Diagnostic stewardship, a new kid on the block

Maurizio Sanguinetti
Institute of Microbiology
Fondazione Policlinico Universitario «A. Gemelli» - IRCCS
Rome - Italy
WHAT IS “DIAGNOSTIC STEWARDSHIP”?

• Diagnostic stewardship involves modifying the process of ordering, performing, and reporting diagnostic tests to improve the treatment of infections and other conditions.

• Within the laboratory community, these steps are referred to as preanalytic, analytic, and postanalytic interventions.
Steps at Which Diagnostic Stewardship May Improve Testing for Common Infectious Disease Tests

<table>
<thead>
<tr>
<th>Ordering (Preaanalytic)</th>
<th>Collection (Preaanalytic)</th>
<th>Processing (Analytic)</th>
<th>Reporting (Postanalytic)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General principles</strong></td>
<td>Test only if clinical presentation is consistent with the infectious etiology (high pretest probability)</td>
<td>Pay attention to sample collection and transport, to optimize yield and reduce contamination</td>
<td>Use adjunctive laboratory tests to distinguish colonization from infection</td>
</tr>
<tr>
<td><strong>Urine cultures</strong></td>
<td>Test only when symptoms suggest urinary tract infection or, if asymptomatic, concordant with guidelines (eg, urologic surgery, pregnancy)</td>
<td>Use aseptic technique—midstream clean catch after periurethral cleansing Obtain catheter sample from collection port (not bag), prefer newly inserted catheter</td>
<td>Only perform urine culture if pyuria present</td>
</tr>
<tr>
<td><strong>Blood cultures</strong></td>
<td>Test only when symptoms of infection present (fever) Avoid repeat cultures unless concern for persistent or endovascular infection</td>
<td>Use aseptic technique—prefer peripheral samples obtained by trained phlebotomists Avoid catheter draws</td>
<td>Consider rapid testing on initial positive results, eg, polymerase chain reaction, PNA-FISH, MALDI-TOF</td>
</tr>
<tr>
<td><strong>Clostridium difficile testing</strong></td>
<td>Test only when disease likely (eg, recent antibiotic exposure, &gt;3 loose stools/d, duration &gt;24 h, and no recent laxative use) Avoid tests of cure</td>
<td>Only collect and send loose stool (ie, that conforms to the container)</td>
<td>Consider use of a testing algorithm that includes toxin immunoassay</td>
</tr>
<tr>
<td><strong>Molecular detection panels (ie, “syndromic testing”)</strong></td>
<td>Test only when pretest probability moderate to high for ≥2 targets on the panel, and when results will influence management</td>
<td>Use recommended collection and transport conditions to reduce contamination and optimize yield</td>
<td>Follow stringent contamination prevention guidance in the laboratory to avoid false-positive results</td>
</tr>
<tr>
<td><strong>Forms of automation</strong></td>
<td>Clinical decision support requiring documentation of symptoms Hard stops for contraindications—eg, laxative use within 48 h of C difficile test</td>
<td>Recording site and method of collection Orders requiring supplementary tests—eg, urinalysis before urine culture</td>
<td>Laboratory support systems performing cascades of tests</td>
</tr>
</tbody>
</table>

Abbreviations: PNA-FISH, peptide nucleic acid–fluorescence in situ hybridization; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight.

Morgan et al., JAMA, 2017
Rapid diagnostic tests: the John Bartlett’s game changers in infectious diseases

• We concur with Dr. John Bartlett, retired infectious diseases physician, who stated in 2010 that rapid diagnostic tests are ‘game changing’ in the management of infectious diseases.

• However, in 2018, we concur with a modification of his statement as suggested by Bauer and Goff, that is: “Rapid diagnostic tests and antimicrobial stewardship economics will ‘change the game’ of managing patients with infectious diseases.”
Every few seconds someone dies of sepsis. Sepsis arises when the body's response to an infection damages its own tissues and organs. It may lead to shock, multiple organ failure, and death, especially if not recognized early and treated promptly.

A global burden: Sepsis is one of the most common but least recognized diseases.

~ 27,000,000 people per year develop sepsis

~ 19,000,000 people per year survive

~ 6,000,000 neonates and children under five die of sepsis

Survivors may face lifelong complications

Maternal Death: Sepsis is one of the most common causes

~ 8,000,000 people per year die

Everybody can develop sepsis following an infection

Sepsis is one of the most expensive conditions treated in the US and Germany

People hospitalized with sepsis in US:

- Up to 417,868 (2011)
- Up to 1,500,000 (2008)

Children hospitalized with sepsis in US:

- ~20,000 to 40,000

Changes in the rates of hospitalizations with severe sepsis among emergency medical services:

- Acute myocardial infarction
- Stroke

Have you ever heard the term "Sepsis"?

NO

YES

50%
44%
29%
7%

Germany
USA
Canada
Brazil

Gemelli
FIG 1 Roles of diagnostic and antimicrobial stewardship in the implementation of rapid molecular infectious disease diagnostics in the clinical setting.
Multistakeholder platform of the antimicrobial, infection prevention and diagnostic stewardship model.

