

Meeting of Experts on Biomarkers to Discriminate Bacterial From Other Infectious Causes of Acute Fever

22-23 September 2015
Geneva, Switzerland

Report prepared by Sabine Dittrich (FIND)



Contents

List of abbreviations.....	3
Executive summary	4
Day 1 – 22 September 2015	5
Session 1: Welcome and meeting objective	5
Session 2: Opening remarks	5
Session 3: Clinical needs in the field	5
Session 4: Target product profiles.....	7
Session 5: Biomarker landscape	8
Session 6: The industry landscape	8
Session 7: Optimal design for clinical trials of fever biomarker assays	12
Session 8: Diagnostic pathway	14
Session 9: Biobanking	16
Session 10: Prioritizing next steps for TPPs, clinical trial design & biobanking.....	17
Day 2 – 23 September 2015	18
Session 11: Important new developments.....	18
Session 12: Improving the diagnosis of fever.....	21
Session 13: Concluding remarks by WHO, FIND, MSF and ReAct	23
Session 14: Closing of the meeting	23
List of participants.....	24

List of abbreviations

AMR	Antimicrobial resistance
AUC	Area under the curve
BMGF	Bill & Melinda Gates Foundation
CRP	C-reactive protein
CSF	Cerebral spinal fluid
DRC	Democratic Republic of Congo
HIC	High-income country
HNL	Human neutrophil lipocalin
IVD	<i>In vitro</i> diagnostic
LMIC	Low- and middle-income country
LOD	Limit of detection
LR	Likelihood ratio
MSF	Médecins Sans Frontières
NPV	Negative predictive value
PCT	Procalcitonin
POC	Point of care
PPV	Positive predictive value
RDT	Rapid diagnostic test
SEA	Southeast Asia
TPP	Target product profile
WHO	World Health Organisation

Executive summary

A meeting convened on 22-23 September 2015 in Geneva by WHO, ReAct, MSF Access Campaign and FIND gathered together more than 50 biomarker scientists, policymakers, implementers, clinical researchers and industry product developers. The meeting assembled a wide diversity of stakeholders representing the full “bench to bedside” pathway for biomarker testing to differentiate between bacterial and viral pathogens. Speakers and participants discussed the critical need for community-level fever diagnostics globally, reviewed initiatives that are currently underway and discussed clinically relevant performance data with the ultimate aim of improved patient care and the reduced use of antimicrobials.

The meeting helped to identify the tools, resources and partnerships needed to accelerate the development of a point-of-care diagnostic assay for the detection of bacterial infections in patients presenting with acute fever. Speakers and participants highlighted the need for new tests that can rapidly distinguish bacterial from non-bacterial causes of acute fever to guide appropriate patient treatment and preserve the effectiveness of existing antibiotics in a context of rapidly increasing antimicrobial resistance (AMR). While several promising biomarkers have been identified for such tests, most of them are still in the proof-of-concept phase and have only been evaluated with a limited number of patients in developed-country settings. Validation with patients in developing countries should thus be done. To facilitate this validation, better standard tools (for design of clinical studies and reference testing) are also needed. Furthermore, it was observed that potential technology platforms are available.

Strengthening multi-sectoral partnerships that include industry, academia, clinicians, NGOs and WHO will contribute to the accelerated development of point-of-care tests that can distinguish bacterial from non-bacterial infections in low-resource settings.

Meeting participants also discussed the unique challenges of bringing biomarker diagnostics to low- and middle-income country settings and agreed that the next steps are the continued development and validation of potential biomarkers to guide patient treatment and reduce the overuse of antimicrobials. For example:

- development of a target product profile (TPP) to guide future diagnostics development;
- development of standardized guidelines for clinical trials of new fever tests;
- harmonization of reference standards; and
- agreement on specifications for building specimen banks to meet identified needs.

The meeting concluded with all organizing partners reiterating the important role of improved diagnostics in reducing inappropriate antibiotic use.

Day 1 – 22 September 2015

Session 1: Welcome and meeting objective

Dr Francis Moussy, Diagnostics Innovation, WHO, opened the meeting by welcoming meeting participants. Dr Moussy highlighted the need to differentiate between viral and bacterial infections and to understand the present diagnostic landscape for biomarkers, developments in the pipeline and development needs. He introduced the goal of the meeting: to encourage closer collaborations between NGOs, academic institutions and industry to speed up diagnostics development and implementation of biomarker tests.

Session 2: Opening remarks

Speaker: Dr Carmen Lucia Pessoa da Silva (Antimicrobial resistance team leader, WHO)

Dr Pessoa da Silva talked about the global action plan on antimicrobial resistance (AMR) agreed on in May 2015 at the World Health Assembly and emphasized the five key points highlighted in the plan:

- Improve awareness and understanding
- Strengthen the knowledge and evidence base
- Reduce the incidence of infection
- Optimize the use of antimicrobial medicines
- Develop the economic case for sustainable investment

Dr Pessoa da Silva closed by outlining the framework of action and indicated that the goal is for all countries to have national action plans to address AMR by May 2017.

Session 3: Clinical needs in the field

Title: *Brief summary of clinical need for biomarker tests to distinguish bacterial from non-bacterial infections in patients presenting with acute fever*

Speaker: Dr Valérie D'Acremont, research group leader at Swiss TPH and consultant in infectious and tropical diseases at the Lausanne University Hospital

Dr D'Acremont opened by noting that the type of tests to be discussed during the meeting should be for patient management, i.e. having clinical impact for the individual

or population rather than being of purely academic value to understand aetiologies. Of the large febrile population at community level, only about a quarter (depending on local health-seeking behaviours) attend a health facility, of which about a tenth will be sent to the outpatient department of a hospital, of which a tenth will be admitted. These last, who require hospitalization for severe disease, thus represent a very small proportion of the febrile population. In order to identify this group at an early stage, a test needs to be highly specific.

