# Detect BSI quickly and identify pathogens with culture-free methods:

## pipe dream, reality or future?



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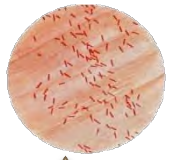
# Timeline of the conventional diagnosis of BSI


Gram stain



~24h

Culture

~24h

Identification

AST (Antibiotic Susceptibility Testing)

MALDI-TOF (SepsiTyper, Bruker)

Positive blood culture

~20 min

Vitek 2 Compact (ID + AST)

~6h

Conventional Diagnosis of sepsis

0 h ~15-20h ~22h ~40-48h ~60-72h

Desired goal: ID pathogens and AST from whole blood

Genotypic & phenotypic methods from positive blood culture



*Quesada MD, *et al*. Clin Microbiol Infect. 2010; 16(2) 137-40

*Giménez M, *et al*. 50 th ICAAC, Boston. 12-15 Septiembre, 2010

# The ideal molecular method for the diagnosis of BSI

Should…

1. Detect a broad range of pathogens.
2. Have high sensitivity and specificity.
3. Have a low turnaround time
4. Detect resistance genes.
5. Allow the analysis of single specimens at any time.
6. Have low hands-on time.
7. Be easy to perform / automatized.
8. Be easy to interpret the results.
9. Be cost-effective.

# Molecular diagnosis from blood: pros and cons

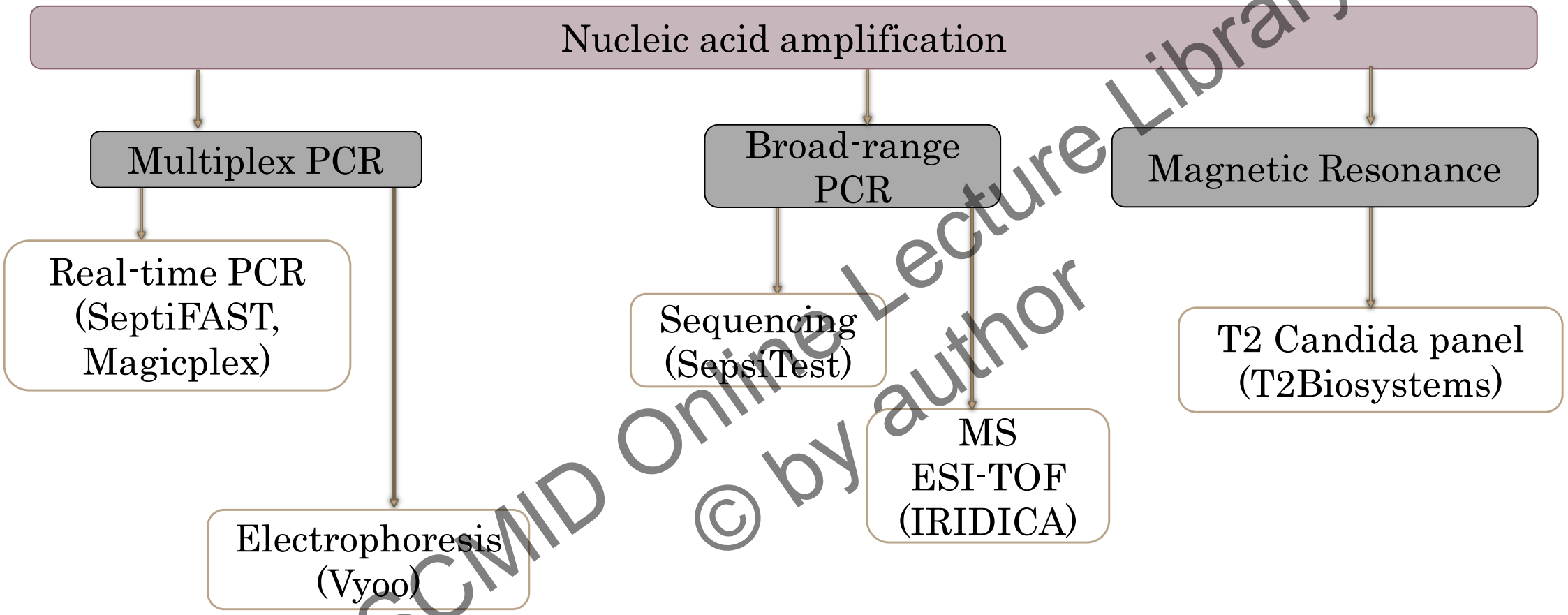
## PROS

- No culture needed→ earlier intervention on patient management.
- High sensitivity.
- Detection of non-culturable, fastidious, intracellular or slow-growing microorganisms.
- Detection of some resistance genes.
- Quantitative detection.

