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| **FULL/LONG TITLE OF THE STUDY** | Incidence and pathogenesis of Invasive Aspergillosis in Intensive care patients with severe Influenza |
| **SHORT STUDY TITLE / ACRONYM** | Aspergillosis in patients with severe influenza (AspiFlu) |
| **PROTOCOL VERSION NUMBER AND DATE** | **2.0, Date 26.11.19** |
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| **STUDY SUMMARY** | | |
| Study Title | Incidence and pathogenesis of Invasive Aspergillosis in Intensive care patients with severe Influenza | |
| Internal ref. no. (or short title) | Aspergillosis in patients with severe influenza (AspiFlu) | |
| Research Question/Aim(s) | **Primary:**  Estimate incidence of influenza-associated invasive aspergillosis (IAA) in critically ill inpatients in London and identify risk factors for infection  **Secondary:**   * Determine morbidity and mortality of IAA * Evaluate utility of AspLFD device for diagnosis of IAA   **Sub-studies:**  Improve understanding of immunopathogenesis of IAA through *ex vivo* PBMC analysis, measurement of serum and bronchoalveolar lavage (BAL) cytokines and targeted immune gene sequencing | |
| Study Design | Prospective observational cohort  Retrospective diagnostic evaluation and immunologic research using stored samples | |
| Study Setting | Intensive care units across four NHS Trusts:   1. Guy's and St Thomas' Hospital NHS Foundation Trust (GSTT) 2. St Georges University Hospitals NHS Foundation Trust (SGH) 3. King’s College Hospital NHS Foundation Trust (KCH) 4. Manchester University NHS Foundation Trust | |
| Study Participants | Intubated adults admitted to intensive care (ICU) with severe influenza | |
| Inclusion/Exclusion criteria | Inclusion:   * Adults > 18 years * Admitted to ICU for respiratory support requiring intubation and ventilation for >24h   **AND EITHER:**   * Positive influenza PCR from nasal, throat swab, BAL or other respiratory specimen taken within 72 hours (of admission to ICU – pre or post)   **OR**   * Influenza suspected but influenza PCR results awaited – under these circumstances the patient can be provisionally enrolled, but later excluded if no specimens taken within 72 hours pre/post admission to ICU is positive as above.   Exclusion:   * Respiratory failure not the primary reason for ICU admission * History of proven/ probable invasive pulmonary aspergillosis | |
| Interventions | Blood draw and storage of additional 30ml blood volume at enrolment for sub-studies  Surplus BAL sample from bronchoscopy performed as part of routine clinical care will be saved for subsequent analysis | |
| Planned sample size | n=75-95 | |
| Objectives/Outcome Measures | **Objectives** | **Outcome Measures** |
| Estimate incidence and risk factors for IAA | Incidence and diagnostic classification of IAA during ICU admission as per modified AspICU incorporating serum galactomannan; BAL fungal culture and galactomannan (mycologic criteria); also incorporating tracheobronchitis at bronchoscopy (as per clinical EORTC/MSG criteria). Risk factors to be elicited from baseline collected clinical data points. |
| Estimate morbidity and mortality associated with IAA | Duration of mechanical ventilation, length of ICU stay, length of hospital stay  ICU all-cause mortality; inpatient all-cause mortality; 90-day all-cause mortality  Survival analysis: Time to death (all-cause mortality) for all patients |
| Evaluation of AspLFD | Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of AspLFD for the diagnosis of IPA using AspICU criteria |
| Immunopathogenesis of IAA | Cytokine/chemokine measurements, ex vivo profiling and stimulation studies and targeted genetic analyses of stored serum, BAL, PBMCs, and DNA samples respectively |
| Follow up duration | 90 days | |
| Planned Study Period | * 1st November 2019 – 31st March 2020: patient enrolment, prospective clinical data and sample collection * 31st March 2020-1st August 2020: Data analysis and evaluation of AspLFD using stored BAL samples. * 1st August 2020-1st August 2022: Immunopathogenesis research using stored blood and BAL samples | |

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| **FUNDING AND SUPPORT** | |
| **FUNDER** | **FINANCIAL AND NON FINANCIAL SUPPORT GIVEN** |
| Gilead UK & Ireland invasive fungal disease fellowship | This competitively awarded, peer-reviewed grant will pay the salary of a full time clinical fellow and consumables (total £65K) for 9 months from Nov 2019- Aug 2020 (primary and secondary aims).  The sub-studies will be contingent upon successful PhD funding applications to the MRC or NIHR for further clinical fellow support |

**ROLES AND RESPONSIBILITIES OF STUDY MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS**

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| **Steering Group** | |
| **Chief Investigator** | Tihana Bicanic, Consultant in Infectious Diseases (St. George’s NHS Foundation Trust and University of London) |
| **Site Principal Investigator** | Jonathan Ball, Consultant Intensivist, St George’s Hospital NHS Foundation Trust |
| **Site Principal Investigator** | Duncan Wyncoll, Consultant Intensivist, Guys and St Thomas’ NHS Foundation Trust |
| **Site Principal Investigator** | Philip Hopkins, Consultant Intensivist, King’s College Hospital NHS Foundation Trust) |
| **Site Principal Investigator** | Timothy Felton, Consultant in Intensive Care and Respiratory Medicine, Manchester University NHS Foundation Trust |
| **Study co-ordinator** | Jonathan Youngs, Clinical research fellow/Infection Specialist Registrar, St Georges University of London |
| **Co-investigators** | Carolyn Hemsley; Jonathan Edgeworth: Consultants in Microbiology, Guys and St Thomas’ NHS Foundation Trust |
| Meera Chand, Consultant Microbiologist Public Health England / Guy's & St Thomas' NHS Foundation Trust |
| Malcolm Richardson and Caroline Moore, Principal Clinical Scientists (Mycology Reference Lab), Manchester University NHS Foundation Trust |
| Amanda Fife; Silke Schelenz, Consultants in Microbiology (King’s College Hospital NHS Foundation Trust) |
| Maximilian Habibi; Derek Macallan (Consultants in Infection), St. George’s University of London & St. Georges Hospitals NHS Foundation Trust |
| Jonathan Youngs; Clare Logan (clinical research fellows) St. George’s University of London |
| **Statistician** | Sile Molloy, Lecturer in Epidemiology, St George’s, University of London |

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| **Patient & Public Involvement** | |
| St Thomas’ Hospital ECMO patient group | Patient and public involvement was sought from St Thomas’ Hospital ECMO patient group: 4 volunteers reviewed protocol and patient information leaflet (three former patients on ECMO for severe infections including influenza and a partner of such a patient). |

**PROTOCOL CONTRIBUTORS**

This study protocol was designed by the chief Investigator in conjunction with the site principal Investigators, co-investigators and input from the statistician.

The funder for this Study (Gilead) had no role in the study design, and will play no role in the conduct of the study, data analysis, presentation or publication of findings

The sponsor (SGUL) will be responsible for study conduct, data analysis and interpretation, manuscript writing, and dissemination of results.

There has been patient and public involvement in the design of the study and patient information sheets (see ‘8.4 Patient & Public Involvement’).

**STUDY Schematic**



**STUDY TIMELINES**

**GANTT chart: (Phases I and II)**

Phase I: Protocol development; Ethical and R&D approvals; prospective enrolment of participants; data and sample collection: Sept 2019-Apr 2020 (8 months)

Phase II: Diagnostic evaluation: Apr 2020-July 2020 (4 months)

Phase III: Immunopathogenesis substudies Aug 2020-Jul 2022 (2 years, subject to further funding)

# LAY SUMMARY

Some patients with seasonal Influenza (‘flu’) develop severe infection requiring admission to the Intensive Care Unit (ICU) to support their breathing. Recent research has suggested that when patients have such severe influenza they may be susceptible to a second infection with a mould (a type of fungus) called *Aspergillus*. The mortality for patients infected with both severe ‘flu and Invasive *Aspergillus* (IA) is high but life-saving antifungal treatments exist and thus it is important that a diagnosis of IA in patients with severe influenza is not missed. Unfortunately, IA can be difficult and lengthy to diagnose in the laboratory and until recently it was only thought to occur in patients whose immune systems were severely impaired. This means that IA in patients with severe influenza may be under-diagnosed currently and the main aim of this study is to establish how common this condition is in UK patients.

This study will take place across three London teaching hospitals during the 2019/2020 influenza season. It will enrol adults admitted to Intensive Care with severe influenza and evaluate what proportion have evidence of IA using routine diagnostic samples sent to the laboratory*.* Clinical information will be recorded and analysed to identify any factors that increase the risk of influenza*-*associated IA.

Ventilated patients with severe lung infection often have a procedure called a bronchoscopy where a small camera is used to look inside the lungs and flush through a small volume of fluid (bronchoalveolar lavage, BAL) to send to the local Microbiology laboratory to diagnose the cause of the infection.

Following informed consent, this study will store surplus BAL samples from patients, and later use them to evaluate a new test called the Aspergillus Lateral Flow Device (AspLFD). This test is very quick and has the potential, if found to be useful, to be incorporated into clinical guidelines to make the diagnosis of IA in ICU much easier. As well as left-over BAL samples, blood samples from patients will also be stored for later immune and immunogenetic studies, to help us understand why certain patients with influenza might be at greater risk of developing IA.