Among the large number of patients presenting with a febrile illness at the community level, only a limited number (possibly less than 5%) will benefit from an antibiotic treatment because most of the causes of (non-malaria) mild fevers are of viral origin. The proportion of patients benefiting from antibiotic therapy increases with disease severity and thus health system level (~5-10% at the primary care level, ~20% at outpatient, ~50% at inpatient).

Unfortunately, antibiotics are widely prescribed at the peripheral level, which creates a huge drug pressure that drives resistance. The highest impact of the development and implementation of a novel test able to distinguish a bacterial from a viral infection would therefore be at peripheral level. Therefore, the TPP needs to be based on the prevalence of disease at this level rather than the hospital level (where most studies have been undertaken). Such a test could also have an impact at hospital level, where antibiotics are often withheld (e.g., because of an incorrect diagnosis of malaria). This impact would not be on resistance (due to the low number of patients) but rather on the mortality rate.

Once the prevalence of disease at primary care level is known (e.g., 3% for typhoid fever), the acceptable post-test probability to rule in and treat (e.g., 20%) or to rule out the disease (e.g., 0.5%) has to be decided. From there the desired positive and negative likelihood ratio (LR) for the test can be decided (for example LR+ of at least 7 and LR- of 0.25). Based on these rules, the best test is not always the one we think (e.g., available rapid diagnostic tests (RDTs) for typhoid have a LR+ of 4, while the clinical predictor abdominal tenderness has a LR+ of 7; blood culture for *Salmonella* has a LR- of around 0.5, while the LR- of typhoid RDT is 0.25).

To develop and validate a novel bacterial test, the next step after the development of an agreed TPP is to set up clinically relevant case definitions and laboratory reference standards for bacterial versus viral disease. The advantages and limitations inherent to each type of diagnostic tool (blood culture, serology, PCR) used to establish the aetiology of an acute episode of fever were presented. One way to overcome limitations is to use a composite reference test combining antibody and antigen or DNA detection.

Dr D'Acremont showed common findings from recent studies specifically designed to establish the aetiology of fever, which differed between children and adults, the latter often being HIV-positive, and according to the level of the health-care system. These

populations need to be specifically considered in clinical studies when evaluating potential biomarkers. Further, because infection does not mean disease and viral-bacterial co-infections are frequent, she suggested that severity markers might be as important as a test that could discriminate between bacterial and viral aetiology, and also for the triage of patients at peripheral levels of the health-care system. Dr D'Acremont concluded with suggestions on using an integrated approach combining several biomarkers and good clinical predictors, as well as host biomarkers with specific pathogen detection. She emphasized that biomarkers should feed into a larger clinical algorithm to guide clinicians and, ultimately, decrease the use of antimicrobials.

Discussion:

Discussion focused on the acceptable diagnostic parameters and the resulting risk of misclassification and negative outcomes due to under-treatment. It was also discussed to what extent the development of one test for all regions might be feasible, particularly in children, in whom a majority of febrile episodes are caused by viral agents.

Session 4: Target product profiles

Title: *Status of target product profile development and next steps to finalize the TPP*

Speaker: Dr Sabine Dittrich, Consultant to FIND

Dr Dittrich outlined the importance of target product profiles (TPP) and described the possible use cases for biomarker testing (severity, triage at different levels of health system, testing for pathogens). She outlined selected test characteristics focusing on the use of a biomarker test at community level to triage patients to treat with antibiotics or not due to likely viral aetiologies. In addition to the presented TPP characteristics (target population, user, performance parameters, instrumentation and multiplexing, sample handling/preparation and costs), she outlined a number of additional features that should be considered.

The speaker explained the next steps in developing useful TPPs, including the implementation of a TPP expert group, stakeholder consultations, a planned Delphi-like survey and a final consensus meeting. Dr Dittrich concluded by inviting meeting participants to join the expert group and highlighted the need for a diverse group of participants to contribute to the TPP document to achieve comprehensive and applicable document.

Discussion:

The discussion following the presentation highlighted the need to discuss similar TPP developments with other organizations (Wellcome Trust, BMGF) and that it would be useful to agree among the expert group on priority scenarios for which TPPs are developed.

Session 5: Biomarker landscape

Title: *Landscape analysis of host biomarkers for diagnosing bacterial and other infectious causes of febrile illness: A brief summary of findings*

Speaker: Dr Tim Rodwell, FIND

Dr Rodwell described the process and methodologies that were used in a landscape analysis conducted by his group to identify promising biomarkers described in the scientific and grey literature. He noted that a number of biomarkers had only been evaluated once without further studies to confirm the results and that a large number of studies on biomarkers had been done in Europe but only 14% in low- and middle-income countries (LMICs). Dr Rodwell explained that in total, seven different types of markers had been identified, and the overall quality of studies, independent of the investigated marker, varied hugely, making the interpretation of the results and accuracy levels more challenging.

The speaker summarized the landscape analysis, which identified three promising markers or combinations with high-quality scores as well as sensitivities and specificities over 85%:

- HBP showed a lot of promise, but current available evidence is limited;
- A combination of CRP+IP+TRAIL showed good diagnostic performance, but studies have so far only been undertaken in high-income countries;
- A combination of MxA and CRP showed good diagnostic performance, but no studies have been conducted in LMICs.

Dr Rodwell closed by concluding that it is likely that not one individual biomarker can be used but that combinations of biomarkers are the way forward.

Discussion:

In the discussion, a number of industry and academic participants confirmed the difficulty in reproducing biomarker results, further highlighting the need for specific studies in the target population. Also, one participant highlighted that a good biomarker is only as good as the platform it is used with. Independent validation of most biomarkers is currently needed. Current surveillance studies could be an appropriate framework for these evaluations. An important data point for these studies would be patient outcome in order to evaluate impact of implementing such tools.

Session 6: The industry landscape

Session Chair: Dr Francis Moussy

The chair introduced the aim of this session as obtaining a better understanding of novel biomarkers in the pipeline, challenges and next steps presented by partners from industry.

