NOI ORIZONTURI IN DIAGNOSTICUL SEPSISULUI- TEHNICI DE BIOLOGIE MOLECULARA

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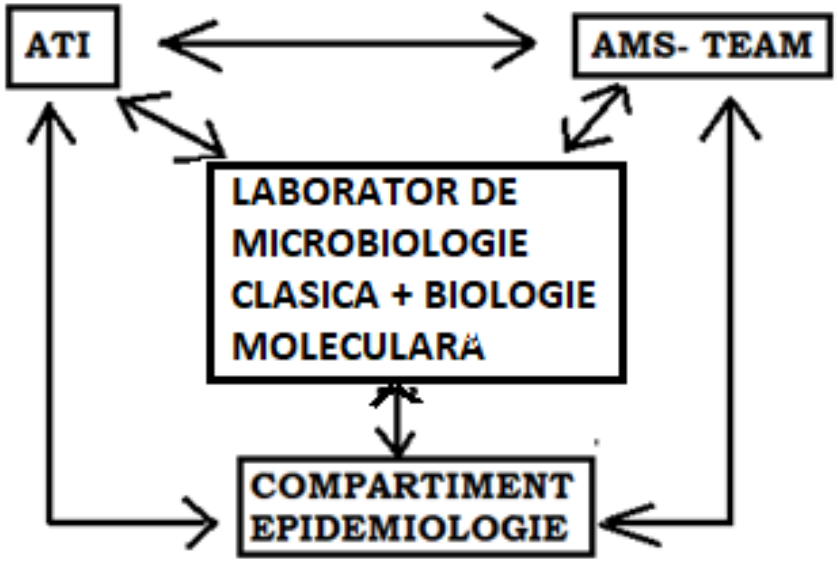
DIAGNOSTIC STEWARDSHIP – EXPERIENTA SJUSV



TEAM - TOGETHER EVERYONE ACHIEVES MORE

POSTULATELE KOCH MOLECULARE

- Fenotipul investigat trebuie asociat cu membrii patogeni ai unui gen sau cu o tulpină patogenă a unei anumite specii
- Inactivarea specifică a genelor asociate cu trăsătura suspectată de virulență ar trebui să conducă la pierderea măsurabilă a patogenității sau virulenței.

Pierderea genei- pierderea functiei

- Inversarea alelică sau înlocuirea genei mutante ar trebui să conducă la

restabilirea patogenității.

Antibioticele au obosit, bacteriile insa nu

- Antibioticele – baza a tot ce a urmat: chirurgia complexa, transplantul si terapia imunosupresiva nu ar fi fost posibile daca nu exista controlul infectiei
- In comunitate, pneumonia pneumococica inca omoara pacientul receptiv
- Pana in 2020 acest edificiu s-a dezvoltat fara provocari virale majore.
    - Pandemia de gripa din 1958-1959 si 1968-1969 s-a terminat prin vaccinare
    - Infectia HIV a castigat teren, dar este preventibila prin precautii personale
    - Pandemia SARS CoV 2 a schimbat dinamica cel putin temporar, daca nu chiar permanent

Rezistenta mediata enzimatic- beta lactamazele

Beta lactamazele sunt enzime stravechi de milioane de ani. In prezent exista aproximativ 2800 de proteine. Chiar daca penicilinazele in bacteriile Gram pozitiv au fost raportate imediat dupa descoperirea penicilinei , beta lactamazele care pot hidroliza cefalosporinele, monobactamele si carbapenemele au devenit extrem de importante la patogenii Gram negativ.

Clasificarea se bazeaza pe profilul de substrat si de inhibitor.In prezent nomenclatura imbina clasificarea biochimica cu cea moleculara – 17 grupe functionale

Hot spot: ESβLA ,serin si metalo β-lactamazele care sunt partial inhibate de noii compusi.