## CONS

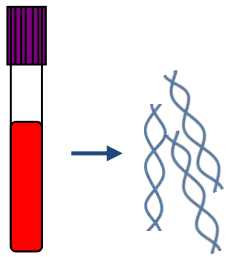
- Whole blood components (haemoglobin, ↑[hDNA], etc.) may inhibit PCR→ false negatives.
- Low volume analysed (1-5 mL vs. 20-30 mL in blood cultures) and the low concentration of microorganisms in blood (<10 CFU/mL)→ false negatives.
- Higher risk of contamination→ false positives.
- Technicians trained in molecular methods.
- Need to isolate the microorganism for the AST.
- Expensive.

# Molecular methods commercially available



# SeptiFAST (Roche)

## Technology



Automated DNA extraction from whole blood

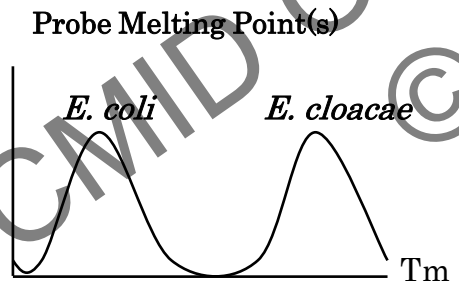
Generic Primers ("pan bacteria")

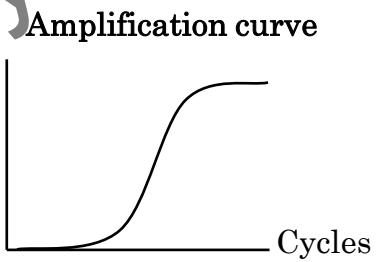

Amplification of the Internal transcribed spacer


Microorganism specific probes

Detection of microorganisms by hybridization of specific probes

Identification of the pathogens by melting curve analysis






## Workflow

DNA extraction (automated) 30'-1h → PCR 2h15' → Results interpretation (automated) → TOTAL 4h30'-6h

# SeptiFAST (Roche)

## Assay characteristics

| | |
|---|---|
| Technology | Real-time PCR, multiplexed probes |
| Nº of pathogens in the panel | 25 |
| Resistance markers | YES (mecA, separate assay) |
| Volume | 1.5 mL |
| Detection limit (CFU/mL) | 3-30 |
| Microbial DNA enrichment | NO |

## Clinical performance

| | Sensitivity | Specificity |
|---|---|---|
| Intensive Care Unit | 64% (54–74%) | 83% (78–86%) |
| Emergency Room | 69% (52–83%) | 93% (86–96%) |
| Onco-hematology | 66% (61–71%) | 86% (84–89%)\ |

Meta-analysis. Dark P., *et al.* Intensive Care Med (2015), 41:21–33



## PROS

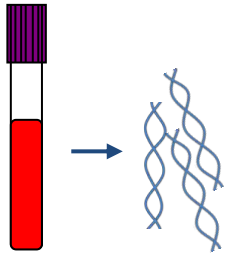
- High number of papers published.
- Mostly automated.
- Easy to interpret the results.

## CONS

- High variability among studies.
- Need of a technician trained in molecular methods (demanding PCR set up).
- Clinical usefulness limited to the 25 microorganisms included.
- Only detects one resistance gene, in a separate assay.

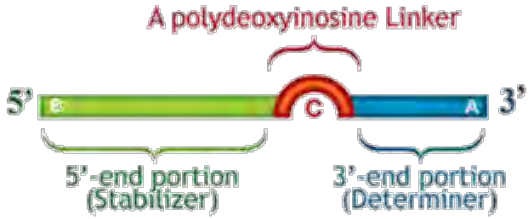
# Magicplex (Seegene)