Title: *Re-defining sepsis using the host immune response*

Speaker: Dr Therese Seldon, Vice President, Immunexpress

Dr Seldon started by introducing the so-called “forgotten cohort” of patients that present with mild disease without severe sepsis signs, the target population of the SeptiCyte assay developed by Immunexpress. The four biomarkers that are used in the SeptiCyte test are specific mRNAs that are differentially regulated in the host immune response during bacterial infection. The company has developed and validated the four-gene algorithm in large-scale clinical trials in the Netherlands. The accuracy in identifying sepsis in systemic inflammatory response syndrome patients that are infection-negative was very good (area under the curve (AUC) of 0.93). The speaker presented the results of further studies (more than 1500 patients from Australia, USA, UK and the Netherlands) in which the four-gene algorithm proved accurate (AUC 0.92 versus AUC [procalcitonin (PCT)] 0.81), which show it to be a useful test for the “forgotten cohort” and can be used at central hospital level.

Dr Seldon shared that the company is currently developing a RDT format and is looking for possible partners to evaluate this methodology. The technology currently requires 200 µl of blood, but Dr Seldon reported that preliminary data from paediatric cohorts suggest that smaller volumes might also be sufficient. The test has been submitted for FDA approval.

Title: *Meeting of experts on biomarkers to discriminate bacterial from other infectious causes of acute fever*

Speaker: Dr Robert P. Sambursky, MD, Founder, Chief Executive Officer and President, RPS Diagnostics (FebriDx test)

Dr Sambursky explained that the aim of the FebriDx rapid test is to help aid primary and urgent care physicians in the outpatient setting to make a rapid assessment of the clinical significance of an acute respiratory infection. Further, the FebriDx test helps to differentiate infections with a systemic host response from local infections or colonization as well as identify patients as having a viral or bacterial infection versus those with a microbiologically unconfirmed respiratory illness. The test uses a combination of two biomarkers, including myxovirus resistance protein A (MxA), a novel viral biomarker, and C-reactive protein (CRP). MxA is an intracellular blood protein that is induced by type 1

interferon and is therefore specific for true viral infections (as opposed to viral carriage). The biomarker is normally very low in blood but elevates rapidly in a viral infection, has a long half-life and stays elevated in the presence of elevated interferon.

The FebriDx test is a single-use disposable test that uses a fingerstick blood (5µl) sample near the bedside. The time to result is approximately 15 minutes and no additional sample processing is required. The read-out of the test is interpreted as either viral infection when MxA is elevated (MxA positive, CRP positive or negative) or as bacterial infection whenever CRP is elevated in the presence of normal MxA (MxA negative, low or high CRP positive).

Dr Sambursky showed data from a prospective, multi-centre clinical trial in the USA where FebriDx demonstrated a sensitivity and specificity of 80% and 96%, respectively, in identifying a bacterial infection, and a sensitivity and specificity of 86% and 94%, respectively, in detecting a viral infection. The test has CE marking, and multicentre studies for FDA clearance are anticipated to start in December 2015.

Dr Sambursky closed by sharing that they are aiming to convert the test to an even simpler format with only one strip that includes MxA and low CRP. However, he also highlighted the challenge in distinguishing true infection from bacterial colonization or a local viral infection without a systemic host response as well as how a change in definition will change the diagnostic parameters and reported performance of a test. He advocated that a new definition be adopted and standardized for a clinically significant respiratory infection that requires confirmation of the presence of a pathogen via antigen, culture or molecular detection in association with a systemic host response.

In response to a question, it was clarified that unconfirmed results would be interpreted as negative. This test has not been evaluated in mixed infections or tropical settings.

Title: *Minicare HNL*

Speaker: Marcel van Kasteel, Vice President & CEO, Handheld Diagnostics

Mr van Kasteel presented the advances made by Philips in the field of medical diagnostics with the development of the MiniCare platform. He started his presentation by introducing Handheld Diagnostics as an arm of Philips. He explained that the aim is to develop an open platform that can be used for numerous diagnostic questions at the primary care facility. The speaker outlined that the product profile of the device will comprise:

- Sample type: fingerprick blood
- Multiplexing of targets
- Handheld device
- Disposable cartridges

- Time-to-result: Less than 10 minutes
- No need for additional reagents

The speaker outlined advances in the biomarker development and described human neutrophil lipocalin (HNL), a marker that is activated in bacterial infections. He shared that the company has recently conducted a large-scale clinical trial with more than 1000 participants that showed promising diagnostic parameters with a PPV of 94% and a NPV of 93%. The speaker observed that a number of studies have shown that the kinetic of HNL is faster than that of PCT, and the resulting diagnostic accuracy is therefore higher (current study population: adults).

Mr van Kasteel concluded by sharing that Philips would be keen to evaluate HNL in a variety of other settings and populations after setting up a simpler ELISA format for research purposes only. The biomarker has been evaluated only in adults to date, and the interest is also to obtain data from paediatric patients. A handheld device with this biomarker would be ready in 2018.

Title: *LabDisk, a multi-purpose, multi-target diagnostic platform for patient management at the point of care*

Speaker: Dr Konstantinos Mitsakakis, Hahn-Schickard & Department of Microsystems Engineering (IMTEK)

Dr Mitsakakis presented the developments of his group focusing on developing a fully automated diagnostic platform based on centrifugal microfluidics technology (the LabDisk). The current tool is a modular, fully automated, open platform that can be adapted to the users' needs in terms of sample and pathogen types, as well as disease panel, and only requires a LabDisk player, which is a disc processing device. The speaker explained that the final aim is to develop a stand-alone battery-operated tool with integrated clinical algorithm.

Dr Mitsakakis described the current prototype of the device, which is a composite sample-to-answer assay with pre-stored reagents, integrated pathogen DNA/RNA and biomarker detection (currently CRP as proof-of-principle, with malaria, dengue and other relevant biomarkers to follow) and the current time-to-result (sample preparation: ~45 minutes, amplification ~15-40 minutes, ELISA ~30 minutes). He reported that the team is working on a concept that allows the simultaneous screening of more than one patient per disc, combining the biomarker component into a lateral flow test, thereby aiming to decrease the cost and increase throughput. So far, the device has been evaluated with inactivated *Salmonella* species, and spiked DNA from *P. falciparum* and *S. pneumoniae*. The limit of detection for these and other pathogens is currently under investigation. Estimated price of the disc including reagents is 7-9 EUR (for an order of more than 1 million discs) depending on the degree of multiplexity and with ongoing work to further

reduce the costs of raw materials. The machine costs about 4000 EUR (for an order of more than 1,000 machines).

