eurobio SCIENTIFIC

cube dx hybCell technology

Let‘s talk Sepsis

Sepsis: Every Minute Matters –
Rapid Detection with compact sequencing

Christoph Reschreiter, Cube Dx

The Eurobio Scientific Group: Global Presence

Headquartered in the Paris region, Eurobio Scientific has established a significant international footprint. With affiliates in the United States and across Europe, our reach is truly global. We are proud to employ over 300 dedicated professionals worldwide, including 50 research and development scientists who are committed to pushing the boundaries of diagnostic innovation.

eurobio did

Based in Milan, Italy, DID distributes in vitro diagnostic products, primarily for microbiology laboratories.

GenBio

A California-based company focused on serology products for infectious and autoimmune diseases.

eurobio UK SCIENTIFIC

Based in Dorking, UK. Supplies a range of products covering Transplantation, Infectious disease, Blood science, Quality management and Life science in the UK and Ireland.

eurobio SCIENTIFIC

Based in Les Ulis, France. Specialises in infectious disease and life science products.

eurobio gendx

Based in Utrecht, Netherlands. Specialises in molecular diagnostics for transplantation, offering advanced HLA typing methods using Sanger and NGS platforms.

eurobio teco

Based in Switzerland and Germany. Focuses on in-vitro tests for medical and veterinary diagnostics, biosafety, and environmental assays.

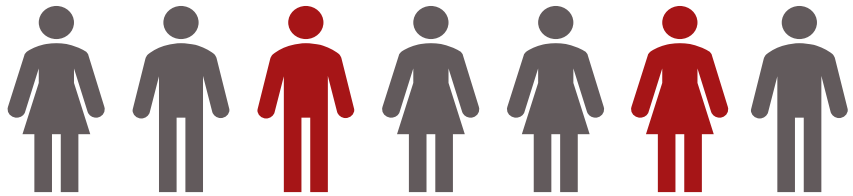
eurobio bmd

Operates in Belgium and the Netherlands, distributing in vitro diagnostic products, specialising in autoimmunity, microbiology, and biotherapy monitoring.

Sepsis and blood culturing

8,000,000 deaths worldwide

1 in every 3 seconds



Median 48 hours to microbial ID

Every hour of delay decreases survival

Hard to grow: fastidious bacteria, fungi, anaerobes..

Many septic episodes without diagnosing causing microorganism

The Cube Dx solution – direct blood test

3 hours from

EDTA blood to result

Broadest molecular panel:

Automated Workflow

95 (144) microorganisms and resistance genes

Especially beneficial in case of…

…fastidious bacteria

Haemophilus influenzae, Streptococcus pneumoniae, Bordetella pertussis, Legionella pneumophilia, Salmonella, Brucella..

…fungi

TTR for C. albicans and C. glabrata 85.8h +/- 30.9h and 154h +/- 43.8h

…anaerobic bacteria

Bacterioides fragilis, Fusobacterium, Clostridium perfringens..

…mixed infections

No bias based in favour of fast-growing microorganisms

…limited availability of sample (500µl or less)

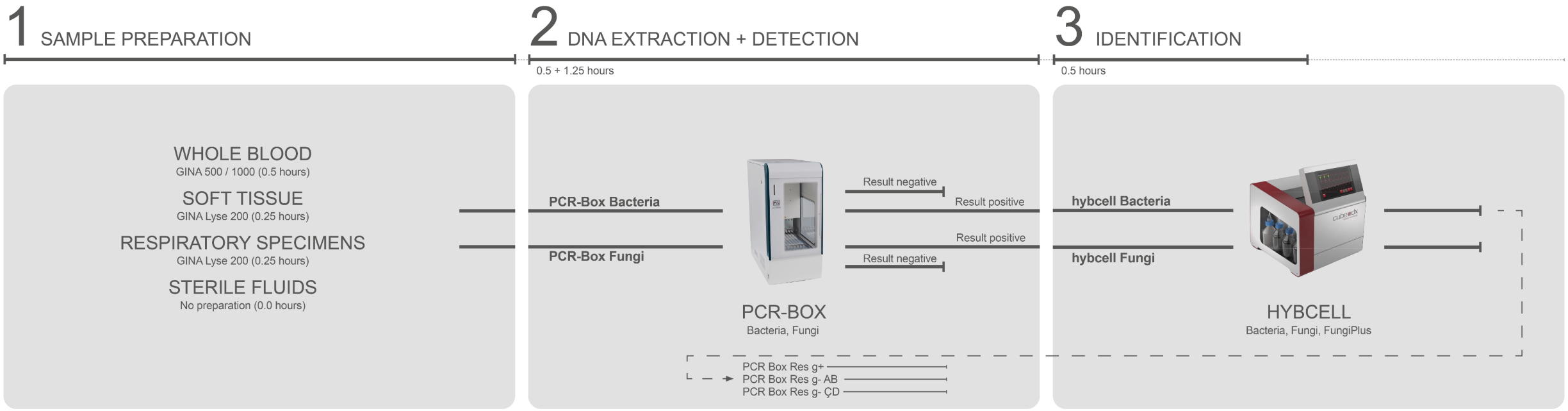
newborns/infants, elderly, severely ill patients…

Key facts about Cube Dx

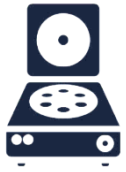
- Established in 2015, in Austria (St. Valentin)
- Focus on molecular dx in infectious disease
- Adressing clinical microbiologies
- ISO13485 certified
- Products distributed in Europe
- „Sepsis customers“ since 2020

Overview of Workflow

WORKFLOW AND PRINCIPLE



Whole blood:
GINA - Depletion of human DNA

1  **11.000g**
5 mins.