## Technology

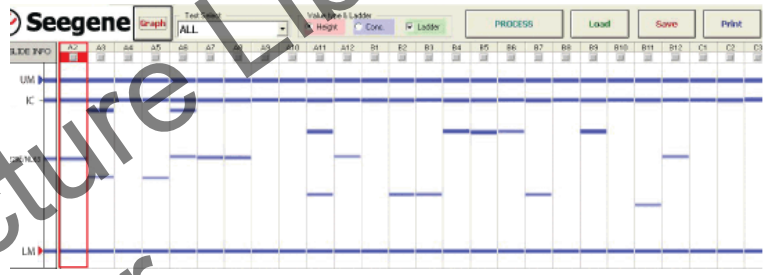


Automated DNA extraction from whole blood

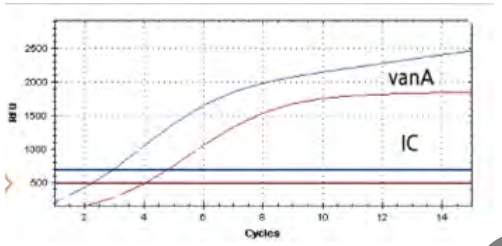
Generic DPO Primers (group of bacteria): highly specific




Screening PCR: 90 m.o detected
Gram+, Gram-, fungi
3 resistance markers




Automated electrophoresis: detection of the positive specimens for further analysis




Identification of the pathogens by melting curve analysis (READ system)

Amplicon bank generated in step 1

Specific Probes

Pathogens identified by specific probes



## Workflow

DNA extraction (automated) 30'-1h → PCR screening (conventional) 2h30' → Electrophoresis ~30' → PCR identification (real time) 30' → Results interpretation (automated) → TOTAL 4-5h

# Magicplex (Seegene)

## Assay characteristics

| | |
|---|---|
| Technology | 2 multiplex PCR (1 conventional, 1 real time) |
| N° of pathogens in the panel | 27 identified (90 initial screening) |
| Resistance markers | YES (mecA, vanA, vanB) |
| Volume | 1 ml |
| Detection limit (CFU/mL) | - |
| Microbial DNA enrichment | YES (prior PCR step) |

## Clinical performance

| | | |
|---|---|---|
| Emergency Room | Sensitivity | 11-65% |
| | Specificity | 77-92% |



## PROS

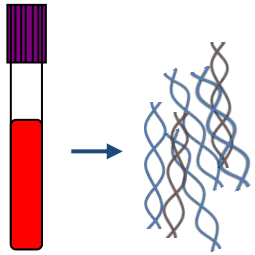
- Easy to perform.
- Mostly automated.
- Easy to interpret the results.
- A positive result in the screening PCR would indicate bacteremia/fungemia.

## CONS

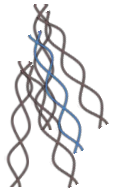
- If the laboratory has separation of areas (PCR and POST-PCR) it would be necessary 2 trained technicians.
- Moderate sensitivity.
- Identification at species level of only 27 among the 90 detected.

# Vyoo (AnalitikJena)

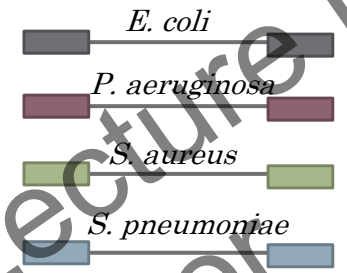
## Technology



Automated DNA extraction from whole blood

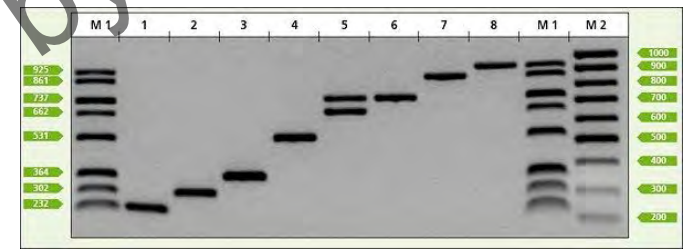


Chromatography column for enrichment of microbial DNA (LOOXTER)




Multiplex PCR using specific primers

Microarray: identification of the pathogens




Electrophoresis: identification of the pathogens



## Workflow

DNA extraction (automated) → Bacterial DNA enrichment → PCR → Electrophoresis/Array hybridization → TOTAL 7 h

# Vyoo (AnalitikJena)

## Assay characteristics

| | |
|---|---|
| Technology | Multiplex PCR + microarray or electrophoresis |
| N° of pathogens in the panel | 34 |
| Resistance markers | YES ( mecA, vanA, vanB, $bla_{SHV}$, $bla_{CTX-M}$ ) |
| Volume | 5 mL |
| Detection limit (CFU/mL) | 5-100 |
| Microbial DNA enrichment | YES (prior PCR step) |