Dr Misakakis concluded by highlighting the need for collaborations with clinical and academic partners with access to patient samples to evaluate and optimize their method prior to taking the tool to the field for a real-life clinical trial.

Session 7: Optimal design for clinical trials of fever biomarker assays

Session Chair: Dr Tim Rodwell, FIND

Title: *The OPPORTUNITY Study: International, multi-centre validation study of a diagnostic to differentiate between bacterial and viral infections*

Speaker (joined by Skype): Dr Louis Bont, UMC Utrecht, The Netherlands

Dr Bont started by introducing the setting in Utrecht at the University Medical Centre and highlighted the global threat of AMR with ~70% of antimicrobials being prescribed inappropriately. He discussed the resulting need for improved diagnostics to differentiate between viral and bacterial infections, which was the starting point for the OPPORTUNITY study in the Netherlands. The study aimed to assess host response-based diagnosis for the differentiation of bacterial from viral infections in lower respiratory tract infections as well as fever without a source (in children under five years). The speaker explained that the commercial diagnostic test under validation targeted three host response proteins (CRP, IP10, TRAIL) and an expert panel of three independent experienced paediatricians was used as the comparative gold standard. Dr Bont highlighted that using an expert panel was superior to what is conventionally used as a gold standard in biomarker studies.

The speaker gave details and results (confidential) of a large multicentre clinical study that showed high sensitivity and acceptable specificity and resulting in a good PPV and a very high NPV. Dr Bont concluded by recommending a blinded and prospective randomized trial to evaluate the utility of the combination of three biomarkers.

Discussion:

The discussion focused on the reference comparator and microbiological confirmation of diagnosis. Microbiological confirmation was not always done. Participants asked about the consensus between expert panel (~80%) as well as which data were available to the experts to make their decision (CRP, chest x-ray, regular blood data).

Title: *From development country prospective, particularly Tanzania*

Speaker: Professor John A. Crump, University of Otago, New Zealand

Professor Crump started his presentation by giving an outline of severe febrile illness aetiologies found in northern Tanzania based on his team's research and around the world based on a systematic review of hospital-based studies. He underlined the lack of data from many regions in the world and lack of standardization among studies. He presented work that showed the clinical over-diagnosis of malaria (~61% of diagnosis) compared to the real malaria burden (~1.6%) and the prevalence of many zoonotic or vector-borne diseases, especially in adults. He highlighted that many of the common pathogens do not respond to antimicrobials recommended by Integrated Management of Adolescent and Adult Illness guidelines for common severe illness syndromes associated with fever. Treatment gaps include tetracycline-responsive infections (~17%), invasive fungal infections and disseminated mycobacterial infections.

The speaker presented recently published data showing the heterogeneity of causative agents between regions and populations (e.g. hospitalized/non-hospitalized patients). He showed further work investigating the minimum performance characteristics of a point-of-care diagnostic test for sepsis in low-resource settings. The work showed that accuracy needed to exceed that of clinical assessment in order to avoid excess deaths and risk for antimicrobial over-use.

Professor Crump concluded his talk by outlining his recommendations for successful study designs:

- Test accuracy matters, as shortcomings of sensitivity drives risk for death while the specificity will drive cost saving by improving the ability to appropriately withhold antimicrobials. Risk for death will be lower if severe disease is triaged out of the cohort.
- Equipose of accuracy with clinical assessment probably needs to be established before a randomized trial using a point-of-care test as the sole arbiter of antimicrobial use could be implemented.
- Understanding prevalence is important to understand the prior probability of aetiologies in various settings. In addition, prevalence will change over time and vary with exposures within populations, adding additional complexity. Host factors (such as HIV or malnutrition) and the underlying infection might be associated with varying accuracy of a biomarker. To understand such variations more studies are needed.
- Considering the high number of non-bacterial and viral diseases (e.g. fungal, parasitic) and the high proportion of tetracycline responsive, empirical treatment guidelines might need to be reconsidered. The role of diagnostics to guide antimicrobial selection is difficult to overlook in the severe febrile illness population.

Discussion:

Subsequent discussion focused on the use of biomarkers to differentiate colonization from infection, the mortality cost and its consequences for the diagnostic accuracy (high sensitivity particularly important in severely sick groups) and the population for which biomarker testing might be most useful (i.e., large outpatient population where risk for death associated with withholding antimicrobials might be low). Appropriate diagnosis would also guide targeted use of antimicrobials that would be cost-effective.

Session 8: Diagnostic pathway

Title: *Understanding the bench-to-bedside pathway for diagnostics*

Speaker: Dr Mark Perkins, Chief Scientist, FIND

Dr Perkins started his presentation by outlining his aim of defining the process of diagnostic test development from concept to impact. He highlighted the importance of concept development as the first stage of product development. Included in that critical stage are the tasks of clearly outlining not only what the test would do, who would use it, and what characteristics it would need to have, but also who would benefit from the implementation of the test, what target population would need to be served, and who would pay for the technology. He further discussed how those considerations need to be reflected in the TPP and how it is important to consider how commercial value of the technology in high-income countries might be used to leverage the initial investment and offset costs to allow affordable use in LMICs.

Dr Perkins noted the challenge of developing biomarker-based diagnostics, the focus of this meeting, and that the rapidly rising amount of biomarker research carried out in the last two decades has not been matched by a concomitant rise in the number of biomarker-based tests being registered. Validation of putative biomarkers is a complex and expensive business, and represents the “valley of death” for many biomarker-based assays. For any biomarker(s) to be validated for global use will require large, multi-country studies across multiple populations with clearly defined enrollment criteria that will inform the indications for use. The speaker emphasized the importance of any diagnostic intended for broad public health use to fit into (or radically improve) existing strategies for disease control; such a fit is often represented by WHO approval and policy generation, which remain important goals for those wishing to develop such assays.