This model represents the complexity of the patients (green for low complex, orange for intermediate complex and red for high complex) that corresponds with the number of patients and the treating staff (width of the pyramid) and the experience level of the treating staff (height of the pyramid).

The more complex a patient, the more he/she requires experienced specialists from multiple disciplines supplemented with correct, on-time performed diagnostics and eHealth tools.

This, together, is needed to adequately deal with the specific infectious problems, whereby the complexity of the patient is not a fixed label, but a continuous changing state which varies over time.

ASP: Antimicrobial Stewardship Program; DSP: Diagnostic Stewardship Program; ISP: Infection Prevention Stewardship Program.
When the Laboratory is the POC: possible outcomes

- Better management of severe infections (e.g., sepsis) and reduction of the associated mortality
- Increased money reducing the length of stay and the use of antibiotics/antifungals
- Better real-time assessment of the local microbial epidemiology of the Hospital and to establish efficacious guidelines for the antimicrobial (empirical) therapy
Diagnostic Stewardship....

The Right patient
**AntiMicrobial Stewardship: Minimum requirements for developing an institutional programme**

Creation of a multidisciplinary inter-professional team which optimally should include but is not limited to:

- an infectious diseases (ID) physician
- a pharmacist with ID training
- a clinical microbiologist
- an infection control professional
- a hospital epidemiologist
AMS Programme

Improved management of infections

**ID specialist**
Assessing clinical signs & symptoms, diagnostic advice, antimicrobial drug selection, duration of treatment

**Clinical microbiologist**
Rapid diagnostic test delivery & interpretation, antimicrobial susceptibility testing, antimicrobial drug selection

**Hematologist/Intensivist**
Risk stratification, assessing clinical signs & symptoms, antimicrobial drug prescribing

**Hospital pharmacist**
Antimicrobial drug dosages, PK issues in specific patient populations, drug-drug interactions, TDM & interpretation
Key diagnostic stewardship considerations for implementation of rapid infectious disease diagnostics

<table>
<thead>
<tr>
<th>Goal</th>
<th>Key question</th>
<th>Key considerations and potential strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right patient</td>
<td>Will the clinical care of the patient be affected by the test result?</td>
<td>Laboratory test utilization committee</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Automatic laboratory reflex</td>
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<tr>
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<td>Appropriate use criteria</td>
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<td>Indication selection</td>
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<td>Prior authorization</td>
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<td></td>
<td>Benchmarking</td>
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<td>Specimen rejection</td>
</tr>
</tbody>
</table>

Messacar et al., JCM., 2017
“Assuming sensitivity of 90% and specificity of 98%, anticipated positive and negative predictive values (PPVs/NPVs) of T2Candida can be calculated”

Thus, “T2Candida performance characteristics enable clinicians to assign clinical settings in which T2Candida is most likely to be useful in guiding antifungal treatment decisions”. Indeed, “PPVs may exceed a threshold that justifies antifungal treatment, while corresponding NPVs render active candidemia extremely unlikely”.

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Representative patient</th>
<th>90% Sensitivity/98% specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4%</td>
<td>Any hospitalized patient in whom a blood culture is collected.</td>
<td>15%</td>
</tr>
<tr>
<td>1%</td>
<td>Patient admitted to critical care unit.</td>
<td>31%</td>
</tr>
<tr>
<td>2%</td>
<td>Patient with febrile neutropenia, baseline rate of candidaemia prior to empirical antifungal treatment</td>
<td>47%</td>
</tr>
<tr>
<td>3%</td>
<td>Patient with sepsis, shock or &gt;3–7 day stay in critical care unit.</td>
<td>67%</td>
</tr>
<tr>
<td>10%</td>
<td>Patient at increased risk of candidaemia based on clinical prediction models.</td>
<td>82%</td>
</tr>
<tr>
<td>20%</td>
<td>Neutropenic bone marrow transplant recipient or leukaemia patient not receiving antifungal prophylaxis</td>
<td>92%</td>
</tr>
</tbody>
</table>
Diagnostic Stewardship....

The Right test
When the Laboratory is the POI: How do we

- Determining how such rapid diagnostic tests (point-of-impact, POI) would best incorporated into clinical diagnostic algorithms
- Determining the clinical and economic benefits of a syndrome-based testing approach
- On this basis we selected the following tests:
  - T2 magnetic resonance
  - Automated microscopy (Accelerate Diagnostics)
  - β-D-glucan detection
  - Multiplex PCR (FilmArray, Unyvero, etc.)
  - LFIA tests (Legionella, S. pneumoniae, RSV)
- And these clinical syndromes:
  - Sepsis (Target 1, implemented)
  - Gastroenteritis (Target 2, implemented)
  - Pneumonitis (Target 3, in progress)
  - Meningitis (Target 4, in progress)
New diagnostic approaches in Clinical Microbiology playing a role in the antimicrobial therapy

- Mass-spectrometry identification of microorganisms (useful for sepsis or bacteremia, less useful for septic shock)
- Rapid susceptibility tests (phenotypic, microscopic or molecular)
- Use of biomarkers for the real-time management of therapies
- Real-time molecular methods able to efficiently detect microorganisms directly from clinical samples
**Target 1: Sepsis**