## Clinical performance

| | | |
|---|---|---|
| Intensive Care Unit | Sensitivity | 38–60% |
| | Specificity | 72-75% |



## PROS

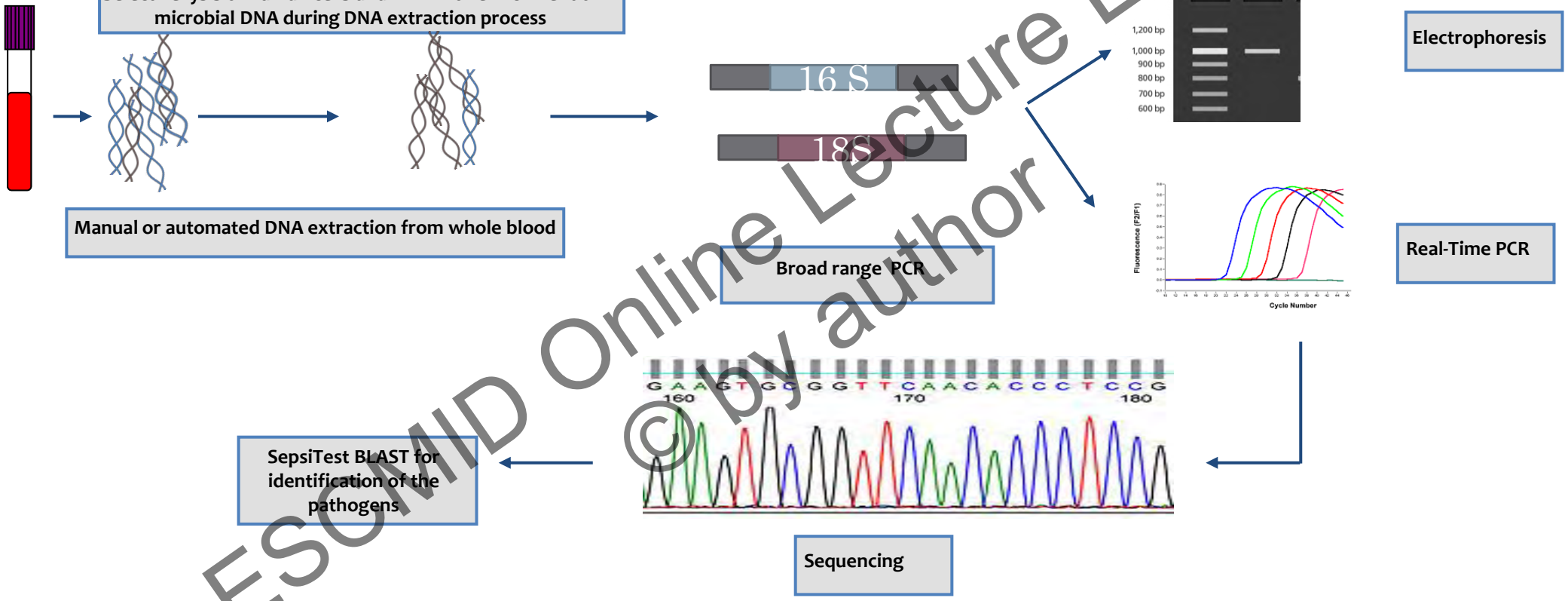
- Easy to perform.
- Mostly automated.
- Adaptable to different thermocyclers.
- Easy to interpret the results.
- Detects up to 5 resistance genes.

## CONS

- If the laboratory has separation of areas (PCR and POST-PCR) it would be necessary 2 trained technicians.
- Moderate sensitivity.
- Clinical utility limited to the 34 microorganisms included.

# SepsiTest (Molzym)

## Technology






## Workflow

DNA extraction with bacterial DNA enrichment (automated) → PCR (detects presence of bacteria/fungi) → Sequencing (identification of the pathogen) → TOTAL 8-12 h

# SepsiTest (Molzym)

## Assay characteristics

| | |
|---|---|
| Technology | Broad-range PCR + sequencing |
| Nº of pathogens in the panel | > 300 |
| Resistance markers | NO |
| Volume | 2 mL |
| Detection limit (CFU/mL) | 20-460 |
| Microbial DNA enrichment | YES (prior PCR step) |

## Clinical performance

| | | |
|---|---|---|
| Intensive Care Unit | Sensitivity | 37–87% |
| | Specificity | 85.5–100% |



