PROJECT FINAL REPORT

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Project acronym: INFECT

Project title: Project title: Improving Outcome of Necrotizing Fasciitis: Elucidation of Complex Host and Pathogen Signatures that Dictate Severity of Tissue Infection

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4.1 Final publishable summary report

4.1.1 Executive summary

The overall goal of INFECT is to advance our understanding of the pathophysiological mechanisms, prognosis, and diagnosis of the multifactorial highly lethal necrotizing soft tissue infections (NSTIs). NSTI's are rapidly spreading infections that may cause extensive soft tissue or limb loss, multiorgan failure and are associated with a considerable fatality rate. It is undisputed that rapid diagnosis and prompt intervention is directly related to survival. The initial presentation may be limited to unspecific symptoms such as tenderness, swelling, erythema and pain. Thus, diagnosis and management are difficult due to heterogeneity in clinical presentation, in co-morbidities and in microbiological aetiology. There is an urgent need for novel diagnostic and therapeutic strategies in order to improve outcome of NSTIs. To achieve this, a comprehensive and integrated knowledge of diagnostic features, causative microbial agent, treatment strategies, and pathogenic mechanisms (host and bacterial disease traits and their underlying interaction network) is required.

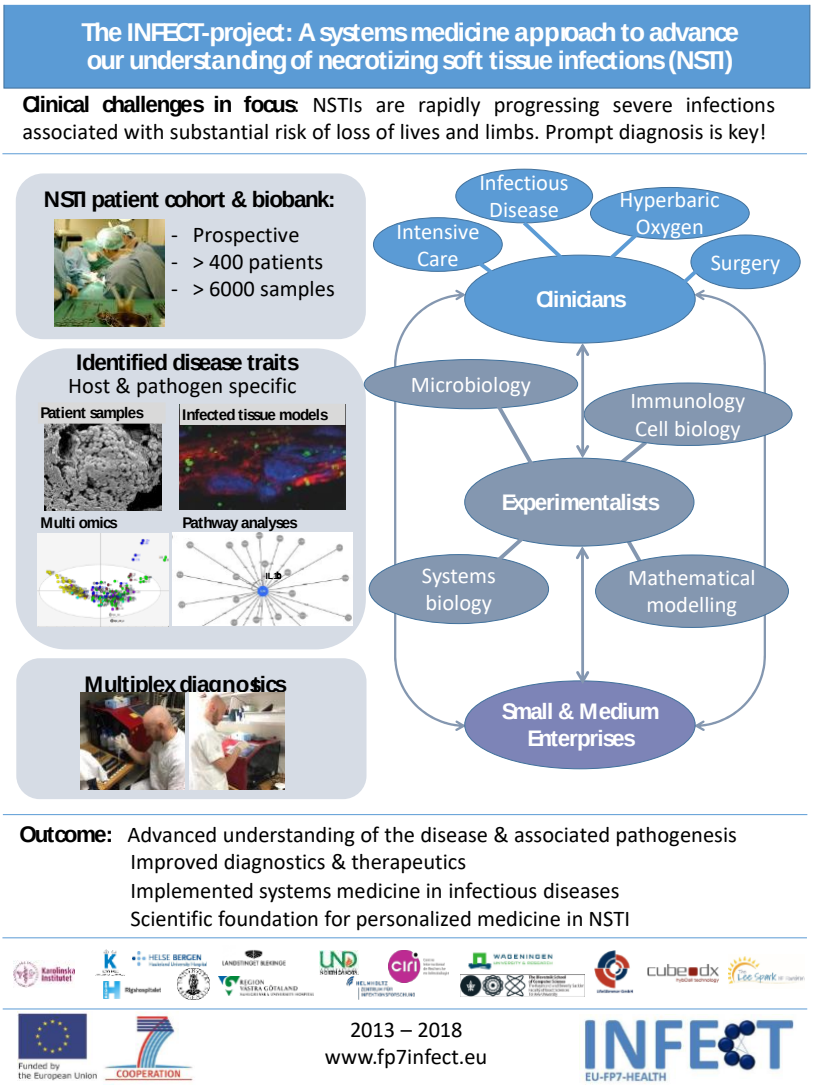
INFECT was designed to obtain such insight through an integrated systems biology approach in patients (WP2) and different clinically relevant experimental models (WP1 and WP6). The work flow includes a comprehensive set of analyses (WP3 and WP5) followed by integration of results in advanced computational platforms, which enabled generation of pathophysiological models of the disease (WP4) and advanced understanding of the underlying mechanisms and hots-pathogen interactions. The results were translated into novel diagnostic tests (WP7) and improved patient management (WP2 & 8). The work was conducted by the INFECT consortium, which consisted of a team of multidisciplinary researchers, clinicians, SMEs and a patient organization, each with a unique expertise, technical platform and/or model systems that together provided the means to successfully conduct the multifaceted research proposed and efficiently disseminate/exploit the knowledge obtained (See figure 1).

Key achievements of INFECT include:

- Establishment of the world's largest NSTI patient cohort with extended clinical registry and associated biobank providing a unique resource for the proposed studies.
- Advanced insight into the clinical aspects of NSTIs providing the basis for evidence-based guidelines for patient management and care.
- The systems medicine analyses within INFECT have substantially advanced our understanding of these life-threatening infections, including the identification of novel pathogenic mechanisms and specific host and bacterial disease traits associated with disease outcome.
- The results demonstrate that the pathophysiology of NSTI are influenced both by the causative microbe and by host factors, underscoring the need for patient stratification and implementation of tailored therapy/personalized medicine in these infections.
- Multiplex diagnostic tools for rapid pathogen identification and monitoring of disease associated biomarkers have been developed and tested in the clinical setting.
- The novel understanding of the disease mechanisms of these infections has resulted in changed clinical practice related to antibiotic usage as well as use of immunomodulatory treatments.
- Fostering the new generation of clinical and preclinical scientists within the field of systems medicine in infectious diseases.

Overall, INFECT has proven the value of systems medicine approaches in acute infectious diseases to achieve improved diagnostics and therapeutics to improve patient disease outcome.

Figure 1.


The INFECT-project: A systems medicine approach to advance our understanding of necrotizing soft tissue infections (NSTI)
Clinical challenges in focus: NSTIs are rapidly progressing severe infections associated with substantial risk of loss of lives and limbs. Prompt diagnosis is key!
NSTI patient cohort & biobank:
- Prospective
- > 400 patients
- > 6000 samples
Identified disease traits
Host & pathogen specific
Patient samples
Infected tissue models
Multi omics
Pathway analyses
Multiplex diagnostics
Infectious Disease
Hyperbaric Oxygen
Intensive Care
Surgery
Clinicians
Microbiology
Immunology Cell biology
Experimentalists
Systems biology
Mathematical modelling
Small & Medium Enterprises
Outcome: Advanced understanding of the disease & associated pathogenesis
Improved diagnostics & therapeutics
Implemented systems medicine in infectious diseases
Scientific foundation for personalized medicine in NSTI
Funded by the European Union
COOPERATION
2013 – 2018
www.fp7infect.eu
INFECT
EU-FP7-HEALTH

4.1.2 Summary description of project context and objectives

The tasks of INFECT were undertaken in 8 highly integrated WPs; all designed to jointly address the *specific objectives* of the project.

A central aspect of the INFECT project is the prospective enrolment of patients with NSTI and collection of biobank samples as well as the causative microbes (**WP2**, clinical **partners 2, 3, 4, 5, 6**). The clinical partners succeeded in enrolling 409 NSTI patients with completed clinical registries (>2000 variables), and collecting associated biobank samples (> 6000). This represents the *world's largest NSTI patient cohort* and is a unique resource for the project.

Results obtained related to objective 1 "Unravel specific mechanisms underlying diseases signatures though a bottom-up* systems *approach applied to clinically relevant experimental settings".
The major tasks here involved establishment of experimental models optimized for NSTI infection, including a model-driven, forward genetics approach using advanced murine models (ARI BXD and HLA class II transgenic mice) (**WP1** headed by **partner 15**), as well as a human 3D artificial tissue model system (**WP6** headed by **partner 1**). The models were successfully established and proven to be robust tools for modelling NSTI. Some key findings obtained through the use of these models were:

- A systems genetics approach using *S. pyogenes* infected ARI BXD mice identified genetic loci and gene networks that strongly predicted critical disease phenotypes, i.e. survival, weight loss and lesion size in NSTI (**WP1**, **partner 15**). The IL1β network was identified as a key regulator of severity of NSTI. This finding was confirmed by studies using the same *S. pyogenes* strain in infections of the human tissue model (**WP6, partner 1**) as well as by analyses of patient tissue biopsies (**WP5, partner 1**).
- A reductionist approach using humanized transgenic mice expressing HLA-Class II genes showed that variations in HLA-II alleles determine severity of NSTI pathogenesis, specifically mice expressing DR3 showed larger lesions and high mortality to NSTI with *S. pyogenes* isolate 5448 and to a lesser extent to the INFECT isolate 2006. Hence, revealing both host genetic factors influencing severity of NSTI but also differences based on the infecting isolate (**WP1, partner 15**).
- Using Ingenuity Pathway Analysis Tools, **partner 15** identified PPARγ as one among the key upstream regulators that drive differential responses whose expression was significantly downregulated during NSTI. Data also revealed that *S. pyogenes* disseminated into adipose tissue and impaired adipogenesis. Based on these findings, novel intervention strategies have been tested (see obj. 4) and also customization of the human tissue model to include adipocytes (**WP6, partner 1**).

Results obtained related to objectives 2 and 3 "Apply a top-down systems biology approach to NSTI patient samples to pin-point key host and pathogen factors involved in the onset and development of infection", and "Identify and quantify disease signatures and underlying networks that contribute to disease outcome".
These two objectives are based on the utilization of the prospective NSTI patient cohort with associated clinical data and linked biobank samples and the causative bacterial isolate(s) (**WP2, partners 2, 3, 4, 5, 6),** as well as the experimental models **(WP1, partner 15, and WP6, partner 1).** The successful collection of the large INFECT cohort and biobank enabled a systems medicine statistical approach **(WP3 and WP4; partners 8, 9, 10, 11, 14).** System-wide analyses (genomics, transcriptomics, proteomics, metabolomics) of both pathogen and infected subjects/models (**WP1, WP3, partners 1, 8, 9, 10, 14, 15**) have been undertaken and analysed through solid multivariate statistics and pathway analyses (**WP4, partners 8, 9, 10, 11**) to delineate factors/pathways/biomarker sets that contribute to disease severity and outcome. The multiple and heterogeneous data sets from **WP1-3, 5** and **6** were aggregated in a dynamic, relational database (**WP4, partner 9, 11**). Some main achievements/findings of this work were:

- On the pathogen side, typing methods and a comprehensive genome database were established for *S. pyogenes*, *S. dysgalactiae* and *S. aureus* NSTI isolates; all prevalent causes of NSTI (**WP3, partner 8, 10**). Statistical analyses of microbiological characteristics and clinical variables revealed a striking link between site of infection and microbial aetiology, e.g. NSTI of the upper or lower extremities was associated with monomicrobial *S. pyogenes* while NSTI located to the abdomen/ano-genital area was associated with polymicrobial infection (**WP2, WP3, WP4; partners 2, 3, 4, 5, 6, 8, 11**).
- The frequent occurrence of multi-species NSTI cases spurred the first comprehensive culture-independent characterization of the NSTI pathobiome, providing insights into the microbial community network and analyses of species distributions/ interactions (**WP3, partner 8**).
- The microbial community profiling was integrated with analysis of host-microbe interactions by RNA-sequencing, which identified differences in the pathophysiology of monomicrobial streptococcal and polymicrobial NSTI (**WP3, WP4, partners 8, 9**). While pathogenic streptococci express a wide range of virulence factors that mediate the different steps of infection, the pathogenicity of polymicrobial NSTIs is dependent on the co-occurrence of multiple bacterial taxa, which complement each other to enhance the virulence of the bacterial community as a whole. These differences result in distinct patterns of molecular host aetiology-dependent molecular pathophysiology.
- Pathway analyses of RNAseq data of *S. pyogenes* NSTI patient biopsies in comparison to healthy controls, were done with a specific focus on the immune responses (**WP3, WP4, partner 1, 8, 9, 11**). Several over-represented pathways were identified in the NSTI patients, including neutrophil degranulation (see below result point), and specific signaling pathways. Furthermore, RNAseq data from S. pyogenes infected ARI BXD mice identified the same set of implicated pathways, and their upregulation was linked to severity of NSTI (**WP1, partner 15**). Similarly, using the human skin tissue model with embedded monocytes revealed an upregulation of the implicated signaling pathways (**WP6, partner 1**). These typical polarizing signals were confirmed in *S. pyogenes* infected patient tissue biopsies by use of multiparameter confocal microscopy analyses (**WP5, partner 1**). The data further implied that the responses are pathogen-specific with different patterns for *S. aureus* and *S. pyogenes*. The differential response by *S. aureus* is in line with its preferential adherence and invasion of particular cell types, where the bacteria also causes substantial cell death that likely is an important contributor to the tissue pathology of *S. aureus* NSTI (**WP3, partner 10**).
- The RNAseq data implicating neutrophil degranulation as a disease trait verifies the *in vitro* studies implicating neutrophil degranulation as a key pathogenic mechanisms contributing to *S. pyogenes* NSTI (**WP3, WP5, WP6, partner 1**).
- Patient tissue biopsies were analysed to verify identified pathogen and host traits (**WP5, partner 1**). One key finding of these analyses were the demonstration of biofilm formation in over 30% of *S. pyogenes* NSTI patients. This has great implications for the antibiotic efficacy and hence choice of antibiotics.
- Also systemic host responses were assessed through analyses of plasma samples using metabolomics and customized multiplex protein assays including panels of inflammatory and metabolic factors (**WP3, WP4: partner 1, 2, 6, 9, 11**). The results of these analyses confirms the dysregulated host response in NSTI and importantly reveals that it is not limited to the tissue site but is also reflected systemically, which is of importance for the diagnostic tool development (**WP7, partner 16**).
- A death prediction model was developed implementing machine learning approaches and using selected "early" clinical variables, like baseline, demographic and early measurements (**WP2, WP4: partner 9, 11, 14**). From those, a set of best predictor variables were selected using a Random Forest algorithm. Both Random Forest and Support Vector Machines were

deployed as machine learning tools for the actual prediction. The prediction model was included as a feature for a prototype mobile app, which allows users/clinicians to input the values for a small set of predictive early parameters in order to receive a probability of patient death/amputation after a certain time period post diagnosis, e.g. after 30 days. In another approach, the set of clinical variables was combined with gene expression data from the RNA-Seq experiments, though with less success than using only clinical parameters.

- Through group-wise principal components analysis of the plasma metabolomics data, key metabolites significantly altered in the NSTI patients as compared to uninfected controls were identified (**WP3, WP4**, **partners 1, 9, 14**). These metabolites have then tested in *in vitro* biofilm assays, and some found to significantly affect bacterial growth and biofilm formation (**WP3, partner 1**). Based on these findings a 3D cellular automata model was developed to dissect the conditions needed for the formation of biofilm of bacteria when in contact with human tissue (**WP4, partner 14**). The 3D cellular automata model is a computational model that simulates the interactions between bacteria in a spatial context, and represents the bacteria using their genome scale metabolic models, as well as taking into account gradients of stress signals, nutrients, and diffusion of metabolites in 3D.

In conclusion the results related to objectives 1-3 provides evidence that the pathogenic mechanisms of NSTI vary depending on microbial aetiology and co-morbidity, and involves distinct dysregulated host immune responses requiring a tailored immunotherapeutic approach in the individual patient. Hence, providing a strong foundation for future work towards personalized medicine in NSTI and in other severe infectious diseases associated with a dysregulated immune response such as sepsis.

Results obtained related to objective 4: "Identify novel therapeutic strategies for NSTI".

A key aim of INFECT has been to improve therapies for NSTI driven by the fact that these infections are associated with significant risk for loss of lives and limbs, even in young previously healthy individuals. This was tackled in two ways: (i) two novel therapeutic strategies (intravenous immunoglobulin; IVIG and hyperbaric oxygen treatment; HBO) were evaluated for clinical efficacy and/or mechanistic action, and (ii) through identification of novel targets revealed by the integrated systems biology approach in **WP1-WP6**. Some key findings include:

- Using the murine experimental model (**WP1**), partner 15 tested several innovative therapeutic strategies targeting the newly identified targets, including PPARγ ligands to upregulate PPARγ expression, which resulted in reduced lesion size as well as significantly improving survival of *S. pyogenes* infected mice. Also, **partner 15** tested interventions for IL1β using a panel of inflammasome inhibitors. The results demonstrated that the NLRP3 inflammasome inhibitor MCC950 in combination with Clindamycin gave the best outcomes by improved survival, significantly low induction of cytokines compared to Clindamycin alone.
- Analyses of patient plasma pre- and post-IVIG therapy revealed that the treatment resulted in inhibition of streptococcal virulence factors (**WP3, partner 8**). In addition, a randomized clinical trial of IVIG-therapy versus placebo in NSTI was conducted by **partner 2** (**WP2**). The results showed that IVIG therapy had no beneficial effect in NSTI patients of all aetiologies. However, the data indicated that specific subgroups of patients, i.e. *S. pyogenes* NSTI, may benefit from the therapy.
- A detailed review of HBO therapy are being finalized to elucidate how HBO-therapy has been applied (target patient population, timing, dosages) at the different INFECT clinical sites. The results will be useful to predict which patients benefit the most, which will guide patient stratification and future clinical trials.

Results related to objective 5 "Exploit identified disease traits for the innovation of optimized diagnostic tools".

This objective aimed to exploit the results obtained in WP1-6 to design, develop and validate a multiplex diagnostic tool (**WP7**) suitable for the clinical needs associated with care of NSTI patients,

i.e. early and rapid identification of pathogens and host immune and organ status. To achieve this, a diagnostic SME (**partner 16**) employed two strategies; one applying compact sequencing for pathogen detection and one compact profiling for host responses. Key achievements included:

- Based on Anagnostics' hybcell technology (**partner 12**), two prototype tests were developed by Anagnostics (**partner 12**) and further developed/redesigned by Cube (**partner 16**).
- A clinical on-site validation of the diagnostic tool for host responses (**partner 16**) was conducted by **partner 2** (**WP7**). This analyses focused on plasma samples from the NSTI patients revealing that some, but not all markers, showed satisfactory correlation with comparative lab results A combination of known and new markers were found to be predictive for key clinical outcomes, i.e. acute kidney failure and 30-day mortality at a level equal to SOFA scores, and better than conventional biomarkers CRP and PCT. The operation of the hybcell technology for plasma samples was found to be easy, fast and reliable with a relatively short staff training period. With further refinement, the system may be useful as a frontline diagnostic tool to imrpove timing and precision of the NSTI diagnosis and interventions.
- To further improve the ease of use, the test was validated for whole blood as well (**partner 16**).
- Combinations of biomarkers (two biomarkers, combined with help of logistic regressions) have been examined to stratify acute kidney failure (AKI) and mortality. The combination of Myoglobin and Cystatin C supersedes the prognostic capability of any single marker (with an AUC of 0,86). The prognosis of mortality could not be improved by any combination.

The high multiplex capability and the outlook to elaborate superior biomarker combinations in addition to the bedside utilization represent advancement in diagnostics and potential for future clinical use.

Results related to objective 6 "Translate the advanced knowledge generated in INFECT into evidence-based guidelines for classification and management of NSTI".

The comprehensive insight of clinical, therapeutic and pathogenic aspects of NSTI provides the foundation for evidence-based guideline for classification and management in NSTI (**WP2, partners 2, 3, 4, 5, 6**). Nonetheless, clinical guidelines must be developed by an independent advisory group. The INFECT consortium has therefore established an intentional agreement with the The Scandinavian Society of Anaesthesiology and Intensive Care Medicine (SSAI).

A number of dissemination activities have been undertaken, including:

- The patient organization (**partner 13**) together with the NSTI clinical partners (**2, 3, 4, 5, 6**) has ensured an efficient dissemination of INFECT's advances to the clinical community as well as to policy makers and the society in large, not the least to patients and their relatives.
- A contact with SSAI has been established for the preparation of clinical guidelines (**WP2).** Also through this collaboration, a 1-day postgraduate training workshop will be held in association with the international SSAI meeting 2019 (**WP8**).
- Other key dissemination activities include:
  - A book volume on NSTI will be published by Springer Nature. The target groups are health care professionals, scientific community and medical students/residents (**WP8, all partners**). This volume is due in 2019.
  - Short videos, including also patients and the patient organization (**partner 13**), targeting the society at large is being finalized and will be published on Youtube (September 2018).
  - 53 publications in well renowned journals has been published.
  - Key contributions at international meetings, such as the Lancefield International symposium on Streptococcal infections and infectious diseases, European conference of Clinical microbiology and infectious diseases, European Association Systems Medicine Conference, and Nordic Society of Clinical Microbiol and Infectious Diseases conference.

4.1.3 Description of the main S&T (science & technical) results/foregrounds.

4.1.3.1 WP1 Systems genetics approach in a murine model of NSTIs

The objectives of WP1 were to through the use of an experimental murine model of NSTI:

- Map quantitative trait loci (QTL) harbouring genes with a high statistical likelihood of modulating susceptibility/outcomes of NSTI,
- Determine host susceptibility/outcome in relation to specific pathogens that cause NSTIs,
- Determine how variations in both the bacteria and in the host genetic content alters the pathogenic strategies and/or the host defense mechanisms, and
- Test clinical efficacy of novel adjunctive therapies.

The results achieved in this WP are described below and relates to the WP specific Tasks:

Task 1.1 Apply forward systems genetics approach to map QTLs harbouring genes with a high likelihood to be involved in modulating susceptibility/outcomes in an NSTI model.

Partner 15 established an NSTI BXD mouse model and using unbiased systems genetics approach, **partner 15** identified genetic loci and gene networks, that strongly predicted survival, weight loss and lesion size in NSTI. **Partner** 15 analysed disease phenotypes in the context of BXD genotypes and identified highly significant QTLs on Chromosomes 2 and 7 that strongly predicts NSTI survival and weight loss respectively. Further **Partner** 15 identified suggestive QTLs on chromosomes 6 and 8 for lesion size. In search of key regulators that modulate NSTI susceptibility, **Partner 15** demonstrated that: a) Host and gender are important regulators of GAS NSTI, b) D2 host were most susceptible than B6 host, and female mice of D2 and other strains of BxD were more resistant than male mice, c) Age and body weight were additional host factors that were significant predictors of survival, d) Host genetics influences GAS burden, bacteraemia and dissemination, and e) forward systems genetics approach revealed IL-1β was the key proinflammatory mediator of susceptibility to GAS NSTI. This work was summarized in the paper of *ChellaKrishnan et al. PLoS Pathog 2016*.

Task 1.2 Determine the host susceptibility/outcomes in infection in relation to specific pathogens that cause NSTI associated with various co-morbidities.