- Objectives of the project
  1. To speed up the “classical” blood culture workflow including POC or POC-like tests (MALDI-TOF mass spectrometry, FilmArray, Accelerate Pheno)
  2. To implement rapid tests for the detection of microbial and fungal pathogens directly from blood
  3. To include the β-D-glucan detection in an integrated diagnostic algorithm for the rapid de-escalation of antifungal therapy
## Direct methods from positive BCs

### Table 1

Methods applied on positive BCs [8,13–20,28–30]

<table>
<thead>
<tr>
<th>Kit/system (manufacturer)</th>
<th>Principle</th>
<th>TAT (h)</th>
<th>Panel</th>
<th>Resistance genes or antibiotics</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Cost Instrument</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microflex LT (Bruker Daltonics)</td>
<td>MALDI-TOF</td>
<td>0.2–0.5</td>
<td>Not limited</td>
<td>—</td>
<td>75</td>
<td>88</td>
<td>€€€</td>
<td>€</td>
</tr>
<tr>
<td>Vitek MS (bioMérieux)</td>
<td>Fluorescent in situ hybridization with PNA probes</td>
<td>1.5–3/0.5</td>
<td>4 different panels (S. aureus/CONS; E. faecalis/Enterococcus spp.; E. coli/K. pneumoniae/P. aeruginosa; Candida spp.)</td>
<td>—</td>
<td>88–100</td>
<td>97–100</td>
<td>€€€</td>
<td>€</td>
</tr>
<tr>
<td>PNA-FISH/QuickFISH (Advantx)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xpert MIRS/SA Blood Culture (Cepheid)</td>
<td>Multiplex real-time PCR</td>
<td>1</td>
<td>S. aureus/CONS</td>
<td>mecA</td>
<td>98–99</td>
<td>99–100</td>
<td>€€€</td>
<td>€</td>
</tr>
<tr>
<td>GenoType StaphSR (BD)</td>
<td>Multiplex real-time PCR</td>
<td>1–1.5</td>
<td>S. aureus/CONS</td>
<td>mecA</td>
<td>94–99</td>
<td>96–98</td>
<td>€€€</td>
<td>€</td>
</tr>
<tr>
<td>Prove-it Sepsis (Mobidiag)</td>
<td>Broad-range PCR + microarray-based detection</td>
<td>3</td>
<td>60 bacteria, 13 fungi</td>
<td>mecA, vraA, vanA</td>
<td>96–99</td>
<td>99–100</td>
<td>€€€</td>
<td>€</td>
</tr>
<tr>
<td>FilmArray Blood Culture Identification (BD) (bioMérieux)</td>
<td>Multiplex real-time PCR</td>
<td>1</td>
<td>8 GP, 11 GN, 5</td>
<td>mecA, vraA, braB, braC</td>
<td>80–87</td>
<td>91–100</td>
<td>€€€</td>
<td>€</td>
</tr>
<tr>
<td>Vengene Gram-Positive Blood Culture [BC-GP] (Lumines)</td>
<td>Multiplex PCR + solid-microarray detection</td>
<td>2.5</td>
<td>11 GP</td>
<td>mecA, vraA, vanA</td>
<td>93–100</td>
<td>94–100</td>
<td>€€€</td>
<td>€</td>
</tr>
<tr>
<td>Vengene Gram-Negative Blood Culture [BC-CN] (Lumines)</td>
<td>Multiplex PCR + solid-microarray detection</td>
<td>2</td>
<td>9 GN</td>
<td>mecA, vraA, braB, braC</td>
<td>89–100</td>
<td>93–100</td>
<td>€€€</td>
<td>€</td>
</tr>
<tr>
<td>Accelerate Phenom System (Accelerate Diagnostics)</td>
<td>FISH (ID) Mophotokinetic cellular analysis (AST)</td>
<td>1.5 (ID)</td>
<td>6 GP, 8 GN, Candida</td>
<td>mecA, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TGP (E, ca); Klebsiella spp.: Proteus spp., AMK, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TGP (E, ca), Klebsiella spp., Proteus spp., AMK, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TGP (E, ca), Klebsiella spp., Proteus spp., AMK, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TGP (E, ca)</td>
<td>89–96 (ID)</td>
<td>99–100 (ID)</td>
<td>€€€</td>
<td>€</td>
</tr>
<tr>
<td></td>
<td>(AST)</td>
<td>7</td>
<td>Candida</td>
<td>mecA, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TGP (E, ca), Klebsiella spp., Proteus spp., AMK, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TGP (E, ca), Klebsiella spp., Proteus spp., AMK, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TGP (E, ca)</td>
<td>91–95 (AST)</td>
<td></td>
<td>€€€</td>
<td>€</td>
</tr>
</tbody>
</table>
Cost-effectiveness plane for rapid identification of blood cultures using molecular methods and MALDI. The y axis represents the average cost of a strategy, while the x axis represents the average effectiveness of a strategy. Cost-effective strategies are depicted with red markers, the baseline strategy is depicted with a black marker, and the remaining strategies that are suboptimal or not cost-effective are indicated with blue markers.