## PROS

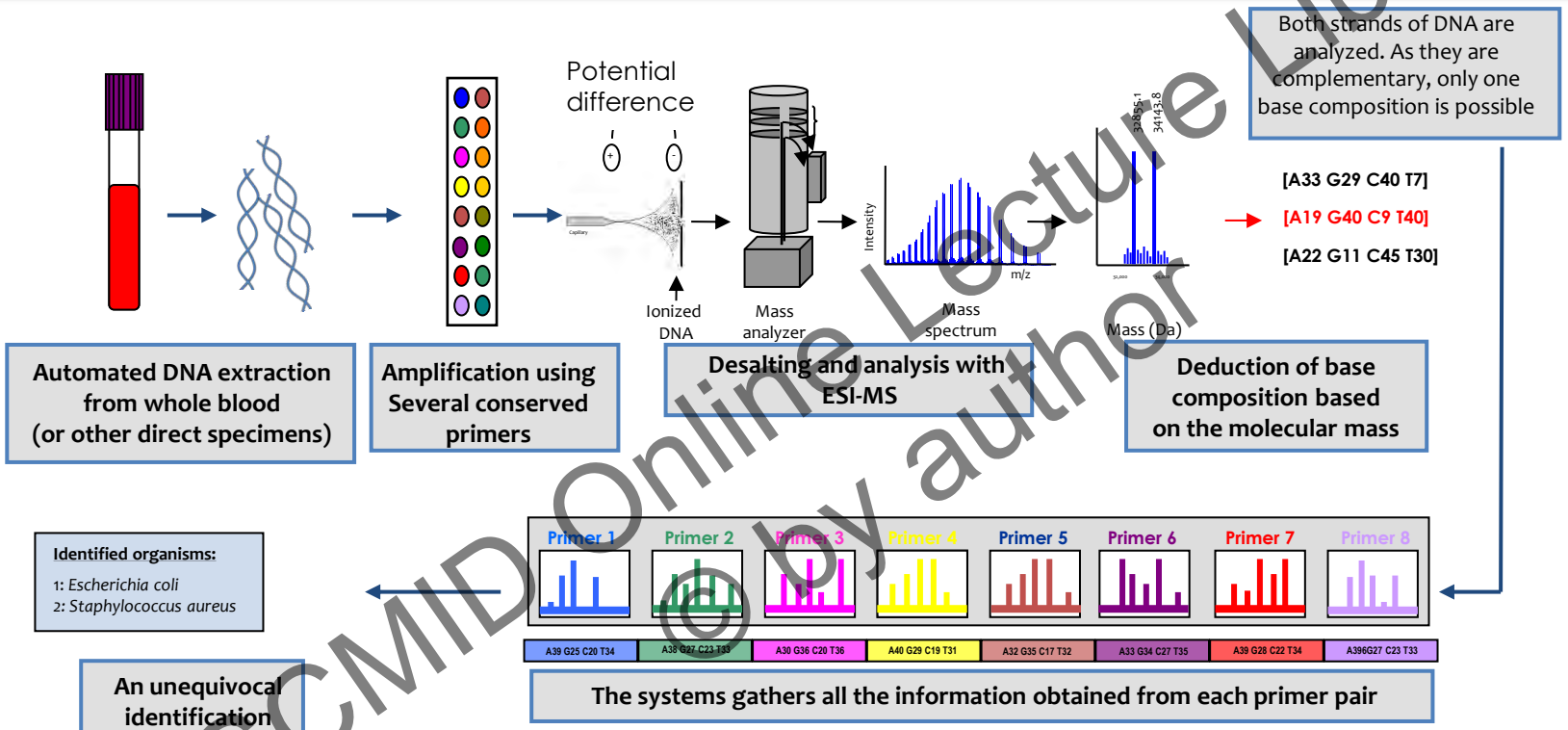
- Easy to perform.
- Mostly automated.
- Adaptable to different thermocyclers.
- Detects a broad range of pathogens.

## CONS

- The need of sequencing in order to obtain the identification delays its diagnostic utility.
- It doesn't detect any resistance gene.
- Lower capability for detecting polymicrobial infections (need of specific software to separate electropherograms (RipSeq)).