In determining how variations in bacteria can alter the bacterial pathogenic strategy (**WP3, partner 1, 10, 15**), **Partner 15** validated using their murine model (**WP1**) of Staphylococcal infections how a single point mutation in phenotypic variants of ST22 MRSA strains can significantly alter virulence properties (*Mairpady Shambat, et al. Scientific Reports 2016*).

Partner 15 made a significant breakthrough in advancing knowledge of NSTI pathogenesis. Transcriptome analysis of skin samples from mice infected with GAS revealed previously unknown niches for GAS adaptations in the host during NSTI that might contribute to NSTI pathogenesis.

Task 1.3 Determine how variations in both the bacteria and the host genetic content, alter the bacterial pathogenic strategy and/or the host defence mechanisms.

In determining how variations in both bacteria and the host genetic content alter bacterial pathogenic strategy, studies in **WP1 (partner 15)** demonstrated:

1. Host responses to GAS isolates with varying virulence were determined on D2 mice most susceptible to NSTI. SpeB activity was determined on representative GAS isolates selected based on their *emm* type.

2. There were survival differences in D2 mice, 2 out 6 D2 mice infected with GAS 2002 (INFECT clinical isolate-M12 type) and 1 out of 4 of the D2 mice infected with GAS 8003 (M3 type, reference strain) were dead by 48 hours post infection.

3. D2 mice infected with GAS 8003 showed lower CFU compared to infections with other GAS isolates. This difference was statistically significant when compared with GAS 2006 and GAS 5448. Levels of IL-1β transcripts in the skin at the site of infection were comparable in response to each of the different GAS isolates. However, at 72h post infection, plasma levels of IL-1β was significantly higher in infection with M1-5448 isolate compared to infections with INFECT isolates 6026 and 6033 (M4 and M63 types respectively) ($P<0.05$).

4. Differential gene expression of upstream regulatory genes that drive the production of IL-1β was investigated by qRT-PCR in infected skin of D2 mice infected with GAS isolates with varying virulence. Not surprisingly, all the GAS isolates with varying virulence effectively induced inflammasome related genes and IL-1β, however, caspase 1 whose expression and activation are associated with IL-1β and IL-18 release was significantly downregulated. IL-18 expression mirrored Caspase-1 expression such that IL-18 was also significantly downregulated.

5. The expression changes in a few GAS genes (M protein, Mga, SpeB, CovR, CovS, SmeZ, HasA and RopA, gyrase, DNAseB) upon *in vivo* infections in D2 host were investigated by Partner 15. Consistently, GAS M protein and Mga showed high levels of expression in the host compared to other GAS genes.

6. In studying how host HLA-II allelic variations modulate susceptibility, **Partner 15** demonstrated distinct polarization into Th1/Treg subsets in their HLA-II transgenic mice models. These findings lay the foundation for an as yet unidentified role for HLA-II alleles in regulating Th1/Treg stability during NSTI.

Task 1.4 Determine the efficacy of novel therapeutic interventions in the NSTI murine model.

Partner 15 undertook several therapeutic interventions in the BxD and HLA-II transgenic murine models of NSTI, including IvIg, Ciprofloxacin, and Clindamycin in conjunction with immunomodulators. **Partner 15** evaluated the effect of the interventions in NSTI susceptible mice and measured their ability to reduce bacterial burden and dissemination, wound healing, lesion area and levels of pro and anti-inflammatory mediators (*Manuscripts under preparation*).

4.1.3.2 WP2 Clinical registry of NSTIs and associated isolate and biobank collection

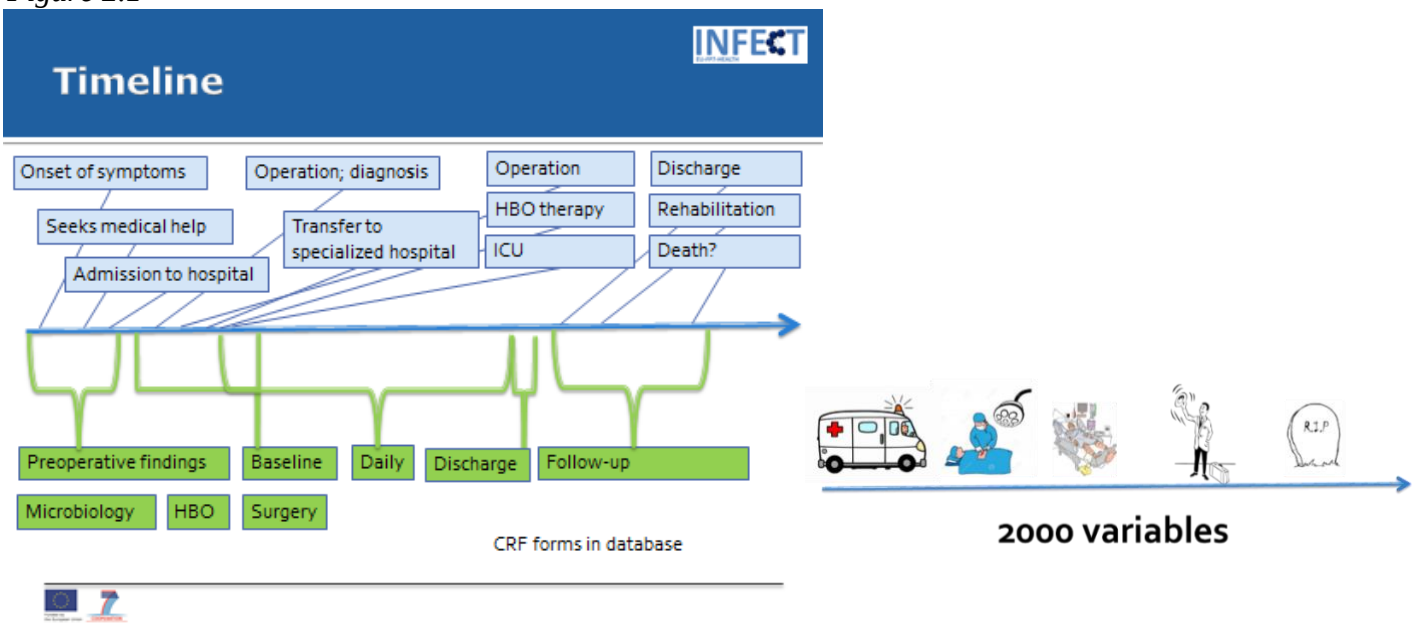
The Scandinavian study group of NSTs (partners2-6) will establish a prospective study of NSTI patients at major referral centers in Denmark, Sweden and Norway to obtain clinical samples that will be linked to detailed clinical information. The specific objectives were to:

- Establish a joint Scandinavian clinical registryMap quantitative trait loci (QTL) harbouring genes with a high statistical likelihood of modulating susceptibility/outcomes of NSTI,
- Prospectively enrol NSTI patients
- Collect clinical isolates and patient samples for the centralized biobank
- Generate evidence-based guidelines for classification and management of NSTIs

The results achieved in this WP are described below and relates to the WP specific Tasks:
During 2013-June 2017, a total of 525 patients were registered and screened within the INFECT cohort. In collaboration with partners in the WP2 group (here especially partner 2 (RH) and partner 6 (UiB) and partners 9 and 11, the database has been cleaned for data entry mistakes, missing data recovered. By re-evaluating and double checking data, uniformity in securing the proper registration of infectious agents (microbiology) in each and every patient has been performed. Cases of patient enrolments where diagnose was doubtful have been re-evaluated by the WP2 group and joint conference decisions made on their recruitment status.

The INFECT registry (electronic case report form) contains clinical data on all the patients, from debut of symptoms, number of surgical interventions- and descriptions, blood samples, treatment modalities, microbiology, length of stay and death (figure 2.1). In total, more than 2000 variables exist for each patient.

Figure 2.1



<u>Final reporting and official closure of database:</u>

The WP2 group (**partners 2-6**) have secured and exported all of the 409 clean patient data sets to the INFECT consortium to be worked with in corporation with **partners 1, 7-11 and 14**.

- Task 2.1b. Corporation with INFECT partners on clinical data analysis.

During 2017/18 the WP2 group team leader have hosted 4 international meetings with the relevant partners 2-6 and 9 and 11 as well as the INFECT consortium. A statistical- and clinical data analysis plan for the main clinical scientific reports has been created with partners 9 and 11 and published. Furthermore, the WP2 group have been cooperating with partners 9, 10, 11 and 14 using clean dataset of the 409 prospectively enroled patients.

Important achievements task 2.1 during this reporting period:

1. All 409 patient data sets have been delivered and the database has proven its operational capacity and feasibility.
2. The clinical data base has been re-verified, checked and declared as clean from data entry mistakes with final closure on the 1st of June 2018.
3. The first scientific reports are emerging – see publication list below.

Task 2.2 prospectively enrol NSTIs patients according to the estimated rate of patients to the different clinical centres.

In tabular form the inclusions of patients into the INFECT cohort were distributed as given in figure 2.2.

Figure 2.2.

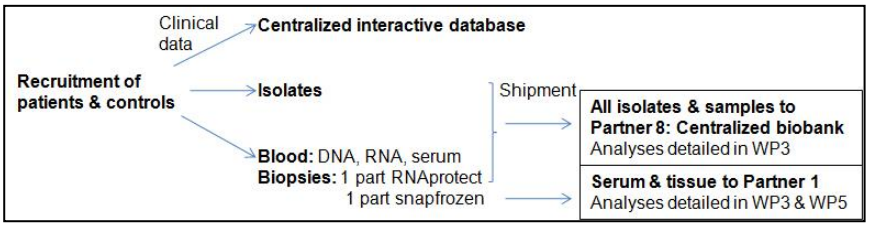
Data from previous retrospective analysis of patient inclusions from clinical partners in WP2				**Expected and ongoing INFECT study sampling from clinical partners in WP2**				
Region	Data collection years	No. of patients Mean per year	Estimated INFECT STUDY enrolments Per Year	INFECT STUDY ENROLMENTS 2013	INFECT STUDY ENROLMENTS 2014	INFECT STUDY ENROLMENTS 2015	INFECT STUDY ENROLMENTS 2016	INFECT STUDY ENROLMENTS 2017/18
Denmark Partner 2 RH	2005-2009	277 Per year: 55	50-60	48	72	61	55	31
Sweden Partner 3 SLL	006-2010	109 Per year: 21	20	4	17	22	11	9
Sweden Partner 4 BLS	2006-2011	27 Per year: 3	5	1	4	3	1	1
Sweden Partner 5 SU	2008-2010	28 Per year: 9	10	6	11	9	9	3
Norway Partner 6 UiB	2000-2009	100 Per year: 10	10-15	14	19	11	10	0

Important achievements task 2.2:

1. To sum up – we achieved our main target of including more than 400 patients during the 4½ year inclusion period. The rate of inclusion was stable throughout the study period.
2. This is the world's largest prospectively enrolled patient cohort from the INFECT multicentre study with 409 patients included, fully monitored with clinical data, microbiome, blood- and tissue samples and one year survival follow-up.

Task 2.3 establish local teams to collect clinical isolates and patient samples for the centralized bio bank on a 24/7/365 time basis for all patients included into the INFECT study.

- Task 2.3 a SOP's for tissue and blood sampling from patients with NSTI (Figure 2.3 below):



The WP2 group have jointly worked together with partners 1 and 8 during 2017 where all clinical partners have contributed. Shipping of Biobank samples have been continuously processed to partner 1 and 8 throughout 2017. Additional control blood samples have been collected during 2017/2018.

Important achievements task 2.3 during the study period:

1. A biobank has been established with **409 individual patient samples** from well-defined, prospectively enroled NSTI patients.
2. We have collected approximately 6000 samples for the entire INFECT biobank.
3. Scientific reports in collaboration between parterns 2-6, 1, 7 and 8 have been published.
4. Enrolment of INFECT controls: Each center contributed with 5 healthy controls – these have been collected at WP2 partners 3, 4 and 6. At Rigshospitalet in CPH (partner 2) we extended the ethical permits for 2018 including the formal use of the following patients included into the INFECT cohort, now as controls:
    - Project BIONEC collected control samples (65 surgical controls consisting of patients undergoing elective orthopedic operations, i.e. in non-infected, non-septic patients where the surgical trauma effect on biomarkers from the human innate immune response can be isolated, described and quantified in comparison to NSTI patients)
    - The project ENDOPAT collected control samples (20 diabetics + 20 non-diabetics) with similar co-morbidities as NSTI patients and receiving elective, hyperbaric oxygen therapy without having sepsis and septic-shock.
    - An additional 10 HBO patients with comparable comorbidities in non-NSTI patients without sepsis and septic-shock.
    - An additional 11 Healthy controls (3 day sampling as for INFECT NSTI patients.
    - An additional 14 patients with cellulitis were entered as controls from partner 8 UiB.

All together we have 155 individuals available as controls with SOP of sampling equal to the INFECT procedures.

Task 2.4 Generation of evidence-based guidelines for classification and management of NSTI's*Developing new clinical guidelines on the management of NSTI patients.*

Our work has identified several important markers for predicting patient outcome that may now form the basis for modelling and machine learing to improve bed-side clinical decision making. In corporation with partner 11 a random forest analysis on predictors for outcome have so far identified baseline INR, lactate, Noradrenalin max infusion, first SOFA score and URINE output to be good predictors. From the data analysis published to this date we have found the following; complement-pathway activation in NSTI implicating only baseline Ficolin-2 was associated with short- and long-term mortality (*Hansen et al J Innate Immun 2016*).; markers of inflammation in NSTI include pentraxin-3 (PTX3) (*Hansen et al Crit Care 2016*); RH) IL-1β and IL-10 had the strongest association with 30-day mortality (*Hansen et al Sci Rep 2017*). In addition, RH partner 2 conducted a clinical trial on the use of IVIG therapy in NSTI (*Bruun Madsen, et al Int Care Med 2017*) showing that IVIG had no benefit in NSTI of all aetiologies, but potentially in NSTI subgroups, such as those caused by streptococci.

Accordingly, this work will continue using the entire INFECT cohort of 409 patients giving more precise and better predictive tools at hand. Furthermore, these data will be integrated in modelling and results be part of management guidelines. In corporation with the Scandinavian Society of Anesthesia and Intensive Care – SSAI - and their international, scientific meeting in Copenhagen 2019 the INFECT consortium will organize a workshop on the subject NSTI – pathophysiology, characteristics and treatment. Now and future directions.

The workshop will be available as part of the pre-congress meetings which will be advocated on the SSAI congress website. The location of the workshop is at the University Hospital in Copenhagen Rigshospitalet on the 27th of August 2019. Organizer is partner 2 (RH) from the INFECT consortium in corporation with partner 6. The INFECT/NSTI workshop at the SSAI meeting will include:
Introduction to NSTI infections
Pathogenesis
Diagnosis, clinical management
The return to life – patient's perspectives
Future aspects: *Systems medicine approach to improve patient management in NSTI*
Guidelines and recommendations in NSTI -development, updates

The INFECT consortium will be hosting a workshop on the below scheduled meeting (figure 2.4) as a pre-congress event on the treatment of NSTI patients. Congress participants will be able to register for this workshop through the SSAI website during this autum 2018. This work will subsequently pave the way for a joint effort in creatingn SSAI based working group – holding significant number of members from the INFECT consortium - new treatment guideline based on the data genereated by the INFECT consortium.

Figure 2.4

4.1.3.3 WP3 Identification of host and pathogen traits affecting disease outcome

The main objective of this work package was to identify specific pathogen and host disease signatures. Specific objectives are to apply systems biology approaches to:

- Identify pathogen traits/pathways associated with disease outcome
- Perform comparative virulence and expression profiling of clinical NSTl isolates
- Explore tissue-specific properties of isolates from blood and tissue of the same patient
- Apply transcriptomics, proteomics and metabolomics to selected sets of host & pathogen samples
- Identify host traits/pathways that contributes to tissue pathology and/or systemic toxicity

A summary of key findings achieved in this WP are described below in relation to WP specific Tasks:

Task 3.1 Identifying pathogen signatures associated with disease outcome.

From the patient cohort (**WP2)**, 143 streptococcal isolates were obtained comprising 85 distinct strains originating from 42 mono- and 38 polymicrobial infections. The epidemiologic typing confirmed not only the dominant role of *S. pyogenes* but also the contribution of *S. dysgalactiae* ssp. *equisimilis* and of opportunistic streptococcal pathogens. Among *S. pyogenes* isolates the serotype M1 was dominating, but also the serotypes M3, M28 and M87 were isolated in considerable high numbers. *S. pyogenes* is able to secrete an arsenal of highly potent exotoxins. To evaluate their role during the acute phase of infection **partner 8** used a multiplex PCR to rapidly detect 21 streptococcal exotoxin genes. Analysis revealed that *speG* was present in the genomes of all analyzed *S. pyogenes* isolates. A minimum of 3 superantigens were present per strain. In contrast to the high prevalence of NSTIs caused by streptococci, only five cases were clearly caused by *S. aureus*. An analysis of 27 strains, including NSTIs, necrotizing cellulitis, myositis and cellulitis, caused by *S. aureus* from the French National Reference Center by a DNA microarray, covering 185 distinct genes did not reveal the presence virulence factors specific for NSTI isolates (**partner 10**). However, the genes encoding the cytotoxin PVL were strongly linked to primary skin infections as compared to colonization isolates.

To characterize the NSTI pathobiome, **partner 8** applied 16S ribosomal DNA profiling to tissue biopsies of 148 patients. Monomicrobial infections were primarily caused by *S. pyogenes*. *S. dysgalactiae* and *S. agalactiae*, pathogens rarely reported as associated with NSTIs, were frequently found, whereas Proteobacteria were rarely observed and only five cases of *S. aureus* infections were identified. Also *Clostridia sensu stricto*, historically considered important causes for clostridial myonecrosis, were only seldomly observed. Polymicrobial NSTIs were associated with varying bacterial communities, however they were typically composed of the Clostridiales genera *Parvimonas* and *Peptostreptococcus*, the Bacteroidales genera *Prevotella*, *Porphyromonas* and *Bacteroides* as well as *Fusobacterium* spp. Phylotypes could usually be identified down to the species level and several of these are common members of the healthy human microbiota. Their contribution to the overall disease etiology is still underestimated as they are difficult to detect by culture-based methods. Consistent with clinical reports, the bacterial diversity of the pathobiome associated with NSTIs of the extremities was significantly lower than of infections localized at the head/neck or anogenital region, indicating a higher frequency of polymicrobial infections in local proximity to the body's orifices. Accordingly, higher abundances of *Bacteroides* spp. were observed in anogenital infections than at those of the extremities, while *Prevotella*/ *Porphyromonas*/*Fusobacterium* exhibited increased abundances in infections of the head/neck area, highlighting the association between natural niches and contribution to NSTI pathophysiology for human pathobionts. To characterize microbial interactions within the NSTI pathobiome, **partners 8 and 9** inferred bacterial co-occurrence patterns via network analysis (**WP3, WP4**) and observed significant negative associations between pathogens causing monomicrobial NSTIs and the diverse genera observed in polymicrobial infections. This indicates competitive exclusion between human pathobionts and pathogens. Using divisive clustering of co-occurring genera, highly interconnected clusters of bacterial genera predominately associated with polymicrobial NSTIs were observed, indicating that synergism is essential for the establishment and progression of polymicrobial necrotic tissue infections.

Task 3.2 Comparative whole genome analysis to identify pathogen- and tissue-specific traits.
To identify bacterial factors associated with the development of NSTI, a comparative whole-genome sequencing approach was employed. During the course of the project, 66 bacterial isolates comprising 35 streptococcal NSTI-associated strains (26 *S. pyogenes*, 6 *S. dysgalactiae* and one *S. anginosus*, *S. constellatus* and *S. oralis*, respectively) and 31 *S. aureus* isolates (including 17 NSTI isolates) were sequenced (**Partners 8, 10**). In accordance with the serological typing, *in silico* typing and multi locus sequence typing of novel NSTI causing *S. pyogenes* isolates highlighted the dominance of the M1 serotype and ST28 in NSTI infections. These types are also the most frequent in northern Europe and in databases. A comparison of virulence factor profiles of NSTI causing isolates and NCBI reference genomes, revealed no obvious differences. A core of potent virulence factors, like, exotoxin *speB*, and anti-proteolytic factor *grab*, is present in most genomes. Accessory virulence factors like certain adhesins and exotoxins are only present in some of the novel and reference *S. pyogenes* genomes. No specific virulence patterns or other genomic features were observed in NSTI causing isolates. A detailed comparison of *S. pyogenes* M1 strains was then performed o identify genomic features causing substantial differences in virulence and pathology. Only minor genomic differences were observed and analysis indicated that the progression from locally restricted to a systemic dissemination and hypervirulence seemed to be caused by multiple copies of prophage-encoded virulence factors and a dysregulation of virulence gene expression by *covRS* and *rofA* mutations.

A comparative genome analysis of 28 *S aureus* isolates from NSTI and bacteremia revealed a high genomic variability, but no apparent discrimination between both invasive strain types (**partners 8, 10**). Phylogenetic analysis based on the core genome confirmed that NSTI and hematogenous strains were phylogenetically related. Further genomic comparison could not identify discriminant markers between the two groups. Due to the high number of variable genomic loci and the limited number of available genomes, distinct genomic features could not be identified. A link between distinct genomic features and the development of NSTI or bacteremia could not be observed and a combination of host factors and fine-tuned bacterial gene expression patterns likely determines disease outcome.

Task 3.3 Functional validation of implicated traits.
Bacterial NSTI isolates (*S. pyogenes, S. dysgalactiae, S. aureus*) were tested for their pathogenicity in appropriate human cell lines. *S. pyogenes* invasion into endothelial cells revealed M-type associated differences in invasion potential of *S. pyogenes* NSTI isolates. Interestingly, the predominant M1 serotype isolates displayed the lowest invasion potential indicating epithelial cell based persistence and spreading to be of the minor importance for pathogenicity of NSTI isolates.
Partner 1 reported the importance of neutrophils in disease pathology (*Snäll et al Sci Rep 2016; Uhlmann et al J Infect Dis 2016*). Analyses showed that *S. pyogenes*.strains were particularly potent triggers of neutrophil activation and degranulation, in particular by SpeB-negative strains. Further analysis, identified phosphoglycerate kinase as a stimulatory factor. This finding is of interest in light of reports of hypervirulent SpeB-negative *S. pyogenes* variants present during invasive infections, also found in NSTI patient biopsies (**WP5, partner 1**).

The analysis of S. *aureus* toxin-mediated tissue pathology revealed that PVL and α-toxin both contribute to tissue damage in cell specific manner (**Partner 1 and 10**). Of importance, toxin-mediated epithelium destruction could be inhibited by IVIG containing antibodies against α-toxin and PVL.

In order to decipher the respective role of *S. pyogenes* and *S. aureus* in the pathogenesis of necrotizing fasciitis and myositis, the virulence of the two species was compared in human keratinocytes and myoblasts (**partner 10**). *S aureus* isolates from NSTI exhibited strong adhesion and internalization rates into human keratinocytes and specifically into myoblasts. *S. aureus* from NSTIs also exhibited extraordinary cytotoxicity toward myoblasts which correlated with high levels of *psm*α and *RNAIII* transcripts. These findings suggest the unique property to invade and kill myoblasts to contribute to *S. aureus* NSTI severity.