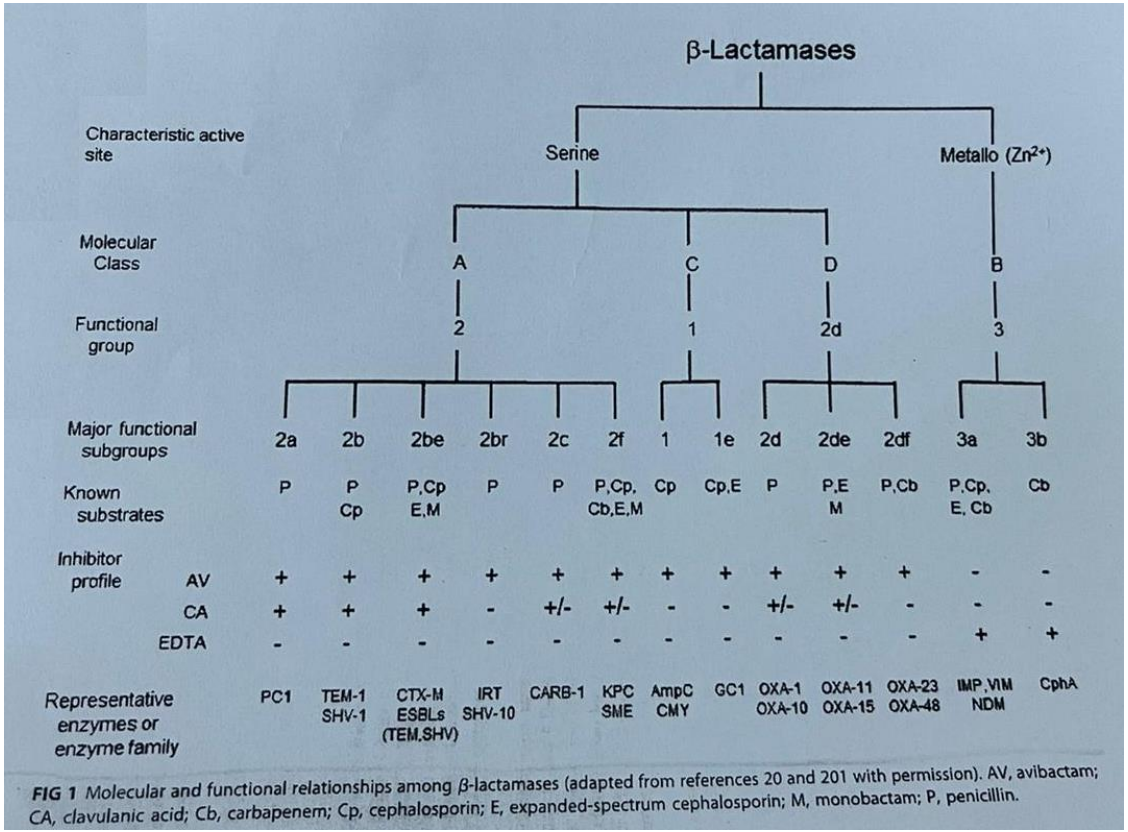


FIG 1 *Molecular and functional relationships among β-lactamases (adapted from references 20 and 201 with permission). AV, avibactam; CA, clavulanic acid; Cb, carbapenem; Cp, cephalosporin; E, expanded-spectrum cephalosporin; M, monobactam; P, penicillin.*

MICROBIOLOGIE CLASICA VERSUS TEHNICI DE BIOLOGIE MOLECULARA

	MICROBIOLOGIE CLASICA	SYNDROMIC MULTIPLEX - PCR
timpul scurs pana la obtinerea rezultatului	cel putin 72 h	cateva ore din momentul recoltarii (2-3 ore)
panel	bacterian, fungic, antibiograma, antifungigrama	viral, bacterian, fungic (panel limitat) + panel de rezistenta
pacienti pretratati cu antibiotic	rezultate fals negative	cuantificarea rezultatului mPCR
contaminarea probelor	importanta culturilor cantitative (10^5 UFC)	flora colonizatoare (disbioza bucala) poate sa contina unele gene de rezistenta; importanta modului de recoltare a esantionului