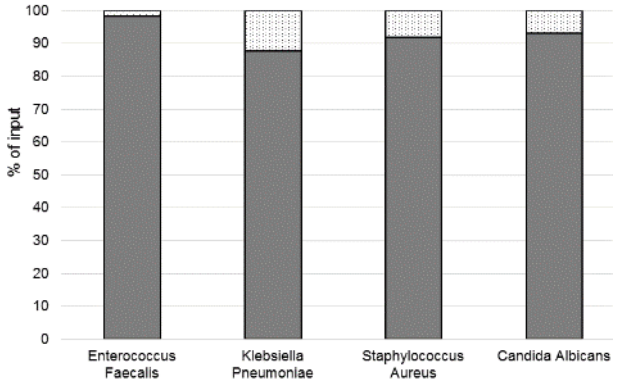
Lysis and separation of human cells

→ 8 mins. →

2  **100°C**
10 mins.

Lysis of microorganisms

→ 20 mins.



Depletion of human DNA / homogenisation of sample

Harsh lysis of microorgansims

Recovery between 88% and 98%

- Gram+, gram-, fungi

compact sequencing

- PCR + Identification =
- **Screening + Typing**

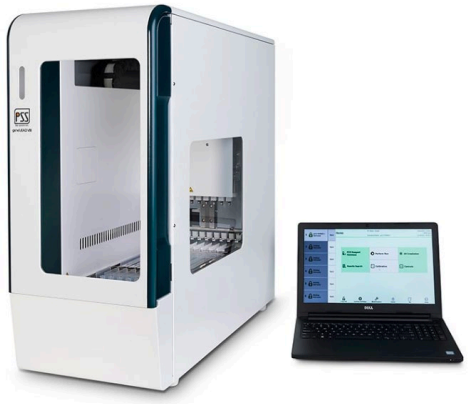
- compact sequencing
  - Sensitive (PCR part)
  - Specific (Primer extension)

Details: [Molecular microbiology | Cube Dx GmbH]

- The targets (DNA oligos) are immobilized on the cylindrical coated surface

- Matching amplicons bind to these immobilized targets

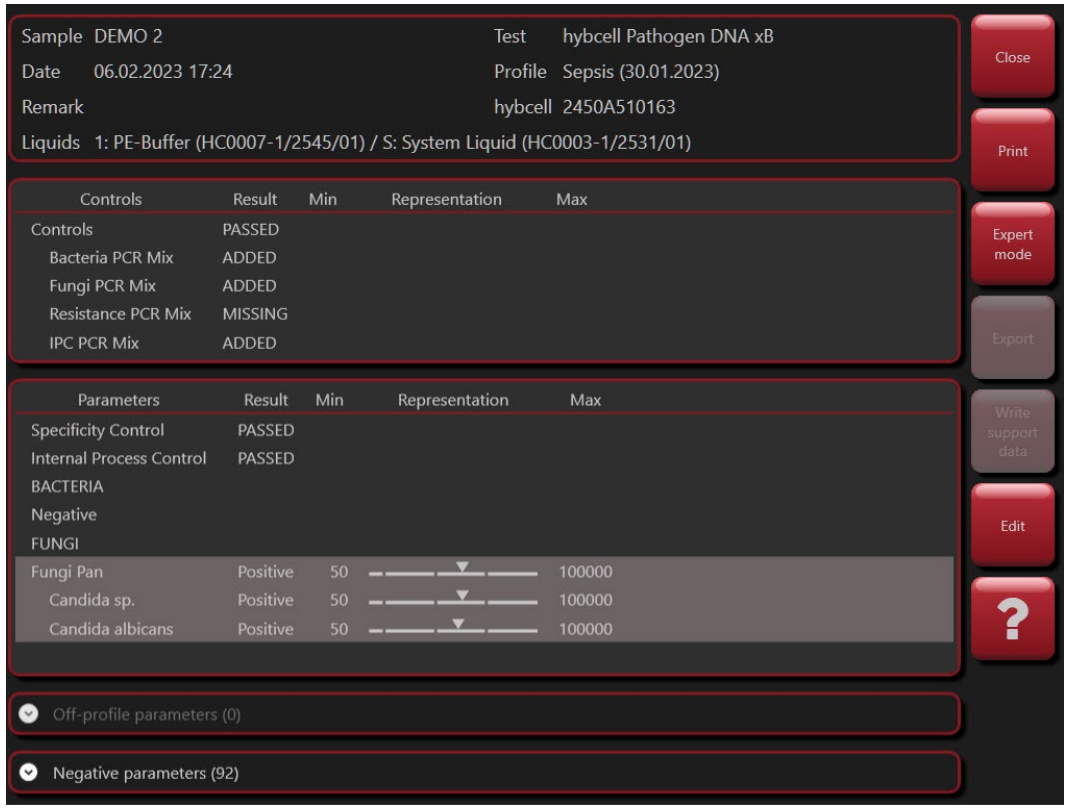
Instruments:
geneLEAD VIII + hyborg Dx RED