Task 3.4 Functional profiling and identification of host traits/pathways contributing to tissue pathology and/or systemic toxicity.

We established a serology screening approach to identify risk factors for the development of a severe NSTI. We purified all 11 known streptococcal superantigens and investigated the role of toxin-specific antibodies in NSTIs caused by *S. pyogenes* and the potential beneficial effect of IVIG treatment. We identified a state of serologic susceptibility in NSTI patients during the earliest stages of the infection. Thus, all studied NSTI patients exhibited a deficiency in specific antibodies directed against the causative *S. pyogenes* strains and the majority of their exotoxins during the initial stage of the infection. We also showed that the clinical application of IVIG during the course of infection compensates the observed antibody deficiency, but is unable to halt the disease progression, once tissue necrosis has developed. These observations emphasize the requirement of pre-existing pathogen-specific antibodies to prevent the irreversible progression of tissue infections into severely spreading NSTI and urge further investigations on the beneficial effect of IVIG-based early phase intervention strategies to prevent the severe effects of this devastating bacterial infection.

We established a protocol for the purification of RNA, DNA and proteins from infected tissue samples, which was used to generate RNAseq data of 117 tissue samples from 91 patients (**Partner 8**). For 47 of these RNAseq datasets that contained a high amount of reads originating from both, the host as well as the infecting pathogen(s) we performed the quality filtering and mapping against the human genome and against a set of bacterial genomes guided by 16S rDNA based amplicon sequencing. All analyzed tissues were characterized by an acute inflammatory signature, however, our analysis showed significantly different gene expression signatures in monomicrobial *Streptococcus* spp. versus polymicrobial NSTI. The core inflammatory signatures comprised genes encoding important inflammatory mediators and key inflammatory mediators were also detectable in plasma. Detailed analysis of the genes differentially expressed between streptococcal and polymicrobial NSTIs showed a strong interferon-mediated immune response in patients infected by *Streptococcus* spp. Respective proteins could also be identified in plasma of these patients. Polymicrobial NSTIs in contrast were characterized by a significantly higher expression of host genes encoding extracellular matrix components. Thus, distinct transcriptional signatures within the infected tissue distinguish streptococcal from polymicrobial NSTIs.

Analysis of transcriptomes also indicates human pathobionts and pathogenic streptococci to utilize different strategies for nutrient acquisition during infection. Whereas streptococci use free carbohydrates, polymicrobial communities exploit host-derived proteins, peptides and amino acids. Besides different using nutritional strategies, differences were also observed in the virulence gene profiles. The transcriptome of *Streptococcus* spp. revealed genes encoding virulence factors as highly expressed during NSTI and clearly adhesion/invasion, immune modulation, proteolysis and toxin producing activities contribute to the pathogenicity of *Streptococcus* spp. NSTIs. In accordance with the diversity of the polymicrobial community a high diversity of virulence-associated domains was expressed in polymicrobial NSTIs. However, the virulence associated domains expressed were restricted to those mediating cellular adhesion and extracellular proteolytic activity. Moreover detailed analysis of the transcriptional profiles of distinct genera in polymicrobial NSTIs showed that they contributed to different extend and with different functionalities to the overall community pathogenicity. Thus, while pathogenic streptococci express a wide range of virulence factors that mediate the different steps of infection comprising colonization and evasion of the host immune response, the pathogenicity of pathobionts is dependent on the complementary activities of multiple bacterial genera, which enhances the virulence of the bacterial community. These differences result in distinct patterns of molecular host pathophysiology. Consequently, a rapid and accurate microbial diagnostic is necessary to optimize clinical treatment and to tailor intervention strategies towards the specific, etiology-dependent molecular pathophysiology.

4.1.3.4 WP4 Systems modelling and integration

The objectives of this WP were to:

- identify and unravel -both in the host and the pathogen- key molecular networks underlying NSTI;
- to develop a modular, physiopathological model framework capturing the main sets of host-pathogen interactions in the onset of disease; and, on the basis thereof,
- to identify biomarker sets of multiple nature for early diagnosis and novel therapeutic targets for intervention.

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The results achieved in this WP within INFECT are described below and relates to the WP specific Tasks:

Task 4.1 Data standardization and data management.
To handle the heterogeneity of the omics data generated through the infect project a Semantic Annotation Platform with Provenance, call SAPP, was developed. The goal of this platform is to handle various kinds of omics data and to integrate them to into a single multi-omics resource. The basis is built upon a Genome Ontology (GBOL) allowing to persistently store high-quality genomics data while making the entire resource FAIR. Through an integrated SPARQL endpoint we were to make the resource human as well as machine interoperable. Through this query language, all data becomes accessible through either an open or a private channel making it more efficient to find and reuse specific data for different research topics. Within INFECT, it enabled us to analyse the data obtained throughout the project and perform in-depth analysis on the host and the microbial environment in an efficient manner while preserving all information. This allowed us to further investigate host-pathogen interaction using different applications.

Task 4.2 Defining a blueprint of the host-pathogen interaction networks in NSTI.
The dual RNASeq from 106 patient biopsies (a concept and strategy proposed by **partner 9** and implemented by **Partner 8** in WP3) was mapped against both human and bacterial genomes to obtain gene expression of both the host and resident pathogens. The tool Kallisto was used to map the sequences against the human genome (GRCh38 release 91). The same sequences were mapped against several bacterial genomes using the published pipeline HUMAnN2. We found the most highly expressed genes in the patient biopsies to be involved in metabolic processes and in immune processes. Concerning pathogens we found no major genera of bacteria that are shared by all the biopsy samples. The 10 most abundant genera are *Streptococcus*, *Escherichia*, *Parvimonas*, *Peptostreptococcus*, *Porphyromonas*, *Propionibacterium*, *Solobacterium*, *Bacillus*, *Prevotella* and *Filifactor*.

The gene expression of the host and that of the pathogens were integrated using multivariate approaches, providing information about the host response to certain bacterial groups or functions. All the 106 samples were used for this integration to build an interaction network, which is highly clustered into sub-networks, with several bacterial genes correlated with one human gene. Analysis of the bacterial genes show that 63% of the bacterial genes in the network are from the species *Streptococcus*

pyogenes which is a known pathogen implicated in NSTI. For instance, there are 5 human genes (NAMPTP1, SRGN, IL18R1, ZPR1, ZNF302) that correlate with this species. The *S .pyogenes genes* in the three clusters correspond to different functions such as biosynthetic processes which could be important for biofilm formation. The process of forming a biofilm is being investigated by **partners 1, 9 and 14** for being an important defence mechanism of the invading pathogens. The human gene correlated with this process is PEAK1, which is known to modulate cell adhesion and is responsive to bacterial load. Several manuscripts exploring host-pathogen interactions are being prepared at the time of reporting.

Data from those 106 biopsies for which gene expression was made available were further analysed to look for any differences in the host response to the NSTI in respect to the presence of comorbidities, in particular type 1 and 2 diabetes. Based on various factors (such as ages, sex, surgery) 17 NSTI patients who do not have diabetes were matched for comparison. We did not find significantly differentially expressed genes but on a gene co-expression network level, we found striking differences between the two groups. Co-expression networks were built from gene expression profile of diabetic and non-diabetic NSTI patients and network modules were then analysed for functional information. We found several modules having similar functional properties even if the genes involved were not the same, indicating dysregulation of the molecular machinery. A module of interest was one pertaining to the GO term 'Striated Muscle Contraction' which is of interest in the context of diabetes because it is known that muscle functions and physiology are altered in diabetic patients.

Task 4.3 Dynamic models of progression of infection and immune defense.

Partners 9 and 14 have deployed a genome-scale metabolic model for the mammalian host as well as the modeling of specific regulatory aspects of the host. A predictive cell-specific model has been realized that can be used to generate valuable predictions. Suitable approaches to handle missing data and data pre-processing were implemented in collaboration with WP2 to ensure the best quality of the final dataset.

Generic metabolic models were also built for the following species based on their genes sequence: Human generic model, Mouse generic model, *S. pyogenes* generic model together with generic compartment based community models for Human (bacteria community model) and Mouse (bacteria community model).

Gene expression data from biopsies from NSTI patients were used to build NSTI vs. normal specific metabolic models for all the generic models we generated before for a total of more 100s of metabolic model which were compared between the generic and the specific metabolic models. Using the flux variability analysis algorithm, we listed the differences in the fluxes of the different species combinations in each of the samples and compared them with the generic metabolic models possible fluxes; results are being interpreted.

To simulate the biofilm formation of *S.* pyogenes we built tools to simulate and graphically show the biofilm formation in a 3D cellular automata model (**Partner 14**). To perform the simulations we used our developed media prediction tools (KOMODO and the growth media prediction tools) we used these media to give predictions based on the generated model and perform manual curation of our *S. pyogenes* metabolic model. The analyses were supported by multivariate statistics (Partner 9).

Statistical algorithms have been tailored for identification of patient-bacteria/treatment associations in clinical record data. Two statistical analysis plans have been published to test hypotheses generated in WP2, one on overall study of the cohort with a particular interest on death outcome and subset of patients with diabetes. The different statistical models used were survival analysis, logistic regression and linear model.

Task 4.4 Building a modular, pathophysiological modelling framework of the host-pathogen signatures.

A death prediction model developed jointly by **Partners 11 and 14** was developed using selected "early" clinical variables, like baseline, demographic and early measurements. From those, a set of best predictor variables were selected using Random Forests. For the actual prediction different machine learning tools were employed: Random Forest as well as Support Vector Machine. The prediction model was included as a feature for a prototype mobile app, which allows users/clinicians to input the values for a small set of predictive early parameters in order to receive a probability of patient death/amputation after a certain time period post diagnosis, e.g. after 30 days. In another approach, the set of clinical variables was combined with gene expression data from the RNA-Seq experiments, though with less success than using only clinical parameters.

During the 5 years of the project, **Partners 9, 11 and 14** have developed multiple bioinformatic tools to handle, visualise and investigate bacterial data and information. In addition to the above-mentioned applications SAPP, GBOL, SynDI, tools developed were:

NInA (Network Integration and Analysis) - a tool for synchronous visualization of multiple biological networks and pathways in NSTI-specific context to facilitate understanding the molecular complexity of the disease. *MTA (Metabolism Transformation Algorithm)* – an algorithm that builds tissue/condition specific models based on Gene Expression. Gene expressions were the data collected in the INFECT project in cases of NSTI; *EDGE* – a research to predict the functional implication of over expression of metabolic genes, we planned to use this research in predicting possible situations within an NSTI situation. *KOMODO (Culturing the unculturables)* - an algorithm that assists in finding growth media for unculturable and difficult to grow bacteria. *Predicting growth media for bacteria* – using metabolic modeling finding minimal media that can enable growth of bacteria since we expected that some of the bacteria we would encounter will be hard to grow in-vitro and we thought of using this algorithm to assist with that together with the next research listed. *Gene promiscuity prediction* – Gene promiscuity is one of the methods of bacteria to gain antibiotic resistance. Knowing which gene's promiscuity can enable such resistance is important when suggesting antibiotic treatment.

Furthermore, **Partner 9** has developed and refined a number of computational and statistical methods and models to be deployed in the analysis of the multidimensional data generated in WP3 and analyzed in WP4. All these are listed in Table 4.

Altogether, the various models and analyses developed in WP4 have enabled to build a modular framework that was (and will be, beyond the duration of the project) pivotal to ascertain the host-pathogen interactions as measured in the experimental and clinicalk WPs and to pin-point and uncover underlying mechanisms in the clinical context.

4.1.3.5 WP5 Host-pathogen interactions at the tissue site of infection (NSTI tissue biopsies)
The objectives of this WP were to validate results obtained in WP1-4, and to identify mechanistic action of novel therapeutic strategies. This was done by **partner 1** throuh analyses of host-microbe interaction at the local site of infection, i.e. in patient tissue biopsies, in particular to:

- test model-driven hypothesis and data on host and pathogen traits from WP1 and WP3-4 by detection of implicated factors in patient tissue biopsies
- determine mechanistic action of the therapeutic strategies HBO and IVIG by analyses of patient biopsies pre- and post-therapy
- elucidate whether there are different host-pathogen interactions in NSTIs caused by varying pathogens.

The results achieved in this WP are described below in relation to the WP specific Tasks. The results are based on analyses of patient tissue biopsies collected from enrolled NSTI patients in **WP2**. As **WP2** identified the main causative agents of NSTI to be *S. pyogenes*, but also group G Streptococcus (SDSE) were a common cause, as well as a few *S. aureus* cases. Therefore, the WP5 analyses have focused on immune-stainings and analyses of the biopsies from patients infected with *S. pyogenes*, group G streptococcus (SDSE), and *S. aur*eus; in total **WP5** has analysed >180 biopsies.

Task 5.1 Assessment of predefined bacterial and host traits hypothesised to be involved in NSTIs.
A panel of markers for bacterial load and host inflammatory responses was selected based on previous publications (*Thulin et al PLoS Med 2006; Sundén-Cullberg et al Crit Care Med 2005)* in which these factors had been implicated as markers of severity of infection. The immunostaining data were analysed in relation to microbiologic aetiology, disease progression and outcome as well as in relation to treatment protocol (further elaborated on in task 5.4).

The results showed that there are variations in different biopsies but there were no significant differences in tissue severity (here defined by HMGB1 levels) or inflammation (here defined by IL8 levels) between the different pathogens. The data illustrates how varying the host response is in individual NSTI even when categorized dependent on microbial aetiology; supporting the concept of patient stratification based on host immune status. The results showed a trend to lower inflammation, yet equal tissue severity, in *S. aureus* infected tissue was noted. This supported our hypothesis that the pathophysiology is microbe-dependent, and was further explored in **WP3**.

A key study of **WP5** here was that of HMGB1 as a potential novel marker for NST1 severity. Partner 1 demonstrated through an analyses of NSTI biopsies and compared these to the uncomplicated tissue infection erysipelas. The data showed that HMGB1 significantly correlate with degree of inflammation and severity of infection. Taken together, the findings provide the first in *vivo* evidence that HMGB1 is abundant at the local site of bacterial soft tissue infections and its levels correlated to severity of infections; hence, indicating its potential as a biomarker for tissue (*Johansson et al Front Cell Infect Microbiol. 2014).*

Task 5.2 Identification of novel bacterial and host protein determinants in tissue identified in the integrated model.

Key findings of this task include:

- Contribution to the study headed by **partner 15** (**WP1**) where systems analyses revealed L1β as a key determinant of NSTI severity in mice. P**artner 1** used gene expression analyses of infected patient tissue as well as protein analyses of plasma samples and thereby provide data

supporting patient data that strengthened the clinical relevance of the murine data. (*Chella Krishnan et al. PLoS Pathogens 2016**)**.

- Analyses of clinical data implied a potential biofilm problem (**WP2**). Through analyses of patient biopsies (**WP5**), partner 1 could provide evidence of *S. pyogenes* biofilm formation in NSTIs. This emphasized the urgent need for biofilm to be considered as a potential complicating microbiological feature of GAS NSTI and, consequently, reconsideration of antibiotic treatment protocols. (S*iemens et al. J Clin Invest INSIGHT. 2016). **This is one finding of INFECT that resulted in changed clinical practice***!
- Comparison of RNA-seq data of biopsies from *S. pyogenes* NSTI patients and skin from healthy donors identified a large number of significantly differentially expressed genes (**WP3,WP4, partners 1, 8, 9, 11**). Reactome pathway analysis on this gene set identified a set of over-represented immune system. Notably, the same host signatures were significantly overrepresented in infected vs uninfected skin tissue model (**WP6**, **partner 1**), as well as in the infected murine model (**WP1, partner 15**).

  The signatures were further verified through a multiparameter imaging workflow established by **partner 1** in **WP5** to enable phenotyping of immune cells at the tissue site. Staining of patient biopsies and infected skin tissue model (**WP6**) provided mechanistic insight to the implicated signaling pathways (*Snäll et al, manuscript in final preparation*).
- Neutrophil degranulation was implicated in the RNAseq analyses (**WP3, WP4**), which was exciting in light of the studies in **WP5** (**partner 1**) demonstrating neutrophils as key players in NSTI and in *S. pyogenes* NTSI in particular (*Snäll et al Sci Rep 2016; Uhlmann et al J Innate Immunity 2016*), including also identification of a novel streptococcal factor PGK with potent neutrophil stimulatory capacity (also relating to task 5.3) (*Uhlmann et al J Infect Dis 2016*).

Task 5.3 Tissue expression of bacterial virulence factors (both protein and gene expression).

- Genes encoding for selected bacterial factors, i.e. the cysteine protease SpeB, streptolysin O (slo) and S (sagA), streptokinase (ska), and the M-protein (emm), as well as key regulatory systems (RofA, Mga, Ihk/Irr) were assessed by qRT-PCR analyses of tissue biopsies of NSTI patients, many of which were upregulated in the tissue setting (*WP5, partner 1*). (*Siemens et al, J Clin Invest INSIGHT, 2016; Siemens et al, Sci Rep 2015*). The results were of importance for the understanding of which factors/regulatory systems promotes biofilm formation and or cytotoxicity resulting in tissue damage. *Siemens et al Sci Rep 2016*, reports that the cytotoxin SLO is a key determinant of tissue damage associated with SDSE NSTI (**WP3, WP5 and WP6**).
- The role of SpeB in infections has long been debated. This is due to the fact that this important protease degrades several important host proteins but also endogenous virulence factors. Here we studied this through culture of tissue biopsies directly on casein plates, which readily identifies colonies expressing SpeB or not. The data showed that all biopsies contained both SpeB+ and SpeB- negative variants identifying a phenotypic variation in the same infectious site (*Siemens et al J Clin Invest INSIGHT 2016*). This is of interest in light of **partner 1**'s studies on neutrophil activation and the identification of a novel neutrophil activator PGK, which proved to be susceptible to SpeB degradation (**WP3**).

Task 5.4 Documentation of therapeutic efficacy.

For this task analyses of snap-frozen tissue biopsies collected pre- and post-IVIG and/or HBO treatment were done to evaluate whether there are variations in host and bacterial factors that might be contributed by the treatment. As there are many potential confounders (among others the fact that the tissue is from necrotic sites involving different tissue types, different surgical sites and collection on different days post enrollment) in this analyses, the results can only provide an indication of

mechanistic action. A large percentage (77%) of the stained S. pyogenes infected NSTI patients had received IVIG as well as HBO (74%). Treatment was started during day 0 or 1. HMGB1, IL8 and bacterial load are shown in patients categorized to treatment with IVIG/HBO (100% received IVIG, n=3, 10% did not receive HBO) versus no IVIG (2 also received HBO, 7 did not). The data show that there was no difference in HMGB-1, IL8 or bacterial load over the treatment days. Samples from the same patient were also analysed in a matched analyses, but also here we did not see any differences over time. This should not be interpreted as the treatments having no effect but likely that the tissue biopsy collection was too diverse to allow for this type analyses, as the tissue collection protocol (and ethical permit) only allowed for collection of tissue that was necrotic and needed to be surgically removed. Hence, a tissue area that had improved and had no necrosis could not be collected.

4.1.3.6 WP6 Artificial tissue modeling

The objectives of this **WP (Partner 1)** were to:

- Elucidate the role of the well-defined bacterial toxins, e.g. Superantigens, cytotoxins, streptolysins, PVL, α-toxin etc., in the pathogenesis of NSTIs.
- Identify and confirm tissue-specific bacterial disease traits.
- Confirm and validate patient and murine data regarding pathogenic mechanisms and factors contributing to severity of NSTIs.
- Test novel therapies (HBO/IVIG) in order to obtain data on dosage and mechanistic actions.
- Identify novel molecular targets and test their therapeutic potential.

To be able to address the WP6's objectives we initiated the work by establishing an organotypic tissue model resembling human skin. This important milestone was achieved at an early stage of the project (D6.1, MS24). The model proved useful for modelling of infection with streptococcal and staphylococcal strains collected in INFECT. 1 x 10^6 colony forming units of bacteria was found to be optimal for infections up to 48 hours, leading to moderate-severe tissue damage. At different time points, 8, 24 and 48 hours post infection; tissue culture supernatants, as well as non-infected or infected tissue models were harvested and subsequently processed for histology and immunofluorescence analyses, protein detection, RNA sequencing and bacterial growth.

A summary of the key findings of **WP6 (Partner 1)** is described in relation to the WP specific tasks:

Task 6.1 Identifying toxins involved in NSTIs
Using *Streptococcus dysgalactiae* subsp *equisimilis* (GGS) isolates to infect the skin tissue model, revealed that three invasive NSTI strains as well as one non-invasive strain derived from an uncomplicated wound infection, were all able to colonize and replicate in the model tissue. While the three invasive strains caused severe tissue damage characterized by substantial epithelial disruption and detachment, which significantly increased over time, the non-invasive strain induced mild to moderate epithelial disruption. Furthermore, higher SLO activity was identified in invasive, as compared to non-invasive strains, whereas the reverse was true for SLS activity. A positive correlation between SLO activity and keratinocyte cytotoxicity was found. Thus, pointing towards important differences in virulence between GGS isolates potentially dictating disease outcome (**Partner 1, 6**) (*Siemens et al, Scientific Reports, 2015*).

Differences in cytotoxicity was also observed between Staphylococcal isolates, as evident by the relatively sever tissue damage caused by *S. aureus* strains harboring tyrosine in position 223 of AgrC. Thus, a naturally occurring single amino acid substitution (tyrosin to cysteine) at position 223 of AgrC determines starkly different *S. aureus* virulence phenotypes, e.g., cytotoxic or colonizing, as evident in both *in vitro* (**partner 1, 10**) and *in vivo* (**partner 1, 15**) skin infections *(Mairpady-Shambat et al, 2016, Scientific Reports*).

Task 6.2 Dosage and mechanistic action of therapeutic strategies
In WP6, the two main therapeutic strategies to be tested were hyper baric oxygen (HBO) and human intravenous immunoglobulin (IVIG) treatments. The skin tissue model tolerated the HBO procedure, and this work will continue post INFECT to evaluate the effect of HBO, by the analyses of in *in vivo* (patients) findings in combination with *in vitro* experiments. To test the effects of IVIG on bacterial exotoxins, we first preformed infections of a well-established 3D human lung tissue system (previously developed by partner 1) with *S. aureus* isolates in the absence and presence of IVIG. This showed that IVIG potently neutralize *S. aureus* exotoxins and reduce the tissue damaging properties of S. aureus

(**Partner 1, 10**) (*Mairpady-Shambat et al, 2016, Disease Models and Mechanisms*). This experimental set was transferred to the skin model, and we investigated the efficacy of IVIG to reduce the cytotoxic activity of GAS and GGS-derived exotoxins. This revealed that IVIG partly reduced, in a concentration dependent manner, the cytolytic effects of GAS-derived exotoxins, but had little effect on GGS-derived exotoxins. Taken together, this indicates that the cytotoxicity mediated by GAS can be targeted by IVIG. However, this needs to be investigated further, and the clinical data analysed in this respect (ongoing studies).