Detectarea carbapenemazelor la *Enterobacterales*

- Testarea difuzimetrica combinate - meropenem asociat cu diferiti inhibitori- dezavantaj: timpul : > 48 ore
- Test biochimic (Colorimetric) -CarbaNP- determina schimbarea pH-ului si a culorii de la rosu la galben in prezenta rosu fenol
- Inactivarea carbapenemelor - hidroliza enzimatica dupa incubarea carbapenemei cu suspensia bacteriana

MALDI-TOF

- Matrix assisted laser desorbtion/ionization time-of - flight mass spectrometry
- Metoda are specificitate si sensibilitate buna
- Dezavantaj: optimizarea si standardizarea trebuie realizata pentru fiecare combinatie antibiotic- enzima

- In ultimii ani au proliferat patogenii oportunisti Gram negativ multirezistenti
- Antibioticele active sunt greu de gasit deoarece:
  - Trebuie sa eludeze mecanismul eflux
  - Rigorule legislative s-au complicat si necesita costurile si complexitatea trialurilor clinic

SOLUTIA:

- Controlul infectiei
- Antibiotic stewardship

Limite: diagnostic tardive, cel putin 2 zile: 1 zi cultivarea, 1 zi testarea rezistentei. Spectrometria de masa a rezolvat partial problema- identificare rapida, dar nu si testarea sensibilitatii

Cum arata viitorul?

- Tendintele previzibile sunt instabile si se pot transforma intr-un eveniment “Black Swan”. In contextul rezistentei la antibiotice, acest eveniment inseamna ceva care nu poate fi anticipat sau prezis.
- Juvenal- rara avis- black swan inseamana imposibilitate
- Rumsfeld- necunoscutul necunoscutului: planificarea pe termen lung este supusa vulnerabilitatii

DIAGNOSTIC MOLECULAR –IVD-T_2 DX IN INFECTII SISTEMICE

- Panel bacterian
  - ➢ permite detectia simultana dintr-o proba de sange (fara necesitatea cultivarii) a urmatoarelor bacterii:

    Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Enterococcus faecium
  - ➢ acest panel bacterian se suprapune foarte bine pe profilul bacterian identificat in hemoculturi in sectia de ATI, la nivelul SJUSV
- Panel *Candida*
  - ➢ permite detectia simultana dintr-o proba de sange (fara necesitatea cultivarii) a urmatoarelor specii de *Candida*:

    C. albicans, C. tropicalis, C. parapsilosis, C. krusei, C. glabrata
- Panel rezistenta
  - ➢ permite detectia simultana dintr-o proba de sange a urmatorilor markeri de rezistenta: KPC, OXA-48, NDM, IMP, CTX-M 14/15, AmpC (CMY/DHA), VanA/B, mecA/C

DIAGNOSTICUL DE LABORATOR

	HEMOCULTURA	IVD – T_2 DX
nivelul bacteremiei	bacteremia redusa (<1 UFC/ml) intermitenta influenteaza rata de pozitivitate	limita de detective < 10 UFC/ml sensibilitate mare: detecteaza pana la 1 UFC/ml
timpul scurs pana la obtinerea rezultatului	cel putin 72 de ore	3-5 ore de la inceputul procesarii probei
tratament anterior cu antibiotice	tratamentul anterior cu antibiotic determina o rata crescuta de rezultate negative	nu interfera cu inceperea tratamentului c u antibiotic
recoltare proba identificare	protocol – hemocultura identificare prin metode standardizate	detectie direct din proba de sange (fara necesitatea cultivarii)
panel	germeni aerobi, anaerobi, fungi	panel bacterian: 6 specii panel fungic: candida spp panel rezistenta: 9 markeri
riscul contaminarii probei	nerespectarea protocolului de recoltare a hemoculturii → rezultate fals pozitive	contaminarea probei SCN-MR poate influenta rezultatul fenotipic pentru SA
fezabilitate	metoda standard, eficienta dovedita	necesita studii viitoare pentru a evalua raportul cost- beneficii
cost	redus	ridicat