Dr Perkins concluded his presentation by advocating for public/private partnerships in diagnostic development and implementation, especially where the goal of addressing health needs of impoverished populations is envisioned. Finally, he reflected that for a test to have successful uptake, it must result in an outcome that is important to the patient – usually in the form of clearly improving health outcomes or saving them money: this is a specific challenge for diagnostics aimed at *decreasing* rather than *increasing*

access to drug treatments, and suggests that the public sector has some distance to go to capture the concrete benefits imagined for such tests in a way that patients will understand and respond to.

Discussion:

Subsequent discussion focused on Dr Perkins' comment regarding the empowerment of patients with test results and how it is important to use a language that makes it clearer to the health-care worker that we are intending to improve health outcomes by targeting treatment rather than increasing non-treatment, *per se*. In response to a question about the market for biomarkers in the fight against AMR, he suggested that success will likely require a market that has utility in broad populations representing diverse groups from HICs to marginalized populations in LMICs.

Title: *Challenges in case definitions and ideas for study designs*

Speaker: Dr Norbert Heinrich, Study coordinator TB and emerging infectious diseases, University of Munich (LMU)

Dr Heinrich started by discussing the desired effects of a biomarker test, which he suggested should be reducing antibiotic use and improving clinical outcomes. In order to obtain an assay that could do this, Dr Heinrich proposed a three-step study design:

1. Proof of concept study: Retrospective testing of well-defined samples possibly derived from a biobank.
2. Prospective study using two diagnostic tools for comparison: Prospective estimation of the clinical sensitivity and specificity after comparison of study test results to a gold standard comparator.
3. Pivotal (licensing) study: Ascertain that test use does not result in worse outcomes.

He outlined the difficulties of defining a true case in the absence of a real gold standard for biomarker testing and how this problem, and the resulting underestimation of positive cases, might lead to a test or marker being discarded due to perceived sensitivity/specificity issues. He highlighted the importance of having special populations (e.g., people living with HIV) sufficiently represented in the study population and to consider that biomarkers used as a reference comparator should not be part of the same biochemical pathway as the biomarker under investigation.

Dr Heinrich concluded his presentation by outlining the current project of the German Centre of Infectious Research (DZIF) in collaboration with four partner institutions in Africa, developing a large fever sample biobank that could be used for the prior evaluation of potential candidate diagnostic tools.

Discussion:

Subsequent discussion focused on the current sample sets that are collected as part of a large fever trial and what cases comply with strict definitions (~60% malaria slide positive, ~15% blood culture positivity during rainy season).

Session 9: Biobanking

Title: *Specimen Banks: Critical tool to accelerate development of diagnostic tests for febrile syndrome*

Speaker: Dr Iveth González, Head of Malaria and Acute Febrile Illness Department, FIND

Dr González started to outline that the effort to develop a specimen library as part of FIND's activities has been of interest by all departments in FIND. She explained that as part of a survey conducted among academic and industry experts, the difficulty of accessing samples for test evaluations was identified as one of the main bottlenecks for assay development. Dr González explained that the survey further showed that industry developers would benefit from fast access to good quality samples, as curating individual biobanks is cumbersome. She highlighted that specimen banks are of importance at any level of the diagnostic value chain from the first proof-of-concept studies to quality assurance activities after deployment of a test and that this applies regardless of the test characteristics.

Dr González outlined the great importance of well-defined samples of high quality from different populations (i.e., children, adults, other subgroups) and geographical regions, collected as part of clinical trials that have appropriate ethical clearance and logistical support in place. She outlined the particular importance for biomarker testing of having standardized reference methodologies and the collection of different sample types from cases and controls, as the lack of an accepted gold standard test means that various pathogen-specific tests need to be performed.

Dr González concluded by inviting experts to help develop targeted TPPs and standardized protocols for clinical trials, and define reference laboratory testing (gold standard/composite) for all biobanked specimens.

Discussion:

The subsequent discussion started with questions about ethical clearance and consent. Participants from industry went on to highlight the need for good quality samples (i.e. avoiding re-freezing) and the concern that frozen samples might not be as good for biomarker testing as access to fresh blood in clinical trials. In addition, the importance of having a defined clinical algorithm and healthy controls from all sites was discussed. In response to queries about who would have access to the samples, Dr González outlined

the current review and approval process (WHO, FIND) for specimens collected as part of the TB and malaria specimen library and how ownership issues are currently handled.

Session 10: Prioritizing next steps for TPPs, clinical trial design & biobanking

Session chair: Dr Anna Zorzet, Coordinator, ReAct Europe, Uppsala University

1) Target product profiles

It was agreed among participants that the first task must be to focus on the most urgent need and to identify where the expert group sees the primary focus – one of the key areas for continued discussion in subsequent sessions and to be agreed by the end of the meeting in order to guide next steps in the development of needed TPPs. Further, the organizing committee agreed to reach out to other organizations that are also developing TPPs for biomarker diagnostics (such as Wellcome Trust, BMGF) to harmonize efforts.

2) Better clinical trial design

Participants agreed that in order to encourage academia and industry to adhere to clinical trial guidelines, funding and publications should be linked to adherence. Based on currently available guidelines¹ for clinical trials for diagnostic test evaluation, a subgroup will work on a harmonized design, including new tools (STARD/QAD) that could then be used as a standardized protocol in the future.

Participants also raised the point that it is important to be clear on the primary aim of the diagnostic tool (i.e., reduced antibiotic use, improved outcome) and the study population (e.g., people living with HIV or malnourished children).

3) Biobanking

Dr Zorzet posed the question of the value of a biobank to industry and academia in order to assess the true need. The response to the open question was diverse, with some industry partners acknowledging the use and importance, and suggesting that access to a defined specimen library would increase the speed of development. In contrast, other industry participants were more critical, highlighting the difficulties when working with biomarkers, as many decay quickly in frozen samples.