Task 6.3 Tissue modeling and systems biology
The first version of tissue model contained human skin keratinocytes and fibroblasts, and was therefore further developed to include also human monocyte-derived macrophages, which are of critical importance in the pathogenesis in bacterial infections. These models were infected with different INFECT GAS isolates, and subsequently processed for RNA sequencing to generate transcriptomic data on human skin infected for different length of time (**Partner 1, 8, 9**). Transcriptional profiling of *S. pyogenes* (GAS)-infected vs non-infected skin tissue models revealed alterations in inflammatory signatures, similar to that identified patient tissue biopsies (**WP5**) (Manuscript in preparation). This unique set-up will be used to gain further knowledge of changes in gene-, protein- and metabolite-expressions during Streptococcus and Staphylococcal infections.

Task 6.4 Host determinants of inflammation.
Using the skin tissue model Partner 1 investigated the inflammatory course during S. pyogenes infection. Since the transcriptional analysis and the subsequent bioinformatic analysis suggested that macrophages are involved in and/or alternatively affected by the course of infection, **partner 1** (**WP5 and WP6**) established the ability to study macrophage's phenotype / functionality with multiparameter imaging at the tissue site. Confocal microscopy analyses of infected 3D skin tissue models revealed a shift in inflammatory cellular status.

The usages of skin models to recapitulate NSTI infections also showed that IL1 beta (mRNA and protein) was increased in infected skin models compared to uninfected skin models. This finding verified the gene expression analysis of *S. pyogenes*-infected mouse tissue performed by **partner 15** and that identified IL-1 beta as a key regulator likely to contribute to NSTIs (**WP1,** PLoS Pathogens, 2016). IL-1 beta was also confirmed to be upregulated in skin tissue and plasma of NSTI patients (**WP2 and WP5**, **partner 1, 2**). Together this has led to the identification of the IL-1 beta network as a key network involved in modulating the differential susceptibility to NSTIs *(Chella Krishnan, et al., PlosPathogens, 2016).*

The skin tissue model was also instrumental in dissecting the properties of *S. pyogens* to form biofilm in skin (**WP2, WP5, WP6, partner 1,2, 6**). The Nra gene regulator was found to be one key component (*Siemens et al., J Clin Invest INSIGHT, 2016*) in biofilm formation. Biofilm was verified in patient tissue biopsies and associated with massive bacterial load, and a pronounced inflammatory response, as well as clinical signs of more severe tissue involvement. This novel finding of GAS biofilm formation in NSTIs emphasize the urgent need for biofilm to be considered as a potential complicating microbiological feature of NSTI and consequently has led to change in clinical practice.

Further exploring determinants of infection, **Partner 15,** found evidence that adipocytes, important for metabolism, are infected with *S. pyogenes* in the experimental model of NSTI. In addition, metabolomics analysis of INFECT plasma samples (**WP3, partner 1,9**) indicated alter metabolism during infection. Therefore, **partners 1** and **15** initiated a collaboration on exploring the role of adipocytes in the inflammatory response in NSTI. As a result, a skin tissue model was developed which supports introduction of adipocytes. Utilizing these 3D skin tissue models, will enable partner 1 and

15, to address the role of adipocytes in the pathogenesis of NSTI. Together this demonstrates how amendable this model system is and can be modified based on different needs by the users.

Main achievements/impact of WP6 :

- Novel tool for biomedical research
    - Recapitulating infection for the generation of gene, protein and metabolite expression
    - Enable testing of therapeutic strategies in a human setting
    - Customization – different cell types can be introduced
- Verification of *in vivo* findings
    - *S. pyogenes* biofilm – awareness, bacterial persistence, treatment, antibiotic stewardship
    - IL-1 beta as a key network in NSTI – novel treatment target

4.1.3.7 WP7 Translation of results into prototype for novel diagnostics

Translation of obtained results (clinically relevant pathogens and pathogenic disease traits) into a clinical compliant diagnostics approach by applying compact sequencing (pathogen detection – DNA) and compact profiling (disease traits – antigens) – both multiplex diagnostic technologies. The tool will be designed to support therapeutical decision as for example antibiotics treatment, decision as for example antibiotic treatment, decision for surgery or closing wounds.

The results achieved in this WP are described below and relates to the WP specific Tasks:

Task 7.1 Test specifications.

All major requirements from clinicians have been collected and summarized in short (preliminary) product specifications which served as guidelines for test development, discussion with interested clinicians or the interested public.

Partner 12 (Anagnostics Bioanalysis GmbH), as the WP leader at that time (later replaced by Cube **partner 16**), intensively communicated with all clinical partners **(2,3,4,5,6**) to outline general requirements for diagnostics in case of (suspected) NSTI. All hospitals (except **partner 5)** were personally visited **partner 12**, the situation on-site evaluated and the project partners interviewed. These personal interviews delivered a clearer picture on what procedures and efforts are acceptable in clinical practice and what questions should be answered. Taking into account technical feasibility and experiences of **partner 12**, initial specifications were outlined (partly as assumptions).

Even if the list of pathogens, the list of necessary antibiotic resistance markers and inflammation markers was to be expected to be extended or altered during the course of the project, the cornerstones of diagnostics tests have been set out in an specification document.

Task 7.2. Implementation of test prototypes.

After specifying the diagnostic tests to be implemented (see above), an iteration of design, implementation and testing (preliminary verification) was executed. Along with the development of the tests themselves, the underlying assay technologies have been (further) developed as well.

The work itself fell into bioinformatics (design of microarray probes and PCR-primers), material testing (above all various antibody – antigen combinations, antibody – secondary antibody combinations), method development (protocol development), (further) development of hard- and software (Cube developed a new device between 2016 and 2017), development of production technologies (coupling of molecules on hybcell surface, printing of microarray onto hybcell surface, drying of reagent mix into cartridge, etc.). So, the work was mainly work related to laboratory experiments.

Along with the development (as a regulatory requirement) a proper documentation of the development and its results – including risk management – had to be done.

To be prepared for verification and later clinical validation, 0-series of the (test)kits had to be produced (figure 7.1). First, the DNA-based tests for pathogen ID and the biomarker test (in an preliminary version) have been developed.

Figure 7.1. Example of testkits developed and produced ready for verification (Pathogens xA)

Later, after Cube **partner 16** took over, new versions of the tests had been developed (broader panel of pathogen ID test (hybcell Pathogens DNA xB); resp. patientmonitoring test with a slightly changed set of markers, with a reduced number of pipetting steps (only pipetting sample into cartridge) calibrated for whole blood to enable the application of the technology on the intensive care ward itself (figure 7.2).

Figure 7.2. Usage of the biomarker test: just pipetting of 100µL whole blood into the hybcell cartridge.

After the above mentioned iterations, the test panels offered as either hybcell Pathogens DNA xB or hybcell Patientmonitoring Blood xA are summarized in Figrue 7.3 and 7.4.

	Family / Classification	Genus	Species
Bacteria	Burkholderiaceae	Burkholderia	***Burkholderia cepacia complex***
			Burkholderia pseudomallei
	Neisseriaceae	Neisseria	***Neisseria meningitidis***
	Campylobacteraceae	**Campylobacter**	
	Heliobacteraceae	Helicobacter	***Helicobacter pylori***
	Enterobacteriaceae	Citrobacter	***Citrobacter koseri***
			Citrobacter freundii complex
		Enterobacter	***Enterobacter aerogenes***
			Enterobacter cloacae complex
		Escherichia	***Escherichia coli***
		Klebsiella	***Klebsiella oxytoca***
			Klebsiella pneumoniae
		Morganella	***Morganella morganii***
		Proteus	***Proteus mirabilis***
		Salmonella	***Salmonella enterica***
		Serratia	***Serratia marcescens***
		Yersinia	***Yersinia enterocolitica***
			Yersinia pseudotuberculosis complex
	Legionellaceae	Legionella	***Legionella pneumophila***
	Pasteurellaceae	Actinobacillus	***Actinobacillus pleuropneumoniae***
		Haemophilus	***Haemophilus haemolyticus***
			Haemophilus influenzae
	Moraxellaceae	Acinetobacter	***Acinetobacter calcoaceticus / baumannii complex***
		Moraxella	***Moraxella catarrhalis***
	Pseudomonaceae	Pseudomonas	***Pseudomonas aeruginosa***
			Pseudomonas syringae
	Xanthomonadaceae	Stenotrophomonas	***Stenotrophomonas maltophilia group***
	Fusobacteriaceae	**Fusobacterium**	
			Fusobacterium nucleatum
			Fusobacterium necrophorum
	Corynebacteriaceae	Corynebacterium	***Corynebacterium diphtheriae***
			Corynebacterium jeikeium
			Corynebacterium ulcerans
	Propionibacteriaceae	**Propionibacterium**	
	Bacillaceae	Bacillus	***Bacillus subtilis***
	Listeriaceae	**Listeria**	
	Staphylococcaceae	**Staphylococcus**	
			Staphylococcus aureus
			Staphylococcus epidermidis
			Staphylococcus haemolyticus
	Enterococcaceae	Enterococcus	***Enterococcus faecalis***
			Enterococcus faecium
	Streptococcaceae	**Streptococcus**	
			Streptococcus pneumoniae
			Streptococcus pyogenes
			Streptococcus anginosus group
			Streptococcus dysgalactiae
			Streptococcus agalactiae
	Peptoniphilaceae	Finegoldia	***Finegoldia magna***
	Prevotellaceae	Prevotella	***Prevotella intermedia***
	Borreliaceae	**Borreliella**	
			Borreliella burgdorferi
Resistance	**Resistance**		**Resistance marker genes**
	Vancomycin resistances		***vanA***
			vanB
	Methicillin resistances		***mecA***
			mecC

	Family / Classification	Genus	Species
Fungi	Ajellomycetaceae	Blastomyces	***Blastomyces dermatitidis***
		Histoplasma	***Histoplasma capsulatum***
	Arthrodermataceae	Arthroderma	
			Arthroderma benhamiae
			Arthroderma otae
		Epidermophyton	***Epidermophyton floccosum***
		Microsporum	***Microsporum gypseum***
			Microsporum audouinii
		Trichophyton	***Trichophyton interdigitale /mentagrophytes***
			Trichophyton rubrum / violaceum
			Trichophyton verrucosum
			Trichophyton tonsurans
			Trichophyton soudanense
	Onygenales incertae sedis	**Coccidioides**	
	Aspergillaceae	**Aspergillus**	
			Aspergillus clavatus
			Aspergillus flavus
			Aspergillus fumigatus
			Aspergillus niger
		Penicillium	***Penicillium digitatum***
	Debaryomycetaceae	Debaryomyces	***Debaryomyces hansenii***
		Candida	
			Candida albicans
			Candida dubliniensis
			Candida parapsilosis
			Candida tropicalis
	Saccharomycetaceae	Nakaseomyces	***Candida glabrata***
		Saccharomyces	
			Saccharomyces cerevisiae
		Zygosaccharomyces	***Zygosaccharomyces rouxii***
	Pichiaceae	Pichia	***Pichia kudriavzevii***
	Microascaceae	**Scedosporium**	
	Nectriaceae	Fusarium	***Fusarium oxysporum species complex***
			Fusarium solani species complex
	Pneumocystidaceae	Pneumocystis	***Pneumocystis jirovecii***
			Pneumocystis murina
	Cladosporiaceae	**Cladosporium**	
	Tremellaceae	Filobasidiella	***Cryptococcus neoformans***
			Cryptococcus gattii

Figure 7.3. Tested pathogens (bacteria, fungi) and resistance genes of hybcell Pathogens DNA xB.

Analyte	Standard value	Working Range	Unit
Ceruplasmin	25 to 60	10 / 500	µg/mL
CRP	< 6	5 / 500	µg/mL
Cystatin C	~ 0.6 to 1.5	0.5 / 10	µg/mL
Myoglobin	25 to 55	10 /100	ng/mL
NGAL (Lipocalin-2)	~ 30	150 / 10,000	ng/mL
Procalcitonin (PCT)	< 0.5	1 / 100	ng/mL
Plasminogen	~ 200	tba	µg/mL
Serum amyloid A (SAA)	< 5	2 / 700	µg/mL
Tachykinin (Substance P)	< 1	1.5 / 50	ng/mL

(*) As limits of quantification can vary from lot to lot, the lot-specific working ranges can be found on our website: www.cubedx.com

Fig 7.4 Tested biomarkers (inflammation, organs, coagulation) of hybcell Patientmonitoring Blood xA

Task 7.3Verification of test prototypes.

Within this task the basic analytical features and the requirements of the specification document(s) of these tests have been tested and verified by Cube (partner 16). The result of this verification was summarized in (verification) reports. Based on these verifications, the tests were improved if necessary and finally released for further clinical validation.

Task 7.4 Clinical validation of tests.

155 plasma samples collected by partner 2 (RH) have were tested with the hybcell Patientmonitoring Blood xA and comparative tests by partner 2 (RH). For the parameters CRP, Cystatin C, Myoblogin, NGAL and PCT, already established tests have been used for comparison. For these biomarkers, the correlation was acceptable to good (Figure 7.5). For the other biomarkers ELISA products were purchased and established by the RH laboratory. No satisfactory correlation could be established.

Figure 7.5.	***Correlation***	***Performance***
CRP	**47%**	**Acceptable**
Cystatin C	**53%**	**Acceptable**
Myoglobin	**84%**	**Good**
NGAL	**60%**	**Good**
PCT	**75%**	**Good**

Further 147 plasm samples samples from the INFECT biobank (from partner 5 (SU) and partner 6 (UiB) have been tested. The collective was basis for clinical examination:

Prognosis of acute kidney injury (AKI):

All biomarkers (and combinations) have been examined with help of a ROC (Receiver Operating Characteristic) curve, how they would prognose acute kidney injury (AKI).

Myoglobin showed the highest AUC for a single marker, Myoglobin and Cystatin C (as combination slightly improve the AUC (from 0,85 to 0,86).

Prognosis of mortality:

The SOFA score shows the highest AUC, followed by the markers myoglobin (cardiac / muscle destruction marker). A combination of markers does not improve the AUC.

From sample to result, cost and ease of use:

The test has a turnaround time of approximately 13 minutes (for all markers), and the cost is € 33 (=9 markers).The usage on the intensive care unit seems feasible.

Task 7.5 Compilation of clinical results.

A public report has been compiled from the clinical results obtained mainly in task 7.4.

4.1.3.8 WP8 Dissemination and exploitation

The ultimate objectives of WP8 was to ensure an efficient dissemination and exploitation of knowledge generated within the INFECT project, the specific aims of WP8:

- prepare and update information for the external open access website
- disseminate clinical guidelines
- disseminate scientific advances to researchers and SMEs
- prepare information material for patients, medical staff and society in large
- provide training for medical staff

The ultimate objectives of work package 8 ("Dissemination and Exploitation") was to ensure that the knowledge produced within INFECT was efficiently disseminated (**all partners**). This has been achieved by using a variety of dissemination activities as detailed in tables A1 and A2. A few key achievements of this WP are described below in relation to the WP specific Tasks:

Task 8.1 and T8.2 *Dissemination of information of the INFECT consortium and project*, and *Dissemination of knowledge generated in INFECT*

- Within the first weeks of the project, and well ahead of schedule, an open access external web site was created and opened for the public. This website has been regularly updated.
- At the same time an informative leaflet to be used e.g. at meetings with health care workers, scientists, decision makers, patients and relatives, was produced.
- All partners have throughout the project period presented their scientific advances through presentations at scientific meetings, patient organization meetings, lectures, scientific publications accepted by well-renowned peer reviewed international journals. Also the INFECT project as such has been presented at several national and international conferences.
- Establishing guidelines (D8.6). It was only into the study period it was realized that medical guidelines based on the novel scientific knowledge produced by INFECT must be created by an independent third parties, in order to be acknowledged, supported and advocated by professional communities such as medical societies. The consortium has established contact with clinical association (e-g- SSAI) with the intention of establishing (national) guidelines.
- The achievement of the entire INFECT project will be summarized in a book invited by Springer Nature publishing group (**edited by partner 1, and co-edited by partner 6; all partners contributing**).
- A YouTube video describing the achievements of INFECT is being produced and will target the society at large, estimated release autumn 2018. **Partner 6** is together with DigiUiB, the media department at UiB responsible for this work.

Task 8.3 Training of medical staff

- This will be offered as a 1-day workshop on NSTI held in association with the Scandinavian Society of Anaesthesiology and Intensive Care Medicine (SSAI) in conjunction with the SSAI 2019 Congress to be held in Copenhagen August 2019. The venue for the one day course will be in the National Hospital, Copenhagen, Denmark, and it will be held one day prior to the conference, at August 27th 2019. Announcement of the conference is in development and the SSAI will ensure the timely announcement of this preconference course.
- The YouTube video described above will promote general awareness of the importance of NSTI, which although targeting the society at large will be useful also for medical staff.

4.1.3.9 WP9 Project management during the period

In summary, the project management activities aimed to achieve/provide:

- an efficient management and administration of the project in line with legal and EC regulations.
- a functional communication between the participants in the project.
- a robust management component to efficiently communicate with the EC.
- support to the project partners regarding administration and reporting.
- identification and handling of problems at early stages.
- tools to monitor and disseminate project progress and results
- productive discussions with advisors and within the Project Steering Committee.

The INFECT consortium is composed of a team of multidisciplinary researchers, clinicians, SMEs and a patient organization, each with a unique expertise, technical platform and/or model systems that together have the means to successfully conduct the research proposed. There are in total 14 partners from 11 different countries that have worked together to achieve the goals set forth in the 8 distinct, highly interrelated, scientific WPs.

A critical component of INFECT has been to fully exploit the multidisciplinary expertise and resources provided by the different partners. Data-sharing has been critical but equally important has been the sharing and exploitation of partners' respective expertise that is needed for optimal data utilization. WP9 has focused on achieving this through a variety of actions (outlined below), as in our view, this is an absolute requirement for a systems medicine approach to be successful, and therefore, this has been highly prioritized in INFECT. The leadership and coordination of INFECT has been based on the principle to always ensure that the actions taken are aligned with the overall aim of the INFECT project, namely to generate advancement that will benefit NSTI patients.

Task 9.1 Management of INFECT and Task 9.2. Coordination of INFECT activities

Key activities have included:

- *Consortium meetings* hold annually as well as a final meetin held in 2017 and 2018). These meetings have included: scientific reports by all partners, presentations of data, discussion of problems and actions needed, as well as ongoing work related to the WPs. Also **partner 1** has used to opportunity to inform about administrative tasks, such as reports, deadlines, etc.
  The *INFECT advisors* (Prof Reuss and Dr. Morgan) have been invited and attended all these meetings. This has been most valuable as they have provided important feedback to the consortium and also to the commission as they performed the mid-term review and always have provided short summaries from the annual meetings. See end of WP9 section for their comments from the final INFECT meeting held in Bergen, June 2018.
- *Project steering committee meetings* has been held annually in conjunction with the annual meetings to discuss INFECT progress and critical issue.
- *Providing support in planning and guiding the project*. This has been done through continuous contact with partners through email correspondence, Skype contacts, and meetings both initiated by the coordinator and by the WP-leaders. Part of these activities aimed to address challenges within the project as soon as they raised.
- *Administration and distribution of the EC funds to partners*. No major deviations and actions according to the grant agreement and associated amendments.
- *Follow-up on work progress in respective WP.* This has been done through meetings, mail and phone communications, and review of deliverable reports submitted for respective WP. An important management tool has been the periodical (every 6 month) DIP (data integration

panel; DIP) report. The purpose of the DIP has been to oversee and ensure the effective creation, management and sharing of samples and data within the consortium, as this would enables fully exploit the potential of INFECT. At regular occasions (meetings, TCs, Skype, email, phone), we discussed which samples and data to focus our analysis and modelling efforts on, to ensure that we work towards the objectives of INFECT, as well how to improve generation and sharing of data. The DIP reports have reviewed the status of patient enrolment and sample shipment, as well as data generation, analyses, processing and sharing. Overall, the aim of the DIP has been to oversee the current status of progress within INFECT and to ensure that INFECT samples and data became accessible to all partners as needed and agreed.

- *Compliance with reporting duties to the commission*. Annual and financial reports have been reviewed and submitted on time.
- *Additional meetings between/within WPs* has occurred continuously. In addition to the meetings listed above, many interactions took place through regular email/skype and TCs. *Coordinating and ensuring administrative information* dissemination to partner members by email, website and to the EC project officer. This has been done throughout without any major issues arising.
- *Ensure full integration and coordination of the research teams.* As needed, necessary contacts have been taken between involved partners. Communication has been through regular Skype, mail or telephone to deal with smaller urgent issues. This has during this period been sufficient to ensure efficient progress and problem solving.

Task 9.3 Dissemination of INFECT.
Due to the central issue of dissemination in INFECT, a special WP (**WP8**) has been responsible for dissemination and exploitation of the results gained in INFECT (See WP8 report). Dissemination activities have been several and of excellent quality. To give a few examples:

1. The INFECT consortium has received a lot of attention in the scientific community, to name a few highlights:
    a. The 20th International Lancefield meeting on Streptococcal infections, September 2018: Partners 1, 6 and 8 participated with INFECT presentations and all received oral presentation, including the coordinator Norrby-Teglund who was invited as plenary speaker. ***In the concluding remark, the organizer highlighted the INFECT project as "The way to do research".***
    b. Systems medicine meeting in Berlin: Two presentations from INFECT were among the 10 presentations (out of 100 abstracts) selected for oral presentation.
2. A book volume will be published by Springer Nature publishing group (INFECT logo on the cover). The contract has been completed and the tentative publishing date is December 2019. The book will focus on NSTI and will target health care professionals and researchers/ clinicians within the fields of infectious diseases, intensive care, microbiology and systems medicine and personalized medicine.
    a. A postgraduate training workshop for medical students and residents will be held in conjunction with the largest Nordic intensive care conference in 2019.
    b. A Youtube video currently being finalized, including clinicians, modellers and patients/relatives. This video is targeting the society at large.

Actions taken to the recommendations provided in the ethical review report

According to the ethical review report, an external ethics expert was assigned who has conducted annual reviews of all ethical aspects. These reports have been submitted with the periodic reports; all reports confirming a sound ethical approach in INFECT.

Figure 9. Remarks from external advisors submitted after the final meeting in Bergen June 2018.

Report Clinical advisor INFECT. **11TH July 2018**

As a clinician with thirty years' experience of necrotising soft tissue infections and toxic shock, it has been a privilege to follow the progress of this massive project.

The incredible vision and effort that has resulted in the recruitment of so many patients and establishing a unique biobank has paid dividends, resulting in a unique and enviable scientific resource and a plethora of clinical information.