DESCRIERE TEHNICA

- permite detectia simultana din probe de sange total (fara necesitatea cultivarii) a minim urmatoarelor bacterii: *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

	Sensibilitate	Specificitate
A. baumannii	97.5%	99.2%
E. coli	90.9%	95.8%
E. faecium	100%	99.5%
K.pneumoniae	100%	98.6%
P. aeruginosa	97.7%	97.7%
S. aureus	89.1%	98.4%
Medie	95.9%	98.2%

- limita de detectie < 10 UFC/ml sange
- sistem automat, permite obtinerea rezultatului in 3- 5 ore de la inceperea procesarii probei
- permite testarea a cate unei probe, fara consum suplimentar de teste (de ex. pentru controale)
- Kitul contine toti reactivii necesari (control intern, reactivi pentru extractie de acizi nucleici, amplificare si detectie produsi de amplificare)
- Reactivii sunt ambalati astfel incat sa se poata utiliza pentru cate o proba, fara piederea sau compromiterea restului reactivilor
- Test validat pentru diagnostic in vitro (IVD)

- permite detectia simultana din probe de sange total (fara necesitatea cultivarii) a speciilor de *Candida: C. albicans, C. tropicalis, C. parapsilosis, C. krusei, C. glabrata*
- sensibilitate si specificitate minim 95%
- limita de detectie 1-3 UFC/ml sange
- sistem automat, permite obtinerea rezultatului in 3-5 ore de la inceperea procesarii probei
- permite testarea a cate unei probe, fara consum suplimentar de teste (de ex. pentru controale)
- Kitul contine toti reactivii necesari (control intern, reactivi pentru extractie de acizi nucleici, amplificare si d etectie produsi de amplificare)
- Reactivii sunt ambalati astfel incat sa se poata utiliza pentru cate o proba, fara piederea sau compromiterea restului reactivilor
- Test validat pentru diagnostic in vitro (IVD)

- permite detectia simultana din probe de sange total (fara necesitatea cultivarii) a minim urmatorilor marke ri de rezistenta la antibiotice asociati cu patogeni ce pot cauza bacteriemie si sepsis: KPC, OXA-48,NDM, VIM, IMP, CTX-M 14/15, AmpC (CMY/DHA), vanA/B, mecA/C
- sensibilitate si specificitate 100%
- limita de detectie 3-11 CFU/mL
- detectie in sistem automat, permite obtinerea rezultatului in 3-5 ore de la inceperea procesarii probei
- permite testarea a cate unei probe, fara consum suplimentar de teste (de ex. pentru controale)
- Kitul contine toti reactivii necesari (control intern, reactivi pentru extractie de acizi nucleici, amplificare si detectie produsi de amplificare)
- Reactivii sunt ambalati astfel incat sa se poata utiliza pentru cate o proba, fara piederea sau compromiterea restului reactivilor
- Test validat pentru diagnostic in vitro (IVD)

ANALIZORUL:

- sistem complet automat de diagnostic molecular, IVD - T2DX, capabil sa ruleze in mod random diverse paneluri multiplex, direct din sange integral si alte lichide biologice.
- sistemul utilizeaza tehnologia Rezonantei Magnetice, care masoara modul in care moleculele de apa interactioneaza in prezenta campului magnetic si

  realizeaza detectia non-optica eliminand astfel necesitatea purificarii probei si a extractiei acizilor nucleici.
- detecteaza diverse tinte intr-o varietate de tipuri de proba nepurificate
- lucreaza direct din sange total
- are sensibilitate foarte mare, detecteaza pana la 1 CFU/mL
- nu interfera cu inceperea tratamentului anti-microbian
- utilizeaza particule superparamagnetice captusite cu molecule de legare specifice tintelor
- metoda utilizata nu presupune purificarea probei, proces in care in mod normal se pierde pana la 90% din cantitatea de tinta.