# IRIDICA (Ibis Biosciences-Abbott Molecular)

## Technology






## Workflow

DNA extraction (automated) 2h10’ → PCR 2h 15’ → Desalt 40’ → ESI-MS 10’ / specimen → TOTAL 6 h

# IRIDICA (Ibis Biosciences-Abbott Molecular)

## Assay characteristics

| | |
|---|---|
| Technology | Broad-range PCR + ESI-MS |
| N° of pathogens in the panel | >700 |
| Resistance markers | YES (mecA, vanA, vanB, KPC) |
| Volume | 5 mL |
| Detection limit (CFU/mL) | 4-16 |
| Microbial DNA enrichment | YES (after PCR step) |

## Clinical performance

| | Sensitivity | Specificity |
|---|---|---|
| Intensive Care Unit | 81-83.3% | 69-78.6% |
| Emergency Room | 72.6-83% | 92-94% |
| Onco-hematology | 45-60.3% | 79.8-93% |

## PROS

- Easy to perform.
- Automated.
- Detects a broad range of pathogens.

## CONS

- Need of an important initial investment.
- Separation of areas is recommended. Need of 2 trained technicians.

# T2 Candida panel (T2Biosystems)

## Technology

Blood cell lysis
Candida concentration

Lysis *Candida* cells

PCR lysate

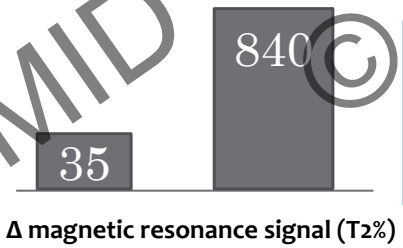
Candia spp.

PCR : amplification of broad-*Candida* genes.

Nanoparticles with specific probes for each specie. They bind at 5' and 3' of the amplicon.

Amplified *Candida* spp. gene

If the pathogen is in the specimen, the probes bind to the DNA clustering. This event alter the magnetic resonance signal of the specimen.




The T2 Dx Intrument reports the pathogen present in the blood.



## Workflow

Insert whole blood tube into the cartridge.

Result

TOTAL 3-5 h

# T2 Candida panel (T2Biosystems)

## Assay characteristics

| | |
|---|---|
| Technology | PCR + T2 Magnetic Resonance (T2MR). |
| Nº of pathogens in the panel | 5 |
| Resistance markers | NO |
| Volume | 3 mL |
| Detection limit (CFU/mL) | 1-3 |
| Microbial DNA enrichment | NO |

## Analytical performance

| | |
|---|---|
| Sensitivity | 91.1-100% |
| Specificity | 98-99% |

Bacterial panel (including 6 bacteria) under development.

## PROS

- Easy to perform.
- Completely automated.
- No need of DNA extraction.
- Point of care device.

## CONS

- Limited range of pathogens.

# Blood culture as gold standard

- Detection of living organisms
- Low sensitivity:
  - slow-growing microorganisms
  - intracellular pathogens
  - fastidious microorganisms
  - **previous ATB therapy**

"False positives" by molecular methods when comparing only with blood culture

**Enhanced gold standard (clinical infection criterion):**

Blood culture result + culture results from other anatomic sites + review of the clinical records of the patient

Better performance of molecular methods

| IRIDICA | | BC | Clin. Inf. crit |
|---|---|---|---|
| Intensive Care Unit | Sensitivity | 81-83.3% | 90.5-95% |
| | Specificity | 69-78.6% | 78-87.2% |
| Emergency Room | Sensitivity | 72.6-83% | 73.6-91% |
| | Specificity | 92-94% | 94-99% |
| Onco-hematology | Sensitivity | 45-60.3% | 74.7% |
| | Specificity | 79.8-93% | 92.8% |

# The ideal molecular method for the diagnosis of BSI

**1. Detect a broad range of pathogens**

IRIDICA > SepsiTest > Vyoo > Magicplex > SeptiFAST > T2 Candida

**2. Have high sensitivity and specificity**

T2 Candida>IRIDICA> SeptiFAST> SepsiTEST>MagicPLEX> Vyoo

**3. Have a low turnaround time**

T2 Candida > SeptiFAST> Magicplex> IRIDICA>Vyoo> SepsiTest

**4. Detect resistance genes**

Vyoo> IRIDICA>Magicplex> SeptiFAST

- SeptiFAST→ separate assay for mecA
- IRIDICA→ Gram-Negative Beta-Lactamase (GNBL) Assay (under development)

# The ideal molecular method for the diagnosis of BSI

**5. Allow the analysis of single specimens at any time**

T2 Candida

Although all methods provide with individual reagents cartridges, specimens are usually organized in batches:

- Long processes (> 2h of some steps) don't allow the analysis of the specimens at any time.