Numerous publications resulting from INFECT have already provided unique insights and some long - awaited answers to contentious areas such as the genesis, pathophysiology and management of these devastating infections. The solid, scientific and clinically applicable content of the empowers clinicians with a better understanding of the manifestations and course of necrotizing infections. Moreover, therapeutic options such as immunoglobulin and hyperbaric oxygen have been systematically analysed for the first time on such a large scale. Seminal work involving the artificial skin model and the demonstration and survival of intracellular organisms has massive implications for early diagnosis and optimal antimicrobials. Dissemination of information resulting from the INFECT work, and the educational materials for patients and clinicians completes the project, with tangible benefits for all.

The logistical and conceptually demanding organisation , encouragement and direction of research streams that has underpinned the success of this amazing enterprise, reflects considerable vision , leadership skills and drive of the project leaders. The coordinated cooperation of internationally renowned research groups working with the one aim -namely better patient outcomes- has already proven immensely successful and clinically invaluable, with the promise of much more to come.

Marina Morgan

Consultant in Medical Microbiology and Infection

Royal Devon & Exeter Hospital

United Kingdom.

Report by Prof. Matthias Reuss:

My assessment of the INFECT-project is overall very positive. The progress has been remarkable in the clinical field as well as in the new paradigm of systems medicine.

The different groups have successfully faced the challenge of managing the difficult link between experimentation and modeling both in terms of mechanistic modeling (e.g. metabolic flux analysis) and data-driven modeling (such as machine learning). As far as the challenge of using the data for modeling and hypothesis driven data acquisition are concerned the participating groups were able to deliver convincing results.

Because most of the projects are nearing the end of their programme, their continuation becomes an issue. The adviser judges that follow-up is very important to build on progress to date. As such, a cooperative integrated multidisciplinary follow-up might create additional synergy and momentum for further developments for clinical use with direct implications for the patients. This visionary view is particularly important for the long - term use of mathematical models and simulations for personalized treatment of the disease.

4.1.4 Impact and the main dissemination activities and exploitation of results.

As outlined in annex I, INFECT had several expected impacts specified linked to the specific areas listed below. Herein, we report ***the actual impact achieved*** for respective area. The different colors indicate the estimated time line; green = done/short-term (within 2018); blue = mid-term (within 2 years); red = long-term (> 2 years).

- ***Novel insights into the pathophysiology of NSTIs (Table 1)***

Results*	Exploitation	End users	Beneficiary
Host trait: IL1β	Potential target for: Diagnostics Intervention Patient stratification Future clinical trials	SMEs Clinicians Researchers	Patients Health care Industry
Host-dependent pathophysiology	Potential target for: Diagnostics Intervention Patient stratification Future clinical trials	SMEs Clinicians Researchers	Patients Health care Industry
Host antibody deficiency	Potential target for: Intervention Patient stratification Future clinical trials	SMEs Clinicians Researchers	Patients Health care Industry
Different bacterial traits influence tissue pathology	Potential target for: Diagnosis Intervention Patient stratification Future clinical trials	SMEs Clinicians Researchers	Patients Health care Industry
Aetiology-dependent pathophysiology	Potential target for: Diagnosis Intervention Patient stratification Future clinical trials	SMEs Clinicians Researchers	Patients Health care Industry
Bacterial biofilm	Diagnosis Antibiotic use	Clinicians Researchers	Patients Health care

*See section 4.1.3 for details of the results/achievements

The integrated systems biology approach used in INFECT identified several host and pathogen traits/pathways that contributed to the severity and outcome of NSTIs. The findings provide critical data to support personalized medicine approaches in infectious diseases, and identifies targets for diagnostics, patient stratification and intervention. Some of which are already being pursued by the consortium in planned future studies (these are highlighted in green). Also some of the findings have *already resulted in a changed clinical practice*, such as in the case of biofilm revealing the need to change type of antibiotics to achieve an efficient bacterial clearance.

The knowledge acquired is not only useful in the field of NSTIs, but will also be useful for other severe invasive infections, such as sepsis and its complication that is causing a significant health burden worldwide.

- ***Patient benefit: superior diagnosis (Table 2)***

Results*	Exploitation	End users	Beneficiary
Hyborg for patient monitoring	Diagnostics	SMEs Clinicians	Patients Health care Industry
Hyborg pathogen ID and enrichment	Diagnostics (blood, tissue)	SMEs Clinicians	Patients Health care Industry

A key goal of INFECT was to promote improved diagnostics, as timely diagnosis is critical for these acute rapidly progressing infections. For this, we developed innovative diagnostic tools based on multiplex technology, and validation in the clinical setting was undertaken, which revealed potential of the new tool but also need for further optimization.

- ***Patient benefit: advanced understanding of the clinical aspects of NSTI and preparation of guidelines for management and care (Table 3).***

Results*	Exploitation	End users	Beneficiary
Advanced understanding of NSTI patients	Diagnostics Treatment strategies Identification of at risk patients Education	Clinicians Students Researchers SMEs	Researchers Health care Industry Patients Students

A key achievement of INFECT was the enrollment of NSTI patients and the creation ***of the world's largest patient cohort and associated biobank***. Analyses of the comprehensive clinical registry generated an advanced understanding of these patients and provided documentation that has previously been lacking. This has been disseminated through scientific conferences and scientific publications, and importantly the process of creating evidence-based guidelines have been initiated through the involvement of proper clinical organisations (*e.g.* SSAI).

- ***Patient benefit: novel therapeutic strategies (Table 4)***

Results*	Exploitation	End users	Beneficiary
IVIG should not be used for all NSTI. Results support the use of IVIG in *S. pyogenes* NSTI.	Patient stratification and tailored therapy	Clinicians	Patients Health care
Biofilm Microbial aetiology is closely linked to body area affected	Appropriate antibiotic use	Clinicians Researchers Industry	Patients Health care Industry

INFECT has utilized the clinical registry containing treatment data, analyses of biobank samples, and even conducted a clinical trial (*Madsen et al Int Care Med 2017*). This has ***resulted in change of clinical practice*** and provided evidence that "one size" does not fit all patients. Further strengthening the importance of patient stratification and tailored therapy in infectious diseases. The results also have an impact on the use of antibiotics as it promotes the timely administration of the right type of antibiotic usage, which is of utmost importance in regards to antibiotic resistance development; a major global health threat.

- ***Design/optimization of future clinical trials (Table 5)***

Results*	Exploitation	End users	Beneficiary
IL1b for patient stratification and intervention	Exploratory clinical trials	Clinicians Researchers	Researchers Health care Industry Patients

The novel insight on host and pathogen traits as detailed above shows the need for patient stratification and tailored therapy. One such host trait is currently being purused by the consortium in planned future trial, and many more are in the pipeline. This is an important step towards achieving personalized medicine in NSTIs and improved patient outcome.

- ***Reduction on health care costs (Table 6)***

Results*	Exploitation	End users	Beneficiary
IVIG should not be used for all NSTI. Results support the use of IVIG in *S. pyogenes* NSTI.	Patient stratification and tailored therapy	Clinicians	Health care
Biofilm Microbial aetiology is closely linked to body area affected	Appropriate antibiotic use	Clinicians	Health care

In addition to the suffering of the patients and their relatives, the costs associated with these infections are substantial and represent a great burden to health care. IVIG as a biological is associated with substantial costs per patient, and hence, our finding that IVIG should not be used for all NSTI irrespective of aetiology but rather on a particular patient subpopulation is associated with direct health cost reductions. Also the results guiding antibiotic use is most valuable as timely and appropriate antibiotic use is critical in infection outcome.

- ***Establishment of the value of systems medicine in solving complex human diseases (Table 7)***

Results*	Exploitation	End users	Beneficiary
Patient outcome prediction model	Clinical decision support	Clinicians	Health care Patients
Pathophysiology-dependent patient stratification	Tailored treatment	Clinicians SME	Health care Patients
Multiplex diagnostic tool based on patient biomarkers	Superior diagnostics Patient stratification	Clinicians SME	Health care Patients
Dedicated Semantic Resource for Data Storage and Management	Clinical decision support Superior diagnostics Patient stratification	Clinicians Academic Institutions SME Patient organisations	Health care Patients

Through the integrated systems biology approach in INFECT utilizing clinical data, different clinically relevant experimental models and computational modeling, INFECT achieve an in-depth understanding of the pathophysiology of the multifactorial NSTIs and their co-morbidities; thereby identifying novel diagnostic and therapeutic targets. Importantly, the data demonstrated the need for patient stratification and tailored intervention and provided the insight necessary to create new concepts for this. Taken together, this shows the value of systems medicine in promoting medical advances in infectious diseases. The results of INFECT has demonstrated the impact of systems medicine to solve important health challenges.

- ***Fostering the competitiveness of SMEs and European innovation*** **(Table 8)**

Results*	Exploitation	End users	Beneficiary
New computational models and bioinformatic tools for:	Clinical decision support Tailored treatment	Clinicians SME	Health care Patients

	Superior diagnostics Patient stratification		
Simple method to enrich pathogens in whole blood samples	Kit to enrich bacteria and fungi in blood samples	Laboratories	Patients
Highly sensitive molecular pathogen diagnostics	Diagnostic tests to identify bacteria and fungi from whole blood	Laboratories	Patients

One of the five objectives of the ambitious Lisbon 2020 agenda is to foster innovation and to starkly improve the competiveness of the European industry, in particular SMEs. The long-term success of SMEs depends on the quality of services that they provided and the importance of these services to potential customers. Cube Dx, as the successor of Anagnostics GmbH, could further develop its highly multiplexed biomarker diagnostics and turn it into a potential point of care tool. The technology now provides a single step usage (filling in the sample) and allows whole blood as a sample. The technology has the potential to quantify more than 100 protein biomarker in about 13 minutes. The technology is therefore perfectly suitable to test whole biomarker profiles in clinical settings. Furthermore, Cube Dx could close the gap in its product range to identify pathogens on a molecular basis (DNA) and can now offer a highly effective and fast method to enrich pathogens from whole blood, beside the test to identify a wide range of relevant bacteria, resistance genes and fungi within less than 3 hours from whole blood. Both (platform) technologies boost Cube Dx' competitiveness in the global diagnostics market. The major activities and commercial goals of the SMEs LifeGlimmer involved in INFECT include providing methods and workflows for the understanding of complex biological systems using systems medicine approaches. As envisaged, the developments in the application of modeling and bioinformatics approaches and tools herein developed have boosted the competitiveness of LifeGlimmer GmbH because they *i)* furthered product development and services of reverse engineering, dynamic, constrained-based and machine learning modeling towards the unraveling the mechanisms underlying disease and to predict potential biomarkers and intervention strategies, *ii)* significantly contributed to improve its product portfolio by creating new specialised tools; *iii)* enabled it to validate its main strategy in developing tools that can be used for analysis and interpretation of complex, high-volume data sets, and clinical data. This has been and will continue to be paramount to strengthen LifeGlimmer's position as high-value service provider in the Systems Medicine and Health landscape.

- ***Training of early stage and experienced researchers (Table 9)***

Results/Activity	Author/Responsible, Title, University, Year	Field
PhD thesis	Anna Linnér, Clinical and Pathophysiological aspects of sepsis, Karolinska Institutet, 2014	Infectious Diseases

PhD thesis	Trond Bruun, Clincial and bacterial diversity in streptococcal skin and soft tissue disease, University of Bergen, 2016	Infectious Diseases
PhD thesis	Oddvar Oppegaard, Trends of *Streptococcus dysgalactiae* subspecies *equisimilis* infections in western Norway – Insight into clinical and microbial aspects. University of Bergen, 2017	Infectious Diseases
PhD thesis	Marco Bo Hansen; Biomarkers of NSTI. Aspects of the innate immune response. University of Copenhagen, 2016.	Intensive care medicine
PhD thesis	Martin Bruun Madsen, "Necrotising Soft Tissue Infections in the ICU to be defended August 30, 2018, University of Copenhagen, 2018	Intensive care medicine
PhD thesis	Peter Polzik: Biomarkers reflecting endothelial dysfunction and prognosis in NSTI. University of Copenhagen 2018.	Intensive Care Medicine
PhD thesis	Jasper Koehorst "FAIR FUNCTIONAL GENOMICS" to be defended on January 25, 2019 at Wageningen University, the Netherlands	Systems medicine
PhD thesis	Jesse van Dam "Semantic Technologies for Systems Medicine" to be defended on January 23, 2019 at Wageningen University, the Netherlands	Systems medicine
PhD thesis	Chella Krishnan, K, Host-Pathogen Interactions Promoting Pathogen Survival and Potentiating Disease Severity & Morbidity in Invasive Group A Streptococcal Necrotizing Soft Tissue Infections, University of Cincinnati, 2015	Molecular Genetics, Biochemistry, & Microbiology
PhD thesis	Srikanth Mairpady Shambat, Host-pathogen interactions in invasive staphylococcal infections, Karolinska Institutet, 2016	Infection Biology
PhD thesis	Julia Uhlmann, Neutrophil interactions with Streptococcus pyogenes and Staphylococcus aureus, Karolinska Institutet, 2017	Infection Biology

PhD thesis	Johanna Snäll, Phagocytic cells and Streptococcus pyogenes in invasive infections: an inflammatory relationship, Karolinska Institutet, 2017	Infection Biology
PhD thesis	Puran Chen, Human organotypic models in biomedical research to be defended in January 2019, Karolinska Institutet	Infection Biology
Postgraduate course	Mattias Svensson, Exploring mechanisms of tissue infection and pathology using innovative human organotypic models, Karolinska Institutet, 2014	Infection Biology
Training at advanced level	Training course in systems analyses approaches and computational modelling, organised by WUR and LG teams, for clinicians and experimentalists in the INFECT consortium, March 2018	Systems Medicine

The INFECT project has included training of clinical and preclinical researchers, including preparation of educational material, training of PhD student as well as residents and medical staff. This training in an outstanding multi-disciplinary research environment has served to support the development of excellent research scientists in the fields of infectious diseases, intensive care, microbiology, and systems medicine. This training does not only offer highly trained research scientists to meet the employment demands of stakeholders in relevant R&D and commercial sectors, but also serves to foster the new generation of scientist in the new field of systems medicine in infectious diseases.

Dissemination activities

In INFECT a great emphasis has been on disseminating the knowledge generated in the project to increasing the awareness of the life-threatening NSTIs, inform about the above reported advances/knowledge created in INFECT, continuously working towards the translation of these results to improve patient care and outcome, as well as working towards the implementation of systems medicine in infectious disease and intensive care, thereby enabling personalized medicine approaches to be applied to also acute life-threatening infections.

The high quality of the work performed and the dissemination activities (as detailed in 4.1.3 and tables below) is evident by the following facts:

1. The high quality publications published in well renowned journals within the fields of infectious diseases, intensive care, microbiology and systems medicine
2. Invitations to leading meetings in infectious diseases, intensive care, microbiology and systems medicine both in Europe and outside (e.g. the Lancefield conferences in 2014 and 2017, European conference on Infectious diseases and clinical microbiology). At these and other meetings, INFECT has gained a lot of positive attention and its efforts acknowledged.
3. Invitation by a major publishing company to edit and produce a book volume on NSTI with a focus on the INFECT project.
4. Established collaborative efforts and support from the clinical scientific community (*e.g* SSAI) for training and guidelines preparations.

4.1.5 The address of the project public website, as well as relevant contact details.

http://www.fp7infect.eu/

We, who have implemented the INFECT Project and who have contributed to this report:

Partner 1	**Karolinska Institutet, Stockholm, Sweden** **Team leader and Coordinator: Anna Norrby Teglund**
Partner 2	**Rigshospitalet, Copenhagen, Denmark** **Team leader: Ole Hyldegaard**
Partner 3	**Karolinska University Hospital, Stockholm, Sweden** **Team leader: Michael Nekludov**
Partner 4	**Blekinge Hospital, Karlskrona, Sweden** **Team leader: Ylva Karlsson**
Partner 5	**Sahlgrenska University Hospital, Gothenburg, Sweden** **Team leader: Per Arnell**
Partner 6	**University of Bergen, Bergen, Norway** **Team leader: Steinar Skrede**
Partner 7	***University of Cincinnati, United States, terminated 2013 – see partner 15*** ***Team leader: Malak Kotb***
Partner 8	**Helmholtz-Zentrum fur infektionsforschung, GmbH, Braunschweig, Germany** **Team leader: Dietmar Pieper**
Partner 9	**Wageningen Universiteit, Wageningen, The Netherlands** **Team leader: Vitor Martins dos Santos**
Partner 10	**Université Lyon, Lyon, France** **Team leader: Francois Vandenesch**
Partner 11	**LifeGlimmer GmbH, Berlin, Germany** **Team leader: Vitor Martins dos Santos**
Partner 12	***Anagnostics Bioanalysis GmbH, St Valentin, Austria, terminated 2015 -see partner16*** ***Team leader: Christoph Reschreiter***
Partner 13	**The Lee Spark Foundation, Preston, United Kingdom** **Team leader: Doreen Marsden**
Partner 14	**Tel Aviv University, Tel Aviv** **Team leader: Eytan Ruppin**
Partner 15	**University of North Dakota, United States** **Team leader: Malak Kotb**
Partner 16	**Cube Dx, GmbH, St Valentin, Austria** **Team leader: Christoph Reschreiter**

4.2 Use and dissemination of foreground

A plan for use and dissemination of foreground (including socio-economic impact and target groups for the results of the research) shall be established at the end of the project. It should, where appropriate, be an update of the initial plan in Annex I for use and dissemination of foreground and be consistent with the report on societal implications on the use and dissemination of foreground (section 4.3 – H).

The plan should consist of:

- Section A

For all partners – add information relevant to your work to the tables (A1 and A2) below.
This section should describe the dissemination measures, including any scientific publications relating to foreground. **Its content will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

- Section B

For all partners– add information relevant to your work to the tables (B1 and B2) below.
This section should specify the exploitable foreground and provide the plans for exploitation. All these data can be public or confidential; the report must clearly mark non-publishable (confidential) parts that will be treated as such by the Commission. Information under Section B that is not marked as confidential **will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

Section A (public)

This section includes two templates

- Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.
- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

These tables are cumulative, which means that they should always show all publications and activities from the beginning until after the end of the project. Updates are possible at any time.

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[2] (if available)	Is/Will open access[3] provided to this publication?
1	Computational evaluation of cellular metabolic costs successfully predicets genes whose expression is deleterious	A. Wagner, R. Zarecki, L. Reshef, C. Gochev, R. Sorek, U. Gophna, E. Ruppin	PNAS	110	National Academy of Science	US	2013	19166-19171	10.1037/pnas.1312361110	yes
2	Necrotizing soft tissue infections caused by Streptococcus pyogenes	T. Bruun , B.R. Kittang , B.J. de Hoog , S. Aardal	Clinical Microbiol.	19	Blackwell Publishing	UK	2013	E545-E550	10.1111/1469-0691.12276	yes

[2] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[3] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

	and Streptococcus dysgalactiae subsp. equisimilis of groups C and G in western Norway	, H.K. Flaatten , N. Langeland , H. Mylvaganam , H.A. Vindenes , S. Skrede	and Infection							
3	HMGB1 in severe soft tissue infections caused by Streptococcus pyogenes	Linda Johansson , Johanna Snäll , Parham Sendi , Anna Linnér , Pontus Thulin , Adam Linder , Carl-Johan Treutiger , Anna Norrby-Teglund	Frontiers in Cellular and Infection Microbiol.	4	Frontiers	Switzerland	2014	1	10.3389/fcimb.2014.00004	yes
4	A Novel Nutritional Predictor Links Microbial Fastidiousness with Lowered Ubiquity, Growth Rate, and Cooperativeness	Raphy Zarecki, Matthew A Oberhardt, Leah Reshef, Uri Gophna, EYtan Ruppin	PLoS Computational Biology	9	Public Library of Science	US	2014	e1003726	10.1371/journal.pcbi.1003726	yes
5	Levels of Alpha-Toxin Correlate with Distinct Phenotypic Response Profiles of Blood Mononuclear Cells and with agr Background of Community-Associated Staphylococcus aureus Isolates	Srikanth Mairpady Shambat , Axana Haggar , Francois Vandenesch , Gerard Lina , Willem J. B. van Wamel , Gayathri Arakere , Mattias Svensson , Anna Norrby-Teglund	PLoS One	8	Public Library of Science	US	2014	e106107	10.1371/journal.pone.0106107	yes
6	Comparative genomics of Streptococcus pyogenes M1 isolates differing in	Fiebig, A. Loof TG, Babbar A, Itzek A,	Int J Med Microbiol	305	Elsevier		2015	532-543	10.1016/j.ijmm.2015.06.002	no

	virulence and propensity to cause systemic infection in mice.	Koehorst JJ, Schaap PJ, Nitsche-Schmitz DP								
7	Modelling staphylococcal pneumonia in a human 3D lung tissue model system delineates toxin-mediated pathology	S. Mairpady Shambat, P Chen, A.T. Nguyen Hoang, H. Bergsten, F. Vandenesch, N. Siemens, G. Lina, I.R. monk, T.J. foster, G. Arakere, M Svensson, A. Norrby-Teglund	DMM Disease Models and Mechanisms	8	Company of Biologists Ltd	UK	2015	1413-1425	10-1242/dmm.021923	yes
8	Increased cytotoxicity and streptolysin O activity in group G streptococcal strains causing invasive tissue infections	Nikolai Siemens, Bård R. Kittang, Bhavya Chakrakodi, Oddvar Oppegaard, Linda Johansson, Trond Bruun , Haima Mylvaganam, Per Arnell, Ole Hyldegaard, Michael Nekludov, Ylva Karlsson, Mattias Svensson, Steiner Skrede, Anna Norrby-Teglund	Scientific Reports	5	Nature Publishing Group	UK	2015	16945	10.1038/srep16945	yes
9	LL-37 Triggers Formation of Streptococcus pyogenes Extracellular	Julia Uhlmann, Manfred Rohde, Nikolai	Journal of Innate Immunity		S. Karger AG	Switzerland	2015	1-15	10.1159/000441896	yes

	Vesicle-Like Structures with Immune Stimulatory Properties	Siemens, Bernd Kreikemeyer, Peter Bergman, Linda Johansson, Anna Norrb-Teglund								
10	Assessing the metabolic Diversity of Streptococcus from a protein Domain Point of View	Edoardo Saccenti, David Nieuwenhuijse, Jasper J. Koehorst, Vitor A.P. Martins dos Santos, Peter J. Schaap	PLoS One	10	Public Library of Service	US	2015	e0137908	10.1371/journal.pone.0137908	yes
11	Effects of Sample Size and Dimensionality on the Performance of Four Algorithms for Inference of Association Networks in Metabonomics	Maria Suarez-Diez , Edoardo Saccenti	Journal of Proteome Research	14	American Chemical Society	UK	2015	5119-5130	10.1021/acs.jproteome.5b00344	no
12	Harnessing the landscape of microbial culture media to predict new organism-media pairings	Matthew A. Oberhardt, Raphy Zarecki, Sabine Gronow, Elke Lang, Hans-Peter Klenk, Uri Gophna, Eytan Ruppin	Nature Commnuications	6	Nature Publishing Group	UK	2015	8493	10.1038/ncomms9493	yes
13	On the use of the observation-wise k -fold operation in PCA cross-validation	Edoardo Saccenti , José Camacho	Journal of Chemometrics	29	John Wiley and Sons Ltd	UK	2015	467-478	10.1002/cem.2726	no
14	Probabilistic Networks of Blood Metabolites in Healthy Subjects As Indicators of Latent Cardiovascular Risk	Edoardo Saccenti, Maria Suarez-Diez, Claudio Luchinat, Claudio	Journal of Proteome Research	14	American Chemical Society	US	2015	1101-1111	10.1021/acs.proteom	no