- Capabil sa detecteze urmatoarele tipuri de paneluri:
  - *Candida* – detecteaza direct din sange integral: *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida glabrata.*
  - Bacteria – detecteaza direct din sange integral: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichia coli*.
  - Resistance Panel – detecteaza urmatorii makeri de rezistenta: KPC, OXA-48, NDM/VIM/IMP, CTX-M 15/14, AmpC (CMY/DHA), vanA/B, mec A/C.

- 6 module de lucru independente si un modul cu functie STAT
- Panel de comanda touchscreen
- Detectie direct din sange integral
- Cititor barcode pentru probe si reactivi
- Conectare la LIS
- Acces random
- Timp de eliberare a rezultatelor 3-5 ore
- Sistem benchtop
- Alimentare 220-240V/ 50/60 Hz

T2Resistance® Panel Critical Workflow Steps

T2Biosystems®

GLOVE CHANGING

Put on fresh gloves prior to:

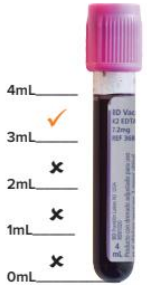
- Work area preparation
- Obtaining samples and opening outer packaging for all Panel components
- Removing Panel components from packaging and assembling Panel for loading
- Unloading completed Panel
- Work area preparation after unload

SAMPLE COLLECTION

Tube Type: 4 mL K_2EDTA or K_3EDTA purple top Vacutainer® tubes (BD #367861, 367862 or equivalent)

Volume: At least 3 mL are required

Sample Preparation: Ensure that the sample is at room temperature and inverted 8-10 times prior to use

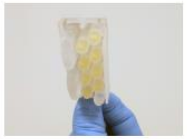


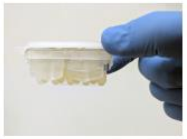
REAGENT TRAY MIXING

Vortex: Securely hold the Reagent Tray upright and push down onto the center of the vortex head cover and vortex at 2400-3200 RPM for 5 seconds until contents are homogeneous

Inspect: Visually inspect contents to ensure all solutions are homogeneous.

Flick: Flick to displace any bubbles – trapped air can negatively impact test results.





PANEL SEATING

When loading the fully assembled Panel onto the T2Dx® Instrument, ensure that it is level and in full contact with the metal rails and seated on the location pins. Location pins are not visible when the Panel is securely seated in the drawer.

PANEL LABEL AND REAGENT TRAY COVER REMOVAL

Follow the T2Dx touchscreen prompts for loading the fully assembled Panel.

Remove the Cartridge Label

Remove the Reagent Tray Cover

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SEPSIS IS A MEDICAL EMERGENCY REQUIRING IMMEDIATE DIAGNOSIS & TREATMENT

Pancreatic Stone Protein (PSP) on the abioSCOPE® is the Earliest Marker of Sepsis

Sepsis is a major healthcare burden claiming more than 11 million lives per year, one death every 2.8 seconds[1].

It is caused by a dysregulated host response to infection which can progress to multiple organ dysfunction, septic shock and death.

It's a medical emergency that requires immediate diagnosis. Unfortunately, current standards of care often lead to sepsis being diagnosed too late.

50m
sepsis cases every year[1]

Every hour antibiotic therapy is delayed, the chances of survival decrease by
8%[2]

The clinical signs and symptoms of sepsis are generic and non-specific, making it extremely challenging to timely identify.

The availability of an early and accurate biomarker at the patient's bedside is key to enabling faster treatment decisions, reduce mortality and lower sepsis-related healthcare costs.