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| **ABBREVIATIONS** | |
| AspICU | Diagnostic criteria for invasive pulmonary aspergillosis in intensive care |
| CI | Chief Investigator |
| CT | Computed Tomography |
| ECDC | European Centre for Disease Prevention and Control |
| ECMO | Extra Corporeal Membrane Oxygenation |
| EIA | Enzyme-linked immunosorbent assay (EIA) |
| EORTC | European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group |
| GCP | Good Clinical Practice |
| GP | General Practitioner |
| HRA | Health Research Authority |
| HSCT | Hematopoietic stem cell transplantation |
| ICU | Intensive Care Unit |
| IDSA | Infectious Diseases Society of America |
| IA | Invasive aspergillosis |
| IAA | Influenza-associated aspergillosis |
| IPA | Invasive pulmonary aspergillosis |
| JRES | (St Georges) Joint Research and Enterprise Services |
| MSG | National Institute of Allergy and Infectious Disease Mycoses Study Group |
| NHS | National Health Service |
| NPV | Negative predictive value |
| PCR | Polymerase chain reaction |
| PHE | Public Health England |
| PI | Principal Investigator |
| PIS | Patient Information Sheet |
| POC | Point of care |
| POCT | Point of care test |
| PPV | Positive predictive value |
| PRR | Pattern-recognition receptor |
| PBMC | Peripheral Blood Mononuclear Cell |
| PSN | Participant Study Number |
| RCT | Randomised control trials |
| REC | Research Ethics Committee |
| RES | Research Ethics Service |
| R&D | NHS Trust Research & Development Department |
| SGUL | St Georges, University of London |
| SGHFT | St Georges, University Hospitals NHS Foundation Trust |
| SOP | Standard Operating Procedure |
| SRF | Severe Respiratory Failure |

**STUDY PROTOCOL**

**Incidence and pathogenesis of Invasive Aspergillosis in Intensive care patients with severe Influenza (AspiFlu)**

# 1 BACKGROUND

* 1. **Background**

**Severe Influenza**

Every winter approximately 5-10% of adults hospitalised with influenza develop severe infection requiring admission to an intensive care unit (ICU) for respiratory support[1]. During the 2018/2019 influenza season 3,157 influenza patients were admitted to ICUs across the United Kingdom (UK)[2]. The case-fatality rate for these patients was 9.3%, highlighting that severe influenza is both common and deadly[2].

The most severely ill patients with influenza are transferred to ‘Severe Respiratory Failure’ (SRF) centres for consideration of ‘Extra Corporeal Membrane Oxygenation’ (ECMO) – a technique that artificially oxygenates the blood outside of the body. There were 96 patients referred to a total of 5 units in England and Scotland last year. The mortality for ECMO patients is higher than those on a standard ICU; 37.1% in one meta-analysis [3].

**Invasive Aspergillosis (IA)**

*Aspergillus* is a type of fungus (specifically a mold) that is ubiquitous in the environment; particularly in soil, compost and also air conditioning units. Inhaled exposure to spores of *Aspergillus* is thus universal but this is usually harmless in those with an intact immune system. In those whose immune system is impaired, however, *Aspergillus* can cause potentially life-threatening infection.

‘Invasive Aspergillosis’ (IA) has long been recognized as a complication of profound immunosuppression, such as that caused by prolonged neutropenia in patients with hematological malignancy. In fact, the very definition of IA in the widely used EORTC / MSG guidelines requires a traditional ‘host factor’ such as neutropenia or recent stem cell transplant (Table 1, below)[4]. In recent years, however, it has emerged that IA is more prevalent than previously realized in critically ill patients without such traditional ‘host factors’[5]. In particular, recent evidence suggests that up to one in five patients with severe influenza on ICU may develop Influenza-associated aspergillosis IAA[7].

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| **Table 1. Definitions of Invasive Pulmonary Aspergillosis (IPA)** | |  |  |  |
| **EORTC/MSG** | |  | **Modified AspICU** | |
| **Proven** | **Any one of** |  | **Proven** | **As per EORTC/MSG** |
|  | Histopathologic, cytopathologic, or direct microscopic evidence of *Aspergillus* hyphae accompanied by tissue damage in a pulmonary specimen |  |  |  |
|  | Positive *Aspergillus* culture from a lung biopsy taken from a clinically or radiologically abnormal site consistent with an infectious disease process |  |  |  |
|  | Positive *Aspergillus* blood culture in the context of a compatible infectious disease process |  |  |  |
|  |  |  |  |  |
| **Probable** | **At least one host factor+ clinical + mycological criterion** |  | **Putative** | **At least one clinical + radiological + mycological criterion** |
|  | **Host factor** |  |  | **Clinical** |
|  | Recent history of neutropenia (<0.5 × 109 neutrophils/L for >10 days) temporally related to the onset of fungal disease |  |  | Fever refractory to at least 3 days of appropriate antibiotic therapy |
|  | Receipt of an allogeneic stem cell transplant |  |  | Recrudescent fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause |
|  | Prolonged use of corticosteroids at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for >3 weeks |  |  | Dyspnoea |
|  | Treatment with other recognized T cell immunosuppressants, such as cyclosporine, TNF-*α* blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days |  |  | Haemoptysis |
|  | Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency) |  |  | Pleural friction rub or chest pain |
|  | **Clinical criteria** |  |  | Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilator support. |
|  | The presence of 1 of the following 3 signs on CT: |  |  | **Radiological** |
|  | Dense, well-circumscribed lesions(s) with or without a halo sign |  |  | Any infiltrate on pulmonary imaging by portable chest x-ray or CT scan of the lungs. |
|  | Air-crescent sign |  |  | **Mycological** |
|  | Cavity |  |  | Histopathology or direct microscopic evidence of dichotomous septate hyphae with positive culture for *Aspergillus* from tissue |
|  | *Tracheobronchitis (IA rather than IPA) :* |  |  | Positive *Aspergillus* culture from BAL |
|  | *Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis* |  |  | Galactomannan optical index on BAL ≥ 1 |
|  | **Mycological criteria** |  |  | Galactomannan optical index on serum ≥ 0.5 |
|  | Direct test (cytology, direct microscopy, or culture) |  |  |  |
|  | *Aspergillus* in sputum, BAL or bronchial brush indicated by 1 of the following: |  | **Colonisation** | **Positive *Aspergillus* culture from BAL but at least one clinical and/or radiological criterion *not* met** |
|  | Presence of fungal elements indicating a mould |  |  |  |
|  | Positive *Aspergillus* culture |  |  |  |
|  | Galactomannan in plasma, serum or BAL or β-d-glucan detected in serum |  |  |  |
|  |  |  |  |  |
| **Possible** | **Host factor + clinical criterion but mycological criteria *not* met** |  | **Not classifiable** | **Clinical, radiological and mycological criteria all met but a *negative* Aspergillus culture from BAL** (the entry criterion in the original Blot et al. AspICU algorithm) |

IA: Invasive Aspergillosis, IPA: Invasive Pulmonary Aspergillosis, BAL: Bronchoalveolar lavage, CT: Computed Tomography

**Influenza-associated aspergillosis (IAA)**

It is well established that patients with severe influenza are prone to bacterial superinfection but the risk of secondary fungal infection with *Aspergillus* has only recently been determined [1][6]. The EORTC/MSG definitions of IA perform poorly in critically ill patients who often lack a traditional ‘host factor’ [7] or classical radiological findings such as a “halo sign”[8]. For this reason Blot et al. developed the ‘AspICU criteria’ to help distinguish IA from *Aspergillus* colonization in critically ill patients (Table 1) [9].

Schauwvlieghe et al. conducted a large retrospective study (2009-16) to investigate the incidence of Influenza-associated aspergillosis (IAA) across seven Belgian and Dutch Intensive Care Units (ICUs)[7]. Employing a modified version of the AspICU criteria they found evidence of IA in 83 of 432 (19%) patients admitted with influenza on ICU respectively [7]. This compared to 16 of 315 (5%) in a control group of patients with bacterial pneumonia. Even amongst non-immunocompromised patients, incidence of IA was 45 of 315 (14%) and only 46% of patients with IA had a traditional EORTC/MSG ‘host factor’.

Whilst this incidence of IA seems surprisingly high, it is supported by other recent small retrospective cohort studies from France and Belgium finding evidence of IA in 5 of 17 (29%) [10] and 9 of 40 (23%)[11] of patients with severe influenza on ICU. A recent UK study found evidence of IA in 5 of 24 (20.8%) of the most severely ill influenza patients requiring ECMO [12].

Martin-Loeches et al. prospectively studied the occurrence of super-infection in 2901 patients with influenza across a number of Spanish ICUs and found evidence of *Aspergillus* in only 35 of 2901 (1.2%). In this study, however, bronchoalveolar lavage (BAL) was not systematically performed during the influenza season and BAL galactomannan (see ‘Diagnosis’ below) was not performed at all[13]. Similarly a large retrospective study by Cavayas et al. retrospectively analyzed risk factors for fungal infection in 19,697 ECMO patients. Of the 2129 with fungal infection or colonization, 301 (14.1%) had influenza. But whilst influenza was found to be an independent risk factor for ‘Aspergillus involvement’ [(OR) 2.48], this term did not distinguish colonization from invasive infection [14]. The reported incidence of aspergillosis in the overall cohort was only 69 of 19697 (0.4%) but this study did not include galactomannan testing either. Neither of these two studies employed the AspICU criteria for IA and both highlight the difficulty in diagnosing invasive aspergillosis in retrospective studies when a diagnosis is not actively pursued and diagnostic criteria are inconsistently applied (see below).