		Santucci, Leonardo Tenori								
15	Determining the number of components in principal component analysis: A comparsion of statistical cross validation and approximated methods	Edoardo Saccenti, José Camcho	Chemometrics and Intelligent Laboratory Systems	149	Elsevier	Netherlands	2015	99-116	10.1016/j.chemolab.2015.10.006	no
16	Biomarkers of necrotising soft tissue infections: aspects of the innate immune response and effects of hyperbaric oxygenation-the protocol of the prospective cohort BIONEC study.	Hansen MB	BMJ Open				2015		10.1136/bmjopen-2014-006995.	yes
17	RDF2Graph a tool to recover, understand and validate the ontology of an RDF resource	esse CJ van Dam, Jasper J Koehorst, Peter J Schaap Vitor AP Martins dos Santos, Maria Suarez-Diez	Journal of Biomedical Semantics	6	BioMed Central	UK	2015	39	10.1186/s13326-015-0038-9	yes
18	Systems-Wide Prediction of Enzyme Promiscuity Reveals a New Underground Alternative Route for Pyridoxal 5'-Phosphate Production in E. coli	Matthew A. Oberhardt , Raphy Zarecki , Leah Reshef , Fangfang Xia , Miquel Duran-Frigola , Rachel Schreiber , Christopher S. Henry , Nir Ben-Tal , Daniel J. Dwyer , Uri Gophna , Eytan Ruppin	PLoS Computational Biology	12	Public Library of Science	United States	2016	e1004705	10.1371/journal.pcbi.1004705	yes

19	A point mutation in AgrC determines cytotoxicity or colonizing properties associated with phenotypic variants of ST22 MRSA strains	S. Mairpady Shambat, N. Siemnes, I.R. Monk, D.B. Mohan, S. Mukundan, K.C. Krishnan, S. Prabhakara, J Snäll, A. Kearns, F. Vandenesch, M. Svensson, M. Kotb, B. Gopal, G Arakere, A Norrby-Teglund	Scientific Reports	6	Nature Publishing Group	UK	2016	31360	10.1038/srep31360	yes
20	Phosphoglycerate Kinase—A Novel Streptococcal Factor Involved in Neutrophil Activation and Degranulation	Julia Uhlmann , Nikolai Siemens , Ylva Kai-Larsen , Tomas Fiedler , Peter Bergman , Linda Johansson, Anna Norrby-Teglund	Journal of Infectious Diseases	214	Oxford University Press	UK	2016	1876-1883	10.1093/infdis/jiw450	no
21	Differential neutrophil resposnes to bacterial stimuli: Streptococcal starins are potent inducers of heparin-binding resistin-release	J. Snäll, A. Linnér, J. Uhlmann, N. Siemens, H. Ibold, M Janos, A. Linder, BKreiemeyer, H. Herwald, L. Johansson, A. Norrby-Teglund	Scientific Reports	6	Nature Publishing Group	UK	2016	21288	10.1038/srep21288	yes
22	Biofilm in group A streptococcal necrotizing soft tissue infections	Nikolai Siemens , Bhavya Chakrakodi , Srikanth Mairpady Shambat , Marina Morgan ,	J of Clinical Invest. INSIGHT	1	The American Society for Clinical Investigation		2016	e87882	10.1172/jci.insight.87882	yes

		Helena Bergsten , Ole Hyldegaard , Steinar Skrede , Per Arnell , Martin B. Madsen , Linda Johansson , Julius Juarez , Lidija Bosnjak , Matthias Mörgelin , Mattias Svensson , Anna Norrby-Teglund								
23	Comparison of 432 Pseudomonas strains through integration of genomic, functional, metabolic and expression data	J.j. Koehorts, J. C.J van Dam, R.G.A. van Heck, E. Saccenti, V.A.P. Martin dos Santos, M. Suarez-Diez, P. Schaap	Scientific Reports	6	Nature Publishing Group	UK	2016		10.1038/srep38699	yes
24	Host Genetic Variations and Sex Differences Potentiate Predisposition, Severity, and Outcomes of Group A *Streptococcus*-Mediated Necrotizing Soft Tissue Infections	Chella Krishnan K, Mukundan S, Alagarsamy J, Laturnus D, Kotb M	Infection and Immunity	*84*	American Society of Microbiology	United States	2016	416 –424	10.1128/ IAI.01191-15.	yes
25	Entropy-Based Network Representation of the Individual Metabolic Phenotype	Edoardo Saccenti, Giulia Menichetti, Veronica Ghini, Daniel Remondini, Leonardo	Journal of Proteome Research	15 (Issue 9)	American Chemical Society	US	2016	3298-3307	10.1021/acs.jproteome.6b00454	no

		Tenori, Claudio Luchinat								
26	Approaches to Sample Size Determination for Multivariate Data : Applications to PCA and PLS-DA of Omics Data	E. Saccenti, M. E. Timmermann	Journal of Proteome Research	15 (Issue 9)	American Chemical Society	US	2016	2379-2393	10.1021/acs.jproteome.5b01029	no
27	Genetic Architecture of Group A Streptococcal Necrotizing Soft Tissue Infections in the Mouse	Chella Krishnan K, Mukundan S, Alagarsamy J, Hur J, Nookala S, Siemens N, Svensson M, Hyldegaard O, Norrby-Teglund A, Kotb M.	PLoS pathogens	12:7	Public Library of Science	United States	2016	e1005732	10.1371/journal.ppat.1005732	yes
28	Pentraxin-3 as a marker of disease severity and risk of death in patients with necrotizing soft tissue infections: a nationwide, prospective, observational study.	Hansen MB	Crit Care	20			2016	40	10.1186/s13054-016-1210-z.	yes
29	The Lectin Complement Pathway in Patients with Necrotizing Soft Tissue Infection	Hansen MB	J Innate Immun	8(5)			2016	507-516	10.1159/000447327	no
30	Immunoglobulin for necrotising soft tissue infections (INSTINCT): protocol for a randomised trial.	Madsen MB	Danish medical journal	63		Denmark	2016	5250		no
31	Thesis: Clinical and bacterial diversity in streptococcal skin and soft tissue disease	Trond Bruun	University of Bergen, Department of Clinical Science		University of Bergen, Bergen, Norway	Norway	2016	91	ISBN 978-82-308-3311-7	yes
32	Association between cytokine response, the LRINEC score and outcome in patients with	Marco Bo Hansen Lars Simon Rasmussen,	Scientific Reports	7	Nature Publishing Group	UK	2017	42179	10.1038/srep42179	yes

	necrotising soft tissue infection: a multicentre, prospective study	Mattias Svensson, Bhavya Chakrakodi, Trond Bruun, Martin Bruun Madsen, Anders Perner, Peter Garred, Ole Hyldegaard, Anna Norrby-Teglund, Michael Nekludov, Per Arnell, Anders Rosén, Nicklas Oscarsson, Ylva Karlsson, Oddvar Oppegaard, Steinar Skrede, Andreas Itzek, Anna Mygind Wahl, Morten Hedetoft, Nina Falcon Bærnthsen, Rasmus Müller, Torbjørn Nedrebø								
33	Emergence of a Streptococcus dysgalatiae subspecies stG62647-lineage ssociated with severe clinical manifestaions	O. Oppegaard, H. Mylvaganam, S. Skrede, P.C. Lindemann, B.R. Kittang	Scientific Reports	7	Nature Publishing Group	UK	2017	1-10	10.1038/s41598-017-08162-z	yes
34	Zoonotic necrotizing myositis caused by Streptococcus equi subsp. zooepidemicus in a farmer	Bård Reiakvam Kittang, Veronika Kuchařová Pettersen, Oddvar	BMC Infectious Diseases	17	BioMed Central	UK	2017	1-8	10.1186/s12879-017-2262-7	yes

		Oppegaard, Dag Harald Skutlaberg, Håvard Dale , Harald G. Wiker, Steinar Skrede								
35	Plasma and Serum Metabolite Association Networks: Comparability within and between Studies Using NMR and MS profiling	M. Suarez-Diez, J. Adam, J. Adamski, S.A. Chasapi, C. Luchinat, A. Peters, C Prehn, C Santucci, A. Spyridonidis, G.A Spyrouöias, L. Tenori, R. Wang-Sattler, E. Saccenti	Journal of Proteome Research	16	American Chemical Society	US	2017	2547-2559	10.1021/acs.jproteome.7b00106	yes
36	Correlation Patterns in Experimental Data Are Affected by Normalization Procedures: Consequences for Data Analysis and Network Inference	Edoardo Saccenti	Journal of Proteome Research	16	American Chemical Society	US	2017	619-634	https://doi.org/10.1021/acs.jproteome.6b00704	no
37	Considering Horn's Parallel Anaysis froma Random Matrix Theory Point of View	E. Saccenti, M.E. Timmerman	Psychometrika	82	Springer New York	US	2017	186-209	10.1007/s11336-016-9515-z	no
38	Group-Wise Principal Component Analysis for Exploratory Data Analysis	José Camacho Rafael A. Rodríguez-Gómez, Edoardo Saccenti	Journal of Computational and Graphical Statistics	26	American Statistical Association	US	2017		10.1080/10618600.2016.1265527	no
39	How biomarkers reflect the prognosis and treatment of necrotising soft tissue infections and the effects of hyperbaric	Polzik P	BMJ Open	5;7(10)			2017	e017805	10.1136/bmjopen-2017-017805	yes

	oxygen therapy: the protocol of the prospective cohort PROTREAT study conducted at a tertiary hospital in Copenhagen, Denmark									
40	Treatment with 24 h-delayed normo- and hyperbaric oxygenation in severe sepsis induced by cecal ligation and puncture in rats.	Bærnthsen NF	J Inflamm (Lond).	14			2017	27	10.1186/s12950-017-0173-4.	no
41	Immunoglobulin G for patients with necrotising soft tissue infection (INSTINCT): a randomised, blinded, placebo-controlled trial.	Madsen MB	Intensive care medicine	43			2017	1585-1593	10.1007/s00134-017-4786-0	no
42	Thesis: Trends of Streptococcus dysgalactiae subsp. equisimilis infections in western Norway	Oddvar Oppegaard	University of Bergen, Department of Clinical Science		University of Bergen, Bergen, Norway	Norway	2017	96	ISBN 978-82-308-3897-6	yes
43	Pivotal Role of Preexisting Pathogen-Specific Antibodies in the Development of Necrotizing Soft-Tissue Infections.	Babbar, A., Bruun T, Hyldegaard O, Nekludov M, Arnell P; Pieper DH, Itzek A.	J Infect Dis	218	Oxford Academic	Oxford	2018	44-52	10.1093/infdis/jiy110	no
44	Diabetes and necrotizing soft tissue infections-A prospective observational cohort study: Statistical analysis plan	A. Rosén, P. Arnell, M. B. Madsen, B. G. Nedrebø, A. Norrby-Teglund, O. Hyldegaard , V. M. dos Santos, F. Bergey, E. Saccent, S. Skrede	Acta Anaesthesiologica Scandinavica	2018 Apr 19	Blackwell Munksgaard	Denmark	2018	1-10	10.1111/aas.13130	yes

45	Exploring the arthritogenicity of Streptococcus dysgalctiae subspecies equisimilis	O. Oppegaard, H. Mylvaganam, S. Skrede, B.K. Kittang	BMC Micobiol.	18	BioMed Central	UK	2018	17	10.1186/s12866-018-1160-5	yes
46	Necrotizing soft tissue infections - a multicentre, prospective observational study (INFECT): protocol and statistical analysis plan	M. B. Madsen , S. Skrede , T. Bruun , P. Arnell , A. Rosén , M. Nekludov , Y. Karlsson , F. Bergey , E. Saccenti , V. A. P. Martins dos Santos , A. Perner , A. Norrby-Teglund , O. Hyldegaard	Acta Anaesthesiologica Scandinavica	62	Blackwell Munksgaard	Denmark	2018	272-279	10.1111/aas.13024	no
47	Group-wise ANOVA simultaneous component analysis for designed omics experiments	E. Saccenti, A. K. Smilde, J. Camacho	Metabolomics	14	Springer New York	US	2018	1	10.1007/s11306-018-1369-1	yes
48	SAPP: functional genome annotation and analysis through a semantic framework using FAIR principles	Jasper J Koehorst , Jesse C J van Dam , Edoardo Saccenti , Vitor A P Martins dos Santos , Maria Suarez-Diez , Peter J Schaap	Bioinformatics	34	Oxford University Press	UK	2018	1401-1403	10.1093/bioinformatics/btx767	yes
49	From correlation to causation: analysis of metabolomics data using systems biology approaches	A. Rosato, L. Tenori, M. Casante, P.D. De Atauri Carulla, V.A.P. Martins dos Santos	Metabolomics	14	Springer New York	US	2018	37-42	10.1007/s11306-018-1355-y	yes

50	Recommended strategies for spectral processing and post-processing of 1D 1H-NMR data of biofluids with a particular focus on urine	Abdul-Hamid Emwas , Edoardo Saccenti , Xin Gao , Ryan T. McKay , Vitor A. P. Martins dos Santos , Raja Roy , David S. Wishart	Metabolomics	14	Springer New York	US	2018	31-37	10.1007/s11306-018-1321-4	yes
51	Age and Sex Effects on Plasma Metabolite Association Networks in Healthy Subjects	A. Vignoli, L. Tenori, C. Luchinat, E. Saccenti	Journal of Proteome Research	17	American Chemical Society	US	2018	97-107	10.1021/acs.jproteome.7b00404	no
52	Group-wise partial least square regression	José Camacho , Edoardo Saccenti	Journal of Chemometrics	32	John Wiley and Sons Ltd	UK	2018	e2964	10.1002/cem.2964	
53	Regulation of Three Virulence Strategies of Mycobacterium tuberculosis: A success Story	N. Zondervan, J. van Dam, P. Schaap, V.A.P Martins dos Santos, m Suarez-Diez	International Journal of Molecular Science	19	Molecular Diversity Preservation International	Switzerland	2018	347	10.3390/ijms19020347	

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES								
NO	Type of activities[4]	Main leader	Title	Date/Period	Place	Type of audience[5]	Size of audience	Countries addressed
1	Organisation of Conference	KAROLINSKA INSTITUTET	INFECT kick-off meeting	15/01/2013	Saltsjöbaden, Stockholm, Sweden	Scientific community (higher education, Research) - Industry	32	Sweden, Norway, Denmark, Germany, The Netherlands, UK, Austria, US, France, Israel
2	Press releases	KAROLINSKA INSTITUTET	Scientists gather to tackle highly lethal destructive soft tissue infections	15/01/2013	www.ki.se	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias		International
3	Oral presentation to a scientific event	VASTRA GOTALANDS LANS LANDSTING	Nec. infections/ INFECT	17/01/2013	Sahlgrenska University Hospital	Scientific community (higher education, Research)	40	Sweden
4	Articles published in the popular press	KAROLINSKA INSTITUTET	Medical Science	12/02/2013	Karolinska Institutet	Scientific community (higher education, Research) - Industry - Civil society -		Predominantly Sweden

[4] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[5] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

						Policy makers - Medias		
5	Presentation	REGION HOVEDSTADEN	INFECT lecures	13/2 2013	Rigshospitalet Dept of Aanesthesia, Rigshospitalet, CPH	Medical staff,	35	DK
6	Presentation	REGION HOVEDSTADEN	INFECT lecture	23/4 2013	Dept. of Urology , Rigshospitalet, Cph	Medical staff, lead doctors, nurses	45	DK
7	TV sequences	KAROLINSKA INSTITUTET	Swedish television, TV4 morning interviews (morgonsoffa)	10/05/2013	Stockholm, Sweden	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias		predominantly Sweden
8	Presentation	REGION HOVEDSTADEN	INFECT lecture	6/6 2013	Dept Thorax Surgery , Rigshospitalet, CPH	Medical staff	40	Dk
9	Presentation	REGION HOVEDSTADEN	INFECT meeting	19-20/9 2013	Haukeland, Bergen	INFECT Partners scientific meeting	35	Sweden, Norway, Denmark, Germany, The Netherlands, UK, Austria, US, France, Israel
10	Oral presentation to a wider public	KAROLINSKA INSTITUTET	Forskarfredag, EU Researcher's night	27/09/2013	Debaser, Stockholm, Sweden	Civil society	100	Sweden
11	Presentation	REGION HOVEDSTADEN	INFECT lecture	5/12 2013	Dept. of Anaesthesia , Rigshospitalet, CPH	Medical staff	200	DK
12	Films	ANAGNOSTICS BIOANALYSIS GMBH	Anagnostics Inflammation Monitoring	28/11/2013	http://www.youtube.com/watch?v=8RQGg42D90w	Scientific community (higher education, Research) - Industry		International

13	Films	ANAGNOSTICS BIOANALYSIS GMBH	Anagnostics Early Pathogen Detection	28/11/2013	http://www.youtube.com/watch?v=TShKrM-GC-A	Scientific community (higher education, Research) - Industry		International
14	Flyers	UNIVERSITETET I BERGEN	Dissemination and exploitation	21/03/2013	Rome, Italy	Scientific community (higher education, Research)	80	International
15	Presentation	REGION HOVEDSTADEN	INFECT	21-03-2013	Dept. of Intensive Care 4131, Rigshospitalet	ICU nurses	25x2	Denmark
16	Oral presentation to a scientific event	UNIVERSITETET I BERGEN	the Scandinavian Society of Anestehesiology and Intensive Care Educational Programme	22/02/2013	Haukeland University Hospital, Bergen, Norway	Scientific community (higher education, Research)	35	Scandinavian
17	Oral presentation to a scientific event	UNIVERSITETET I BERGEN	Educational session Dept Plastic Surgery HUH	15/02/2013	HUH	Scientific community (higher education, Research)	20	Norway
18	Oral presentation to a scientific event	UNIVERSITETET I BERGEN	Study presentation and NSTI educational session	12/11/2013	Dept Internal Medicine/Dept Cardiology/Dept Pulmonary Medicine	Scientific community (higher education, Research)	70	Norway
19	Oral presentation to a scientific event	UNIVERSITETET I BERGEN	Plenary educational session HUH	24/05/2013	HUH	Scientific community (higher education, Research)	350	Norway
20	Oral presentation to a scientific event	UNIVERSITETET I BERGEN	Study and NSTI educational programme, Nurse staff	05/12/2013	HUH, Dept Medicine	Scientific community (higher education, Research)	30	Norway

21	Organisation of Workshops	UNIVERSITETET I BERGEN	CLINICAL PARTNERS CONFERENCE	19/09/2013	Haukeland University Hospital	Scientific community (higher education, Research)	12	Norway, Sweden, Denmark
22	Presentation	REGION HOVEDSTADEN	INFECT	21-03-2013	Dept. of Intensive Care 4131, Rigshospitalet	ICU nurses	25x2	Denmark
23	Presentation	REGION HOVEDSTADEN	INFECT	02-05-2013	Weekly conference, departments of anaesthesiology and intensive care, Rigshospitalet	Aneashesiologists and Intensivists	50	Denmark
24	Presentation	REGION HOVEDSTADEN	INFECT	14-05-2013	Research Forum, Abdominal Centre, Rigshospitalet	Researchers	15	Denmark
25	Presentation	REGION HOVEDSTADEN	INFECT Database	11-06-2013	Dept. of Orthopaedics, Hvidovre Hospital	Doctors	15	Denmark
26	Presentation	REGION HOVEDSTADEN	INFECT/INSTINCT – status and preliminary results	03-10-2013	Weekly conference, departments of anaesthesiology and intensive care, Rigshospitalet	Anaesthesiologists and Intensivists	50	Denmark
27	Oral presentation to a scientific event	NFSUK	Necrotising Fasciitis - The Patients Story	21/02/2013	Belfast	Scientific community (higher education, Research)	25	Northern Ireland
28	Oral presentation to a scientific event	NFSUK	The Aftermaths of Necrotising Fasciitis	14/03/2013	London	Scientific community (higher education, Research)	200	UK
29	Oral presentation to a scientific event	NFSUK	Necrotising Fasciitis - PTSD	10/04/2013	St Thomas Hospital - London	Scientific community (higher education, Research)	35	UK
30	Organisation of Workshops	NFSUK	Education of Necrotising Fasciitis	04/05/2013	Birmingham - Uk	Scientific community (higher	15	UK

						education, Research)		
31	Oral presentation to a scientific event	NFSUK	A Patients Story and PTSD	21/05/2013	Nottingham - UK	Scientific community (higher education, Research)	120	UK
32	Oral presentation to a scientific event	NFSUK	IGAS Necrotising Fasciitis	25/06/2013	Southampton Hospital - UK	Scientific community (higher education, Research)	90	UK
33	Oral presentation to a scientific event	NFSUK	Two Survivors Present their Experience	26/06/2013	Sheffield General Hospital	Scientific community (higher education, Research)	120	Uk
34	Oral presentation to a scientific event	NFSUK	IGAS Necrotising Fasciitis	02/07/2013	Infection Prevention Welsh Annual Conference.Cardiff Stadium- South Wales	Scientific community (higher education, Research)	220	UK
35	Oral presentation to a scientific event	NFSUK	Necrotising Fasciitis in child birth	04/07/2013	Birkinshaw - Yorkshire	Scientific community (higher education, Research)	30	UK
36	Oral presentation to a wider public	NFSUK	Charity Summer Ball	06/07/2013	Shrewsbury - UK	Civil society	200	UK
37	Media briefings	NFSUK	Raising Awareness of Necrotising Fasciitis	04/09/2013	Houses of Parliament - Westminster- London	Medias	1500	International
38	Interviews	NFSUK	Raising Awareness Campagn	05/09/2013	Manchester TV Studio's	Medias	1500	UK
39	Exhibitions	NFSUK	Infection Prevention Society Glasgow Annual Conference	30/10/2013	Glasgow	Scientific community (higher education, Research)	150	UK