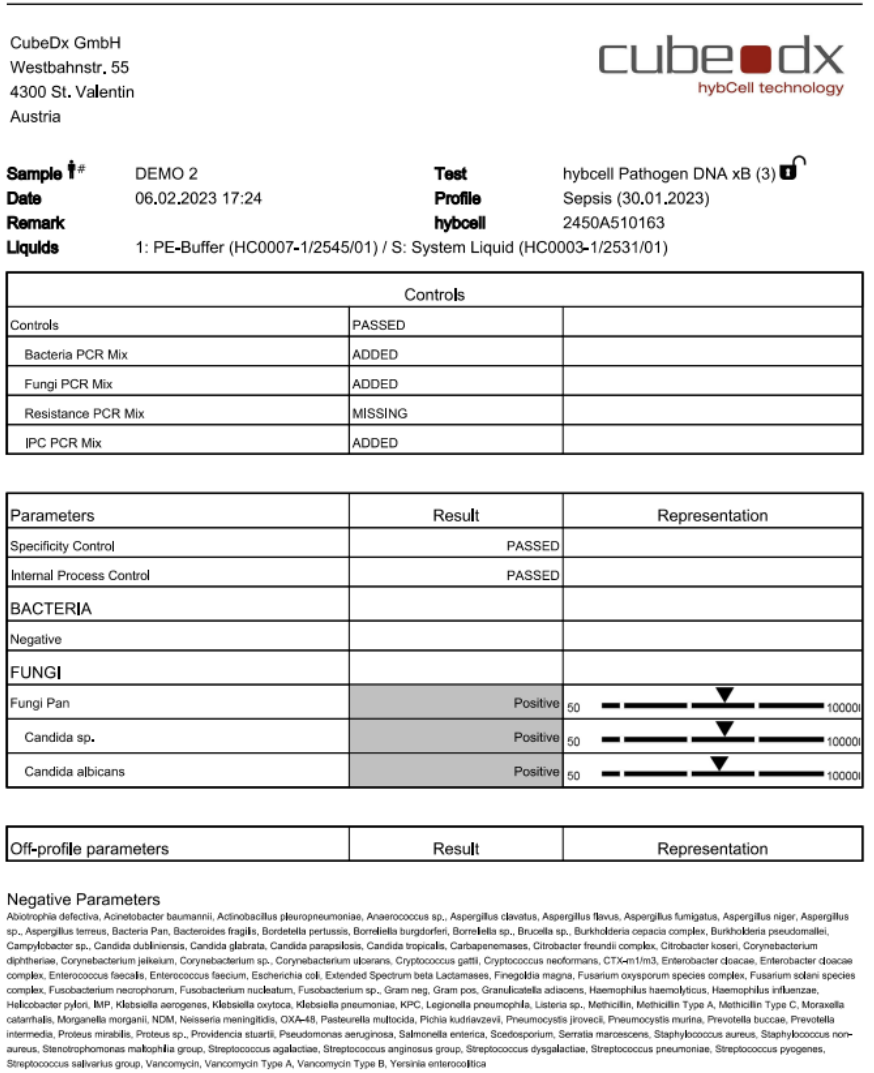
- Magtration technology (beads)
- SV200 cartridges
  - 200/400µl input
  - 100µl eluate
- 8 independent lanes
- Pre-defined protocols (Cube Dx)

- Processes hybcells fully automated
- Up to 24 hybcells can be loaded
- Automated reports
- LIMs connectivity

Example of hyborg Dx RED Report



Software version of the hyborg report



CubeDx GmbH
Westbahnstr. 55
4300 St. Valentin
Austria

cube dx hybCell technology

Sample DEMO 2 **Test** hybcell Pathogen DNA xB (3)
Date 06.02.2023 17:24 **Profile** Sepsis (30.01.2023)
Remark **hybcell** 2450A510163
Liquids 1: PE-Buffer (HC0007-1/2545/01) / S: System Liquid (HC0003-1/2531/01)

Controls		
Controls	PASSED	
Bacteria PCR Mix	ADDED	
Fungi PCR Mix	ADDED	
Resistance PCR Mix	MISSING	
IPC PCR Mix	ADDED	

Parameters	Result	Representation
Specificity Control	PASSED	
Internal Process Control	PASSED	
BACTERIA		
Negative		
FUNGI		
Fungi Pan	Positive	50 100000
Candida sp.	Positive	50 100000
Candida albicans	Positive	50 100000

Off-profile parameters	Result	Representation

Negative Parameters

Abiotrophia defectiva, Acinetobacter baumannii, Actinobacillus pleuropneumoniae, Anaerococcus sp., Aspergillus clavatus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus sp., Aspergillus terreus, Bacteria Pan, Bacteroides fragilis, Bordetella pertussis, Borreliella burgdorferi, Borreliella sp., Brucella sp., Burkholderia cepacia complex, Burkholderia pseudomallei, Campylobacter sp., Candida dubliniensis, Candida glabrata, Candida parapsilosis, Candida tropicalis, Carbapenemases, Citrobacter freundii complex, Citrobacter koseri, Corynebacterium diphtheriae, Corynebacterium jeikeium, Corynebacterium sp., Corynebacterium ulcerans, Cryptococcus gattii, Cryptococcus neoformans, CTX-m1/m3, Enterobacter cloacae, Enterobacter cloacae complex, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Extended Spectrum beta Lactamases, Finegoldia magna, Fusarium oxysporum species complex, Fusarium solani species complex, Fusobacterium necrophorum, Fusobacterium nucleatum, Fusobacterium sp., Gram neg, Gram pos, Granulicatella adiacens, Haemophilus haemolyticus, Haemophilus influenzae, Helicobacter pylori, IMP, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, KPC, Legionella pneumophila, Listeria sp., Methicillin, Methicillin Type A, Methicillin Type C, Moraxella catarrhalis, Morganella morganii, NDM, Neisseria meningitidis, OXA-48, Pasteurella multocida, Pichia kudriavzevii, Pneumocystis jirovecii, Pneumocystis murina, Prevotella buccae, Prevotella intermedia, Proteus mirabilis, Proteus sp., Providencia stuartii, Pseudomonas aeruginosa, Salmonella enterica, Scedosporium, Serratia marcescens, Staphylococcus aureus, Staphylococcus non-aureus, Stenotrophomonas maltophilia group, Streptococcus agalactiae, Streptococcus anginosus group, Streptococcus dysgalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus salivarius group, Vancomycin, Vancomycin Type A, Vancomycin Type B, Yersinia enterocolitica