Pliakos et al., CMR, 2018
Molecular detection of resistance

- Identification of microbial genes related to drug resistance directly from clinical specimens or from positive blood cultures (to reduce the time-to-result)
- Reduction of the time to appropriate antibiotic treatment (escalation or de-escalation therapy)
- Easy to use
- Relatively few genes included in the proposed panels
- Expensive (?)
Rapid Identification of *Escherichia coli* and *Klebsiella pneumoniae* Producing CTX-M and Carbapenemases in Positive Blood Cultures Combined with Bedside Infectious Disease Consultation is Associated with Very Early Initiation of Adequate Antimicrobial Therapy

- We evaluated the performance and the advantages of a PCR-based rapid detection of *bla*<sub>CTX-M</sub>, *bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, *bla*<sub>OXA-48</sub> and *bla*<sub>NDM</sub> genes (Eazyplex® SuperBug CRE) after direct detection of *E. coli* and/or *K. pneumoniae* using MALDI BioTyper and/or FilmArray BCID panel.
- We analyzed 425 cases of bloodstream infections with positive blood cultures (BCs).
- Resistance genes were detected in 43.5% (191/439) of the isolates with an overall agreement with the culture results of 100%.
- Total time to identification (median) of the direct method was 18 h (IQR, 8-23 h) versus 46 h (IQR, 36-68.8 h) (P<0.001) with the culture-based method.
- The therapeutic impact of the rapid strategy was evaluated in 327 cases.
- All these patients received an empirical treatment after BC collection, but it was inadequate in 58.1% of the cases.
- After real-time communications and bedside infectious disease interventions, 82.1% of the patients with inadequate therapy were switched to an effective coverage at a median time of 20 h (IQR, 10.3 to 25 h) from the starting of diagnostic procedure.

Spanu et al, unpublished
Updated UCSC protocol for the management of positive blood cultures

Positive BC bottle

Gram staining

Monomicrobial

Direct MALDI BioTyper system

K. pneumoniae

NO

Stop

Polymicrobial

Direct AST

Rapidec Carba NP

Yes

Pos./doubtful

Enterobacteriaceae

ERT/MEM MIC > 0.12 µg/ml

yes

no

Stop

Phenotypic and/or molecular tests

Result reporting

Eazyplex Superbug CRE

Result reporting
The GRES (Gestione Rapida Emocolture Sepsi) study

<table>
<thead>
<tr>
<th></th>
<th>Total N=743</th>
<th>Study period 1 N=197</th>
<th>Study period 2 N=233</th>
<th>Study period 3 N=313</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean time to start antibiotic therapy (SD), days</td>
<td>0.70 (1.4)</td>
<td>0.92 (1.7)</td>
<td>0.71 (1.4)</td>
<td>0.55 (1.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Percentage of effective therapy at the start of antibiotic therapy (SD)</td>
<td>92.6 (18.2)</td>
<td>93.9 (19.0)</td>
<td>91.4 (21.1)</td>
<td>92.6 (15.2)</td>
<td>0.37</td>
</tr>
<tr>
<td>Percentage of optimal therapy at the start of antibiotic therapy (SD)</td>
<td>62.2 (38.9)</td>
<td>59.0 (42.0)</td>
<td>51.9 (43.0)</td>
<td>71.9 (30.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean duration of antibiotic therapy (SD), days</td>
<td>19.3 (13.3)</td>
<td>21.9 (15.4)</td>
<td>19.3 (13.3)</td>
<td>17.7 (11.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Mean length of hospital stay (SD), days</td>
<td>26.5 (24.5)</td>
<td>29.7 (29.3)</td>
<td>26.8 (24.7)</td>
<td>24.2 (20.7)</td>
<td>0.04</td>
</tr>
<tr>
<td>Died at 30 days, n (%)</td>
<td>118 (15.9)</td>
<td>31 (15.7)</td>
<td>39 (16.7)</td>
<td>48 (15.3)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Study Period 1: the ID physician was called by a ward physician when patients had positive blood cultures.

Study Period 2: the ID physician were called directly by the microbiologist immediately after a pathogen was isolated from blood culture.

Study Period 3: the ID physician was called by the microbiologist immediately after a pathogen was identified from the blood culture, and all cases were re-evaluated at bedside 72 h after starting antibiotic therapy in order to optimize therapy.

Murri et al., Diag. Microbiol. Infect. Dis., 2018
• The T2Dx, an automated instrument platform, uses T2 magnetic resonance (T2MR) technology, to provide a “sample-to-result” clinical diagnostic test. The test was designed to enable multiplex detection, of ESKAPE pathogens, which comprise *Escherichia coli* instead of *Enterobacter* species, and are hereafter designated as ESKAPE* pathogens, with a single whole blood sample.

• In addition, T2MR is able to accurately identify *Candida* pathogens in whole blood faster and easier than blood culture-based diagnostics.

• T2Candida and T2Bacteria are cleared by the US FDA and EMA for the diagnosis of bloodstream infections.
T2Dx *Candida* panel

- T2MR demonstrated an overall specificity per assay of 99.4% with a mean time to negative result of 4.2 ± 0.9 hours. The overall sensitivity was found to be 91.1% (96.6% considering also other studies) with a mean time of 4.4 ± 1.0 hours for detection and species identification.