40	Oral presentation to a scientific event	NFSUK	Education of Necrotising Fasciitis	11/12/2013	Infection Prevention Society Team Neville Hall Hospital	Scientific community (higher education, Research)	20	Wales - UK
41	Oral presentation to a scientific event	VASTRA GOTALANDS LANS LANDSTING	Intensivvårdssällskapet	15/11/2013	Johannesbergs slott	Scientific community (higher education, Research)	50	Sweden
40	Oral presentation to a wider public	VASTRA GOTALANDS LANS LANDSTING	Patientexperience/INFECT	05/04/2013	Gothenburg	Scientific community (higher education, Research)	80	Sweden
42	Oral presentation to a scientific event	VASTRA GOTALANDS LANS LANDSTING	Necrotizing soft tissue infections / INFECT	08/11/2013	Jönköping	Scientific community (higher education, Research)	200	Sweden
43	Articles published in the popular press	VASTRA GOTALANDS LANS LANDSTING	Article with patient perspective	06/04/2013	Göteborgs Posten	Medias	500000	Sweden
44	Oral presentation to a scientific event	VASTRA GOTALANDS LANS LANDSTING	INFECT	10/12/2013	Gothenburg	Scientific community (higher education, Research)	40	Sweden
45	Oral presentation to a scientific event	VASTRA GOTALANDS LANS LANDSTING	INFECT	18/11/2013	Sahlgrenska University Hospital	Scientific community (higher education, Research)	20	Sweden
46	Oral presentation to a wider public	REGION HOVEDSTADEN	British Hyperbaric Association, Annual Meeting	08/11/2013	Plymouth, UK	Scientific community (higher education, Research)		International

47	Flyers	UNIVERSITETET I BERGEN	Dissemination of flyers	11/09/2013	53rd ICAAC, Denver, Colorado, USA	Scientific community (higher education, Research)		International
48	Presentation	REGION HOVEDSTADEN	INFECT – status and preliminary results	09-04-2014	Weekly conference, departments of anaesthesiology and intensive care, Rigshospitalet	Anaesthesiologists and Intensivists	50	Denmark
49	Presentation	REGION HOVEDSTADEN	INFECT lecture	25/3 2014	Rigshospitalet, Dept. of Anesthesia, Rigshospitalet, CPH	Medical staff – INFECT team	7	DK
50	Presentation	REGION HOVEDSTADEN	INFECT lecture	27/3 2014	Society of Urology, Denmark, Hotel Scandic, Glostrup	Medical staff, training course for urologist specialst doctors	40	DK
51	Presentation	REGION HOVEDSTADEN	Inverview	2/4 2014	Ekstrabladet, Politikken NewMagazine, Copenhagen	Journalist	Public	DK
52	Presentation	REGION HOVEDSTADEN NOVARTIS, course for GP's	INFECT lecture	3/4/2014	Rigshospitalet, CPH	General practitioners doctors in capitol reagion	30	DK
53	Conference	REGION HOVEDSTADEN	Necrotising soft tissue infections	27-09-2014	Danish Paediatric Infectious Diseases Symposium 2014, Comwell, Korsør	Doctors	50	International
54	Oral presentation to scientific event	WU	ICRM 2014 – International Chemometrics Research Meeting	2014	Nijmegen, The Netherlands	Scientific Community (higher education, Research)	>150	The Netherlands
55	Oral presentation to scientific event	WUR	SB@NL2014 Systems Biology Symposium, December 201	2014	Maastricht, The Netherlands	Scientific Community (higher education, Research	>500	The Netherlands

56	Oral presentation to a wider public	KAROLINSKA INSTITUTET	ForskarFredag, EU Research Friday	26/09/2014	Debaser, Stockholm	Civil society	50	Sweden
57	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	ECCMID 2014	10/05/2014	Barcelona	Scientific community (higher education, Research)		International
58	Poster presentation to a scientific event	ANAGNOSTIC /CUBE	ECCMID 2014	10/05/2014	Barcelona	Scientific community (higher education, Research)		International
59	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	Seminar INFECT	09/09/2014	University of North Dakota, US	Scientific community (higher education, Research)		US
60	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	Talk at the Mayo Clinic	12/09/2014	Rochester, US	Scientific community (higher education, Research)		US, Sweden
61	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	Nordic Society of Clinical Microbiology and Infectious Diseases (NSCMID)	25/09/2014	Bergen, Norway	Scientific community (higher education, Research)	400	Nordic
62	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	Intensive care meeting	07/10/2014	Aarhus, Denmark	Scientific community (higher education, Research)		Denmark
63	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	MODELLING BACTERIAL INFECTION AND PATHOLOGY IN ORGANOTYPIC MODELS OF HUMAN TISSUE	20/10/2014	University of Pittsburgh	Scientific community (higher education, Research)	50	US

64	Presentation	REGION HOVEDSTADEN	Necrotising soft tissue infections	30-10-2014	Dept. of thoracic surgery and anaesthesia, Rigshospitalet	Doctors	50	Denmark
65	Presentation	REGION HOVEDSTADEN	INFECT lecture	8/11 2014	British Hyperbaric Association yearly scientific conference , Hull and East Yourkshire Hospitals NHS Trust – Hull Hyperbaric Unit	Medical staff	120	UK
66	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	MODELLING EARLY EVENTS OF GRAM POSITIVE BACTERIAL INFECTION IN AN ORGANOTYPIC MODEL OF HUMAN SKIN	11/11/2014	Lancefield Conference, Buenos Aires	Scientific community (higher education, Research)	300	International
67	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	NEUTROPHIL RESPONSES DIFFER DEPENDING ON BACTERIAL STIMULI: STREPTOCOCCAL STRAINS ARE POTENT INDUCERS OF HEPARIN-BINDING PROTEIN AND RESISTIN-RELEASE	11/11/2014	Lancefield Conference, Buenos Aires	Scientific community (higher education, Research)	300	International
69	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	Clinical efficacy of polyspecific intravenous immunoglobulin therapy in patients with streptococcal toxic shock syndrome a comparative observational study	12/11/2014	Lancefield Conference, Buenos Aires	Scientific community (higher education, Research)	300	International
69	Posters	KAROLINSKA INSTITUTET	The INFECT-project: A systems medicine approach to advance our understanding of	10/11/2014	Lancefield Conference, Buenos Aires	Scientific community (higher education, Research)	300	International

			necrotizing soft tissue infection					
70	Articles published in the popular press	KAROLINSKA INSTITUTET	Dagens Medicin	12/11/2014	Sweden	Scientific community (higher education, Research) - Civil society - Policy makers - Medias		Predominantly Sweden
71	Flyers	UNIVERSITETET I BERGEN	INFECT Flyer 2014	25/09/2014	Nordic Society of Clincial Microbiology and Infectious Diseases	Scientific community (higher education, Research)	300	International
72	Oral presentation to a scientific event	UNIVERSITETET I BERGEN	Clinical challenges in severe soft tissue infections	25/09/2014	Nordic Society of Clinical Microbiology and Infectious Diseases, Bergen Norway	Scientific community (higher education, Research)	300	internationall
73	Organisation of Workshops	REGION HOVEDSTADEN	Clincal partners conference	11/12/2014	Rigshospitalet (National Hospital), Copenhagen	Scientific community (higher education, Research)	7	Denmark, Sweden, Norway
74	Posters	HELMHOLTZ-ZENTRUM FUER INFEKTIONSFORSCHUNG GMBH	Identification of pathogen traits and host immune responses affecting the outcome of necrotizing soft tissue infections	12/11/2014	XIX Lancefield international symposium on streptococci and streptococcal diseases, Buenos Aires, Aa	Scientific community (higher education, Research)	300	International
75	Articles published in the popular press	UNIVERSITETET I BERGEN	Våre infeksjonsavdelinger, Haukeland Universitetssjukehus, pest-Posten, 2014;20(1):19-25	31/01/2014	Norway	Scientific community (higher education, Research)	200	Norway

76	Flyers	UNIVERSITETET I BERGEN	INFECT Flyer 2014	12/11/2014	XIX Lancefield International Symposium on Streptococci and Streptococcal Diseases, Buenos Aires, Arg	Scientific community (higher education, Research)	300	International
77	Oral presentation to a scientific event	NFSUK	IGAS and necrotising fasciitis	23/01/2014	Homerton Hospital London UK	Scientific community (higher education, Research)	25	UK
78	Oral presentation to a scientific event	NFSUK	IGAS and necrotising fasciitis	23/04/2014	Imperial College London..uk	Scientific community (higher education, Research)	250	UK
79	Oral presentation to a scientific event	NFSUK	The aftermaths of necrotising fasciitis	07/05/2014	Nova Hotel - Infection Prevention - Wakefield Yorkshire UK	Scientific community (higher education, Research)	150	UK
80	Exhibitions	NFSUK	Promotional	16/05/2014	Clydebank	Medias	200	Scotland
81	Exhibitions	NFSUK	Promote INFECT	28/05/2014	Park Inn Hotel - IPS Conference Glasgow	Scientific community (higher education, Research)	200	Scotland
82	Oral presentation to a scientific event	NFSUK	The aftermaths of necrotising fasciitis	03/06/2014	Billingshurst Hospital - Sussex	Scientific community (higher education, Research)	200	UK
83	Oral presentation to a wider public	NFSUK	Survivors meeting	07/06/2014	Birmingham	Civil society	24	UK
84	Oral presentation to a scientific event	NFSUK	The aftermaths of necrotising fasciitis	10/06/2014	Rougement Hotel - Exeter UK	Scientific community (higher education, Research)	120	UK

85	Oral presentation to a scientific event	NFSUK	IGAS and necrotising fasciitis	02/07/2014	Kingsgate Conference Centre - Peterborough UK	Scientific community (higher education, Research)	120	UK
86	Organisation of Workshops	NFSUK	Necrotising fasccitis - our work	23/07/2014	London	Scientific community (higher education, Research)	50	UK
87	Oral presentation to a wider public	NFSUK	Promote INFECT and support	03/08/2014	Braintree - Essex UK	Civil society	250	UK
88	Media briefings	NFSUK	Promote INFECT and NF	02/09/2014	Houses of Parliament - Westminster London	Medias	250	UK
89	Oral presentation to a wider public	NFSUK	Necrotising fasciitis in Pediatric's	12/09/2014	Edgebaston Birmingham	Civil society	350	UK
90	Exhibitions	NFSUK	Infection Prevention Awareness Conference	24/09/2014	Olympia - London UK	Scientific community (higher education, Research)	300	UK
91	Posters	NFSUK	Rheumatology Conference	25/09/2014	School of Oriental & African Studies - London	Scientific community (higher education, Research)	120	International
92	Oral presentation to a scientific event	NFSUK	IGAS and necrotising fasciitis	06/11/2014	Hillingdon Hospital Trust - London	Scientific community (higher education, Research)	100	UK
93	Oral presentation to a scientific event	NFSUK	Necrotising fasccitis - our work	13/11/2014	Novatel - Southampton	Scientific community (higher education, Research)	150	UK
94	Organisation of Workshops	UNIVERSITET I BERGEN	Role of Aspirate in diagnostics	23/01/2014	University of Bergen	Scientific community	3	Norway, Austria

						(higher education, Research)		
95	Oral presentation to a scientific event	UNIVERSITETET I BERGEN	Presentation of the INFECT study	17/10/2014	Haraldsplass Deaconal Hospital, Bergen, Norway	Scientific community (higher education, Research)	7	Norway
96	Oral presentation to a scientific event	UNIVERSITY OF NORTH DAKOTA	The INFECT Project - Unerstanding, Preventing And/or Curing NSTI Infections	01/07/2014	Altru Health	Scientific community (higher education, Research)		US
97	Oral presentation to a scientific event	UNIVERSITY OF NORTH DAKOTA	Necrotizing Soft Tissue Infections (NSTI): The INFECT Project	01/02/2014	Sanford Health	Scientific community (higher education, Research)		US
98	Exhibitions	ANAGNOSTICS BIOANALYSIS GMBH	Congress of the Austrian Society of Infectious Diseases 2014	02/04/2014	Saalfelden	Scientific community (higher education, Research)		Austria
99	Exhibitions	ANAGNOSTICS BIOANALYSIS GMBH	European Congress of Clinical Microbiology and Infectious Disease 2014 (ECCMID)	10/05/2014	Barcelona	Scientific community (higher education, Research)		Europe
100	Exhibitions	ANAGNOSTICS BIOANALYSIS GMBH	Annual Meeting of the German Society of Hygiene and Microbiology 2014	05/10/2014	Dresden	Scientific community (higher education, Research)		Germany
101	Oral presentation to scientific event	WUR	Molecular Interactions - Free University Berlin	2015	Berlin, Germanty	Scientific Community (higher education, Research	>200	Germany

102	Oral presentation to scientific event	WUR	4th conference of the Dutch/Flemish Classification Society	2015	Nijmegen, The Netherlands	Scientific Community (higher education, Research	>500	The, Netherlands
103	Oral presentation to scientific event	WUR	BioSB 2015 – Dutch conference of bioinformatics and systems biology	2015	Lunteren, The Netherlands	Scientific Community (higher education, Research	>500	The Netherlands
104	Oral presentation to scientific event	WUR	14th Scandinavian Chemometrics Conference	2015	Chia, Sicily	Scientific Community (higher education, Research	>200	Italy
105	Oral presentation to scientific event	WUR	Benelux Bioinformatics Conference	2015	Antwerpen, Belgium	Scientific Community (higher education, Research	>300	Belgium
106	Oral presentation to scientific event	WUR	ISMB/ECCB	2015	Dublin, Ireland	Scientific Community (higher education, Research	>500	Ireland
107	Presentation	REGION HOVEDSTADEN	INFECT presentation	20-21/01 2015	Swedish Hyperbaric Soc, Karlskrona Navy Base, Karlskrona	Medical staff	40	Sweden, Norway, Dk
108	Media briefings	NFSUK	EU INFECT Project meeting	27/01/2015	Social Media	Medias	2500	International
109	Flyers	NFSUK	Promote INFECT and NF	02/03/2015	Yorkshire UK	Scientific community (higher education, Research)	6	UK
110	Presentation	REGION HOVEDSTADEN	INFECT presentation	24/2 2015	Soc.of Dermatology, Post-Doc Course, Gentofte Hospital, DK	Medical staff	30	DK
111	Flyers	NFSUK	Promote INFECT and NF	04/03/2015	Kent UK	Scientific community	4	UK

						(higher education, Research)		
112	Flyers	NFSUK	Promote INFECT and NF	05/03/2015	Orlando USA	Scientific community (higher education, Research)	10	USA
113	Meeting organizer	REGION HOVEDSTADEN	INFECT WP2 meeting	27/4 2015	Dept. of Aneasthesia, Rigshospitalet, CPH	Scientific community (higer education Research)	8	Sweden, Norway, Denmark, Germany, The Netherlands,
114	Poster	UND	Systems Genetics Approach in a Murine Model of Streptococcal Necrotizing Soft Tissue Infections (NSTIs)	April 2015	35th Annual Frank Low Research Day, University of North Dakota	Scientific Community (higher education, Research)	>500	USA
115	Oral Presentation	UND	Systems Genetics Approach in a Murine Model of Streptococcal Necrotizing Soft Tissue Infections (NSTIs)	May 31, 2015	115th American Society for Microbiology, New Orleans, Louisiana, US	Scientific Community (higher education, Research)	>1000	International
116	Poster	CUBE	Value of single and combined inflammatory and kidney-dependent parameters for prediction of prognosis in septic shock		ASA 2015	San Diego		International
117	Presentation	REGION HOVEDSTADEN	INFECT presentation	10/6 2015	Dept. of Plastic Surgery, Herlev Hospital, Capitol Region	Medical staff	25	DK
118	Presentation-guideline working group	REGION HOVEDSTADEN	INFECT/NSTI presentation	12/6 2015	Dept. of Orthopedic Surgegry, Rigshospitalet.	Medical staff	15	DK

119	Flyers	NFSUK	Promote INFECT and NF	08/03/2015	Oregon USA	Scientific community (higher education, Research)	15	USA
120	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	INFECT: A systems medicine approach to advance our understandig of necrotizing soft tissue infection	24/06/2015	Djuronäset/Stockholm	Scientific community (higher education, Research)	20	International
121	Oral presentation to a wider public	KAROLINSKA INSTITUTET	ForskarFredag	25/09/2015	Debaser/Stockholm	Civil society	20	Sweden
122	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	Biofilm meeting	07/05/2015	Stockholm, Sweden	Scientific community (higher education, Research)	70	International
123	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	6th European conference on bloodstream infections	05/06/2015	Athens, Greece	Scientific community (higher education, Research)	100	International
124	Presentation	REGION HOVEDSTADEN	INFECT presentation	8/9 2015	Danish HBO society Odense University Hospital	Medical staff	8	DK
125	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	LuSep: International sepsis meeting in Lund	17/09/2015	Lund, Sweden	Scientific community (higher education, Research)	150	International
126	Oral presentation to a wider public	KAROLINSKA INSTITUTET	Lee Spark Foundation conference, Necrotising Fasciitis in Depth	16/10/2015	Blackpool, UK	Scientific community (higher education, Research) - Civil society	100	International
127	Oral presentation to	KAROLINSKA INSTITUTET	Seminar about NSTI for clinicians	13/11/2015	Stockholm, Sweden	Scientific community	60	Sweden

	a scientific event					(higher education, Research)		
128	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	Seminar about severe Gram-positive infections, Lund university	17/12/2015	Lund, Sweden	Scientific community (higher education, Research)	50	Sweden
129	Oral presentation to a scientific event	UNIVERSITET I BERGEN	The Infect Study	03/11/2015	Solstrand Hotell	Scientific community (higher education, Research)	35	Norway
130	Oral presentation to a wider public	VASTRA GOTALANDS LANS LANDSTING	Research presentation dept of surgery Sahlgrenska University Hospital	07/10/2015	Sahlgrenska Hospital	Scientific community (higher education, Research)	25	sweden
131	Oral presentation to a scientific event	VASTRA GOTALANDS LANS LANDSTING	INFECT Update	09/11/2015	St Jörgen Park Hotel	Scientific community (higher education, Research)	40	Sweden
132	Oral presentation to a wider public	VASTRA GOTALANDS LANS LANDSTING	INFECT	19/11/2015	Sahlgrenska University Hospital	Scientific community (higher education, Research)	50	Sweden
133	Organisation of Conference	REGION HOVEDSTADEN	WP2-WP4 meeting	25/06/2015	Rigshospitalet	Scientific community (higher education, Research)	10	International
134	Organisation of Conference	REGION HOVEDSTADEN	WP2-WP4 meeting	27/08/2015	Rigshospitalet, Copenhagen	Scientific community (higher education, Research)	10	International
135	Organisation of Conference	UNIVERSITET I BERGEN	WP2-WP8 meeting	23/04/2015	Bergen, Norway	Scientific community	7	Norway, Sweden

						(higher education, Research)		
136	Oral presentation to a wider public	VASTRA GOTALANDS LANS LANDSTING	Svåra mjukdelsinfektioner	23/10/2015	Sahlgrenska University Hospital	Scientific community (higher education, Research)	80	Sweden
137	Oral presentation to a scientific event	VASTRA GOTALANDS LANS LANDSTING	INFECT	01/04/2015	Hyperbaric dept Sahlgrenska University Hospital	Scientific community (higher education, Research)	15	Sweden
138	Oral presentation to a scientific event	UNIVERSITETET I BERGEN	Severe soft tissue infections	13/05/2015	Haukeland University Hospital	Scientific community (higher education, Research)	50	Norway
139	Organisation of Conference	NFSUK	Necrotising fasciitis - in depth	16/10/2015	Blackpool	Scientific community (higher education, Research) - Civil society	100	UK/International
140	Oral presentation to a scientific event	STOCKHOLMS LAENS LANDSTING	INFECT and necrotising fasciitis	07/01/2015	Karolinska Universitetssjukhuset	Scientific community (higher education, Research)	30	Sweden
141	Oral presentation to a scientific event	STOCKHOLMS LAENS LANDSTING	INFECT and necrotising fasciitis	22/05/2015	Karolinska Universitetssjukhuset	Scientific community (higher education, Research)	100	Sweden
142	YouTube video	Cube	Pathogen ID detection, https://www.youtube.com/watch?time_continue=8&v=CEaiDdzgXxI	5 aug 2015	St. Valentin, Austria	Health care professionals	>500	International
143	YouTube video	Cube	Patient monitoring, https://www.youtube.c	5 aug 2015	St. Valentin, Austria	Health care professionals	>500	International

			om/watch?time_continue=4&v=4_OGYp_fDxI					
144	Oral presentation to a scientific event	STOCKHOLMS LAENS LANDSTING	INFECT and necrotising fasciitis	03/10/2015	Södersjukhuset Stockholm	Scientific community (higher education, Research)	25	Sweden
145	Presentation	REGION HOVEDSTADEN	HBO and INFECT presentation	2/12 2015	Herlev Hospital, dept. of onkology , University hospital of Herlev	Medical staff	30	DK
146	Presentation	REGION HOVEDSTADEN	INFECT presentation	3/12 2015	Rigshospitalet, Juliane Marie Center , Rigshospitalet, CPH	Medical staff	20	Dk
147	Presentation	REGION HOVEDSTADEN	INFECT – microbiology of nevrotising soft tissue infections	10-12-2015	Dept. of Intensive Care 4131, Rigshospitalet	Intensivists	20	Denmark
148	Oral presentation to scientific event	WUR	Chemometrics in analytical chemistry	2016	Barcelona, Spain	Scientific Community (higher education, Research	>200	Spain
149	Oral presentation to scientific event	WUR	JDS	2016	Montpellier, France	Scientific Community (higher education, Research	>200	France
150	Presentation	REGION HOVEDSTADEN	INFECT preliminary results	10-01-2016	Copenhagen	INFECT WP2 group	15	International
151	Organisation of Conference	LIFEGLIMMER GMBH	INFECT 3rd Annual Meeting	12/01/2016	Berlin, Germany	Scientific community (higher education, Research) - Industry	35	Sweden, Germany, Austria, Netherlands, Norway, US, Denmark, UK, Israel, France
152	Oral presentation to	KAROLINSKA INSTITUTET	CASyM/SYSMedIBD joined workshiop:	18/02/2016	Brussels, Belgium	Scientific community (higher	40	International, European countries

	a scientific event		European Systems Medicine			education, Research) - Policy makers		
153	Presentation	REGION HOVEDSTAD	INFECT/INSTINCT trial updates	06-03-2016	Daily conference, departments of anaesthesiology and intensive care, Rigshospitalet	Aneashesiologists and Intensivists	50	Denmark
154	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	Organotypic models of human tissue and their application in biomedical research	09/03/2016	Pharmacity, Turku University	Scientific community (higher education, Research)	20	Finland
155	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	Superbugs & Superdrugs	16/03/2016	London, UK, 16-17 March	Scientific community (higher education, Research) - Industry - Policy makers	100	International
156	Presentation	REGION HOVEDSTADEN	INFECT presentation	15-17/3 2016	SMI – Super bugs and super drugs , London, UK	Doctors, scientist, pharmaceutical industry	50	UK-EU
157	Presentation	REGION HOVEDSTADEN	INFECT presentation/NSTI treatment	11/3 2016	Danish Society for Wound Care , Korsør, Denmark	Doctors, nurses, pharmaceutical industry	250	DK, Scandinavia
158	Presentation	Int. conf. on Emergency medicine	INFECT presentation on ph.d.pub	April 2016	Cape Town South-Africa	Doctors, nurses, paramedics	50	Global audience
159	Presentation	REGION HOVEDSTADEN	INFECT presentation	26-27/4 2016	Scand. Hyperbar Medicinsk Selskab, Dykeri- och HBO kurs, Karlskrona, Sweden	Doctors, nurses, Navy	50	Sweden
160	Presentation	REGION HOVEDSTADEN	Necrotising soft tissue infections	15-06-2016	Herlev Hospital	Doctors and nurses	150	Denmark
161	Oral presentation to	UNIVERSITETET I BERGEN	Soft tissue infections	06/06/2016	Haukeland university hospital	Scientific community (higher	7	Norway, Sweden