A pdf version of the hyborg report

Tested bacteria

A		*Abiotrophia defectiva*
		Acinetobacter baumannii *Acinetobacter calcoaceticus complex*
		Actinobacillus pleuropneumoniae
	Anaerococcus	
B		*Bacteroides fragilis*
		Bordetella pertussis
	Borreliella	*Borreliella burgdorferi*
	Brucella	
		Burkholderia cepacia complex *Burkholderia pseudomallei*
C	**Campylobacter**	
		Citrobacter koseri *Citrobacter freundii complex*
	Corynebacterium	*Corynebacterium diphtheriae* *Corynebacterium jeikeium* *Corynebacterium ulcerans*
E		*Enterobacter cloacae* *Enterobacter cloacae complex*
		Enterococcus faecalis *Enterococcus faecium*
		Escherichia coli
F		*Finegoldia magna*
	Fusobacterium	*Fusobacterium nucleatum* *Fusobacterium necrophorum*
G		*Granulicatella adiacens*
H		*Haemophilus haemolyticus* *Haemophilus influenzae*
		Helicobacter pylori
K		*Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae*
L		*Legionella pneumophila*
	Listeria	
M		*Moraxella catarrhalis*
		Morganella morganii
N		*Neisseria meningitidis*
P		*Pasteurella multocida*
		Prevotella buccae *Prevotella intermedia*
	Propionibacterium	*Propionibacterium acnes*
	Proteus	*Proteus mirabilis*
		Providencia stuartii
		Pseudomonas aeruginosa *Pseudomonas non-aeruginosa*
S		*Salmonella enterica*
		Serratia marcescens
	Staphylococcus	*Staphylococcus aureus*
		Stenotrophomonas maltophilia group
	Streptococcus	*Streptococcus anginosus group* *Streptococcus agalactiae* *Streptococcus dysgalactiae* *Streptococcus gordonii* *Streptococcus mitis group* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Streptococcus salivarius group*
Y		*Yersinia enterocolitica* *Yersinia pseudotuberculosis complex*

xC:

Tested bacteria in a few months

A		*Abiotrophia defectiva / Granulicatella elegans*
		Acinetobacter baumannii *Acinetobacter nosocomialis / pittii*
		Actinobacillus pleuropneumoniae
		Aerococcus urinae *Aerococcus viridans*
	Aeromonas	
		Aggregatibacter actinomycetem-comitans *Aggregatibacter aphrophilus*
	Alcaligenes	
	Anaerococcus	
B		*Bacillus cereus*
		Bacteroides fragilis
	Bartonella	*Bartonella bacilliformis / quintana*
		Bordetella pertussis / parapertussis
	Borrelia	
	Borreliella	
	Brucella	
		Burkholderia cepacia complex *Burkholderia pseudomallei*
C	**Campylobacter**	
	Capnocytophaga	
		Cardiobacterium hominis *Cardiobacterium valvarum*
		Citrobacter freundii *Citrobacter koseri*
		Clostridium perfringens
		Corynebacterium diphtheriae *Corynebacterium jeikeium* *Corynebacterium ulcerans*
		Coxiella burnetii
		Cronobacter sakazakii
		Cutibacterium acnes *Cutibacterium avidum*
E	**Ehrlichia**	
		Eikenella corrodens
	Enterobacter cloacae complex	*Enterobacter asburiae/ cancerogenus* *Enterobacter cloacae* *Enterobacter hormaechei* *Enterobacter kobei/ludwigii* *Enterobacter roggenkampii*
		Enterococcus faecalis *Enterococcus faecium*
		Escherichia coli
F		*Finegoldia magna*
		Francisella tularensis
	Fusobacterium	*Fusobacterium nucleatum* *Fusobacterium necrophorum*
G		*Granulicatella adiacens*
H		*Haemophilus haemolyticus* *Haemophilus influenzae* *Haemophilus parainfluenzae*
		Helicobacter pylori
K		*Kingella kingae*
		Klebsiella aerogenes *Klebsiella michiganensis* *Klebsiella oxytoca* *Klebsiella pneumoniae complex*
L		*Legionella pneumophila*
	Leptospira	
	Listeria	
M		*Micrococcus luteus*
		Moraxella catarrhalis
		Morganella morganii
		Mycoplasmoides pneumoniae
N		*Neisseria meningitidis*
	Nocardia	
P		*Pantoea agglomerans*
	Parvimonas	
		Pasteurella multocida
		Prevotella intermedia
	Proteus	*Proteus mirabilis*
		Providencia stuartii
		Pseudomonas aeruginosa group
R	**Rickettsia**	
S		*Salmonella enterica*
		Serratia marcescens
	Staphylococcus	*Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus haemolyticus* *Staphylococcus lugdunensis* *Staphylococcus saprophyticus*
		Stenotrophomonas maltophilia group
	Streptococcus	*Streptococcus agalactiae* *Streptococcus anginosus group* *Streptococcus dysgalactiae* *Streptococcus equinus* *Streptococcus gordonii* *Streptococcus mitis* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Streptococcus salivarius*
V		*Vibrio cholerae* *Vibrio vulnificus*
Y		*Yersinia enterocolitica subsp. enterocolitica* *Yersinia enterocolitica subsp. palearctica* *Yersinia pseudotuberculosis complex*

Tested fungi

A	**Aspergillus**	*Aspergillus clavatus* *Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus niger* *Aspergillus terreus*
C	**Candida**	*Candida albicans* *Candida dubliniensis* *Candida parapsilosis* *Candida tropicalis*
		Candida auris
	Cladosporium	
		Cryptococcus neoformans *Cryptococcus gattii*
F		*Fusarium oxysporum species complex* *Fusarium solani species complex*
N		*Nakaseomyces glabratus*
P		*Pichia kudriavzevii*
		Pneumocystis jirovecii *Pneumocystis murina*
S	**Saccharomyces**	*Saccharomyces cerevisiae*
	Scedosporium	