- Significant reduction of the time to appropriate therapy (from 20 to 28 hours)

- Significant reduction of the time to detection of *Candida*

- Significant reduction of the ICU length of stay

- Significant reduction in antifungal consumption

- Strong indicator of complications and poor outcomes

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1. Mylonakis E, et al. CID. 2015
2. Wilson et al, ID Week 2016
3. Patch et al., CMI, 2018
4. Muñoz et al., CMI, 2018
Positive results more than 4.5 times faster: Positive results for a bacterial infection with T2Bacteria were available in 5.5 hours, compared to a mean time of 25 hours for blood culture. Every hour of providing targeted therapy to patients faster can reduce patient mortality rates by 7.6%.

Negative results 20 times faster: A negative result took 120 hours (5 days) for blood culture, compared to 6.1 hours with the T2Bacteria Panel.

Excellent accuracy: The T2Bacteria Panel had 89.5% sensitivity and 98.4% specificity in comparison with clinical criterion of a true infection. The authors point out that blood culture-based diagnostics have a sensitivity as low as 65% for the first blood culture draw.

Potential impact on therapy: The authors found that 66.7% of the clinically infected patients missed by blood culture and correctly identified by T2Bacteria were being inappropriately treated at the time of the T2Bacteria result.
Clinical Case Study: Rapid identification of polymicrobial infection by T2MR

M 68 year old patient, miastenia gravis, admitted to ICU for respiratory failure. High fever, chills, flush skin, tachycardia and rapid breathing. Suspect focus of infection was abdomen. Started therapy with meropenem and vancomycin.

~41 hours to Species Identification

- **DAY0**
  - 3:00pm: BCs collected
  - 3:50pm: start BCs incubation
  - 9:26pm: BCs positive (TTP - 5h30’)
- **DAY1**
  - 8:10am: BCs bottle unload
  - 10:00am: *E. coli* by direct MALDI-TOF
- **DAY2**
  - 8:00am subculture show also *K. pneumoniae*

© ESCMID eLibrary by author

~4 hours to Species Identification

- **DAY0**
  - 3:00pm: T2 sample collected
  - 3:25pm: T2 start time
  - 7:07pm: *E. coli* + *K. pneumoniae*
Objectives:
- To determine the effects of a strategy that uses (1,3)-β-D-glucan (BDG) results for antifungal treatment of ICU patients at high risk of invasive candidiasis.

Methods:
- Patients were included in the analysis if they exhibited sepsis at the time of BDG testing, and they met Candida-score components (i.e., severe sepsis, total parenteral nutrition, surgery, or multifocal Candida colonization) to reach a ≥3 value.

Results:
- 198 patients were studied (63 positives and 135 negatives)
- Of 63 BDG-positive patients, 47 with candidemia and 16 with probable Candida infection, all received antifungal therapy
- Of 135 BDG-negative patients, 110 (55.5%) did not receive antifungal therapy, whereas 25 (12.6%) were initially treated. In 14 of these 25 patients antifungals were discontinued as negative BDG results were notified. Candidemia was subsequently diagnosed only in one patient who did not receive prior antifungal therapy
- The median antifungal therapy duration in candidemic patients differed from that in non-candidemic patients (14 days [IQR, 6–18 d] vs 4 days [IQR, 3–7 d]; \( p < 0.001 \))
- Thus, unnecessary antifungal therapy was avoided in ~73% of potentially treatable patients and it was shortened in another ~20%
- A rough cost analysis showed also that adopting a BDG-based therapeutic approach might lead to a not negligible cost reduction by saving approximately €3500 per patient.
• Positive predictive value (PPV) and negative predictive value (NPV) for candidemia of (1,3)-β-D-glucan (BDG) and procalcitonin (PCT) considered both separately and in combination (BDG+PCT).

• The reported PPV for candidemia (also readable as NPV for bacteremia) was obtained when both markers were concordant in indicating candidaemia (BDG ≥80 pg/ml and PCT <2 ng/ml), while the reported NPV for candidemia (also readable as PPV for bacteremia) was obtained when both markers when concordant in indicating bacteremia (BDG <80 pg/ml and PCT ≥2 ng/ml).

Time Lapse Microscopy

Piperacillin-Tazobactam

Colistin
This study describes results from a multicenter study evaluating the Accelerate Pheno system. A combination of fresh clinical and seeded blood cultures were tested, and results from the Accelerate Pheno system were compared to Vitek 2 results for identification (ID) and broth microdilution or disk diffusion for AST.

Six common Gram-positive cocci and from the 4,142 AST results, the overall essential agreement (EA) and categorical agreement (CA) were 97.6% and 97.9%, respectively. Overall very major error (VME), major error (ME), and minor error (mE) rates were 1.0%, 0.7%, and 1.3%, respectively.

Eight species of Gram-negative rods were evaluated and from the 6,331 AST results, overall EA and CA were 95.4% and 94.3%, respectively. Overall VME, ME, and mE rates were 0.5%, 0.9%, and 4.8%, respectively.