	a scientific event					education, Research)		
162	Oral presentation to a scientific event	UNIVERSITETET I BERGEN	The Infect Study	06/06/2016	Department of Clinical Science	Scientific community (higher education, Research)	30	International
163	Oral presentation to a wider public	VASTRA GOTALANDS LANS LANDSTING	INFECT	27/08/2016	Hyperbaric dept Sahlgrenska University Hospital	Scientific community (higher education, Research)	20	sweden
164	Oral presentation to a scientific event	HELMHOLTZ-ZENTRUM FUER INFEKTIONSFORSCHUNG GMBH	Investigating the serological background of Necrotizing Soft Tissue Infections	14/09/2016	Ulm, Germany	Scientific community (higher education, Research)	500	Germany, United States, Russia, Australia, India
165	Posters	KAROLINSKA INSTITUTET	Cell Symposium: 100 years of Phagocytes	19/09/2016	Sicily, Italy	Scientific community (higher education, Research)	300	International
167	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	3rd German Pneumococcal and Streptococcal Symposium	09/09/2016	Bruanschweig, Germany	Scientific community (higher education, Research)	60	Germany, Sweden, Australia, India, US, UK, China, Spain
168	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	Local mediators of pathology in invasive bacterial tissue infections	02/09/2016	The Medical School, University of Sheffield	Scientific community (higher education, Research)	30	United Kingdom
169	Conference	REGION HOVEDSTADEN	Intravenous polyspecific immunoglobulin G for patients with necrotizing soft tissue infection: Results of the randomised,	03-10-2016	Presidents Session, ESICM LIVES yearly congress 2016, Milano	Intensivists	1000	International

			blinded, placebo-controlled INSTINCT trial					
170	Organisation of Conference	KAROLINSKA INSTITUTET	INFECT meeting WP1, 3-6	26/10/2016	Berlin, Germany	Scientific community (higher education, Research)	10	Sweden, Germany, Netherlands, US
172	Presentation	REGION HOVEDSTADEN	INFECT presentions	8/9 2016	Dept. of Anaesthesia- and Surgery, RIgshospitalet , Rigshospitalet, CPH	INFECT team, doctors, nurses	10	DK
173	Presentation	REGION HOVEDSTADEN	INFECT presentation	22/9 2016	Dept. orthopedic Surg, Rigshospitalet, Rigshospitalet, CPH	Doctors, nurses	45	DK
174	Presentation	REGION HOVEDSTADEN	INFECT presentation/HBO meeting	29/9 2016	Århus University Hospital	Doctors, nurses	8	DK
175	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	First conference of EASyM	26/10/2016	Berlin, Germany 26-28 October	Scientific community (higher education, Research)	150	International
176	Poster	UND	European Association of Systems Medicine, Transcriptome Analysis Of Skin Lesions Reveals a New Role For Adipose Tissue in Necrotizing Soft Tissue Infections Caused by Group A Streptococcus,	October 26-28, 2016	Berlin, Germany	Scientific Community (higher education, Research)	~100	International
177	Organisation of Workshops	UNIVERSITETET I BERGEN	INFECT workshop	03/11/2016	Haukeland university hospital	Scientific community (higher education, Research)	12	Norway

178	Oral presentation to a wider public	MARSDEN DOREEN	Infection Prevention Nurses	09/03/2016	Shrewsbury	Civil society	50	UK
179	Oral presentation to a wider public	MARSDEN DOREEN	Infection Prevention Nurses	15/04/2016	Cardiff	Civil society		UK
180	Oral presentation to a wider public	MARSDEN DOREEN	INFECT project and patients experiences	12/02/2016	Cardiff	Civil society		UK
181	Oral presentation to a wider public	MARSDEN DOREEN	Infection Prevention Nurses	21/07/2016	North Wales	Civil society		UK
182	Oral presentation to a wider public	MARSDEN DOREEN	INFECT project and patients experiences	14/09/2016	North West Branch Meeting	Civil society		UK
183	Conference	CUBE	European Concress of Clinical Microbiology and Infectious Diseases	9-12 April 2016	Amsterdam	Scientific, Industry	>10.000	Europe, USA, worldwide
184	Conference	CUBE	8th International Congress "Sep“is and Multiorgan Dysfunction”	6-8 Sept. 2017	Weimar	Scientific	872	Germany (604), Europe and Rest of World (268)
185	Presentation	REGION HOVEDSTADEN	INFECT presention on ph.d.-pub	November 2016	Danis Soc for Anaesthesiology and Intensive Care , Copenhagen	Doctors	50	DK
186	Oral presentation to scientific event	WUR	15th Scandinavian Chemometrics Conference	2017	Naantali, Finland	Scientific Community (higher education, Research	>200	Finland
187	Oral presentation to scientific event	WUR	ICRM 2017 – International Chemometrics Research Meeting September 2017	2017	Nijmegen, The Netherlands	Scientific Community (higher education, Research	>200	The Netherlands
188	Organisation of Conference	REGION HOVEDSTADEN	INFECT 4th annual meeting	11/01/2017	Copenhagen, Denmark	Scientific community (higher	35	Denmark, Sweden, Norway, Austria, Germany,

						education, Research)		Netherlands, US, Israel, France, UK
189	Presentation	REGION HOVEDSTADEN	Necrotising soft tissue infections	19-01-2017	Dept. of Ophthalmology, Glostrup Hospital	Doctors	40	Denmark
190	Presentation		Biofilm in GAS NSTI	26-02-2017	Rigshospitalet	Aneashesiologists and Intensivists	50	Denmark
191	Presentation		Necrotising soft tissue infections	06-03-2017	Herlev Hospital	Urologists	30	Denmark
192	Presentation	REGION HOVEDSTADEN	INFECT presentation	6/4 2017	Rigshospitalet, CPH	Doctors	30	DK
193	Poster	UND	Downregulated PPAR□ and impaired adipogenesis during invasive Group A Streptococcal infections	April 2017	37th Annual Frank Low Research Day, University of North Dakota, Grand Forks, ND, USA	Scientific Community (higher education, Research)	>500	USA
194	Presentation		Necrotising soft tissue infections	04-05-2017	Rigshospitalet	PhD students	15	Denmark
195	Presentation		Necrotising soft tissue infections	05-05-2017	Gyn-obs Hvidovre	Doctors	30	Denmark
196	Presentation, organizer	REGION HOVEDSTADEN	INFECT presentaions	1/6 2017	Dept. of Anaesthesia- and Surgery, Rigshospitalet,m Scandic Hotel, CPH	Scientific community WP2 (higher education research)	11	Sweden, Norway, Denmark, Germany, The Netherlands
197	Presentation, organizer	REGION HOVEDSTADEN	INFECT presentions	21/9 2017	Dept. of Anaesthesia- and Surgery, Rigshospitalet, Admiral Hotel, CPH	Scientific community WP2 and partners (higher education research)	12	Sweden, Norway, Denmark, Germany, The Netherlands
198	Presentation	REGION HOVEDSTADEN	INFECT administration	28/9 2017	Dept. anaesthesia- and surgery, Rigshospitalet-Glostrup, CPH	Adm.staff	2	DK
199	Presentation		Incidence of NSTI	02-10-2017	ESICM LIVES 2017, Vienna	Intensivists	20	Denmark

200	Presentation	REGION HOVEDSTADEN	INFECT presentation/HBO	25/10 2017	onk. Dept. – Hillerød Hosp.	Doctors	20	DK
201	Oral presentation to a scientific event	KAROLINSKA INSTITUTET	The INFECT-project; a multicentre prospective study on necrotizing soft tissue infections:from clinics to pathogenesis to intervention	19/10/2017	Fiji, Lancefiled	Scientific community (higher education, Research)	300	International
202	Oral presentation to a scientific event	HELMHOLTZ-ZENTRUM FUER INFEKTIONSFORSCHUNG GMBH	A serological evaluation of the host immune response during necrotizing soft tissue infections caused by Streptococcus pyogenes	16/10/2017	Fiji, Lancefield	Scientific community (higher education, Research)	300	International
203	Oral presentation to a scientific event	UNIVERSITET I BERGEN	Clinical features of necrotizing soft tissue infections caused by beta-hemolytic Group A, C and G streptococci: analysis of the Scandinavian INFECT study cohort	19/10/2017	Fiji, Lancefield	Scientific community (higher education, Research)	300	International
204	Oral presentation to a scientific event	UNIVERSITET I BERGEN	The role of streptococcus pyogenes and other beta-hemolytic streptococci in erysipelas and cellulitis	16/10/2017	Fiji, Lancefield	Scientific community (higher education, Research)	300	International
205	Presentation	REGION HOVEDSTADEN	INFECT presentation/HBO	25/10 2017	Hillerød onk. Department, Capitol region, onk. Dept. – Hillerød Hosp.	Doctors	20	DK
206	Presentation	REGION HOVEDSTADEN	Results from INFECT	10-11-2017	DASAIM congress 2017	Intensivists and anaesthesiotologists	25	Denmark

207	Presentation	REGION HOVEDSTADEN	INFECT presentation	16/11 2017	Dept. of Plastic Surgery, RIgshospitalet , Rgshospitalet, CPH	Doctors	25	DK
208	Organisation of Conference	KAROLINSKA INSTITUTET	INFECT 5th Annual Meeting	29/11/2017	Stockholm, Sweden	Scientific community (higher education, Research)	30	Sweden, Denmark, Norway, Netherlands, Germany, Israel, France, UK, US, Austria
209	Presentation	REGION HOVEDSTADEN	INFECT presentation/HBO	6/12 2017	Danish soc dermatology, Gentofte Hospital, Capitol region	Doctors	34	DK
210	Presentation	REGION HOVEDSTADEN	Results from INFECT	12-04-2018	Rigshospitalet	ICU nurses	50	Denmark
211	Oral presentation to scientific event	WUR	MINI Arctic Symposium,	2018	Fluddir, Iceland	Scientific Community (higher education, Research	100	Iceland
212	Oral presentation to scientific event	WUR	BioSB 2018 – Dutch conference of bioinformatics and systems biology	2018	Lunteren, The Netherlands	Scientific Community (higher education, Research	>500	The Netherlands
213	Organisation of Conference	UNIVERSITETET I BERGEN	INFECT Final Meeting	18/06/2018	Grand Hotel Terminus, Bergen	Scientific community (higher education, Research)	30	International
214	Oral presentation to a wider public	UNIVERSITETET I BERGEN	Presicion medicine in Necrotizing soft tissue infections	24/05/2018	NTNU; St.Olav's Hospital, Trondheim, Norway	Scientific community (higher education, Research)	80	Norway, Sweden, UK, USA
215	Oral presentation to a scientific event	VASTRA GOTALANDS LANS LANDSTING	INFECT Study	01/03/2018	Sahlgrenska sjukhuset, Gothenburg, Sweden	Scientific community (higher education, Research)	20	Sweden

216	Conference (Annual Conference 2018 of the Association for General and Applied Microbiology)	HZI	Combining multiple sequencing technologies to investigate mono- and multispecies communities in necrotizing soft tissue infections	15.-18. april 2018	Wolfsburg. Germany	Scientific community	500	Germany, EU wide
217	Poster	UND	Group A Streptococcus induces neuroinflammatory responses in HLA-II transgenic mice model of Necrotizing Soft Tissue Infections	April 2018	38th Annual Frank Low Research Day, University of North Dakota, Grand Forks ND, USA	Scientific Community (higher education, Research)	>500	US
218	Press release	HZI	Neue Therapieansätze bei nekrotisierender Fasziitis	27. june 2018	Braunschweig, Germany	Civil Society	2000	Germany
221	YouTube video	Cube	Pathogen enrichment, https://www.youtube.com/watch?v=qMtccEWM0iY	10 July 2017	St. Valentin, Austria	Health care professionals	>500	International
222	Presentation	REGION HOVEDSTADEN	INFECT presentation /HBO	22-28/9 2018	TRICON 2018 meeting, European Underw. And Baromedicl Soc, South-Paficif Underw Med Soc. Int. meeting Durban, South-Africa	Doctors, nurses, medical staff, pharmaceutical industry	450	Global int. meeting.
223		UNIVERSITETET I BERGEN	INFECT Final meeting	18-20/06/2018	Grand Hotel Terminus, Bergen, Bergen, Norway	Scientific community (higher education, Research)	30	International
224	YouTube video clip	All partners	The INFECT project	Fall 2018		Society at large	TBD	worldwide

Section B (Confidential[6] or public: confidential information to be marked clearly)

Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.						
NO.	Type of IP Rights[7]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)

[6] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[7] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2

Please complete the table hereafter:

NO.	Type of Exploitable Foreground[8]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[9]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
1	*General advancement of knowledge,*	*Biomarkers for identification of infecting agent*	*yes*		*Biomarker test*	*Q86.2.2 - Specialist medical practice activities*	*in planning phase*	*in planning phase*	*Partner 8 (HZI)**
2	*General advancement of knowledge,*	*Biomarkers for identification of susceptability to develop NSTI*	*yes*		*Biomarker test*	*Q86.2.2 - Specialist medical practice activities*	*in planning phase*	*in planning phase*	*Partner 8 (HZI)*
3	*General advancement of knowledge,*	*Microbiota analysis for rapid identification of infecting agents*	*yes*		*Education and consulting*	*Q86.2.2 - Specialist medical practice activities*	*in planning phase*	*in planning phase*	*Partner 8 (HZI)*
4	Commercial exploitation	Biomarker test (based on whole blood)	NO		hybcell Patient monitoring Blood xA	Medical	2019	Patents on biomarker combination are planned	Partner 16 (Cube)**

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[9] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

NO.	Type of Exploitable Foreground[8]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[9]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
5	Commercial exploitation	Early pathogen identification test	NO		GINA 500 (pathogen enrichment) hybcell Pathogens DNA xB (and variants)	Medical	2017	Patents on internal control and pathogen enrichment planned	Partner 16 (Cube)

***Partner 8**

1. Different bacteria/communities are capable to cause necrotizing fasciitis and these causing agents cause distinct patterns of molecular host pathophysiology, emphasizing the importance of rapid and accurate bacterial diagnosis to optimize clinical treatment and the potential benefits of intervention strategies tailored towards the specific, etiology-dependent molecular pathophysiology.

We have shown that NSTIs caused by polymicrobial communities and those caused by Streptococcus sp. can be differentiated based on the chemokine profile in blood. Future research will focus on the use of specific chemokine profiles as biomarkers to allow a rapid identification of NSTI causing bacteria.

2. We have shown that NSTI patients exhibited a deficiency in specific antibodies directed against the causative *S. pyogenes* strains and the majority of their exotoxins during the initial stage of the infection. We also showed that the clinical application of IVIG during the course of infection compensates the observed antibody deficiency, but is unable to halt the disease progression, once tissue necrosis has developed.
There is, thus, an urgent need, to first define the infecting bacteria (see above) but also to identify the susceptibility to develop NSTI by a rapid antibody profiling test.
Future research will focus on the development of an appropriate antibody profiling test.

3. Different bacteria/communities are capable to cause necrotizing fasciitis and these causing agents cause distinct patterns of molecular host pathophysiology, emphasizing the importance of rapid and accurate bacterial diagnosis to optimize clinical treatment and the potential benefits of intervention strategies tailored towards the specific, etiology-dependent molecular pathophysiology.
We have shown here that microbial community structure analysis by Illumina next generation sequencing is a rapid and reliable tool for cost effective analysis which allows species identification for all genera known to be important in causing necrotizing fasciitis
It is, expected that the main exploitation is not by selling but analysis for medical doctors and training.

****Partner 16**

As a commercial SME, Cube focused on the translation of results into diagnostic tests. So, the exploitable results are per definition different tests (and associated products) and there underlying assay technologies.

For the early identification of pathogens, Cube has developed a suite of products comprising of:

- Products for enrichment of pathogens (in human samples, above all whole blood): GINA 500 (+ DNA Purification),
- Tests for the detection of bacterial and fungal DNA: PCR-Box Bacteria, PCR-Box Fungi, PCR-Box Resistance,
- Tests for the identification of bacteria, resistance genes and fungi: hybcell Bacteria DNA xB / Fungi DNA xB / Pathogens DNA xB,
- an internal control especially to differentiate between negative results and a failure of the analytic process and
- external quality controls to periodically demonstrate the capability of testing.

In April 2018 the products for early identification of pathogens have been declared to be a CE-IVD. Therefore the marketing in Europe and the Middle East can start and the products will be marketed in Europe and Middle East, later in the USA and worldwide.In the German speaking countries Cube distributes the products on its own expenses with its own salesforce. In Europe and the Middle East, distributors are contracted, who distribute the products in their name and at their expenses. Even if already marketed, more studies – especially on the clinical and societal impact (health economics) – have to be conducted. The first such study will start in August 2018 at the Medical University of Vienna. If an improvement of the usage of antimicrobials can be shown, and if such improvement leads to better outcomes, Cube will have a good marketing position and can approach payers of health services directly. The sepsis diagnostics market itself is a growing market and expected to reach $ 564,1 million by 2021 from $ 370 million in 2016, at a CAGR of 8.8%.

For rapid point-of-care patientmonitoring, Cube has developed following test:

- hybcell Patientmonitoring Blood xA.

The CE-IVD mark for this biomarker test will follow in Q4 2018 (based on the data generated with RH (partner 2) and internal data, as well as data from Inselspital in Bern). Business development for this test will start in selected European countries (Austria, Germany, Denmark, Switzerland, Sweden, Italy). However, the clinical impact of such test has to be further elaborated. A co-operation with the largest intensive care unit of Switzerland at the Inselspital in Bern will start in August and up to 1000 patients should be tested in the coming 2 years. Beside that, Cube tries to set-up a spin-off company focussing on the biomarker (protein) testing. A first step has been made, as the trademark Celeras Dx has been created (www.celerasdx.com). Crucial for success will be the identification of clinical benefits of applying a regular patientmonitoring (once or twice a day) and its link to therapy decisions.

Quantifying the impacts of both product groups will be possible after large data sets with the outcome of the tests and the associated clinical outcome are compiled. Therefore the collection of such data is the focus of further research / studies.
Apart from the developed products, the INFECT project facilitated the development of basic assay technologies for Cube: compact sequencing for broad, sensitive and highly specific DNA tests, as well as compact profiling for broad, dynamic and fast biomarker tests. Both technologies can spread into other fields of application like oncological tests, autoimmune disease testing, etc.

4.3 Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	305340
Title of Project:	INFECT (Improving Outcome of Necrotizing Faciitis: Elucidation of Complex Host and Pathogen Signatures Dictate Severity of Tissue Infections
Name and Title of Coordinator:	Anna Norrby-Teglund, Professor

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements (Ethics reports have been included in periodic reports 1-5).*	YES
2. Please indicate whether your project involved any of the following issues (tick box) :	YES
RESEARCH ON HUMANS	
• Did the project involve children?	
• Did the project involve patients?	√
• Did the project involve persons not able to give consent?	√
• Did the project involve adult healthy volunteers?	√
• Did the project involve Human genetic material?	√
• Did the project involve Human biological samples?	√
• Did the project involve Human data collection?	√
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	√
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	√

• Were those animals transgenic small laboratory animals?	√
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	No
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	No
DUAL USE	
• Research having direct military use	No
• Research having the potential for terrorist abuse	No

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	1	
Work package leaders	3	6
Experienced researchers (i.e. PhD holders)	12	28
PhD Students	5	11
Other	15	10

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	18
Of which, indicate the number of men:	12

D Gender Aspects

5.	**Did you carry out specific Gender Equality Actions under the project**?	○ √	Yes No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	○	○	○
❑	Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

√ Yes- please specify

We observed sex-dependent differences in both patient and the experimental in vivo model.

No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

√ Yes- please specify

Lectures, Presentations on Research Friday.

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

√ Yes- please specify

Videos that are in production, where the project and achievements are presented, will be launched shortly. A book volume on the INFECT achievements will be published. Both these will be of value as educational material. An external website was opened for public access spring 2013.

○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

√ Main discipline[10]: 3.2

√ Associated discipline[Error! Bookmark not defined.]: 3.1, 3.3, 1.5, 1.1

○ Associated discipline[Error! Bookmark not defined.]:

G Engaging with Civil society and policy makers

11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	√	Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No

√	Yes- in determining what research should be performed	
○	Yes - in implementing the research	
√	Yes, in communicating /disseminating / using the results of the project	
11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	√	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

○	No
○	Yes- in framing the research agenda
○	Yes - in implementing the research agenda
√	Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- √ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		**Public Health**	√
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		**Research and Innovation**	√
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

[10] Insert number from list below (Frascati Manual).

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- √ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	53
To how many of these is open access[11] provided?	**34**
How many of these are published in open access journals?	**23**
How many of these are published in open repositories?	**11**
To how many of these is open access not provided?	18
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available X no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[12]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**0**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		1
Indicate the approximate number of additional jobs in these companies:		3

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

√	Increase in employment, or	√	In small & medium-sized enterprises
√	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*
Difficult to estimate / not possible to quantify	X

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes √ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

√ Yes ○ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

√	Press Release	√	Coverage in specialist press
❑	Media briefing	√	Coverage in general (non-specialist) press
√	TV coverage / report	√	Coverage in national press
√	Radio coverage / report	❑	Coverage in international press
√	Brochures /posters / flyers	√	Website for the general public / internet
√	DVD /Film /Multimedia	√	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	√	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

[11] Open Access is defined as free of charge access for anyone via Internet.

[12] For instance: classification for security project.

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

2. FINAL REPORT ON THE DISTRIBUTION OF THE EUROPEAN UNION FINANCIAL CONTRIBUTION

This report shall be submitted to the Commission within 30 days after receipt of the final payment of the European Union financial contribution.

Report on the distribution of the European Union financial contribution between beneficiaries

Name of beneficiary	Final amount of EU contribution per beneficiary in Euros
1. Karolinska Institutet	
2. Region Hovedstaden	
3. Stockholms Lans Landsting	
4. Blekinge Lans Landsting	
5. Västra Gotalands Lans Landsting	
6. Universitetet Ii Bergen	
7. *University of Cincinnati (terminated 2013)*	
8. Helmholtz-Zentrum fur Infektionsforschung	
9. Wageningen Universiteit	
10. Université Lyon	
11. LifeGlimmer GmbH	
12. *Anagnostics Bioanalysis GmbH (terminated 2015)*	
13. The Lee Spark Foundation	
14. Tel Aviv University	
15. University of North Dakota (replaced partner 7)	
16. Cube Dx, GmbH (replaced partner 12)	
Total	