Innovation in point-of-care diagnostics for sepsis and bloodstream infections

Guest speakers: Till T. Bachmann, Amrita Sukrity, David Anderson
Moderator: Caroline Purslow (Nesta Challenges)
Host: Astrid Pentz-Murr (GARDP)
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- Innovation in point-of-care diagnostics for sepsis and bloodstream infections (26 November 2019)
- Converting Gram-positive-only compounds into broad-spectrum antibiotics (7 November 2019)
- Natural product antibiotics: from traditional screening to novel discovery approaches (3 October 2019)
- Models for antimicrobial R&D: Advanced and complex in vivo models for infectious disease research (10 September 2019)
- Models for antimicrobial R&D: Computational modelling for population PK and PKPD (20 August 2019)
- Enabling academia to fill the discovery gap – Learnings from industry and funders (18 July 2019)

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Today’s speakers

Innovation in point-of-care diagnostics for sepsis and bloodstream infections

Till T. Bachmann
University of Edinburgh, UK

Amrita Sukrity
SpotSense, India

David Anderson
Burnet Institute, Australia

Moderator:
Caroline Purslow
Nesta Challenges, UK
Today’s speakers

Till Bachmann is Professor of Molecular Diagnostics and Infection and Deputy Head of Infection Medicine at Edinburgh Medical School: Biomedical Sciences at the University of Edinburgh. Till is an expert in point of care (POC) detection, infectious diseases and antimicrobial resistance, conducting research at the interface of clinical microbiology and diagnostics.

Amrita Sukrity is the founder of Spot Healthcare Solutions Pvt Ltd which is a start-up in India working in the field of affordable diagnostics. The start-up aims to develop multiple solutions in the space of maternal and child health and is currently piloting an algorithm for detection and monitoring of neonatal sepsis.

David Anderson is Deputy Director (Partnerships) and co-head of the Global Health Diagnostics Laboratory at the Burnet Institute in Melbourne, Australia, where he has spent his entire professional career. His work has led to diagnostic innovations including the Visitect® CD4 POC test for monitoring of CD4+ T-cells in HIV, which serves as a paradigm for future cell-based POC tests including CD64 in sepsis and severe infection, and novel POC tests for liver disease.
A £8 million global prize that will reward a transformative, rapid, accurate, and affordable point-of-care diagnostic test that can significantly reduce antibiotic misuse or overuse, anywhere in the world.
What do competitors need to do to win?
Who are our teams?

57 Teams

19 UK
13 India
10 US
3 Australia
3 Sweden
LONGITUDE PRIZE

longitudeprize.org

@longitude_prize

www.facebook.com/longitudeprize
Innovation in point-of-care diagnostics for sepsis and bloodstream infections

Introduction

Till T. Bachmann
Infection Medicine, Edinburgh Medical School: Biomedical Sciences
University of Edinburgh

GARDP Webinar 26 November 2019
Introduction & Contact

Till T. Bachmann, PhD FRSG

- Deputy Head of Infection Medicine
- Professor of Molecular Diagnostics and Infection
- Programme Director Clinical Microbiology and Infectious Diseases
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WORLD SEPSIS DAY INFOGRAPHICS

A GLOBAL HEALTH CRISIS

27 000 000 - 30 000 000 people per year develop sepsis

7 000 000 - 9 000 000 die - 1 death every 3.5 seconds

Survivors may face lifelong consequences

WORLD SEPSIS DAY INFOGRAPHICS

SOURCES OF SEPSIS

The Most Common Sources of Sepsis

Meningitis

Infection of unknown source

Pneumonia

Bloodstream Infection

Abdominal Infections, e.g.
- Appendicitis
- Infectious Diarrhea
- Gallbladder infection etc.