On the basis of these results the Accelerate Pheno system seems to be able to identify and provide phenotypic MIC and categorical AST results in a few hours directly from positive blood culture bottles and support accurate antimicrobial adjustment.
Antimicrobial susceptibility testing of pathogens isolated from blood culture: a performance comparison of Accelerate Pheno™ and VITEK® 2 systems with broth microdilution method

<table>
<thead>
<tr>
<th>Species (n)</th>
<th>Accelerate Pheno™ system AST</th>
<th>VITEK® 2 system AST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of AST results³</td>
<td>No. (%) of CA</td>
</tr>
<tr>
<td><strong>K. pneumoniae (11)</strong></td>
<td>63</td>
<td>58 (92.0)</td>
</tr>
<tr>
<td><strong>E. coli (9)</strong></td>
<td>23</td>
<td>21 (91.3)</td>
</tr>
<tr>
<td><strong>P. aeruginosa (8)</strong></td>
<td>36</td>
<td>35 (97.2)</td>
</tr>
<tr>
<td><strong>A. baumannii (7)</strong></td>
<td>21</td>
<td>21 (100)</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci (5)</td>
<td>4</td>
<td>4 (100)</td>
</tr>
<tr>
<td><strong>E. faecium (3)</strong></td>
<td>5</td>
<td>5 (100)</td>
</tr>
<tr>
<td><strong>S. aureus (3)</strong></td>
<td>3</td>
<td>3 (100)</td>
</tr>
<tr>
<td><strong>P. mirabilis (2)</strong></td>
<td>8</td>
<td>8 (100)</td>
</tr>
<tr>
<td><strong>Enterobacter species (1)</strong></td>
<td>2</td>
<td>2 (100)</td>
</tr>
<tr>
<td><strong>E. faecalis (1)</strong></td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><strong>S. marcescens (1)</strong></td>
<td>1</td>
<td>1 (100)</td>
</tr>
<tr>
<td><strong>Total (51)</strong></td>
<td>166</td>
<td>158 (95.2)</td>
</tr>
</tbody>
</table>

Accelerate Pheno™ system and VITEK® 2 systems’ results for the isolates found to be resistant to at least one antimicrobial agent by the reference BMD method

De Angelis et al., JAC, submitted
<table>
<thead>
<tr>
<th>Method</th>
<th>TTR (hrs)</th>
<th>Targets</th>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>FilmArray® (bioMérieux)</td>
<td>1 h</td>
<td>KPC</td>
<td>Positive blood culture</td>
</tr>
<tr>
<td>Verigene® (Nanosphere)</td>
<td>2 h</td>
<td>CTX-M, IMP, KPC, NDM, OXA-48, VIM</td>
<td>Positive blood culture</td>
</tr>
<tr>
<td>GeneXpert® (Cepheid)</td>
<td>1 h</td>
<td>IMP, KPC, NDM, OXA-48, VIM</td>
<td>Rectal swab</td>
</tr>
<tr>
<td>Unyvero® (Curetis)</td>
<td>5 h</td>
<td>CTX-M, IMP, KPC, NDM, OXA-48, VIM, OXA-23, OXA-24/40, OXA-58</td>
<td>positive blood culture, respiratory samples, implant and tissue samples</td>
</tr>
<tr>
<td>Eazyplex® (Amplex)</td>
<td>0.5 h</td>
<td>KPC, NDM, OXA-48 like, VIM</td>
<td>colony, positive blood culture, rectal swab</td>
</tr>
<tr>
<td>Check-Direct CPE (Check-Points)</td>
<td>2 h</td>
<td>KPC, OXA-48 like, VIM, NDM</td>
<td>colony, rectal swab</td>
</tr>
</tbody>
</table>
SepsiScan workflow

✔ Preclinical study
Positive blood cultures
1:1000 dilution in NaCl

✔ Clinical study
Whole blood sample

1) 23°C cycle: Rnasi internal control
2) 35°C cycle: Positive result for E. faecalis
<table>
<thead>
<tr>
<th>Gram-negative bacteria</th>
<th>Gram-positive bacteria</th>
<th>Yeast</th>
<th>Resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td><em>Staphylococcus</em> spp.</td>
<td><em>C. albicans</em></td>
<td>mecA</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td><em>S. aureus</em></td>
<td><em>C. glabrata</em></td>
<td>bla\textsubscript{KPC}</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td><em>E. faecalis</em></td>
<td><em>C. krusei</em></td>
<td>vanA/B</td>
</tr>
<tr>
<td><em>S. marcescens</em></td>
<td><em>E. faecium</em></td>
<td><em>Candida</em> spp.</td>
<td></td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td><em>Streptococcus</em> spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. cloacaee</em></td>
<td><em>S. pneumoniae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood culture</td>
<td>Clinical information</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>--------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Matched positives, n</td>
<td>15</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Matched negatives, n</td>
<td>72</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Sepsiscan overdetections, n</td>
<td>15</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Sepsiscan misses, n</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Overall agreement, %</td>
<td>80.5</td>
<td>92.0</td>
<td></td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>71.4</td>
<td>82.0</td>
<td></td>
</tr>
<tr>
<td>Specificity, %</td>
<td>82.8</td>
<td>96.0</td>
<td></td>
</tr>
<tr>
<td>Positive predictive value, %</td>
<td>50.0</td>
<td>90.0</td>
<td></td>
</tr>
<tr>
<td>Negative predictive value, %</td>
<td>92.3</td>
<td>92.3</td>
<td></td>
</tr>
</tbody>
</table>
Target 2: Gastroenteritis