EARLY SEPSIS DETECTION UP TO 72 HOURS BEFORE THE STANDARD OF CARE IN 5 MIN*

The PSP Test on the abioSCOPE® Can Save Millions of Lives

The **IVD CAPSULE PSP** on the **abioSCOPE®** is the first CE-marked *in vitro* diagnostic test to enable fast, reliable and early sepsis detection at the point-of-care from a single drop of blood in only 5 minutes*.

Get accurate results in
5min

A multicentric study published recently in *Critical Care*, proves that bedside measurement of PSP on the abioSCOPE® clearly correlates with the onset of sepsis, **enabling personalized clinical management of patients in the ICU**[3].

An increasing PSP concentration in the days preceding the clinical diagnosis of sepsis, offers a unique window of opportunity for clinicians to initiate timely the right treatment.

Reducing time to treatment by up to
72h
could dramatically improve patients' outcome

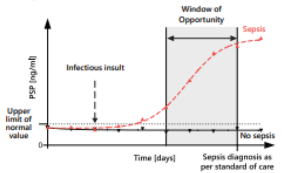

Figure 1. Schematized daily PSP biomarker readings in patients who develop nosocomial sepsis (dashed red line) or not (solid black line).

TRUE ENABLER OF EARLY SEPSIS DETECTION
SEE EARLIER - ACT FASTER

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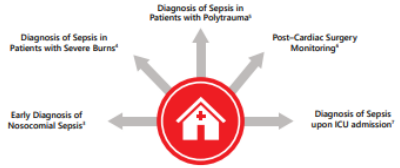
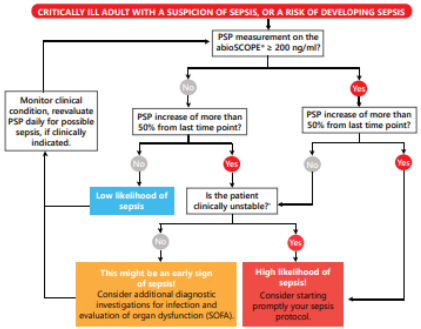
IVD CAPSULE PSP ON THE ABIOSCOPE DESIGNED FOR ON-DEMAND USE IN THE ICU

Scope of Use for the PSP Biomarker in the Diagnosis of Sepsis in Adults



Compact, robust, and intuitive to use, Abionic's PSP test on the **abioSCOPE®** is fully compatible with hospital information systems and fits seamlessly into the workflow. When sepsis is suspected, an immediate access to reliable test results is essential. Serial bedside PSP measurements every 24 hours can aid the clinical management and early identification of sepsis in patients at risk. A constantly low PSP value is also a strong indicator of a patient's stability, supporting the decision to not start or withhold unnecessary antibacterial treatment.

DECISION TREE FOR THE INTERPRETATION OF SERIAL PSP MEASUREMENTS



TRUE ENABLER OF EARLY SEPSIS DETECTION

SEE EARLIER - ACT FASTER

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CLINICAL EVIDENCE

Early Diagnosis of Sepsis in Hospitalized Patients

It is important for intensive care physicians to be able to differentiate between patients suffering from a systemic inflammatory response without infection, compared to those suffering from sepsis. This differential diagnosis is imperative to administer the appropriate treatment.

The multicentric study on critically ill patients published in *Critical Care*, showed that PSP was the only biomarker able to identify sepsis 72 hours before clinical diagnosis according to an external adjudication committee (Fig. 2)[1]. Providing a large window of opportunity to adapt the patient's clinical management.

PSP shows similar performances that have been previously reported in studies looking at a variety of critically ill patients, including those with severe burns[2], polytrauma[3], post-cardiac surgery[4] and on admission to the intensive care unit (ICU)[5].

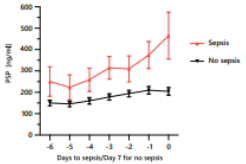


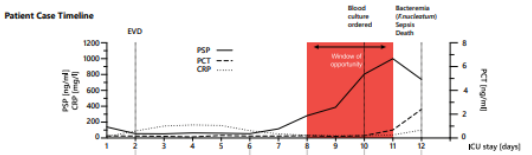
Figure 2. Average daily PSP concentration with standard error of the mean preceding the diagnosis of nosocomial sepsis (day 0, red line) or ICU discharge for patients who did not develop sepsis (black line).