Skin or Soft Tissue Infection

Catheter-related infection

Urinary tract infection

www.world-sepsis-day.org
www.global-sepsis-alliance.org

https://www.global-sepsis-alliance.org
Rapid Appropriate Antimicrobial Therapy is Essential for Survival


**Figure 1.** Cumulative effective antimicrobial initiation following onset of septic shock-associated hypotension and associated survival. The x-axis represents time (hrs) following first documentation of septic shock-associated hypotension. Black bars represent the fraction of patients surviving to hospital discharge for effective therapy initiated within the given time interval. The gray bars represent the cumulative fraction of patients having received effective antimicrobials at any given time point.
Clinical Response to Sepsis - Sepsis 6 Bundle

### Action (complete ALL within 1 hour)

<table>
<thead>
<tr>
<th>Action</th>
<th>Why we do this</th>
<th>Why we do this</th>
<th>Why we do this</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Administer oxygen</strong>&lt;br&gt;Aim to keep saturations &gt;94% (88-92% if at risk of CO₂ retention e.g. COPD)</td>
<td>To improve the oxygen content of the blood, and therefore its delivery to the tissues</td>
<td>To improve preload to the heart by correcting hypovolaemia, improving cardiac output and BP</td>
<td></td>
</tr>
<tr>
<td><strong>2. Take blood cultures</strong>&lt;br&gt;At least a peripheral set. Consider e.g. CSF, urine, sputum. <strong>Think source control!</strong> Call surgeon/ radiologist if needed</td>
<td>To help identify pathogens, to determine likely source of infection &amp; guide antimicrobial therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>3. Give IV antibiotics</strong>&lt;br&gt;According to Trust protocol&lt;br&gt;Consider allergies prior to administration</td>
<td>To control the underlying infection, removing the trigger for immune overreaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>4. Give IV fluids</strong>&lt;br&gt;If hypotensive/ lactate &gt;2 mmol/l, up to 30ml/kg&lt;br&gt;Give 500ml stat if not hypotensive and lactate normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5. Check serial lactates</strong>&lt;br&gt;Corroborate high VBG lactate with arterial sample&lt;br&gt;If lactate &gt;4 mmol/L, recheck after each 10ml/kg challenge and call Critical Care</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>6. Measure urine output</strong>&lt;br&gt;May require urinary catheter&lt;br&gt;Ensure fluid balance chart commenced &amp; completed hourly</td>
<td></td>
<td></td>
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</tbody>
</table>

### Why we do this

- To improve the oxygen content of the blood, and therefore its delivery to the tissues
- To help identify pathogens, to determine likely source of infection & guide antimicrobial therapy
- To control the underlying infection, removing the trigger for immune overreaction
- To improve preload to the heart by correcting hypovolaemia, improving cardiac output and BP
The Global Threat of Antimicrobial Resistance

AMR now 700,000 (low estimate)
AMR in 2050 10 million

Tetanus 60,000
Road traffic accidents 1.2 million
Cancer 8.2 million
Measles 130,000
Cholera 100,000 – 120,000
Diarrhoeal disease 1.4 million
Diabetes 1.5 million

“I call on the governments of the richest countries to mandate now that by 2020, all antibiotic prescriptions will need to be informed by up-to-date surveillance information and a rapid diagnostic test wherever one exists.”

“... be able to report on the percentage of prescriptions supported by a diagnostic test or decision support tool by 2024.”

There has been very little progress on the review’s central and most expensive recommendations for transforming research and development incentives for antibiotics, vaccines and diagnostics.

Review of Progress on Antimicrobial Resistance: Background and Analysis, Charles Clift, Centre on Global Health Security | October 2019, Chatham House
Variability of Antibiotic Prescription for Febrile Children


Figure 3: Variability in antibiotic prescription across countries for the most frequent foci of infection in 4560 children without comorbidities

Unmet Need in Clinical Microbiology

Blood

~ 10 CFU/mL

~ 1,000,000 CFU/mL

Pathogen

Drug Resistance

Therapy

Rapid Diagnostic
- Therapy Decision Support
- Patient Management
- Surveillance
- Drug Development
The Diagnostic Spectrum in Clinical Microbiology

Phenotypic
- Gram staining
- Culture
- Biochemistry

Non phenotypic
- Immunoassays
- Molecular
- Mass Spectrometry
- Imaging
- Sequencing