*C. difficile* infection

- Objectives of the project
  1. To implement a diagnostic procedure including a CLIA test for GDH and Toxins A and B, and a confirmatory molecular test (BDMax platform)
  2. To setup a clinical-diagnostic algorithm at the Hospital level to manage the *C. difficile* infection
  3. To include the Fecal Microbiota Transplantation (FMT) in this algorithm
Sample Multistep Algorithm for Rapid Diagnosis of *Clostridium difficile* Infection

**Detailed Diagnostic Steps**

1. Perform EIA for GDH in stool sample
2. Perform EIA for Toxin A and B in stool sample

**GDH +/Toxin +**
- Perform PCR for *tcdB* Gene
  - *tcdB* Gene +
    - Testing consistent with *C difficile* infection
  - *tcdB* Gene -
    - Testing not consistent with *C difficile* infection

**GDH +/Toxin -**
- GDH -/Toxin +
- GDH -/Toxin -

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Bagdasarian et al., JAMA, 2015
## Steps at Which Diagnostic Stewardship May Improve Testing for Common Infectious Disease Tests

<table>
<thead>
<tr>
<th>Steps at Which Diagnostic Stewardship May Improve Testing</th>
<th>Ordering (Preanalytic)</th>
<th>Collection (Preanalytic)</th>
<th>Processing (Analytic)</th>
<th>Reporting (Postanalytic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General principles</td>
<td>Test only if clinical presentation is consistent with the infectious etiology (high pretest probability)</td>
<td>Pay attention to sample collection and transport, to optimize yield and reduce contamination</td>
<td>Use adjunctive laboratory tests to distinguish colonization from infection</td>
<td>Report results in a format that guides appropriate practice</td>
</tr>
<tr>
<td>Urine cultures</td>
<td>Test only when symptoms suggest urinary tract infection or, if asymptomatic, concordant with guidelines (e.g., urologic surgery, pregnancy)</td>
<td>Use aseptic technique—midstream clean catch after periurethral cleansing Obtain catheter sample from collection port (not bag), prefer newly inserted catheter</td>
<td>Only perform urine culture if pyuria present</td>
<td>Text interpreting result, eg, &quot;multiple organisms indicating likely contamination&quot;; &quot;no pyuria, culture not performed&quot; Selective reporting of antibiotic susceptibilities—display preferred antibiotics only</td>
</tr>
<tr>
<td>Blood cultures</td>
<td>Test only when symptoms of infection present (fever) Avoid repeat cultures unless concern for persistent or endovascular infection</td>
<td>Use aseptic technique—prefer peripheral samples obtained by trained phlebotomists Avoid catheter draws</td>
<td>Consider rapid testing on initial positive results, eg, polymerase chain reaction, PNA-FISH, MALDI-TOF</td>
<td>Text interpreting result, eg, &quot;likely skin contaminant&quot;; &quot;Staphylococcus aureus, likely pathogen consider infectious diseases consult&quot; Selective reporting of antibiotic susceptibilities</td>
</tr>
<tr>
<td><em>Clostridium difficile</em> testing</td>
<td>Test only when disease likely (e.g., recent antibiotic exposure, &gt;3 loose stools/d, duration &gt;24 h, and no recent laxative use) Avoid tests of cure</td>
<td>Only collect and send loose stool (i.e., that conforms to the container)</td>
<td>Consider use of a testing algorithm that includes toxin immunoassay</td>
<td>Text interpreting result, eg, &quot;toxin+/PCR+ indicating possible colonization rather than disease&quot;</td>
</tr>
<tr>
<td>Molecular detection panels (i.e., &quot;syndromic testing&quot;)</td>
<td>Test only when pretest probability moderate to high for ≥2 targets on the panel, and when results will influence management</td>
<td>Use recommended collection and transport conditions to reduce contamination and optimize yield</td>
<td>Follow stringent contamination prevention guidance in the laboratory to avoid false-positive results</td>
<td>Selective suppression of results for tests on panel if other testing approach used in the laboratory (e.g., C. difficile testing on stool pathogen panel) Text interpreting results discussing colonization</td>
</tr>
<tr>
<td>Forms of automation</td>
<td>Clinical decision support requiring documentation of symptoms Hard stops for contraindications—e.g., laxative use within 48 h of <em>C difficile</em> test</td>
<td>Recording site and method of collection Orders requiring supplementary tests—e.g., urinalysis before urine culture</td>
<td>Laboratory support systems performing cascades of tests</td>
<td>Prepopulated reports that can be reviewed and modified by laboratory personnel</td>
</tr>
<tr>
<td>Clinician education</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Abbreviations:** PNA-FISH, peptide nucleic acid–fluorescence in situ hybridization; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight.
Adopted procedure for the management of *C. difficile* infection at the “A. Gemelli” Hospital