In addition to individual studies, a large meta-analysis including more than 600 patients also confirms the high diagnostic performance of PSP for diagnosing infection in the ICU and emergency department (ED), with an accuracy of 81%[6].

Patient Case Report

Patient history
A 71-year-old male patient was hospitalized for a traumatic brain injury, which required immediate ICU admission with invasive mechanical ventilation.

Patient Case Timeline



Day 2
The patient underwent external ventricular drainage (EVD).

Day 6
Blood culture no pathogen identified.

Day 8
No blood culture was considered necessary, patient's SOFA score was stable.

Day 10
A pulmonary aspiration identified a *Corynebacterium propinquum* infection.

Day 12
A bacteremia caused by *Fusobacterium nucleatum* was found. The patient suffered a severe mesenteric infarction and died that day.

Biomarkers
The patient's PSP, CRP, and PCT levels were relatively low on admission. The CRP level was non-specifically elevated from day 2 onward, peaking on day 4. The PCT level was <0.2 ng/ml through day 10, then only increased later on day 11 and on day 12.

PSP remained stable and low until day 7, after which, it began to increase and eventually surpassed the 200 ng/ml cut-off. The continuous increase in the PSP concentration between days 7 and 10 was associated with the development of bacteremia, and anticipated the **diagnosis of sepsis by >72h.**

TRUE ENABLER OF EARLY SEPSIS DETECTION
SEE EARLIER - ACT FASTER

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UNIQUE NANOTECHNOLOGY-BASED PLATFORM

Abionic's Patented Nanofluidic Immunoassay Revolutionizes Point-of-Care Diagnostics

Abionic's technology enables quantitative results for up to 14 specific parameters in a single capsule.

Molecules are forced into a nanochannel, limiting their travel distance to a few hundred nanometers and reducing incubation time to 2 minutes[6].

A washing step is not needed as the surface-to-volume ratio is extremely high, and non-specific background is negligible[6].

PSP level can thus be efficiently quantified within an ultra short assay time, with high precision and accuracy on a closed, small, easy-to-operate platform, providing lab quality results at the point-of-care.

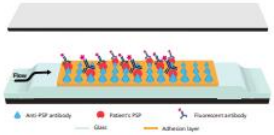

Figure 3. Cross-section through a nanofluidic biosensor

The abioSCOPE®: True Game Changer for the Future of Diagnostics

Rapid results
5-minute measuring time to get accurate actionable results

Easy to use
4 simple steps with 50 µl of blood from a fingerstick or venous blood

No maintenance
Contamination-free device, no washing step required

Laboratory quality results
Performances equivalent to those obtained in a laboratory

Connectivity options
Input: Barcode scanner, remote software upgrade
Output: HL7, ethernet to HIS/LIS, QR code

Complementary menu in development
Available tests: cSOFA test, a severity score for COVID-19 patients
Coming soon: CRP, D-Dimer

TRUE ENABLER OF EARLY SEPSIS DETECTION
SEE EARLIER - ACT FASTER

DIAGNOSTICUL DE LABORATOR

- Se vor recolta concomitent probe de sange atat pentru hemocultura cat si pentru biologie moleculara – T_2
- Se vor recolta probe pentru laboratorul de microbiologie si din alte situsuri

TEHNICA	ACELASI GERMENE	FARA IDENTIFICARE	GERMENE IDENTIFICAT DOAR PRINTR-O METODA		GERMENI DIFERITI
PCR – T2	DA	DA	DA	NU	DA
CLASIC	DA	DA	NU	DA	DA

- INTERPRETAREA REZULTATELOR
- CONTAMINANTII
- CUANTIFICAREA REZULTATELOR