having the ultimate authority to decide the appropriate choice of medical journal(s).

The manuscript of the main publication will be prepared by the PI or a study member and sent to other parties for comments and revision. The contributions to the project will be taken into account in a fair manner. Persons qualify for authorship if they have contributed significantly to the trial. If a contributor will not qualify for authorship, her/his contribution will be mentioned as an acknowledgment.

All financial support will be disclosed in any publication of study results.

9 Protocol amendment

Background and rationale

The management of patients with acute infection and suspected sepsis needs rapid understanding of the etiology and implications of a clinical condition, in order to make clinical decisions such as prescription and timing of antibiotics and level-of-care decisions [23].

In order to provide timely and accurate help to diagnose and to make prognosis in acute infection, several host-immune-response-based tests are currently being developed. One of them is InSep™ (Inflammatix, Inc.). This test analyzes a 29-host-mRNA-pattern from a 2.5 ml whole blood sample. The 29 mRNAs analyzed by the test panel have been chosen based on their biological and pathophysiological significance in the context of bacterial infection, viral infection and development of sepsis. Two algorithms interpret the mRNA levels using machine learning. The test provides a likelihood for of bacterial infection, the likelihood of viral infection and the risk of physiological decompensation, i.e. the risk for need for ICU care within seven days [24, 25].

The test has been shown to accurately predict bacterial infection and viral infection in two prospective studies [26, 27]. Further studies are needed to assess its validity in clinical practice.

Study procedures

We plan to obtain the probability scores delivered by InSep™ in 200 individuals included in the study. Blood samples will be obtained on the day of bronchoscopy/BAL (day 1), and on the consecutive days during hospitalization. The InSep™ test will be performed on every blood sample.

Statistics and methodology

We will explore associations of the sores delivered by InSep™ with the final diagnosis, the clinical severity and the results of microbiology studies in the BAL.

The hypothesis is that the scores delivered by InSep™ are associated with disease severity.

Risk for the participants

There is no additional risk for the participants since the collection of blood sample is a routine procedure in hospital care.

## 9 REFERENCES


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