- **Principal steps of the procedure**
  1. Identification of the patient with suspected *C. difficile* infection
  2. Microbiological real-time evaluation of the patient
  3. Management of the patient on the basis of microbiological results (including the possible institution of FMT)
Outcomes of the implementation of *C. difficile* procedure at the “A. Gemelli” Hospital

**Length of stay (days)**

- 2008-2012 (before procedure)
- 2012-2016 (after procedure)

**Mortality (%)**

- 2008-2012 (before procedure)
- 2012-2016 (after procedure)

De Belvis et al., manuscript in preparation
Fecal Microbiota Transplantation at the “A. Gemelli” Hospital in Rome (Italy)

Overall Preliminary Data (years 2013–2017)

- 130 patients treated (mean age, 71 years; range, 29–94 years)
- Mean number of recurrences of *Clostridium difficile* infection (rCDI) = 3 (range = 2–7)
- Mean Charlson Comorbidity Index score = 3
- Inpatients/Outpatients = 90/40
- Overall 192 infusion procedures (71 patients received one infusion, 59 multiple infusions)
- Fresh material/Frozen material = 126/66

**FMT procedures/year**
- June–December 2013 = 9 (7 pts)
- 2014 = 24 (15 pts)
- 2015 = 36 (25 pts)
- January–July 2016 = 32 (18 pts)
- July 2016–July 2017 = 91 (65 pts)

**RESULTS:**
- Severe colitis (pseudomembranous colitis) in 51 patients
- Follow-up range = 1–59 months
- Resolution of rCDI in 127/130 treated patients (98%)
- No patients experienced further recurrences after FMT
- No more surgery for CDI in our hospital since 2014

Cammarota et al., Aliment Pharmacol Ther, 2018
Diagnostic Stewardship....

The Right time
Why the 24-hour laboratory is an important value in the Hospital

• To provide in real-time results to the clinicians to reduce mortality
• To enhance the efficacy of the obtained results that otherwise would be un-useful if the tests are used few times during the week
• To permit a continuous adjustment of the treatment on the basis of the obtained results and to reduce the empirical therapy
This retrospective study investigates the potential benefits from the introduction of point-of-care tests for rapid diagnosis of infectious diseases.

- The authors analysed a sample of 441 hospitalized patients who had received a final diagnosis related to 18 pathogenic agents.

- The length of hospital stay was partitioned into pre- and post-laboratory diagnosis stages.

- Regression analysis and elementary queueing theory were applied to estimate the impact of quick diagnosis on the mean length of stay and the utilization of healthcare resources.

- The analysis suggests that eliminating the pre-diagnosis times through point-of-care testing could shorten the mean length of hospital stay for infectious diseases by up to 34 per cent and result in an equal reduction in bed occupancy and other resources.
Rapid metagenomic sequencing-based investigation of hospital-acquired and ventilator-associated pneumonia

Dr Hollian Richardson
Norwich Medical School
University of East Anglia

This presentation on ECCMID live
Optimised MinION workflow

- Streamlined DNA depletion – **accelerated**
- Optimised extraction - **bead beating**

- Number of PCR cycles- **20/25 cycles**
- PCR extension time reduced from 6 min (~2.5 h) to 4 min (~2 h)

<6 h from sample collection to result
Conclusions

• Respiratory pathogens and acquired antimicrobial resistance genes were identifiable within 6 h

• MinION metagenomic sequencing has potential for the rapid and accurate microbiological investigation of HAP/VAP

• WIMP and ARMA data analysis ongoing

• First clinical evaluation of rapid metagenomics in infection
Conclusions

• More than ever, the primary role of a clinical microbiology laboratory is to assist clinicians in the diagnosis and treatment of infectious diseases, and to support infection control specialists in their tasks.

• In many instances, however, the delay between the collection of the specimen and the results of the microbial culture makes the latter unhelpful for the clinician.

• Therefore, advances in diagnostic methods (such as POC or POC-like tests) and/or procedures might satisfy the ‘need for speed’, particularly when clinicians, infection control specialists, and microbiologists confront with a septic patient or, in general, with a patient with a systemic infection, or deal with an emerging epidemic.
• With the implementation of rapid point-of-impact technologies, subsequent rapid decision-making will be beneficial for the optimal use of resources (for instance bed management and isolation room capacity).
• These diagnostic assays are mostly based on molecular technologies and are therefore more expensive compared with classical culture-based methodology.
• They are however faster, delivering results within hours, thereby enabling a theragnostic approach for infection management.
• From a managerial point of view and to support health economical decision-making, the so-called ‘€hr concept’ can easily make turnaround times visible in relation to the overall costs (such as costs for unnecessary isolation).
• By multiplying costs (€) and turnaround time (h), it provides a quick, understandable figure, assuming that quality remains high and therefore equals one.

Dik et al., Future Microbiol., 2015
The UCSC Fungal and Bacterial Rapid Diagnostic Team

... and special thanks to

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