.. and in a few months (xC)

A	**Aspergillus**	*Aspergillus clavatus* *Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus niger* *Aspergillus terreus*
C	**Candida**	*Candida albicans* *Candida dubliniensis* *Candida parapsilosis* *Candida tropicalis*
		Candidozyma auris *Candidozyma duobushaemulonii* *Candidozyma haemulonii*
	Cladosporium	
		Cryptococcus neoformans *Cryptococcus gattii*
F		*Fusarium oxysporum* *Fusarium solani*
L		*Lomentospora prolificans*
N		*Nakaseomyces bracarensis* *Nakaseomyces glabratus* *Nakaseomyces nivariensis*
P	**Pichia**	*Pichia kudriavzevii*
		Pneumocystis jirovecii
S	**Sacharomyces**	*Saccharomyces cerevisiae*
	Scedosporium	

Target list for Pathogens xC follow-up (after bacterial ID) resistance testing

PCR-Box Resistance g+ xC:

mecA, mecC	*FAM*
vanA	*JOE*
vanB	*Cy5*

PCR-Box Resistance g- AB xC:

NDM, VIM, IMP	*FAM*
KPC	*JOE*
CTX-M (Group 1)	*Cy5*

PCR-Box Resistance g- CD xC:

OXA48	*FAM*
AmpC	*JOE*
mcr-1	*Cy5*

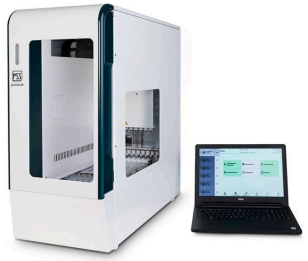
Coverage of incidence (xC)?

	All HA infections	systemic infections	pneumonia	
Nr of total specified groups reported (Bac/Fun):	**92**	**30**	**66**	
Nr. of groups covered on CDX panel:	**72**	**28**	**56**	
% of reported groups (Bac/Fun) covered:	**78**	**93**	**85**	%
% of incident microorganisms covered:	**88**	**98**	**96**	%

Source of Data: https://www.ecdc.europa.eu/en/healthcare-associated-infections-acute-care-hospitals/database/microorganisms-and-antimicrobial-resistance/list

Refer to ECDC PPS protocol for case definitions. Data from the ECDC point prevalence survey of healthcare-associated infections and antimicrobial use in acute care hospitals (ECDC PPS) in the period 2011-2012 as reported to TESSy as of 2013-02-06 14:06:48.)

Product configuration



1 ***Prep + DNA***

2 ***PCR***

PCR, qPCR result
Result positive

3 ***ID***

GINA

PCR-Box Bacteria (16S) → **hybcell Bacteria xC**

PCR-Box Fungi (28S) → **hybcell Fungi xC / hybcell FungiPlus xC**

PCR-Box Res g+

PCR-Box Res g-

Positive for bacteria

qPCR result
Result negative

- No ID if PCR negative
- Reduced cost / time

Same test – additional intended uses

2 **Patho ID from sterile fluids**
(synovial fluid, pleura fluid,…)

Joint infections / pleurisy

3 **Patho ID from soft tissue**
(tissue and implants..)

Endocarditis / implant and tissue infections

Further possibilities (sample matrices):

Empyema, CSF, urine, ascites, BAL, sputum, sperm…

Validation results for Pathogens xB

SMARTDIAGNOS – next generation molecular sepsis diagnosis technology for whole blood samples

[1]Helena Enroth, [1]Lilla Marki, [2]Jan Zavora, [2]Vaclava Adamkova, [3]Helena Brodska, [4]Viktoria Weber, [5]Anna-Karin Pernestig, [5]Diana Tilevik, [6]Bernhard Ronacher, [6]Christoph Reschreiter

[1]Unilabs laboratory medicine, SkaS, Skövde, Sweden, [2]Clinical Microbiology and ATB Centre, General University Hospital, Prague, Czech Republic, [3]Department of Medical Biochemistry and laboratory diagnostic, General University Hospital, Prague, Czech Republic, [4]Department for Biomedical Research, Danube University Krems, Austria, [5]Systems Biology Research Centre, School of Biosciences, University of Skövde, Skövde, Sweden, [6]Cube Dx GmbH, St. Valentin, Austria

	Blood culture positive (reference)	Blood culture negative (reference)
LAB positive	True positive: 28 (8%)	False positive: 5 (1%)
LAB negative	False negative: 10 (3%)	True negative: 309 (88%)

- Validated in 2019, published ECCMID 2021
- 352 cases / samples, 3 centres

- LOD = 20 CFU / ml (and less)
- Sensitivity = 28 / (28 + 10) = 73,68 %
- Specificity = 309 / (309 + 5) = 98,41%
- NPV = 309 / (309 + 10) = 96,87 %
- PPV = 28 / (28 + 5) = 87,50 %