**Mortality in Influenza-associated aspergillosis**

In the Dutch-Belgian study the 90-day mortality in those with IAA was 51%, almost twice the 28% found in those with influenza *without* evidence of IPA (p=0·0001). Similarly high mortality rates in patients with IAA have been reported in other studies[1][6]. It is therefore important to discern whether the incidence of IAA reported in the Dutch-Belgian study can be replicated in other settings, such as ICUs in England, in order to facilitate early diagnosis and treatment. IA was diagnosed at a median 3 days after admission in the Dutch-Belgian study, so a prompt diagnosis is essential [7][15].

**1.2 Diagnostics**

**Diagnosis of Influenza**

Diagnosing severe Influenza on clinical grounds alone is extremely challenging because typical symptoms such as rhinorrhea and sore throat are present in fewer than a third of hospitilised patients [16]. For this reason a liberal testing policy is employed at many hospitals during influenza season to include any patient with suspected respiratory infection or exacerbation of chronic lung disease[17].

Once testing is performed, confirming a diagnosis of influenza is usually straightforward. Polymerase chain reaction (PCR) is used to detect viral RNA in nasal and throat swabs (or other respiratory specimen) with extremely high degree of sensitivity and specificity[18].

# Diagnosis of Invasive Aspergillosis

Whilst respiratory sample PCR makes the diagnosis of Influenza simple, the diagnosis of IA is notoriously challenging. This may be one reason why the potentially high incidence of IA in severe influenza has gone largely unrecognized until recent years [6].

There are two principal difficulties in diagnosing IPA. Firstly, evidence of the presence of *Aspergillus* must be found and secondly IA must be distinguished from clinically irrelevant *Aspergillus* colonization. Focusing on the second problem, the ‘AspICU’ criteria developed by Blot et al. take as their starting point an Aspergillus-positive lower respiratory tract specimen culture. The AspICU criteria were then modified in the Dutch-Belgian study by removing the requirement for a positive culture, which has a sensitivity of only 29-58% when compared against ‘proven’ cases of IA.[5][8]

The ‘modified AspICU’ definition of IA requires at least one ‘clinical’, ‘radiological’ and ‘mycological’ criterion to be met (Table 1). It can be seen that these criteria are fairly broad and non-specific; in particular the ‘clinical’ and ‘radiological’ criteria might be met by many patients on ICU with severe influenza. Thus the diagnosis of IA in critically ill patients relied heavily upon the ‘mycological’ criteria, in particular the use of biomarkers such as galactomannan (below).

Of note, the Dutch-Belgian study assessed for the prevalence of Invasive *Pulmonary* Aspergillosis (IPA) rather than Invasive Aspergillosis (IA). For this reason, the presence of tracheobronchitis at bronchoscopy (an EORTC / MSG ‘clinical criterion’ for IA) did not feature in their modified AspICU criteria (Table 1). In one review, 19 (15%) of patients with IAA had tracheal plaques noted at bronchoscopy[1]., as opposed to the angio-invasive pulmonary disease seen in patients with classic EORTC / MSG host factors. Given that radiological manifestations of tracheobronchitis are subtle or may be absent [1], in order to capture this patient group, for this study we have further modified the AspICU to include the presence of tracheobronchitis (an EORTC/MSG clinical criterion) together with a mycological criterion (*not* requiring a radiological criterion) in the definition of putative IA.

**Galactomannan**

Because of the low sensitivity of *Aspergillus* culture, galactomannan is crucial in providing evidence of IA. Using the EORTC criteria, proven IA is only rarely diagnosed (usually post mortem) and diagnosis is most commonly ‘probable’, based on a host factor with suggestive radiological findings and a positive galactomannan in serum or BAL fluid.

Galactomannan (GM) is an exo-antigen released from Aspergillus hyphae as they invade host tissue, so should distinguish infection from colonization[5]. Its presence can be measured using an Enzyme-linked immunosorbent assay (EIA) - a one-stage sandwich microplate assay[19]. Heat-treated BAL and conjugate is added to microplate wells treated with a monoclonal antibody and incubated. If galactomannan is present it will form a monoclonal antibody-peroxidase complex that is detected through an enzyme-mediated *blue to yellow* colour reaction measured by a spectrophotometer.

In one prospective study including 26 cases of proven IPA in ICU patients (EORTC / MSG criteria) a BAL cut off of >1 produced a sensitivity of 85 and specificity of >90% [5]. In another prospective study of patients with IA as per AspICU criteria, those with a positive galactomannan in BAL (≥0.5) had similar patient characteristics, ICU scores, radiological findings and outcomes when compared to those with a positive Aspergillus culture suggesting that the prevalence of ‘true’ IPA was similar between groups [20]. Unfortunately, galactomannan is not available on-site at most UK hospital laboratories and prolonged turn-around-times of up to 2 weeks impede its utility[21].

***Aspergillus* lateral-flow device (AspLFD)**

A new CE-marked POC *Aspergillus* lateral-flow device (AspLFD, OLM Diagnostic, Newcastle UK) that detects extracellular glycoprotein antigen produced by thefungus during active growth provides a locally implementable alternative to galactomannan [22]. It is a rapid immunochromatographic test that involves pipetting untreated serum or centrifuged BAL sample direct into the port of a cassette and leaving for 30 minutes[23]. If Aspergillus antigen is present, it will form a monoclonal antibody complex which will appear as red line on the strip.

Figure 1 example of AspLFD device readout showing double red line, sample plus control, produced if positive (reproduced from [23])



In a prospective study of 106 patients with haematological malignancy BAL AspLFD had a sensitivity and specificity of 73% and 87% respectively for differentiating probable IA versus no IA using EORTC / MSG criteria [24]. Furthermore, in a study of 82 patients *without* underlying haematological malignancy, BAL AspLFD had a sensitivity of 69% and specificity of 71% in those with putative/proven IA using a modified AspICU criteria (Table 1)[8]. Only 15 (18.3%) were ICU patients, however, and none had influenza and thus the AspLFD urgently requires validation in this cohort.

Use of galactommanan on BAL (with a cut-off of ≥ 1) is endorsed in the most recent 2017 ESCMID *Aspergillus* guidelines, but the AspLFD is not fully discussedas it was not yet commercially available at the time of writing [25]. The guidelines also endorse the use of serum galactomannan (with a cut-off of ≥ 0.5) but note that it is less sensitive in non-neutropenic patients.

**1.3 Standard of Care**

**Standard of care: Severe influenza**

Treatment of severe influenza revolves around supportive care, treatment with neuraminidase inhibitors (NAIs) and identification and treatment of secondary complications. A proportion of patients with respiratory failure secondary to severe influenza will require intubation ventilation. Where respiratory failure is unresponsive to conventional measures, referral to an ECMO centre may be considered if the respiratory failure is considered potentially reversible[3]. Large meta-analysis of cohort data suggest that NAI use (especially within 48H of symptoms) may significantly reduce mortality in patients with severe influenza[26][27]. As such, both PHE [28] and Infectious Diseases Society of America (IDSA) [29] guidelines advocate their use. IDSA guidelines recommend that clinicians should investigate and empirically treat bacterial coinfection in patients who either present with severe disease, deteriorate after initial treatment, or fail to improve after 3-5 days of NAI therapy [29]. For patients intubated on ICU investigation of such bacterial co-infection would usually involve a BAL.

**Standard of care: Invasive Aspergillosis (IA)**

RCT evidence supports the use of Voriconazole over Amphotericin B for the treatment of IA [30] and so it is the current standard of care according to ECIL/ESCMID-ECMM-ERS[25] and IDSA guidelines[31]. Voriconazole can cause adverse effects such as photosensitivity, rash, visual disturbance and derangement of liver function. It has multiple drug interactions and unpredictable Pharmacokinetics (PK): therapeutic drug monitoring is required. Liposomal amphotericin B or Isavuconazole, a newer azole with a more favorable toxicity profile, are alternatives [25]. Once the diagnosis of IA is suspected, timely treatment is essential [1]. In one retrospective study of IAA, patients who survived had received antifungal therapy at a median of 2 days after influenza diagnosis, compared with 9 days in patients who had died [32].

Azole use for primary prophylaxis against Aspergilloses is currently only recommended in those with traditional EORTC / MSG host factors such as profound and prolonged neutropenia or following HSCT [25]. The Dutch-Belgian group are currently conducting a trial of Posaconazole prophylaxis for patients with severe influenza (POSA-FLU study, NCT03378479, clinical trials.gov). An alternative strategy would involve serial screening for, and prompt treatment of, patients that meet AspICU criteria [1]. This has the advantage of improved antifungal stewardship, an important consideration with the emergence of voriconazole resistant *Aspergillus*[21].

**Standard of care: Influenza-associated Aspergillosis (IAA)**

The 2018 IDSA guidelines consider fungal co-infection as “rare” in influenza and thus make no specific recommendations [29]. Following publication of the Belgian-Dutch study, the European Centre for Disease Prevention and Control (ECDC) published a rapid risk assessment in Nov 2018 which included the following reccomendations[15]:

1. Public health authorities should raise awareness of IAA among ICU physicians and clinical microbiologists.
2. IA should be considered as a possible complication of severe influenza, and appropriate diagnostic procedures (including BAL) should be considered to be performed in time.
3. Antifungal treatment should be initiated along the relevant national and international guidelines, when indicated, based on careful clinical judgement and also taking into account the side effects of antifungal drugs

A recent review article similarly advocated early BAL and biomarker use to diagnose IAA as soon as possible and also suggests this strategy be repeated in the event of a respiratory deterioration[1].