¹ Peeling RW, Smith PG, Bossuyt PM. A guide for diagnostic evaluations. *Nat. Rev. Microbiol.* 2006 Dec;4(12 Suppl):S2-6

Day 2 – 23 September 2015

Session 11: Important new developments

Title: *Flow cytometric bacterial infection kit for distinguishing between bacterial and viral infection in less than 30 minutes*

Speaker: Dr Jari Nuutila, University of Turku

Dr Nuutila started by outlining that in his opinion the ideal diagnostic marker/test would use a single technology, incorporate three to four variables, have rapid turnaround, have a diagnostic sensitivity and specificity greater than 90%, and require minimal sample and data handling. He continued with a basic introduction to the human host response to bacterial and viral infection and how these unique response profiles (changes in leukocyte cell surface receptor expressions) can be utilized to differentiate bacterial from viral infection. Dr Nuutila described eight different infection markers that were identified by his group and can be assessed by flow cytometer. He continued to describe the development of different methodologies and corresponding clinical trials with increasing diagnostic accuracy. Dr Nuutila reported that his latest and most refined methodology (BI INDEX) to this point incorporated four cell surface markers (receptors), used only 30 μ L of whole blood and resulted in a final sensitivity and specificity of greater than 90% without the need for the external calibration beads normally needed in quantitative flow cytometric receptor analysis.

Dr Nuutila concluded with preliminary results from an ongoing clinical study in Finland that suggests that a clear distinction between viral and bacterial infections is possible using the BI INDEX.

Discussion:

The discussion following Dr Nuutila's presentation focused on the possibility of adapting current flow cytometer devices used in the field (CD4 count assessments; Partec CyFlow Counter or portable flow cytometers, such as Sysmex CyFlow® Cube 6 and CyFlow® miniPOC, for example) to the described methodology. It was further suggested that an adaption of the even simpler Pima™ CD4 (Alere) to his methodology would allow use in the field. In response to a question regarding potential differences in markers according to population, Dr Nuutila responded that since BI INDEX variables (receptors) played fundamental roles in cell-mediated immunology, it was unlikely that race or geographic location would have significant effects on phagocyte receptor expression.

Title: *Biomarkers for management of respiratory infections*

Speaker: Dr Ann R Falsey, University of Rochester

Dr Falsey started by discussing whether the diagnosis of a viral infection really leads to a change in treatment. She showed data from her work that suggested that only 40% of all patients hospitalized with viral respiratory infections had an additional bacterial infection that would justify antimicrobial therapy. The study question of her trials was whether the use of increased viral testing combined with the use of a bacterial biomarker might reduce the use of antimicrobial therapy. She outlined a study that enrolled patients in an intervention (PCT and viral testing result made available) and non-intervention arm to investigate whether clinicians followed the guidance of the PCT and viral diagnostic. Dr Falsey reported that overall, no significant difference between the two groups was detected. However, a significant difference was seen in the patients with low PCT versus high PCT. She also showed that overall, the PCT testing was well received among the clinicians, suggesting that biomarker-assisted care can be envisioned.

In the second part of her presentation, Dr Falsey described the use of microarray data to distinguish between viral and bacterial infections, and reported that different expression patterns were observed between patients with viral, bacterial and co-infections (viral and bacterial) and that the diagnostic sensitivity and specificity of 15 identified classifier genes was calculated to be 95% and 91%, respectively.

Discussion:

The discussion following Dr Falsey's talk focused on questions on the detailed training that was provided to clinical staff prior to the study and how this educational effect might have been responsible for the study outcome.

Title: *Overview of FDA review of diagnostic devices/tests*

Speaker: Dr Kate Simon, Senior Consultant, Biologics Consulting Group, Inc.

Dr Simon started by highlighting that the FDA evaluation process for an *in vitro* diagnostic (IVD) device is more data-driven than technology-driven. The FDA looks at the performance data to determine whether it supports the proposed indication for use statement for the device. Diagnostic test developers should provide a detailed proposed indication for use statement when submitting a new diagnostic device to FDA, which should include the following:

- Trade name;
- Description of measurand/analyte (what is being detected);
- Type of testing technology;
- Type of read-out (qualitative, semi-qualitative, quantitative);
- Patient population;
- Clinical indication and specifically designed clinical trials; and
- Specimen used with the test (every specimen needs validation).

The speaker explained that the IVD labelling (package insert) is also a centrepiece of the FDA review. The labelling should provide a detailed description of the IVD device and how the test is performed (protocol), among other details (such as warnings and limitations, storage and handling), which all need to be in place for the final validation

study. Dr Simon then outlined a number of important elements of a clinical study that need to be specified in detail for successful FDA review, including:

- Inclusion/exclusion criteria in as much detail as possible;
- Appropriate comparator;
- Steps taken to avoid bias in the dataset;
- Site monitoring during the trial.

The speaker concluded by mentioning an upcoming workshop to be held by the FDA on biomarker diagnosis (Title: Public workshop – non-microbial biomarkers of infection for *in vitro* diagnostic device use) on 16 October 2015 and noted that the outcome of this public workshop might be of great importance to the participants of the biomarker meeting, particularly with regard to the issue of appropriate comparator testing.

Discussion:

The discussion following Dr Simon's presentation focused on the issues with gold standard testing and the use of an appropriate comparator in trials. In Dr Simon's opinion, the FDA would currently not allow a comparator test that is related (e.g., same biochemical pathway) to the biomarker under investigation. However, she did point the participants to the upcoming workshop, as possible exceptions to this policy could potentially be discussed (such as when the biomarker under investigation is a composite biomarker containing many analytes and the comparator includes only one of these analytes).

Title: *Host responses distinguish paediatric bacterial, viral and malarial origins of paediatric clinical pneumonia*

Speaker: Dr Michael Gillette, Broad Institute, Massachusetts General Hospital

Dr Gillette started by describing that the aim of the work he and his team are doing is to define the host response (in both gene expression and proteome space) of children presenting with pneumonia syndromes associated with microbiologically confirmed bacterial, viral and parasitic (malarial) infections, with the objective of working towards a field-deployable rapid diagnostic test to guide triage decisions and therapeutic interventions. He outlined the extensive work that has been done to identify potential markers using gold standard clinical samples (with acknowledgment of the limitations of existing diagnostics) and a range of analytical methods including RNA sequence-based gene expression analysis as well as mass spectrometry-, antibody array- and aptamer array-based (Somalogic) proteomics approaches. Collectively these strategies have led to the identification of a substantial number of target genes and proteins with diagnostic potential.