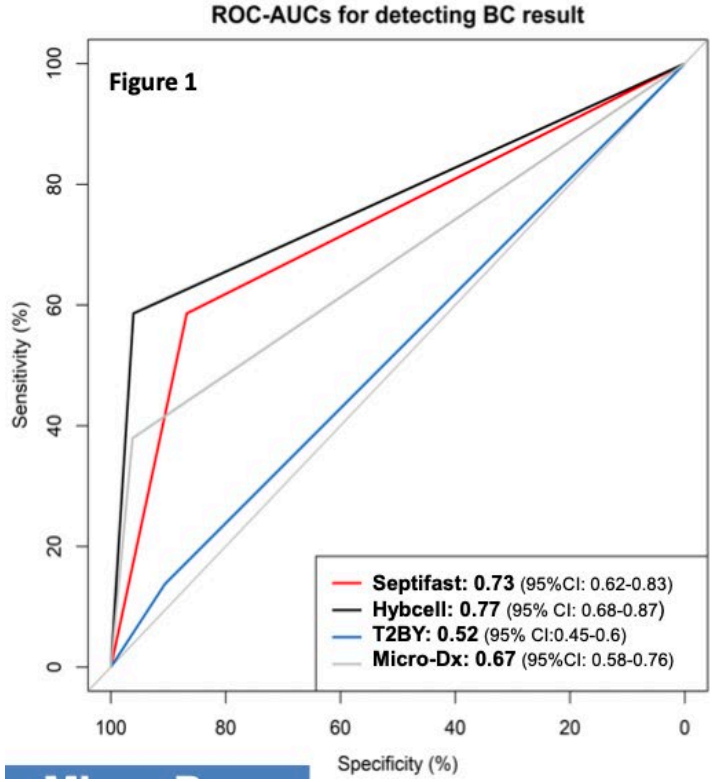
Validation results for Pathogens xB

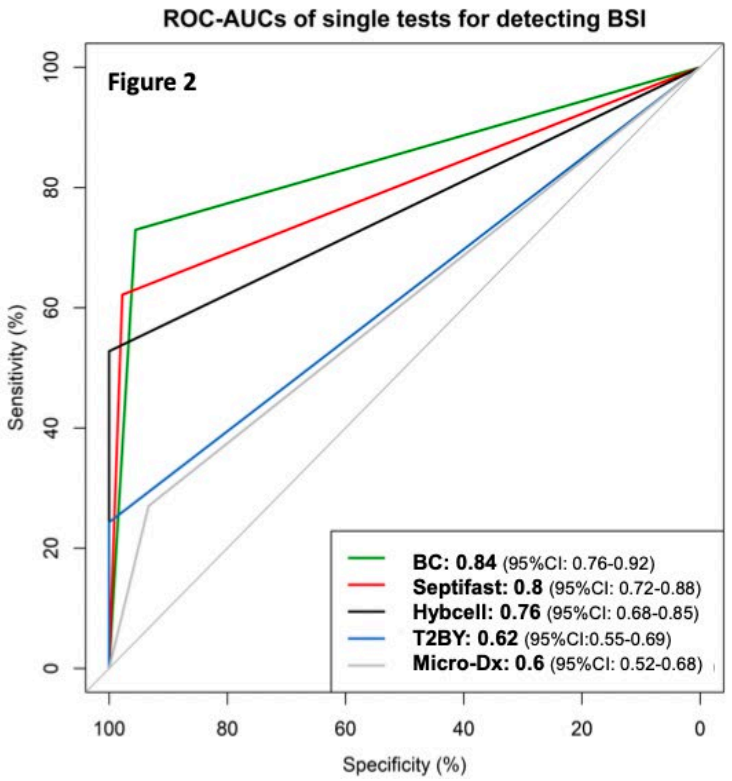
MEDICAL UNIVERSITY OF VIENNA

Diagnostic value of blood culture independent molecular tests for the diagnosis of bloodstream infections in neutropenic patients with fever

P. Starzengruber[1], I. Camp[1], M. Kussmann[2], F. Ratzinger[3], W.R. Sperr[4], K. Gleixner[4], B. Selitsch[1], H. Burgmann[2], B. Willinger[1], A. Makristathis[1]

1 Division of Clinical Microbiology, Department of Laboratory Medicine, Medical University Vienna, Austria
2 Division of Infectious Diseases and Tropical Medicine, Department of Medicine I, Medical University Vienna, Austria
3 IHR LABOR, Vienna, Austria
4 Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University Vienna, Austria.

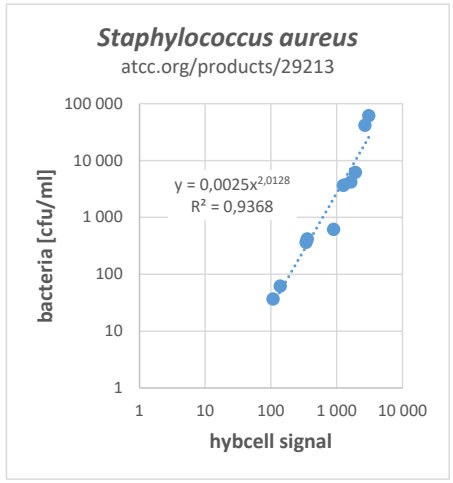


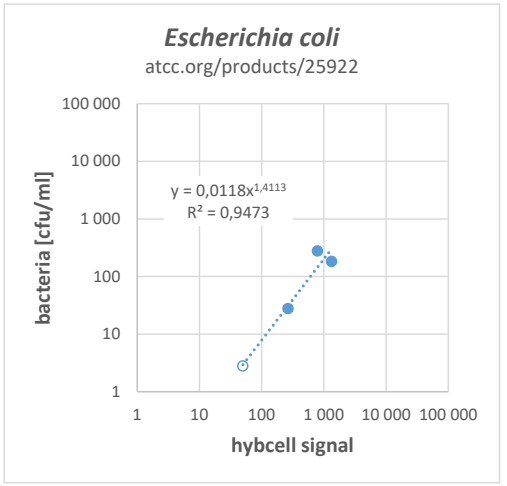


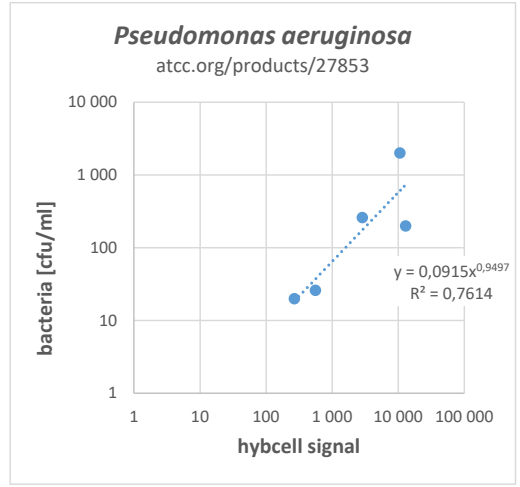
- Data gathered in 2019 and 2020 (improvements made in the meantime)
- → Highest concordance with BC
- → Highest rate of identification of BSI (except SeptiFast)