There are no adjunctive treatments of proven benefit in severe influenza and the use of steroids is actively discouraged[29]. This is because a Cochrane meta-analysis has found that corticosteroid therapy use in influenza is associated with increased mortality (OR 3.06, 95% confidence interval (CI) 1.58 to 5.92) [33].

# 1.4 Pathogenesis of influenza-associated aspergillosis

The reason for the association between severe influenza and IA is not fully understood but likely results from a combination of physical, immunological, microbiological and iatrogenic factors.

**Physical factors**

The inflammation caused by severe influenza infection causes physical damage to the respiratory tract epithelial lining. This impedes mucocilary clearance and likely leaves the host vulnerable to invasive infection by *Aspergillus*[1]. See discussion regarding tracheobronchitis in ‘Diagnosis of Invasive Aspergillosis’ above.

**Immunological factors**

The immune response to severe influenza can be broadly divided in to an ‘early’(innate) and ‘late’(adaptive) phase. In the early/innate phase (first week of symptoms) there is a sometimes excessive pro-inflammatory immune response with upregulation of Th1 proinflammatory cytokines such as TNF-α, IL-1 and IL-6 [34][35]. This leads to recruitment and activation of neutrophils with resulting tissue damage. In those with severe infection these high levels of proinflammatory cytokines persist, but there is also a failure to down-regulate immunosuppressive cytokines such as IL-10. The result is ongoing inflammation alongside an impaired adaptive response characterised by lack of T helper cell differentiation and B cell development [36][37][35]. This in turn allows for persistent viral replication, creating a vicious cycle.

Figure 2. Model of host immune deficiency in severe influenza, adapted from {36].



Long pentraxin 3 (PTX3) is an acute phase protein released by phagocytes and non-immune cells at sites of inflammation[38]. It is a pattern-recognition receptor (PRR) that forms complexes with *Aspergillus* enhancing recognition of the fungus by the innate immune system. Genetic polymorphisms in PTX3 production have been associated with increased incidence of IPA in haematology patients[38][39]. A key cytokine induced by PTX3 is IL-8: significantly raised levels of IL-8 were found in BAL fluid from hematology patients with IPA compared to matched controls [40]. Measuring the proportion of patients with IAA with such PTX3 polymorphisms, as well as cytokine production in BAL fluid could contribute to understanding of immunopathogenesis [36].

Another PRR thought to play an important role in the pathogenesis of aspergillosis is Dectin-1 [41]. Dectin-1 is a C-type lectin receptor present on immune cells that recognizes beta-1,3-glucan; a polysaccharide present on the cell walls of *Aspergillus* (and other fungi). As with PTX3, genetic polymorphisms influencing the production of Dectin-1 have been associated with increased incidence of invasive aspergillosis in haematology patients but its role in severe influenza requires study[39].

**Microbiological factors**

Influenza has long been associated with bacterial superinfection with pathogens such as *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Streptococcus pyogenes* [29]**.** But recent work suggests influenza may have more subtle effects on the lung microbiome, especially in patients intubated on ICU. It may be that influenza not only leads to IAA directly, but also through changes in the bacterial microbiome[42]. Severe infection with H1N1 Influenza A appears to carry a higher risk of IAA[1] than other strains of influenza. The effect of influenza strain and duration of viral shedding on the risk of IAA needs further characterization.

**Iatrogenic factors: Steroids**

In the Dutch-Belgian study, corticosteroid use (0.1 mg/kg/day prednisone equivalent) in the 4 weeks prior to ICU admission was independently associated with IAA (aOR 1.59; 95% CI 1.30–1.99; p<0.0001) [7] . Similar findings are replicated elsewhere[1] and supported by the Cochrane meta-analysis (mentioned above) that found that steroid use in influenza was associated with increased mortality [33].

# 2 RATIONALE

# 2.1 Potential for improved patient care

The incidence of IA in patients with severe influenza may be much higher than previously realized. IAA is associated with significant mortality but antifungal therapy may be life-saving if initiated promptly. Unfortunately the diagnosis of IA can be elusive if appropriate diagnostic procedures (such as BAL and galactomannan testing) are not performed. This means the IAA is likely to remain under-diagnosed without increased awareness of IAA among ICU physicians and clinical microbiologists.

By evaluating the incidence of IAA across three London trusts, this study has the potential to increase awareness of IAA within the NHS and could inform future PHE policy. Determination of risk factors associated with IAA could help identify those patients most likely to benefit from further investigation or, potentially, antifungal prophylaxis.

The validation of the AspLFD has the potential to improve NHS patient care by streamlining diagnostic pathways and improving turnaround time as part of implementation of the NHS England antifungal CQUINN 2019/20 (<https://www.england.nhs.uk/publication/pss1-meds-optimisation-pss-cquin-indicator/> published Mar 2019).

# 2.2 Hypothesis

The incidence of IA in ICU patients with severe influenza will be comparable to the 20% found in a recent large retrospective study in the Netherlands/Belgium. IA will be associated with excess mortality and morbidity in those with severe influenza. Steroid use will emerge as an independent risk factor for the development of IA.

# 2.3 Need for a study

The Dutch-Belgian study provides the strongest evidence to date of a significant burden of IA in patients with severe influenza. But the incidence of IA might vary widely between hospitals and countries [15]. An estimate of the incidence if IAA in the UK is necessary to guide NHS policy. Furthermore the Dutch-Belgian study was retrospective. By being prospective, this study will be less liable to bias by ensuring systematic data and sample collection.

Central to the diagnosis of IAA is biomarker testing, but galactomannan testing for many ICUs suffers from prolonged turn-around times. AspLFD offers an attractive solution but validation is urgently needed in this cohort of patients.

IAA is a disease distinct from IPA in patients with traditional EORTC / MSG host factors. Research is urgently needed to understand the biological mechanisms that underpin it, to inform future prevention and treatment strategies [15].

**3 THEORETICAL FRAMEWORK**

Due to the difficulties in diagnosing IA as outlined above, a prospective observational study is required to accurately evaluate the incidence of IA in UK NHS patients with severe influenza. This is to ensure that information pertinent to the AspICU classification is systematically recorded, and that the relevant diagnostic tests are systematically performed.

A multi-centre study is required to recruit a sufficient number of ventilated patients with severe influenza during a single influenza season. Increasing the number of patients enrolled and sites involved will ensure that the results are generalisable to a wider population of patients with severe influenza.

For the purpose of the AspLFD validation, serum and BAL samples will be retrospectively tested in parallel by both galactomannan EIA and the AspLFD after the influenza season. In the event that there is sample degradation over time, parallel testing will reduce the chance that this effect will impact on the measured performance of one test more than the other.

A detailed outline of future sub-studies to be performed on samples collected and stored as part of this study lies outside the scope of this protocol.

# 4 RESEARCH QUESTION/AIM(S)

**4.1** **Objectives**

**Primary:** Estimate incidence of influenza-associated invasive aspergillosis (IAA) and identify risk factors for infection

**Secondary:** Determine morbidity and mortality of IAA

Evaluate utility of AspLFD device for diagnosis of IAA

**Sub-studies:** Improve understanding of immunopathogenesis of IAA through *ex vivo* PBMC analysis, measurement of serum and bronchoalveolar lavage (BAL) cytokines and targeted immune gene sequencing

**4.2** **Outcomes**

**Primary:**

Diagnostic classification of IAA during ICU admission as per modified AspICU criteria.

The modified AspICU criteria will be further modified for our study in two ways (Table 1):

The presence of tracheobronchitis at bronchoscopy (an EORTC/MSG clinical criterion) will replace the need for a radiological and clinical criterion to meet the definition of **putative IA** (i.e. tracheobronchitis plus mycological criterion)

1. The ‘not classifiable’ group, namely ‘Clinical, radiological and mycological criteria all met but a *negative* Aspergillus culture from BAL’ will be classified as Putative IA in our study.

Patients will therefore be classified as evidence of invasive aspergillosis (subdivided into proven/putative), and no evidence of invasive aspergillosis (subdivided into those with and without *Aspergillus* colonisation).

The outcome of interest is evidence of IA at any point during study ICU stay, including on admission. The strongest classification for each patient will be recorded. For example, some patients may have no evidence of IA on their admission blood tests / BAL, but later clinically deteriorate and have a second blood test / BAL that places them in the ‘Putative IA’ AspICU group. This patient would be classified as ‘Putative IA’. If this patient later died and a biopsy from a post mortem placed them in the ‘Proven IA’ group they would be classified as ‘Proven IA’.

Risk factors to be elicited from baseline collected clinical data points (see Table 3), e.g. EORTC / MSG host factors, steroid use and other co-morbidities.