The speaker showed that a model based on the identified gene targets from RNA analyses demonstrated sensitivity/specificity of 100%/70% for determination of bacterial

aetiology in a first independent test set. Protein-level targets appear especially promising in preliminary review, but analyses are at an earlier stage and, in particular, efforts are ongoing both to synthesize data from multiple sources and to determine a minimum marker set that retains strong predictive accuracy. While pointing out that evolving platforms (including some presented at this meeting) might allow RNA-based diagnostics to be used in resource-poor settings in the near future, Dr Gillette suggested that protein markers are likely to be more easily deployable in the field given current established technologies, and that marker candidates from proteomic discovery have a higher chance of success in protein-level verification studies. Dr Gillette closed by inviting future collaborations to further evaluate a test developed at Broad Institute in resource-poor settings.

Session 12: Improving the diagnosis of fever

Session chair: Dr Arlene Chua, MSF Access Campaign

Title: *CRP for the management of fevers and acute respiratory infection in community/primary settings in Southeast Asia*

Speaker: Dr Yoel Lubell, Mahidol and Oxford University Research Unit, Thailand

Dr Lubell noted that in many communities, malaria village health workers are under-utilized due to the decline in malaria cases and that while this population of health-care staff could be used for the treatment of other diseases, this will remain difficult as long as limited data on the epidemiology of infections are available. He explained that the aim of the large studies conducted by his team was to assess the usefulness of CRP and PCT in areas in Southeast Asia where malaria is no longer a major cause of fever. He presented the results from three different countries (Cambodia, Lao, Thailand), which showed that CRP outperformed PCT in all sites with AUCs of 0.8-0.9 to differentiate viral from bacterial infections. Further, CRP was slightly elevated in subclinical parasitaemia (malaria) in rural Cambodia but still low, and these and other findings were consistent when using the NycoCardIII reader. He also presented very good results using the currently available CRP lateral flow assay in remote settings in Southeast Asia, which suggests that the current technology could already be used in routine clinical care.

Dr Lubell showed the results of a modelling study that suggested biomarker detection is more robust compared to pathogen-specific testing, hence would reduce antibiotic use further. He also outlined the details of a recent study in Vietnam that demonstrated a reduction in the use of antibiotics in patients with acute respiratory infection using a CRP-guided treatment algorithm, with partial patient and physician adherence to the test. The trial showed that the antibiotic treatment frequency went down in the CRP group (63%→43%) without any evidence of inferior clinical outcomes. Dr Lubell reported that his team aims to confirm those findings in a planned randomized trial in Thailand,

Myanmar and DR Congo, where patients will be randomized into CRP-guided treatment arms using different thresholds to classify the need for antibiotics. The team is also exploring the development of a combined malaria and CRP test. Dr Lubell concluded that CRP seems to be an effective tool that could be used in the community by health-care workers.

Discussion:

The discussion following Dr Lubell's presentation focused on the routine use of CRP tests in a study site in Shoklo Malaria Research Unit (in the Thailand-Myanmar border area) and how the implementation could be rolled out in a way that would allow later analysis to study the effect. Questions were also asked about capturing the social benefits of reducing AMR.

Title: *Evaluations of biomarkers of bacterial infections in MSF settings*

Speaker: Dr Anne-Laure Page, Epicentre, MSF Operational Research Centre

Dr Page outlined the interests of MSF in using CRP in their settings and investigating how CRP performs in populations with high prevalence of malnutrition, malaria, as well as malaria and bacterial co-infection. She described two studies. The first, set in Niger, focused on severely ill children with acute malnutrition, in which both CRP and PCT testing showed limited accuracy (AUC~0.6-0.7). The other, set in Uganda, focused on a paediatric population with central nervous system infections in which serum CRP did not perform as well as traditional CSF markers (e.g., CSF leukocytes) and where CRP was elevated not only in children with bacterial infections, but also in those with malaria. Dr Page summarized the findings from both studies by saying that in the investigated settings, CRP and/or PCT did not perform well, likely because biomarkers in malnourished children are not rising in similar levels compared to otherwise healthy children and are elevated in response to malaria, making the markers less useful in settings with high prevalence of malaria.

Dr Page concluded her talk by calling for a biomarker that can not only differentiate between viral and bacterial infections but can also be used for meningitis/encephalitis. She noted that such a biomarker-based test should not be limited for use with general febrile patients, as ~30% of malnourished children never developed a fever, and particularly that all biomarker-based assays require thorough evaluations in different populations in LMICs.

Discussion:

In the discussion following Dr Page's presentation, it was suggested that monitoring the progression of biomarkers in malnourished children and at different time points to monitor the kinetics of biomarkers in this population might be useful. In addition, the reasons for the differences between the results from Southeast Asia and Africa were

discussed. It was concluded that results are difficult to compare due to the very different populations investigated, with patients in the African studies being more severely sick compared to the studies in Lao, Cambodia and Thailand.

Session 13: Concluding remarks by WHO, FIND, MSF and ReAct

Speaker: Dr Iveth González, FIND

On behalf of the organizing committee, Dr González summarized the conclusions of the meeting: that the focus should be on a biomarker test for use at the community level, prioritizing a biomarker test to distinguish between viral and bacterial infections. She outlined the next steps needed to continue the development and validation of potential biomarkers for appropriate diagnostics to guide patient treatment and reduce the overuse of antimicrobials:

1. Draft a TPP for a biomarker test for use at the community level to differentiate viral from bacterial infections. The draft TPP will be circulated for review and discussed in a small expert group. Controversial points will be further discussed during a final TPP meeting.
2. Develop agreed guidelines for future clinical trials to evaluate biomarker assays.
3. Agree on a gold standard or a number of reference tests.
4. Develop specifications for a specimen bank that can aid pre-clinical trial evaluation.