**6. Have low hands-on time**

T2 Candida > IRIDICA > SeptiFAST> SeptiTest= MagicPlex=Vyoo

**7. Be easy to perform / automatized**

T2 Candida > IRIDICA > SeptiFAST> SeptiTest= MagicPlex=Vyoo

# The ideal molecular method for the diagnosis of BSI

**8. Be easy to interpret the results**

IRIDICA = SeptiFAST=MagicPlex=Vyoo=SeptiTest= T2 Candida

**9. Be cost-effective**

Lack of studies for some of the methods.

IRIDICA> SepsiTest> SeptiFAST

(Stevenson et al, Health Technologu Assesment, 2016, Vol. 20, no. 46)

# Molecular methods: pipe dream, reality or future?

## Limitations of the current molecular methods

- Long processes undermine the capability to test single specimens→ Longer turnaround time if analysis in batches.
- Insufficient information regarding the antibiotic susceptibility.
- False negatives don't rule out an infection.
- Polymicrobial infections may be underestimated.
- Insufficient information regarding the real clinical impact of the molecular methods.

# Molecular methods: future

- NGS would give information of both ID and resistance genes. But platforms have to be adapted to be implemented in the lab routine and powerful databases must be constructed first.

- The new technologies to be developed should be able to avoid the DNA extraction and/or perform all the steps in a single cartridge.

- Nanotechnology and biosensors: emergent research fields.

- Devices that would be commercially available soon:
  - DNAe: full automated NGS and genomic analysis in 2-3h, direct from blood.
  - GeneWEAVE: DNA-delivery bio-particles that causes the bacteria to express luciferase. Turnaround of 4h.

# Take home messages

- Among the currently available molecular methods, IRIDICA would be the most complete.
- T2 Biosystems is a promising technology, although it currently detects a narrow spectra of microorganisms.
- The gold standard for evaluating molecular methods should include other positive cultures and reviewing the clinical records of the patient rather than comparing only with the blood culture.
- More systematic studies are needed in order to asses the real clinical impact of those technologies.
- In the present, molecular methods are a useful tool complementary to the blood culture.
- The new methods to be developed should be able to perform all the steps in a single cartridge (PoC), be highly sensitive/specific and have a shorter turnaround time (ideally 1-3h).

# Acknowledgments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Intensive Care Unit

Germans Trias i Pujol Hospital

Abbott Molecular

IBIS Biosciences-Abbott Molecular

# Comparison of the molecular methods commercially available

| | SeptiFast (Roche) | Magicplex Sepsis (Seegene) | Vyoo (Analitik jena) | SepsiTest (Molzym) | IRIDICA (Ibis Biosciences-Abbott Molecular) | T2 Candida (T2 Biosystems) |
|---|---|---|---|---|---|---|
| Technology | Real-time PCR | 2 multiplex PCR (1 conventional, 1 real time) | Multiplex PCR + microarray | Broad-range PCR + sequencing | Broad-range PCR + ESI-MS | PCR + T2 Magnetic Resonance (T2MR). |
| N° of pathogens in the panel | 25 | 27 identified (90 initial screening) | 34 | > 300 | >700 | 5 |
| Resistance markers | YES (mecA, separate assay) | YES (mecA, vanA, vanB) | YES ( mecA, vanA, vanB, $bla_{SHV}$, $bla_{CTX-M}$ ) | NO | YES (mecA, vanA, vanB, KPC) | NO |
| Volume | 1.5 mL | 1 ml | 5 mL | 2 mL | 5 mL | 3 mL |
| Detection limit (CFU/mL) | 3-30 | - | 5-100 | 20-460 | 4-16 | 1-3 |
| Microbial DNA enrichment | NO | YES (prior PCR step) | YES (prior PCR step) | YES (prior PCR step) | YES (after PCR step) | NO |
| Turnaround time | 4.5-6 h | 4 h | 8 h | 8-12 h | 6h | 3-5h |
| Population studied | ER, ICU, Hemato-oncology, others | ER, ICU, Hemato-oncology | ICU | ICU | ER, ICU, Hemato-oncology | ER, ICU |
| Sensitivity | 68-69% | 11-65% | 38–60% | 37–87% | 45-83.3% | 91.1-100% |
| Specificity | 83-93% | 77-92% | 72-75% | 85.5–100% | 69-94% | 98-99% |