Host Response
- Biomarkers
- Gene expression

Specimen - Culture
Whole Blood - Blood Culture
Automation, Integration, Sample to Answer
ID, ID/AST, AMR
Lab - POCT
Some Molecular Diagnostic Systems in Use or With Potential for Infectious Disease Diagnostics


# WHO AMR Diagnostics Landscape & Gap Analysis

## Table 1. Gaps in syndromic testing at Level I and Level II healthcare facilities

<table>
<thead>
<tr>
<th>Purpose Syndromes</th>
<th>Fever without a known source</th>
<th>Sepsis</th>
<th>Sore throat, cough, URTI</th>
<th>Pneumonia, LRTI</th>
<th>Diarrhea</th>
<th>Visible skin/soft tissue infection</th>
<th>Wounds (traumatic and chronic)</th>
<th>Urethral and vaginal discharge</th>
<th>UTI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Level I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria vs other</td>
<td>A</td>
<td>NA</td>
<td>A</td>
<td>A</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>A</td>
<td>A1</td>
</tr>
<tr>
<td>Bacterial ID (culture, RDT, ...)</td>
<td>NA</td>
<td>NA</td>
<td>A, B</td>
<td>NA</td>
<td>A, B</td>
<td>NA</td>
<td>NA</td>
<td>A, B</td>
<td>NA</td>
</tr>
<tr>
<td>Antibiotic Susceptibility</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>A, B</td>
<td>NA</td>
</tr>
<tr>
<td>Resistance Testing</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>A, B</td>
<td>NA</td>
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<tr>
<td><strong>Level II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bacteria vs other</td>
<td>A</td>
<td>NA</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>NA</td>
<td>A1</td>
</tr>
</tbody>
</table>

If test desired: Available | Not fully available or ideal | Not available

A: Reduce unnecessary antibiotic prescriptions,
B: Guidance for appropriate treatment of drug-resistant infections,
C: Surveillance

*Based on informal consensus of participants attending the Technical Consultation on In Vitro Diagnostics for AMR.

**Notes:**
1. MTB, the cause of human tuberculosis, was not subjected to review for inclusion in this prioritization exercise as it is already a globally established priority. And although priority TPPs to stimulate product development have been developed, more innovative new TB diagnostics are urgently needed. The section on TB was provided by the WHO Global TB Programme.
2. In case it is needed in special populations.
3. Infection marker.

[https://www.who.int/medicines/access/antimicrobial_resistance/en/](https://www.who.int/medicines/access/antimicrobial_resistance/en/)
Emerging BSI Tests & Technologies

www.t2biosystems.com
Josephson L et al Angew Chem 2001

www.acceleratediagnostics.com

DNA-achiever: novel technology to detect clinical pathogens efficiently, accurately

Lancet, UK and Carlsbad, CA, 2016 – A new diagnostic platform that demonstrates the power of pathogen DNA sequencing technology, promises to revolutionise the ability of healthcare to detect microbial infection.

www.dnae.com
Commercial Pathway to Develop Antimicrobial Susceptibility Testing Systems

Many Thanks for Your Attention

Visit our Surveys

AMR Diagnostics-Teaching and Training
https://edin.ac/2Qk37Vb

AMR Diagnostics Teaching & Training Resource
https://edin.ac/376Vd7N

@BachmannTill
Creating point-of-care diagnostics for neonatal sepsis.

Amrita Sukrity
Founder, SpotSense
GARDP-REVIVE Webinar 26th Nov 2019
Current diagnostics landscape for neonatal sepsis.

Modules in treatment

1) Antimicrobial therapy to fight an underlying infection.
2) Supportive care to maintain physiology

Questions in Diagnostics

1) Is there an infection?
2) How severe is the underlying condition?

Chart 1: Current diagnostic protocols, their Time-to-positivity, accuracy and accessibility parameters. The radius of bubbles indicate accessibility of these tests at community health level.

*NAAT - nucleic acid amplification test
CRP – C-reactive protein
PCT - Procalcitonin
Requirements for a point-of-care test to be used at the community.