**Secondary:**

* Duration of mechanical ventilation; ICU stay; hospital stay
* ICU all-cause mortality; inpatient all-cause mortality; 90-day all-cause mortality
* Survival analysis: Time to death (all-cause mortality) for all patients
* Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of AspLFD

**Sub-studies:**

* *Ex vivo* PBMC analysis, measurement of serum and bronchoalveolar lavage (BAL) cytokines/ chemokines and targeted immune gene sequencing

|  |  |
| --- | --- |
| **Table 2. AspiFlu Study Definitions of Invasive Aspergillosis (IA)** | |
| **Proven** | **As per EORTC/MSG, any one of** |
|  | Histopathologic, cytopathologic, or direct microscopic evidence of *Aspergillus* hyphae accompanied by tissue damage in a pulmonary specimen |
|  | Positive *Aspergillus* culture from a lung biopsy taken from a clinically or radiologically abnormal site consistent with an infectious disease process |
|  | Positive *Aspergillus* blood culture in the context of a compatible infectious disease process |
|  |  |
| **Putative** | **At least one clinical + radiological + mycological criterion OR Tracheobronchitis + at least one mycological criterion** |
|  | **Clinical** |
|  | Fever refractory to at least 3 days of appropriate antibiotic therapy |
|  | Recrudescent fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause |
|  | Dyspnoea |
|  | Haemoptysis |
|  | Pleural friction rub or chest pain |
|  | Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilator support. |
|  | **Radiological** |
|  | Any infiltrate on pulmonary imaging by portable chest x-ray or CT scan of the lungs. |
|  | **Tracheobronchitis** |
|  | Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis |
|  | **Mycological** |
|  | Histopathology or direct microscopic evidence of dichotomous septate hyphae with positive culture for *Aspergillus* from tissue |
|  | Positive *Aspergillus* culture from BAL |
|  | Galactomannan optical index on BAL ≥ 1 |
|  | Galactomannan optical index on serum ≥ 0.5 |
|  |  |
| **Colonisation** | **Positive *Aspergillus* culture from BAL but Putative criteria *not* met** |

# 5 STUDY DESIGN, METHODS, DATA COLLECTION AND ANALYSIS

**5.1 STUDY DESIGN**

AspiFlu is a multicentre prospective observational cohort study, incorporating retrospective evaluation of a novel POC diagnostic device and scientific sub-studies on stored patient samples.

**5.2 OUTLINE OF METHODS**

# *See also ‘Study Schematic’ above.*

Between 1st November 2019 and 31st March 2020, intubated adults on the study ICUs will be assessed for eligibility and consented for enrolment (see ‘7.3 Recruitment’). Based on historic data, an estimated 75-95 patients with severe influenza are expected to be recruited over the study period (see ‘7.2 Sampling’).

Enrolled patients will have baseline blood sampling performed within 72 hours of ICU admission (see ‘7.3.4 Biological Sample Handling’). Surplus BAL from bronchoscopy performed as part of routine clinical care will also be stored alongside leftover serum. This is a non-interventional study and patients will receive routine clinical care throughout the study.

Clinical data (see 5.3 Data Collection) will be collected at baseline, during the patients ICU stay, and after ICU discharge - up to 90 days or hospital discharge (whichever is longer). No patient will be enrolled after 31st March 2020. To achieve 90-day follow-up the end of patient data collection will thus be approximately 30th June 2020 (it may be longer if some enrolled patients remain an inpatient, in order to record inpatient mortality and re-admission to ICU).

After the influenza season, the stored BAL and serum samples will be tested *retrospectively* in parallel by both galactomannan EIA and by the AspLFD (see ‘7.3.4 Biological Sample Handling’). This testing will be done by a member of the research team blinded to the AspICU IA classification of the patient.

For the primary analysis the incidence of IA in the study cohort during ICU stay will be determined as per modified AspICU criteria (Table 2) incorporating clinical and radiologic data and prospective galactomannan results from BAL and serum samples (see 4.2 Outcomes). Risk factors for the development of IA and morbidity of IA will also be assessed (see 5.5 Data analysis).

As a secondary analysis, an evaluation of the AspLFD will be performed by comparing AspLFD results on serum and BAL to the modified AspICU classifications of IA in the cohort. For this analysis, if there is a discrepancy between a BAL/serum galactomannan result performed prospectively as part of routine clinical care and the result from the retrospective testing, then the retrospective result will be used. In the event that there is sample degradation over time, parallel testing will reduce the chance that this effect will impact on the measured performance of one test more than the other.

Subsequent to a successful MRC/NHIR funding grant application clinical data and stored blood/BAL samples will be used in a series of sub-studies to improve understanding of immunopathogenesis of IAA. This will involve *ex vivo* PBMC analysis using flow cytometry and stimulation with Aspergillus antigens, measurement of serum and bronchoalveolar lavage (BAL) cytokines and targeted immune gene sequencing.

**5.3 DATA COLLECTION**

Demographic and clinical data, including inclusion and exclusion criteria, EORTC and AspICU criteria, IA risk factors, influenza factors and critical illness severity will be collected at baseline (Table 3). Outcome measures to be collected will include investigation dates and results (mycological criteria); morbidity and mortality outcome data. Risk factors to be elicited from baseline collected clinical data points (see Table 3), including EORTC / MSG host factors, steroid use and other co-morbidities. All-cause mortality rates at 90 days will be collected using NHS Digital and the Intensive Care National Audit and Research Centre for those discharged prior to 90 days.

Patients enrolled will be allocated a Participant Study Number (PSN). Clinical and microbiological data will be entered into an NHS compatible REDCap™ database using the PSN. A separate log will be kept securely at each site linking the PSN to patient identifiable information (name, date of birth, NHS number); this will take the form of a password-protected, Microsoft Excel spreadsheet saved on a secure server at each site. Research blood samples and left over BAL samples will be stored using the PSN. The PSN will not reveal the identity of the patient or the diagnostic classification to laboratory staff undertaking the subsequent laboratory diagnostic evaluation (see ‘7.3.3 Data collection tool’).

|  |  |
| --- | --- |
| **Table 3: Data to be collected** | |
| **Baseline Assessments** | |
|  |  |
| Patient identifiers | Age and gender |
|  |  |
| Inclusion Criteria | >18 years old |
|  | Admitted to intensive care for respiratory support requiring intubation and ventilation for >24h |
|  | Positive influenza PCR from nasal, throat swab, BAL or other respiratory specimen taken within 72 hours (of admission to ICU – pre or post.  OR  Influenza suspected but influenza PCR results awaited – under these circumstances the patient can be provisionally enrolled, but later excluded if no specimens taken within 72 hours pre/post admission to ICU is positive as above. |
|  |  |
| Exclusion Criteria | Respiratory failure not the primary reason for ICU admission |
|  | History of proven/ probable invasive pulmonary aspergillosis |
|  |  |
| EORTC / MSG host factors | Neutropenia <0.5 x 10(9) for >10 days temporally related to the onset of fungal disease |
|  | Allogeneic stem cell transplant |
|  | Corticosteroid before ICU admission (mean dose > 0.3mg/kg/day of prednisolone > 3 weeks) |
|  | Treatment with recognized T cell immunosuppressants (e.g. cyclosporine, TNF-*α* blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days |
|  |  |
| Other IA risk factors | Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency |
|  | Haematological Malignancy |
|  | Solid organ transplant |
|  | Solid organ malignancy |
|  | Chronic Obstructive Pulmonary Disease |
|  | Smoker in the past year |
|  | Steroid use (not meeting EORTC/MSG criteria), including inhaled |
|  |  |
| Other co-morbidities | BMI >30, Liver cirrhosis, Diabetes, CKD, Chronic lung disease |
|  | APACHE II vs SOFA score on ICU admission |
|  |  |
| Influenza Factors | Symptom Onset |
|  | Date of first PCR positive influenza specimen |
|  | Influenza strain (if known) |
|  | Neuraminidase inhibitor start date |
|  | Vaccination status |
|  |  |
| Fungal factors | Azole use - either prophylaxis or other exposure in last 28 days |
|  |  |
| **Data collected during ICU stay** | |
|  |  |
| Diagnosis of Invasive aspergillosis (IA) | Diagnosis of IA made by clinical team? |
|  | BAL performed? Date |
|  | Biomarker performed? Date |
|  |  |
| EORTC / MSG 'Clinical' | Dense well circumscribed lesion(s) with/without halo |
|  | Air-crescent |
|  | Cavity |
|  | Tracheobronchitis (ulceration/nodule/pseudomembrane/plaque/eschar on bronchoscopy) |
|  |  |
| AspiFlu 'Radiological' | Pulmonary infiltrate (any) |
|  | Tracheobronchitis (ulceration/nodule/pseudomembrane/plaque/eschar on bronchoscopy) |
|  |  |
| EORTC / MSG / AspICU 'Mycological' | Evidence of Aspergillus hyphae and tissue damage in histopath/cytopath sterile specimen |
|  | Aspergillus positive culture |
|  | Galactomannan positive >0.5 (serum) |
|  | Galactomannan positive >1 (BAL) |
|  |  |
| Coinfections | Other respiratory virus |
|  | Bacteria isolated in BAL |
|  |  |
| Therapeutics/Interventions | Azole use (treatment) and indication |
|  | Antibiotic use duration |
|  | Neuraminidase inhibitor duration |
|  | Date of first intubation |
|  | Date of ECMO |
|  |  |
| Outcome measures | Length of intubation |
|  | Length of ECMO |
|  | Length of ICU stay |
|  | ICU all-cause mortality |
|  |  |
| **Data collected after ICU discharge** | |
|  |  |
| Influenza Factors | Crude estimate of duration influenza PCR positive (last positive swab +5 days) |
|  |  |
| Other outcome measures | Inpatient all-cause mortality |
|  | 90-day all-cause mortality |
|  | Re-admission to ICU |

**5.4 STUDY INTERVENTIONS**

This study is observational and patients will be investigated and managed by the local clinical teams.