Dr González observed that all organizations represented at the meeting had expressed their commitment to these next steps and to obtaining funding for the planned activities.

Session 14: Closing of the meeting

Speaker: Dr Francis Moussy, WHO

Dr Moussy thanked attendees for the productive meeting and reiterated the full commitment of WHO, as the fight against AMR is of very high priority to the agency.

List of participants

(listed alphabetically by surname, with organizing committee member names in bold)

Quique Bassat
Paediatrician
Associate Research Professor
ISGlobal
Barcelona Institute for Global Health
Hospital Clínic - Universitat de Barcelona
Spain

Noémie Boillat Blanco
CHUV Lausanne, Switzerland

Louis Bont
UMC Utrecht, Netherlands

Emmanuel Bottieau
Head of Unit Tropical Diseases
Department of Clinical Sciences
Institute of Tropical Medicine, Antwerp, Belgium

François Chappuis
Head of Division of Tropical and Humanitarian Medicine
Geneva University Hospitals
6, rue Gabrielle-Perret-Gentil
1211 Geneva 14, Switzerland

Arlene Chua
Antimicrobial Resistance Advisor
Diagnostics Advisor
Medecins Sans Frontières, Access Campaign
Geneva, Switzerland

John A. Crump, MB ChB, MD, DTM&H
McKinlay Professor of Global Health and Co-Director
Centre for International Health
Dunedin School of Medicine
PO Box 56
University of Otago
Dunedin 9054
New Zealand

Isra Cruz, PhD
Senior Scientific Officer
Neglected Tropical Diseases Programme
FIND, Switzerland

Valérie D'Acremont, MD, PhD
Senior consultant in tropical diseases
Swiss Tropical and Public Health Institute | Basel | Switzerland
Department of Ambulatory Care and Community Medicine | University hospital of
Lausanne | Switzerland

Claudia Denking
Head of Tuberculosis & HCV Programme
FIND, Switzerland

Sabine Dittrich, Dipl. Ing., PhD
Consultant to FIND

Sergey Eremin, MD, PhD
Antimicrobial Resistance
Pandemic and Epidemic Diseases
WHO

Ann R. Falsey, M.D.
Rochester General Hospital
Rochester, USA

Laurence Flevaud
MSF, SPAIN

Michael Gillette
Senior Research Fellow, Proteomics and Biomarker Discovery, Broad Institute of MIT
and Harvard
Attending Physician, Pulmonary and Critical Care Medicine, Massachusetts General
Hospital
Assistant Professor of Medicine, Harvard Medical School
Boston, MA, USA

Iveth González
Head of Malaria & AFS
FIND, Switzerland

Matthew G. Hall, PhD
CSO and co-founder

SimplicityBio SA
Route de l'Île-aux-Bois 1A
1870 Monthey, Switzerland

Norbert Heinrich
Senior Scientist, Division for Infectious Diseases and Tropical Medicine, Medical Center,
University of Munich, Germany

Heidi Hopkins, MD
ACT Consortium
Senior Lecturer, London School of Hygiene & Tropical Medicine
Uganda and UK

Marcel van Kasteel
Vice President
CEO Handheld Diagnostics
Philips Healthcare Incubator
Philips Group Innovation
High Tech Campus 29.P.618a
5656 AE Eindhoven
The Netherlands

Philippe Leissner
Global and Tropical Infectious Diseases Program Director
Medical Diagnostic Discovery Department
bioMérieux
Chemin de l'Orme – 69280 Marcy l'Etoile, France

Laura Lovejoy
Acting Director of Corporate Communications at RPS Diagnostics
(through Saranova, LLC)
USA

Yoel Lubell
Mahidol Oxford Tropical Medicine Research Unit (MORU)
Thailand

Konstantinos Mitsakakis
Hahn-Schickard & IMTEK, University of Freiburg
Project Coordinator, LabDisk diagnostic platform
Georges Koehler Allee 103, 79110 Freiburg, Germany

Richard L. Moore
Molecular Analyst, BD Diagnostic Systems

BD Life Sciences
7 Loveton Circle
Mail Code 618
Sparks, MD 21152
USA

Francis Moussy
Diagnostics Innovation
WHO, Geneva, Switzerland

Jari Nuutila
University of Turku, Finland

Alexandre Pachot
Director of Medical Diagnostic Discovery Department (MD3)
bioMérieux
Chemin de l'Orme – 69280 Marcy l'Etoile – France

Anne-Laure Page, PhD
Epicentre
Paris, France

Mark Perkins, MD
CSO
FIND, Switzerland

Dr Carmen de Pessoa
WHO, Switzerland

Dr Nicolas Peyraud
MSF, Switzerland

Albert Picado, DVM, Ph.D.
Senior Scientific Officer
Neglected Tropical Diseases Programme
FIND, Switzerland

Maria Pránting, MSc, PhD
Scientific Officer
ReAct, Sweden

Clotilde Rambaud-Althaus
Innovation Unit
Médecins Sans Frontières, Switzerland

Andy Ramsay
WHO/TDR, Switzerland

Tim Rodwell
FIND, Switzerland

Robert Sambursky, MD
Co-founder, CEO, and President
RPS Diagnostics, USA

Nicole Schneiderhan-Marra
Project Manager / Deputy Head Biochemistry
NMI Natural and Medical Sciences Institute
Markwiesenstrasse 55, 72770 Reutlingen, Germany

Therese Seldon PhD
Vice President - Operations
Immunexpress, USA

Micaela Serafini
MSF, Switzerland

Kate Simon
Senior Consultant
Biologics Consulting Group, Inc.
400 N. Washington St., Suite 100
Alexandria, VA 22314 USA

Roberto de la Tour
MSF, Switzerland

Catherine Van Overloop
MSF, Brussels

Anna Zorzet, MSc, PhD
Coordinator ReAct Europe
University of Uppsala, Sweden