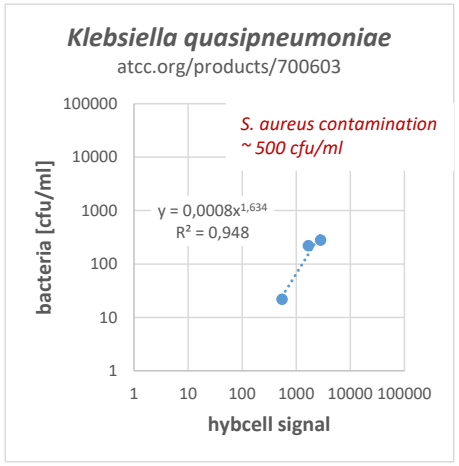
xC: preliminary LOD data - bacteria

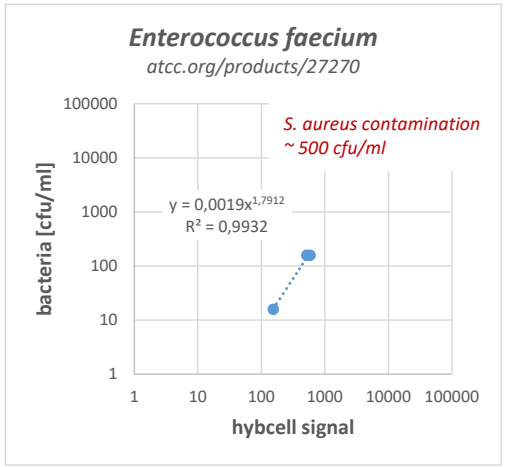
	Calculated LoD cfu/ml
Staphylococcus aureus	27
Escherichia coli	8
Pseudomonas aeruginosa	7
Klebsiella quasipneumoniae	1
Enterococcus faecium	7
Streptococcus pneumoniae	4

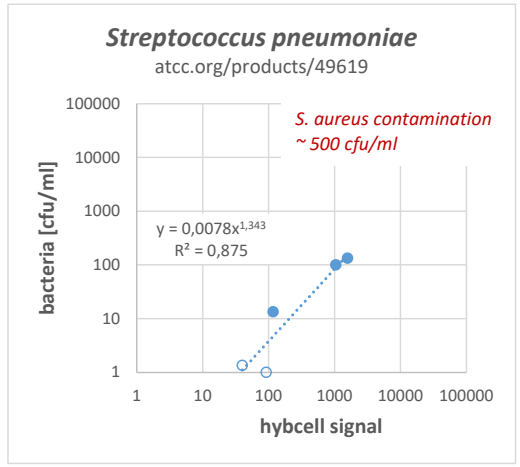






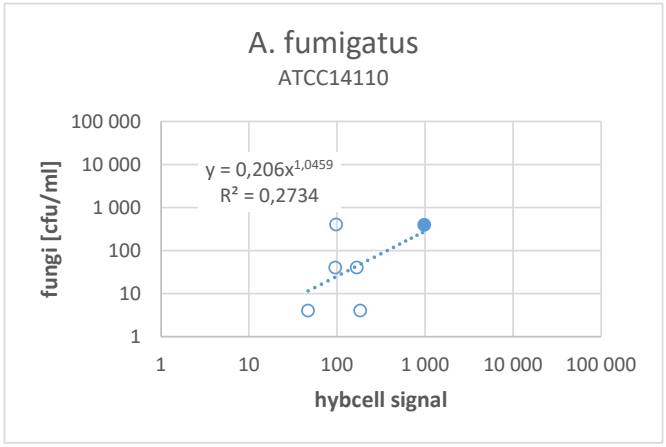


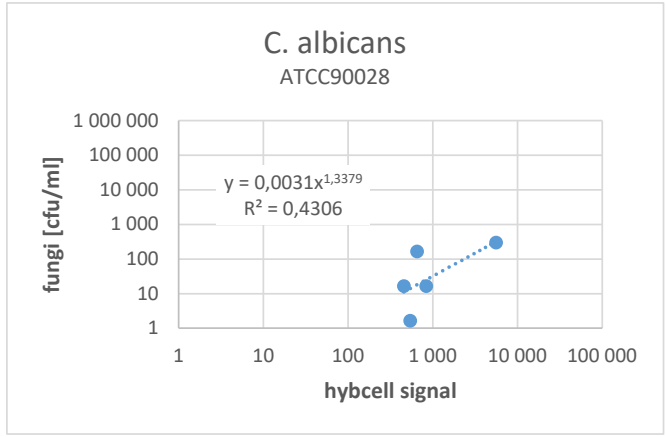


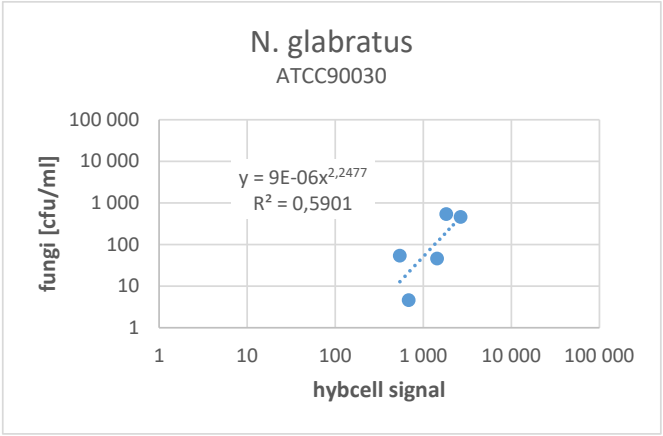


xC: preliminary LOD data - fungi

	Calculated LoD cfu/ml
Aspergillus fumigatus	53
Nakaseomyces glabratus	1
Candida albicans	4