The only additional procedure in this study will be the taking and storing of blood samples (including PBMC, serum and DNA) for subsequent analysis. Approximately 30-40ml of blood will be taken within 72H of ICU admission. Many patients will have a central venous catheter or arterial line through which blood will be taken without the need for venepuncture. In those who do not have a central line, we will minimise discomfort by taking the blood sample at same time as routine blood tests. Many patients will also be sedated.

Where a patient is enrolled prior to a bronchoscopy (and BAL) performed as part of routine clinical care, a 30ml research sample of surplus BAL fluid will be taken and stored. BAL will only be performed at the discretion of the treating team as per standard clinical care – a BAL will not be performed, or delayed, for the purpose of this study. Some patients may have more than one BAL, surplus material from any subsequent BAL will also be stored following the same procedure as for the first. Leftover BAL samples will also be stored (see 7.3.4 Biological Sample Handling). The local ICU SOP for BAL will be followed at each site. The presence or absence of evidence of tracheobronchitis at Bronchoscopy will be recorded in the clinical notes.

**5.5 DATA ANALYSIS**

The primary analysis will be to determine the incidence of IA in the study population (as per modified AspICU criteria, table 2) and identify risk factors for infection.

Univariable analyses using Kruskal-Wallis, χ2 tests, Fisher exact, Mann Whitney U tests or t tests, as appropriate, will be performed to detect factors from Table 3 associated with an increased risk of IA. A multivariable analysis using binary logistic regression will be performed to identify *independent* risk factors for infection. The dependent variable will be the presence of IA (including proven and putative) and independent variables risk factors for IA selected using a predictive backward selection modelling approach.

Variables selected for inclusion in the model will be based on *a priori* knowledge from previous studies (e.g. EORTC/MSG host factor, steroid use[7]) and variables identified as associated with IA infection following univariable analysis (P ≤ 0.1). Variables will be retained based on likelihood ratio testing, if they significantly improve model fit, to obtain the most parsimonious model. The strength of the association between independent risk factors and IA will be expressed as adjusted odds ratio (aOR) with corresponding 95% confidence intervals.

The morbidity of patients with and without IA will be compared using the following outcomes measures: Duration of mechanical ventilation, length of ICU stay, length of hospital stay. 90-day all-cause mortality will be compared for patients with and without IA. ICU all-cause mortality and inpatient all-cause mortality will be compared also.

A survival analysis (time-to-all-cause mortality) to 90 days, will also be performed comparing those with and without IA. Kaplan Meier curves will be produced and compared using log-rank tests and Cox regression models to control for any potential confounding.

For the AspLFD diagnostic test validation, the sensitivity, specificity, PPV and NPV of BAL AspLFD against the modified AspICU criteria in this cohort will be determined. The performance of AspLFD on serum or BAL or both (combined) will be assessed.

Data will be analysed using Stata version 15.

**6 STUDY SETTING**

Study participants will be enrolled across 4 trusts comprising 324 ICU beds total:

1. Guy's and St Thomas' Hospital NHS Foundation Trust (Guy’s Hospital and St Thomas’ Hospital) – x1 ICU at Guy’s (13 beds), x3 at St Thomas’ (42 beds)
2. St Georges University Hospitals NHS Foundation Trust (St Georges Hospital) – x3 ICUs (60 beds)
3. King’s College Hospital NHS Foundation Trust (King’s College Hospital)– x4 ICUs (144 beds)
4. Manchester University NHS Foundation Trust (65 beds)

At each site potential participants will be identified and enrolled by the local clinical teams, site PIs and/or site research nurses (see ‘7.3 Recruitment’)

A full time clinical research fellow (with honorary research contracts at each site) will work across all the sites in collaboration with and under the supervision of the site PIs. The research fellow will oversee:

* Data entry into the REDCap™ database (see ‘7.3.3 Data collection tool’)
* Baseline blood sampling and storing of BAL and serum samples (see’7.3.4 Biological Sample Handling’)

**7 PATIENT RECRUITMENT AND SAMPLING**

**7.1 Eligibility Criteria**

Intubated adults on the study ICUs will be screened against the inclusion/exclusion criteria for potential participation.

**7.1.1 Inclusion criteria**

* Adults > 18 years
* Admitted to intensive care for respiratory support requiring intubation and ventilation for >24h

**AND**

* Positive influenza PCR (nasal, throat swab or other respiratory specimen) within 72 hours of admission to ICU

**OR**

* Influenza suspected but influenza PCR results awaited – under these circumstances the patient can be provisionally enrolled, but later excluded if no specimens taken within 72 hours pre/post admission to ICU is positive as above.

Two of the study sites are ECMO referral centres. This means that during winter patients with severe influenza are transferred to these study site ICU from other hospitals. A BAL is usually performed shortly after admission, often before influenza PCR results from the referring hospital are available. It is therefore preferable to provisionally enrol these patients so that BAL can be stored appropriately. If no respiratory specimen taken within 48 hours pre/post admission is positive, then the patient can be subsequently excluded.

**7.1.2 Exclusion criteria**

* Respiratory failure not the primary reason for ICU admission
* History of proven/ probable invasive pulmonary aspergillosis

**7.2 Sampling**

All potentially eligible patients who consent to be enrolled will be included in the study.

Extracting data from laboratory databases of ICU influenza diagnoses and following discussion with the PIs for each site of this study we estimate the following number of patients with severe influenza will be enrolled at each site: St George’s (n=10-15), Kings College Hospital (n=15-20), Guys and St Thomas’ Hospitals (n=45-50), Manchester (n =5-10) total (n=75-95).

**7.3 Recruitment**

**7.3.1 Participant identification**

Intubated adults admitted to intensive care for respiratory support will be screened for eligibility by the clinical team, site PIs and/or site research nurses (providing NIHR portfolio adopted). Potential participants will identified through two methods:

1. Hospital Virology laboratory viral respiratory PCR result worksheets
2. Liaison with the clinical teams on each ICU.

Each potential participant must satisfy all the approved inclusion and exclusion criteria of the protocol to be eligible for enrolment. Once a potential participant has been deemed eligible they will be approached by the clinical team, site PIs and/or site research nurses to initiate the consent process.

**7.3.2 Consent**

The consent process will be undertaken by the clinical team, site PIs and/or site research nurses (providing NIHR portfolio adopted). The clinical research fellow can also undertake consent in circumstances where the potential participant has already been identified and approached by the local clinical/ research teams.

In many cases it will not be possible to obtain prospective informed consent from the patient at the time of enrolment. This is due to the fact that many patients will have a reduced level of consciousness due to their illness or due to sedative medications used as part of their treatment.

**Patient Consent**

If possible, informed consent will be obtained directly from the patient. The patient will be informed about the study by the responsible clinician or a member of the research (co-investigator) team and given a copy of the Participant Information Sheet (PIS). Patients will be given an adequate amount of time to consider their participation in the study. If the patient decides to participate in the study they will be asked to sign the Patient Consent Form, together with the responsible clinician/researcher. The patient will retain one copy of the signed Consent Form. Another copy will be placed in the patient’s medical records whilst the original will be retained in the study Site File. Patients are expected to have been transferred and/or discharged up until the 90-day data collection point and will be assumed (if not withdrawn before this point) to have given consent up to this data collection point. Fluctuating capacity is anticipated for this patient group. Any patients who are initially able to consent will be asked to identify a person who knows them well in order to support with monitoring of their ongoing consent, alongside standard care.

**Personal Consultee Declaration**

If the patient is unable to give consent then advice will be sought from the patient’s Personal Consultee, who may be a relative, partner or close friend. This is in line with the legal requirements for obtaining consent in patients without capacity in England and Wales (Mental Capacity Act 2005). The Personal Consultee will be informed about the study by the responsible clinician or a member of the research team and provided with a copy of the Consultee Information Sheet and asked to give an opinion as to whether the patient would object to taking part in medical research. The Consultee will be given adequate time to consider the patient’s participation in the study. If the Consultee decides that the patient would have no objection to participating in the study they will be asked to sign the Consultee Declaration Form together with the consenting clinician / researcher. The Consultee will retain a copy of the signed Declaration/Assent Form. A second copy will be placed in the patients’ medical records whilst the original will be retained in the Study Site File. Patients are expected to have been transferred and/or discharged up until the 90-day data collection point, consent will be assumed under the signed Declaration/Assent Forms (unless withdrawn before this point) to continue to data collection points.

**Nominated Consultee Declaration**

If the patient is unable to give informed consent and attempts to meet and discuss with a Personal Consultee have failed then a nominated Consultee, who is not connected with the conduct of the Study may act as a nominated/Professional Consultee.

It is expected that the majority of patients eligible for this study will have a BAL performed within the first 24-48 hours after admission to the study ICU. It would be preferable that patients are enrolled prior to this BAL so that a research sample of surplus BAL can be obtained and stored. Therefore, if attempts to meet and discuss with a Personal Consultee fail by the time of this BAL then a nominated Consultee will be approached. Further attempts to meet with a Personal Consultee will continue for 1-2 working days because it would be preferable for Personal Consultee involvement prior to the baseline blood sampling (which is to occur <72 hours after study ICU admission).