# Microorganisms included in the different molecular methods

| | Grampositives | | Gramnegatives | | Fungi | Resistance markers |
|---|---|---|---|---|---|---|
| SeptiFAST | *Staphylococcus aureus* | *Streptococcus pyogenes* | *Escherichia coli* | *Proteus mirabilis* | *Aspergillus fumigatus* | mecA (different assay) |
| | *Staphylococcus epidermidis/ haemolyticus* | *Streptococcus agalactiae* | *Klebsiella pneumoniae/oxytoca* | *Pseudomonas aeruginosa* | *Candida albicans* | |
| | *Streptococcus pneumoniae* | *Enterococcus faecalis* | *Serratia marcescens* | *Acinetobacter baumannii* | *Candida tropicalis* | |
| | *Streptococcus viridans* | *Enterococcus faecium* | *Enterobacter cloacae/aerogenes* | *Stenotrophomonas maltophilia* | *Candida parapsilosis* | |
| | | | | | *Candida krusei* | |
| | | | | | *Candida glabrata* | |
| Vyoo | *Clostridium perfingens* | *Streptococcus agalactiae* | *Acinetobacter baumannii* | *Morganella morganii* | *Aspergillus fumigatus* | mecA |
| | *Enterococcus faecalis* | *Streptococcus bovis* | *Bacteroides fragilis* | *Neisseria meningitidis* | *Candida albicans* | vanA |
| | *Enterococcus faecium* | *Streptococcus dysagalactiae* | *Burkholderia cepacia* | *Proteus mirabilis* | *Candida dubliniensis* | vanB |
| | *Staphylococcus aureus* | *Streptococcus mutans* | *Enterobacter aerogenes* | *Pseudomonas aeruginosa* | *Candida glabrata* | $bla_{CTx\text{-}M}$* |
| | *Staphylococcus epidermidis* | *Streptococcus pneumoniae* | *Enterobacter cloacae* | *Serratia marcescens* | *Candida krusei* | $bla_{SHV}$* |
| | *Staphylococcus haemolyticus* | *Streptococcus pyogenes* | *Escherichia coli* | *Stenotrophomonas maltophilia* | *Candida parapsilosis* | |
| | *Staphylococcus hominis* | *Streptococcus sanguinis* | *Haemophilus influenzae/(CTb)* | *Prevotella buccae* | *Candida tropicalis* | * Several variants detected |
| | *Staphylococcus saprophyticus* | | *Klebsiella oxytoca* | *Prevotella intermedia* | | |
| | | | *Klebsiella pneumoniae* | *Prevotella imelaninogenica* | | |
| Magicplex | *Streptococcus agalactiae* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Klebsiella oxytoca* | *Aspergillus fumigatus* | mecA |
| | *Streptococcus pyogenes* | *Staphylococcus epidermidis* | *Acinetobacter baumannii* | *Klebsiella pneumoniae* | *Candida albicans* | vanA |
| | *Streptococcus pneumoniae* | *Staphylococcus haemolyticus* | *Stenotrophomonas maltophilia* | *Proteus mirabilis* | *Candida tropicalis* | vanB |
| | *Enterococcus faecalis* | | *Serratia marcescens* | *Escherichia coli* | *Candida parapsilosis* | |
| | *Enterococcus faecium* | | *Bacteroides fragilis* | *Enterobacter cloacae* | *Candida krusei* | |
| | *Enterococcus gallinarum* | | *Salmonella typhi* | *Enterobacter aerogenes* | *Candida glabrata* | |
| T2 Biosystems | *Staphylococcus aureus* * | | *Pseudomonas aeruginosa* * | | *Candida albicans* | |
| | *Enterococcus faecium** | | *Acinetobacter baumannii* * | | *Candida tropicalis* | |
| | | | *Klebsiella pneumoniae* * | | *Candida parapsilosis* | |
| | | | *Escherichia coli* * | | *Candida krusei* | |
| | | | | | *Candida glabrata* | |

** Under development*

# References


**SeptiFAST**

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3. Chang, S. S. *et al.* Multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis - a systemic review and meta-analysis. *PLoS One* **8,** e62323 (2013).

**Magicplex**

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**Vyoo**

1. Fitting, C. *et al.* DNAemia detection by multiplex PCR and biomarkers for infection in systemic inflammatory response syndrome patients. *PLoS One* **7,** e38916 (2012).
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**SepsiTEST**

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