xC: preliminary data whole blood testing

	BC relevant Bacteria	BC relevant Fungi
n	154	154
TN	129	144
TP	13	2
FN	2	0
FP	6	4
n.a.	4	4
Sensitivity	**87%**	**100%**
Specificity	**96%**	**97%**
Correct Classification	95%	97%
Positive predictive value (PPV)	**68%**	**33%**
Negative predictive value (NPV)	**98%**	**100%**
Accuracy	95%	97%
Prevalence	10%	1%

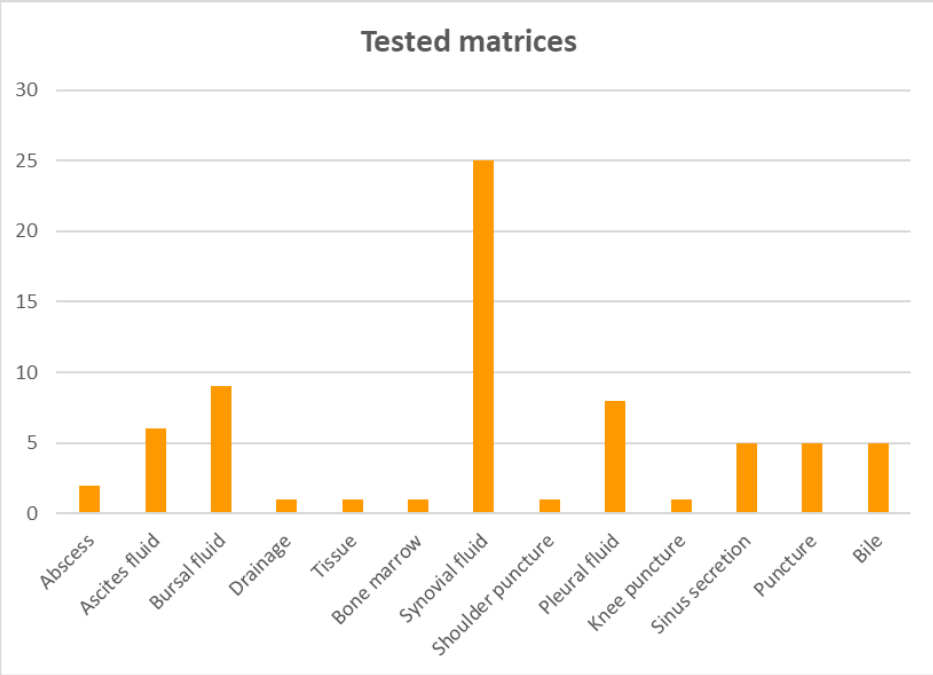
xC: preliminary data whole blood testing

Bacteria	BC	CUBE DX	Remarks
E. coli	8	6	
K. pneumoniae	2	2	
P. aeruginosa	3	2	
E. faecalis	1	1	
E. faecium	1	0	1 of 2 BC positive
Str. pneumoniae	1	1	
Staph. epidermidis	8	0	
Staph. haemolyticus	1	0	**Not on sepsis**
Str. gordonii	1	0	**profile**
Staph. hominis	2	0	
Bacillus cereus	1	0	
Clostridium septicum	1	0	**Not on test panel**
Str. parasanguinis	1	0	
Corynebacterium ulcerans	0	1	
A. baumannii	0	4	
Str. agalactiae	0	1	

Fungi	BC	CUBE DX	Remarks
C. parapsilosis	1	1	
N. glabratus	1	1	
C. albicans	0	3	Confirmed: 3rd party
A. fumigatus	0	2	PCR / repetition

xC: preliminary data tissue + sterile fluids

Specimen	Quantity
Abscess	2
Ascites fluid	6
Bursal fluid	9
Drainage	1
Tissue	1
Bone marrow	1
Synovial fluid	25
Shoulder puncture	1
Pleural fluid	8
Knee puncture	1
Sinus secretion	5
Puncture	5
Bile	5
	70



Classification	
TN	26
TP	35
FN	1
FP	5 **
Discordant	3
Total	70

Sensitivity	97,2%
Specificity	83,9%

**FP	BC	CUBE DX
Staphylococcus aureus	0	1
Fusobacterium nucleatum	0	1
Pseudomonas aeruginosa group	0	1
Proteus spp.	0	1
Burkholderia cepacia complex	0	1

Summary / Conclusions

Complementary DNA based „molecular microbiology“

Testing wide range of bacteria and fungi from whole blood and other matrices in 3 hours

Reducing time to result

Whole blood: 3 hours and less for 70 to 80% of all relevant positive samples

Widening the scope of results

Additional positive results for culture negative samples (fungi, fastidious bacteria, anaerobes..)

Low sample volume (500µl to 1000µl)

Allows for example re-testing patients on consecutive days or testing infants..

Versatility in testing

Different sample matrices to test in case of suspected “severe infections”

Further resources

- Eurobio Scientific UK
  https://eurobio-scientific.co.uk/

- General Cube Dx information
  www.cubedx.com

STAND B33

Britannia Suite

Discuss with Us

- Specific information on molecular microbiology solution + publications
  www.cubedx.com/en/molecular-microbiology

- Video tutorials, IFUs, guidelines..
  www.cubedx.com/en/support