The Nominated Consultee will have received information about the study, by a member of the research team, prior to considering study participation for an individual patient. A copy of the CIS will be provided each time an individual patient is to be considered. The patient’s treating physician, with the support of the research team will determine the patient’s eligibility to enter the study and a Nominated Consultee will advise as to whether the patient would decline to take part if he/she had capacity. The patient’s lead treating physician may act as a Nominated Consultee only if they have no connection with the conduct of the study and therefore would have a dual role in both determining eligibility and considering the patient’s participation. The Nominated Consultee, together with the researcher will sign and retain one copy of the signed Consultee Declaration/Assent Form, the original will be retained in the Study Site File. If a relative, partner or close friend should subsequently visit the patient, after enrolment and before the patient has regained capacity, they should be informed about the patient’s participation and invited to take over the role of Consultee and be informed about the retrospective consent process. Patients are expected to have been transferred and/or discharged up until the 90-day data collection point, consent will be assumed under the signed Declaration/Assent form (unless withdrawn before this point) to continue to data collection points.

**Recovered Capacity**

Patients, for whom an opinion is given by a close relative, will be monitored in line with GCP and if they gain capacity by the time of primary hospital discharge, they will be informed of their participation in the study by the responsible clinician or a member of the research team. The clinician / researcher will discuss the study with the patient and the patient will be given a copy of the Recovered Capacity PIS to keep. The patient will be asked for consent to continue follow-up in the study or will be supported if they wish to withdraw, it will be confirmed that data already collected will be retained by default unless the participant or their Guardian/Welfare Attorney or Relative requests otherwise. If consent is given, the patient will be asked to sign the Recovered Capacity Consent Form. The patient will retain one copy of the signed Consent Form. Another copy will be placed in the patient’s medical records whilst the original will be retained in the Study Site File. If the patient does not want to continue follow-up in the study, no further clinical data beyond that time-point or new samples will be collected.

The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the study without giving reasons and without prejudicing further treatment.

## **Withdrawal of Participants**

Participants may withdraw from the study at any time without prejudice. This could be because the participant, or their Consultee/Guardian/Welfare Attorney withdraws consent, or because of Lead clinician opinion.

According to the design of the study, participants may have the following two options for withdrawal:

1. Participants may withdraw from any further communication but allow the study team to continue to access their medical records and any relevant hospital data that is recorded as part of routine standard of care; i.e., CT-Scans, blood results and disease progression data etc. No further samples will be taken/stored but any samples already taken/stored will still be included in the study.
2. Participants can withdraw completely from the study and withdraw the data and samples collected up until the point of withdrawal. The data and samples already collected would not be used in the final study analysis. Stored samples would be destroyed.

The type of withdrawal and reason for withdrawal will be recorded

**7.3.3** **Data collection tool**

Clinical, radiographic and microbiologic data relevant to the measures outlined in Table 3 will be collected from the electronic notes systems and hospital databases at each site and entered into an NHS compatible REDCap™ database (Research Electronic Data Capture). Any information collected during the study will be kept strictly confidential and will only be used for research purposes (see ‘8.6 Data protection and patient confidentiality’).

**7.3.4 Biological** **Sample Handling**

**Blood samples**

Following informed consent, approximately 30-40mls of blood will be drawn from each patient for research purposes. This will include heparin/EDTA (25ml total) for PBMC isolation, serum (1 x 4ml), and Paxgene tubes (DNA) (1 x 2.5ml). Samples will be taken within 72h of study ICU admission.

PBMCs will be separated from freshly drawn samples within 24h of collection and stored in liquid nitrogen. Serum and DNA tubes will be frozen stored on site (-20C/ -80C as appropriate) and shipped on dry ice from the local hospital site to St Georges University of London at the end of the study.

For the immunopathogenesis sub-studies, PBMCs will undergo flow cytometry and *ex vivo* antigen stimulation. Host DNA will be extracted for targeted sequencing of known genetic polymorphisms associated with an increased risk of IA (e.g. PTX3; Dectin-1 synthesis).

**BAL samples**

Surplus material (30mls) of bronchoalveolar lavage (BAL) will be taken for research purposes at the time of each bronchoscopy. BAL will be divided into 2-3 separate sterile Falcon tubes and stored on site at -80C. If a bronchoscopy was undertaken during the patient’s study ICU stay, but before enrolment, the clinical laboratory will be asked to provide any residual BAL from these once routine clinical testing has been completed on them.

It is anticipated that most patients in the study will have a BAL performed as part of routine clinical care within 72 hours of ICU admission. Patients that remain intubated for a prolonged period may have more than one BAL. Surplus material from any subsequent BAL will also be stored following the same procedure as for the first.

After the influenza season BAL samples will be transferred by batch shipped on dry ice from the local hospital site to St Georges University of London.

After the influenza season both serum and BAL will undergo retrospective testing by galactomannan EIA and AspLFD (see 5.2 ‘Outline of Methods’ and ‘Study Schematic’). Chemokine/ cytokine analyses will be performed as part of the immunopathogenesis sub-studies.

**Pathogen samples**

Respiratory swabs/ NPA transport medium will be stored to allow subsequent influenza typing. Any *Aspergillus* isolates cultured from BAL samples will be stored for subsequent resistance genotyping.

Only members of the research team under the supervision of the sponsor will have access to the stored biological samples. Analyses will only be performed within the scope described in this protocol. If there is a desire to use the stored samples for a subsequent research project a new IRAS application will be made.

# 8 ETHICAL AND REGULATORY CONSIDERATIONS

**Ethical Considerations**

Most patients with severe influenza or other respiratory on ICU will be unable to give informed consent due to alterations in consciousness and cognition caused by illness and, in some cases, therapeutic sedation. Consent will therefore be obtained in line with legal requirements for obtaining consent in patients without capacity in England & Wales (Mental Capacity Act 2005) (see ‘7.3.2 Consent’).

Some patients will have capacity to consent, either during their acute illness or after they recover. Under these circumstances the consent process will include: assessment and documentation of capacity by a trained member of a research team; providing written information about the study; allowing sufficient time for the patient to understand the material and ask questions; obtaining written informed consent.

## **Declaration of Helsinki**

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

## **Guidelines for Good Clinical Practice**

The Chief Investigator will ensure that this study is conducted in accordance with Good Clinical Practice and that local PIs and co-investigators are up-to-date with GCP training.

## **Approvals**

Ethical approval for the study will be sought using the Integrated Research Application System (IRAS). The informed consent form and PIS will both be submitted to IRAS.

Before enrolling patients into the trial, each trial site must ensure that the local conduct of the trial has the agreement of each NHS Trust Research & Development (R&D) department.

The CI will submit a final report to the required authorities with the results, including any publications, within one year of the end of the study.

## **8.1 Assessment and management of risk**

Apart from the taking of blood samples this study is non-interventional. The taking of a 30ml sample of surplus BAL during a bronchoscopy already being performed as part of routine clinical care is not expected to entaill any additional risks. Study enrolment is thus not associated with physical risk to the patient.

There will be no direct information gathering from participants. All information will be gathered from the electronic medical notes, and limited to that information outlines in Table 3 (‘5.3 Data Collection’). This means there is minimal scope that a researcher could come into information that has safeguarding implications.

In the event that during the enrolment process the potential participant reveals information that has safeguarding implications this would be brought to the attention of the clinical team caring for the patient on the ICU.

**8.2 Research Ethics Committee (REC) and other Regulatory review & reports**

Before the start of the study, a favourable opinion will be sought from an appropriate REC for the study protocol, informed consent forms and other relevant documents e.g. advertisements.

**For HRA- NHS REC reviewed research**

* Substantial amendments that require review by NHS REC will not be implemented until that review is in place and other mechanisms are in place to implement at site.
* It is the Chief Investigator’s responsibility to produce the annual reports and submit the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended.
* The Chief Investigator will notify the REC of the end of the study within one year after the end of the study.
* If the study is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination.

**Regulatory Review & Compliance**

Before any site can enrol patients into the study, the Chief Investigator/Principal Investigator or designee will ensure that appropriate approvals from participating organisations are in place. Specific arrangements on how to gain approval from participating organisations are in place and comply with the relevant guidance.

Amendments

For any amendment to the study, the Chief Investigator or designee, in agreement with the sponsor will submit information to the appropriate body in order for them to issue approval for the amendment. The Chief Investigator or designee will work with sites (R&D departments at NHS sites as well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment and confirm their support for the study as [amended](http://www.hra.nhs.uk/resources/after-you-apply/amendments/).

**8.3 Peer review**

The research protocol was written and reviewed by members of Study steering group (CI, Principal Investigators and Co-investigators above). This group comprises at least one Infection and one Intensive care specialist for each site.

Funding for the study was awarded via a competitive, peer-reviewed scheme open to applicants in the UK and Ireland via the Gilead Fellowship Scheme <https://www.ukifellowshipprogramme.com/>

The protocol (Version 0.2) was also given a favorable external expert peer review using a standardized feedback form.

**8.4 Patient & Public Involvement**

Patient and public involvement was sought at a fundraising event for a St Thomas’ Hospital ECMO patient group.

A number of people kindly volunteered. This included three people who had previously been patients on ECMO at St Thomas’ for severe infections including influenza, plus a partner of one such patient.

A copy of the patient information sheet (PIS) and study protocol (version 0.2) was provided to all volunteers alongside a ‘Patient & Public Feedback Form’ to complete.

All found the PIS easy to read and understand. All felt that the PIS made clear both the purpose of the study and what participation would involve. None reported that they would not take part in the study after reading the PIS.

The study was deemed worthwhile and no concerns were raised regarding the study design, management, or ethical aspects.