- Blood culture, considered a gold standard for sepsis can indicate false negatives in 20-40% of sepsis cases.
- While physicians do want to know the causative organism, the choice of antibiotics doesn’t always depend on the result.
- Prolonged early empirical antibiotic therapy is also known to be associated with increased risk of sepsis, NEC* and death.

- Most sepsis protocols focus on initiating treatment within 60 min of presentation of sepsis.
- Newborns with sepsis who are treated with antibiotics within 60 min of presentation have significantly better outcomes than those with delayed treatment.

- The delay in start of therapy is very often attributed to a delay in referral, especially when clinical presentation isn’t conclusive for sepsis.
- After start of treatment, continuous monitoring is a challenge as frequent blood draws are not advisable.

*NEC – Necrotizing enterocolitis
Understanding the role of Interleukin 8/IL8 (CXCL8) in neonatal immunology.

Why did we choose IL8 as our ‘biomarker of choice’?

- There isn’t a significant physiological change in IL8 production based on gestational age at birth.

- IL8 production is evident in babies with gestational age as low as 23 weeks and then remains mostly stable for healthy newborns.

- Previous literature has shown a marked rise in IL8 production which precedes a rise in C-Reactive Protein aiding in fast recognition of sepsis.

- Some previous studies have shown that IL8 can be correlated with severity of sepsis.

However, in case of early onset sepsis

- Significantly lower amounts of monocytes and neutrophils are seen to be recruited during infection.

- A reduction in expression of adhesion molecules is also noticed leading to difficulties in migration of neutrophils to the site of infection.

- IL8 production can still be very readily seen to be provoked by antigen receptor engagement of T cells in preterm infants, but levels remain low for first 3 days.
Predicting late onset culture positive sepsis (bacteremia) using a combined score of salivary IL8 and salivary CRP.
What are some of the trends for early onset sepsis?

**Variation of salivary IL8 and salivary CRP levels with gestational age**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age and salivary IL8 levels</td>
<td>0.0045</td>
</tr>
<tr>
<td>Gestational age and salivary CRP levels</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

**Variation of salivary IL8 and salivary CRP levels with physiological age**

Variations across CRP (top) and IL8 (bottom) levels against physiological age for healthy babies without any comorbidities.

Variations across CRP (top) and IL8 (bottom) levels against physiological age for babies with early onset sepsis.
Predicting early onset culture-positive sepsis (bacteremia) using a combined score of salivary IL8 and salivary CRP.

For early onset, especially for babies who are 48 hours old, a CRP and IL8 salivary test may not work for screening and should be used in addition with clinical symptom analysis.
Where do we go from here?

Current ‘standard-of-diagnostic’ for neonatal sepsis

- Baby has clinical symptoms of infection
  - Prophylactic antibiotics are started
  - Therapy is continued and baby is kept under observation.
  - Blood tests are performed.

Proposed ‘standard-of-diagnostic’ for neonatal sepsis

- Baby has clinical symptoms of infection
  - Salivary infection screening test is done
  - Serial tests are done to assess therapy effectiveness
  - Prophylactic antibiotics are started.
  - Blood tests are performed (if needed).
  - Therapy is continued and baby is kept under observation.

Some unanswered questions

- Would there be a difference in the cut-off points based on demography/ethnicity of the newborn?
- How do we separate out the effects arising from developmental immune disorders?
- What clinical parameter should be compared to quantitatively define the severity of sepsis?
Thank You
Going against the Flow: towards a lateral flow, point of care test for sepsis/severe infections

A/Prof David Anderson, Deputy Director, Burnet Institute

GARDP Webinar, 26 November 2019
“Going against the Flow” – can we use lateral flow technology (POC) instead of Flow Cytometry to measure cell-associated biomarkers?