Volunteers provided some excellent feedback which helped improve the PIS (subsequent modifications italicised below). This included:

* Making the observational nature of the study clear from the outset of the PIS - *sentence added to introductory paragraph.*
* Having a brief synopsis at beginning allowing people to read on to find out more if they wish *: ‘short summary’ box added to first page.*
* Make clear that blood samples could be taken alongside routine bloods and that a bronchoscopy wouldn’t be unnecessarily done for the purpose of the study: *added to brief synopsis and bold type used in sentences highlighting this.*
* Use pictures in the PIS, including a picture of the AspLFD : *pictures of bronchoscopy and AspLFD added.*
* Make clear publications would contain anonymised data only*: sentence added to this effect.*

Some excerpts from comments include:

|  |  |
| --- | --- |
| Do you think the study is worthwhile and why? | “Absolutely, prediction would allow earlier diagnosis and thus potentially save lives” |
| After reading this PIS, would you be inclined to participate in the study? If not why? Is there anything in the PIS that puts you off? | “Yes and especially with what I’ve been through I’d definitely be more inclined to take part” |
| Any other comments or feedback in terms of feedback of the PIS? | “I was glad to see that those that consent have the PIS to take home, as I would’ve read it again with a clear head and be very pleased that I was participating.” |

**8.5 Protocol compliance**

Protocol deviations, non-compliances, or breaches are departures from the approved protocol.

All protocol deviations must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately.

Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

### 

**8.6 Data protection and patient confidentiality**

All data will be handled in accordance with the Data Protection Act 2018 (UK implementation of the EU General Data Protection Regulation (GDPR)).

Participants will be allocated a personal study number (PSN) which will be used to protect their identity. The secure (password protected) REDCap™ database will only be accessed by members of the research team. The REDCap™ database will be coded with the PSN only. A separate log will be kept securely at each site linking the PSN to patient identifiable information (name, date of birth, NHS number); this will take the form of a password-protected, Microsoft Excel spreadsheet saved on a secure server at each site. This system (sometimes called ‘pseudonymised’ data) ensures that confidentiality is protected during routine collection of data. Only the pseudonymised database will be used during analysis.

All data will be stored in line with the EU General Data Protection Regulation (GDPR) 2016/679, Data Protection Act (1998) and Caldicott principles. St. Georges University of London (SGUL) is the data controller for this study. St. George’s University of London (SGUL) is a REDCap™ consortium partner with a dedicated secure server hosting the database. The legal basis we are applying in order to process personal data under GDPR regulations is that ‘processing is necessary for the performance of a task carried out in the public interest’ (Article 6(1)(e)). The following web address will be provided on the PIS: [www.hra.nhs.uk/information-about-patients/](http://www.hra.nhs.uk/information-about-patients/)

The electronic database will be kept securely in pseudonymised form for 5 years following the end of the study in accordance with relevant legislation and the SGUL Research Data Management Policy. All information will be held in compliance with NHS Digital data confidentiality and security arrangements, the Data Protection Act (1998) and the GDPR.

Physical patient identifiable information will be stored in areas where there is restricted access including only people involved in research. For example, the paper patient consent and Consultee declaration forms will be stored securely in the research nurses office at each site. This information will be kept securely at each site for 5 years following the end of the study.

8.7 Indemnity

**St George’s University of London sponsored research:**

St George’s University of London holds insurance to cover participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that St George’s has been negligent. This includes negligence in the writing of the protocol, or selection of trial resources.

Where the Trial is conducted in a hospital, the hospital has a duty of care to participants. St George’s University of London will not accept liability for any breach in the hospital’s duty of care, or any negligence on the part of hospital employees. .

If a participant indicates that they wish to make a claim for compensation, this needs to be brought to the attention of St George’s University of London immediately.

Failure to alert St George’s University of London without delay and to comply with requests for information by the sponsor or any designated Agents may lead to a lack of insurance cover for the incident.

**8.8 Access to the final study dataset**

Only the Chief Investigator, full time clinical research fellow responsible for data entry, and the statistician will have access to the full dataset. Only the chief Investigator and clinical research fellow will have access to the separate electronic database linking the PSN to patient identifiable information.

### 9 DISSEMINATION POLICY

All co-investigators will be involved as co-authors of the main study manuscript. Any other publications arising from the scientific sub-studies will include the CI, site PIs, collaborating scientists as well as any co-investigators meeting authorship criteria. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. Authors will acknowledge that the study was funded by Gilead Fellowship Programme, but the funder will have no role in study design analysis, presentation or publication of findings

The study findings will be presented at national and relevant international meetings with abstract publication, and published in high impact peer-reviewed journals, with media coverage where applicable, so that findings and their implications quickly reach all of relevant UK clinical communities and can be translated into policy. This will be facilitated by our investigator group which includes key individuals linked to Infection and Intensive Care societies, professional bodies, the Department of Health and patient/relative groups across a wide range of responsibilities relevant to the planning of the management of influenza, fungal infection and intensive care in the NHS.

### 9.1 Dissemination policy

Publication: “Any activity that discloses, outside of the circle of trial investigators, any final or interim data or results of the Trial, or any details of the Trial methodology that have not been made public by the Sponsor including, for example, presentations at symposia, national or regional professional meetings, publications in journals, theses or dissertations.”

All scientific contributors to the Trial have a responsibility to ensure that results of scientific interest arising from Trial are appropriately published and disseminated. The Sponsor has a firm commitment to publish the results of the Trial in a transparent and unbiased manner without consideration for commercial objectives.

To maximise the impact and scientific validity of the Trial, data shall be consolidated over the duration of the trial, reviewed internally among all investigators and not be submitted for publication prematurely. Lead in any publications arising from the Trial shall lie with the Sponsor in the first instance.

**Before the official completion of the Trial**

All publications during this period are subject to permission by the Sponsor. If an investigator wishes to publish a sub-set of data without permission by the Sponsor during this period, the **Steering Committee/the Funder** shall have the final say.

Exempt from this requirement are student theses that can be submitted for confidential evaluation but are subject to embargo for a period not shorter than the anticipated remaining duration of the trial.

**Up to 180 days after the official completion of the Trial**

During this period the Chief Investigator shall liaise with all investigators and strive to consolidate data and results and submit a manuscript for peer-review with a view to publication in a reputable academic journal or similar outlet as the Main Publication.

* The Chief Investigator shall be senior and corresponding author of the Main Publication.
* Insofar as compatible with the policies of the publication outlet and good academic practice, the other Investigators shall be listed in alphabetic order.
* Providers of analytical or technical services shall be acknowledged, but will only be listed as co-authors if their services were provided in a non-routine manner as part of a scientific collaboration.
* Members of the Steering Group shall only be acknowledged as co-authors if they contributed in other capacities as well.
* If there are disagreements about the substance, content, style, conclusions, or author list of the Main Publication, the Chief Investigator shall ask the Steering Group to arbitrate.

**Beyond 180 days after the official completion of the Trial**

After the Main Publication or after 180 days from Trial end date any Investigator or group of investigators may prepare further publications. In order to ensure that the Sponsor will be able to make comments and suggestions where pertinent, material for public dissemination will be submitted to the Sponsor for review at least sixty (60) days prior to submission for publication, public dissemination, or review by a publication committee. Sponsor’s reasonable comments shall be reflected. All publications related to the Trial shall credit the Chief and Co-Investigators as co-authors where this would be in accordance with normal academic practice and shall acknowledge the Sponsor and the Funders.

**9.2 Archiving Arrangements**

Each site will be responsible for their onsite level study archiving. The trial essential TMF along with any central trial database will be archived in accordance with the sponsor SOP.

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### 11. APPENDICIES

**11.1** **Appendix 2**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Schedule of Procedures (See Table 3 above for data to be collected )** | | | | |
| **Procedures** |  | | | |
| **Screening** | **Baseline** | **During ICU stay** | **Until hospital discharge/90 days** |
| Consent | x |  |  |  |
| Blood sampling |  | x |  |  |
| Left over BAL sampling |  |  | x |  |
| Demographics |  | x |  |  |
| Clinical data (Table 3) |  | x | x | x |

**13.3** **Appendix 3**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Amendment Log** | | | | |
| **Amendment No.** | **Protocol version no.** | **Date issued** | **Author(s) of changes** | **Details of changes made** |
| 1 | 0.2 | 23/08/19 | Tihana Bicanic, Carolyn Hemsley, Jonathan Edgeworth, Jonathan Ball, Duncan Wyncoll, Jonathan Youngs. | Addition of Sile Molloy as named statistician |
|  |  |  |  | Inclusion of a control group removed. Inclusion criteria now: patients with influenza only. |
|  |  |  |  | Inclusion Criteria now: Intubated patients only |
|  |  |  |  | Specify blood sampling within 72H of ICU admission. |
|  |  |  |  | Flu vaccination status added as recorded data. |
|  |  |  |  | Potential patient identification and screening for eligibility will be done by clinical team and research nurses if NHIR portfolio adopted |
|  |  |  |  | Further clarification of when physician consent will be obtained rather than consultee. |
| 2 | 0.3 | 04/10/19 | Tihana Bicanic,  Jonathan Youngs,  Sam Hollingworth. | Post External review and R&D review. Minor adjustments only. |
| 3 | 1.0 | 04/10/19 | Tihana Bicanic,  Jonathan Youngs,  Sam Hollingworth. | Submitted to IRAS with signatures. |
| 4 | 2.0 | 26/11/19 | Tihana Bicanic,  Jonathan Youngs,  Sam Hollingworth | Manchester added as a participating site |