- CD4 T-cells (Visitect® CD4 and Visitect® CD4 Advanced Disease – Omega Diagnostics, UK)
  - CE Mark approval, and recent Global Fund endorsement for procurement of the CD4 Advanced Disease test

- Neutrophil CD64 as a marker of sepsis/severe infections
  - Well documented as a sepsis biomarker using flow cytometry, with elevated levels of CD64 on both neutrophils and monocytes during severe infections, but available flow cytometry test based on neutrophils only
From Visitect® CD4 to sepsis/severe infections

- Burnet R&D that led to the Visitect® CD4 T-cell tests (Omega Diagnostics) solved two critical questions that are also relevant to our development of a test for sepsis/severe infection:

  1) Can you measure cell-associated molecules in lateral flow, as an alternative to Flow Cytometry?

  2) Can you remove monocytes from whole blood in a lateral flow device?
1. Measuring cell-associated antigen (CD4) vs Flow

>90% accuracy for the Visiitect® CD4 and CD4 Advanced Disease test using visual readout in multiple trial sites across India, UK, and Sub-Saharan Africa.
CD64 in sepsis/severe infections

- Sepsis kills around 8 million people each year, mostly neonates and young children plus the elderly – #1 cause of death in hospitalised patients, and major cause of death in HIV patients with Advanced Disease (CD4 <200)

- Urgent need for rapid diagnosis to identify severe bacterial infections requiring antibiotic treatment, while reducing over-treatment / prophylaxis

- Neutrophil CD64 expression (by flow cytometry) is one of the most robust single biomarkers, but utility is severely limited by access to flow cytometry

  - Monocytes show high basal level of CD64, up-regulated by IFN-gamma etc during infection

  - Neutrophils show negligible basal CD64, but high levels during infection in MOST patients, when tested using flow cytometry
Can we adapt “neutrophil CD64” for POC?

- Adaptation of the neutrophil CD64 index test (Leuko64) to point of care using the same approach as CD4 T-cell test
  - But surprising discoveries along the way….

- Measuring NE as an indication of neutrophil numbers (just like CD4), and the total amount of CD64 in whole blood

- Ongoing clinical study (lab-based ELISAs) in healthy and ICU controls and sepsis patients (Sepsis-3 criteria), Alfred Hospital, Melbourne; and in neonates (referred to lab for suspicion of sepsis or severe infection), Royal Children’s Hospital, Melbourne, and Royal Hobart Hospital, Hobart
CD4 vs CD4 T-cells is linear

Linear Correlation of pooled samples: November ELISA Burnet Institute: Flow vs ELISA (CD4 counts)
n = 28

Estimated CD4 counts (ELISA) cells/ul vs CD4 counts (Flow) cells/ul

R² = 0.9243
CD4 vs CD4 T-cells is linear

Linear Correlation of pooled samples: November ELISA Burnet Institute: Flow vs ELISA (CD4 counts)

\[ n = 28 \]

CD64 vs Neutrophils (NE) should be the same (?)
BUT IT’S NOT!
Cell lysis
Sample addition
Monocyte depletion
Buffer addition (gold conjugate)

Procedural control
Reference
Test line(s)

CD64 (FcγR1)
NE (Neutrophil Elastase)

CD4

VISITECT CD4

A
B
C

CD64/NE

aXXin

INNOVATION TO IMPACT
Thanks

- Mary Garcia, Robyn Lloyd, Suzanne Crowe, Simone van de Waarsenburg, Jocelyn Diaz, Nadine Barnes, Alan Landay (Rush U.), Tom Denny (Duke U.)

- Stanley Luchters, Minh Duc Pham (Burnet), Dr Pachamuthu Balakrishnan (YRG Care, Chennai), Matthew Chersich (WITS, Johannesburg) – CD4 test validation and implementation studies

- Andrew Shepherd, Edward Valente, John Bannister and teams, Omega Diagnostics, UK – Visitect® CD4 commercialisation

- Riya Palchaudhuri, Suzanne Crowe, Clovis Palmer, Mary Garcia, Shuning Zheng, Nobin Khan, Grace Anderson, Serina Cucuzza, Viv Gleeson, Naomi Spotswood (Burnet), Steve McGloughlin, Shirley Vallance, Emma Martin and team (Alfred ICU Melbourne) and lab team at RCH/RHH – CD64/sepsis
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