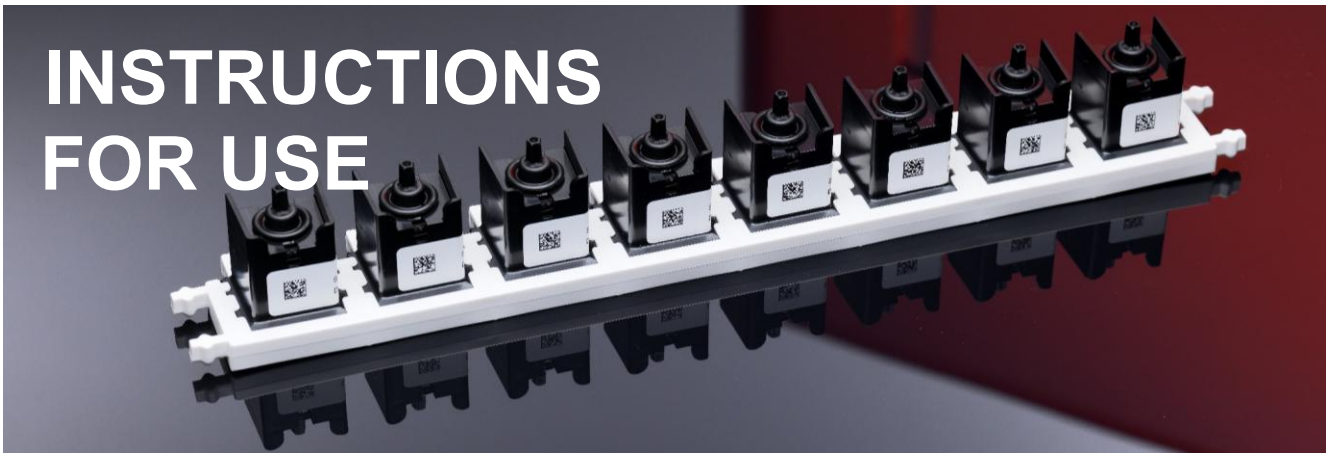
INSTRUCTIONS FOR USE



Pathogens xC_instructions for use_E_2026_02_13

EVAL

Products belonging to Risk Class A (according to IVDR – EU 2017/746)

GINA 500	*Basic UDI-DI 912012773GINA7H*	*REF / UDI-DI 09120127730244*
GINA 500 + DNA Purification		*REF / UDI-DI 09120127730145*
GINA 1000		*REF / UDI-DI 09120127730480*
GINA Lyse 200		*REF / UDI-DI 09120127730664*
Kits for enriching bacterial and fungal cells from human blood and / or microbial lysis including / not including DNA purification		
LINA	*Basic UDI-DI 912012773LINA8L*	*REF / UDI-DI 09120127730152*
Modulation buffer for extraction-free testing of positive Blood Culture (BC) media		

Products belonging to Risk Class C (according to IVDR – EU 2017/746)

PCR-Box Bacteria xC 24rx-s	*Basic UDI-DI 912012773P-BxC3R*	*REF / UDI-DI 09120127730701*
PCR-Box Fungi xC 24rx-s	*Basic UDI-DI 912012773P-FxC4D*	*REF / UDI-DI 9120127730633*
hybcell Bacteria DNA xC	*Basic UDI-DI 912012773h-BxCAA*	*REF / UDI-DI 09120127730336*
hybcell Fungi DNA xC	*Basic UDI-DI 912012773h-FxCAW*	*REF / UDI-DI 09120127730404*
hybcell Fungi Plus DNA xC		*REF / UDI-DI 09120127730367*
Multiplex DNA tests for detection of bacterial 16S DNA and of fungal 28S DNA from human samples with an indication of homologies to known bacterial and fungal type strains, including internal control.		
PCR-Box Res g+ xC 24rx-s	*Basic UDI-DI 912012773P-RxC69*	*REF / UDI-DI 09120127730640*
PCR-Box Res g- AB xC 24rx-s		*REF / UDI-DI 09120127730657*
PCR-Box Res g- CD xC 24rx-s		*REF / UDI-DI 09120127730695*
Multiplex PCR tests for detection of bacterial antibiotic resistance marker genes from human samples, including internal control.		

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RUO/EVAL

Content

Version information

Version	Document and Changes	Modified	Released
001	Pathogens xC_instructions for use_E_2025_02_06, First consolidated draft		06.02.2025; CRE
002	Pathogens xC_instructions for use_E_2026_02_13, General updates, streamlining of text, updates of intended uses and technical description...	12.02.2026; BRO, CRE	13.02.2026; CRE

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1. Explanation of symbols

Symbol	Explanation
CE IVD	CE mark. In vitro diagnostic medical device.
	Manufacturer.
EXP	Expiry date.
REF	Catalog number, UDI-DI.
SN	Serial number.
	Reference to the instructions for use.
	Only use it once. Do not reuse.
	Use by date.
	Temperature limit for storage.
	Sufficient for <n> tests.
CONTROL	Control material.
H225	Highly flammable liquid and vapour.
H301	Toxic if swallowed.
H315	Causes skin irritation.
H318	Causes serious eye damage.
H319	Causes serious eye irritation.
H371	May cause damage to organs.
H412	Harmful to aquatic life with long lasting effects.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P273	Avoid release to the environment.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P310	IF SWALLOWED: Immediately call a POISON CENTER/doctor.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice/attention.
P370+P378	In case of fire: Use sand, carbon dioxide or powder extinguisher to extinguish.
P403+P235	Store in a well-ventilated place. Keep cool.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

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2. Intended Use(s)

GINA 500

The *GINA 500 + DNA Purification* kit and its variants *GINA 500* and *GINA 1000* are sample preparation kits designed for manual sample preparation for follow-up extraction of microbial (bacterial/fungal) DNA/RNA from EDTA whole blood. The kits are a set of reagents that deplete human cells and DNA from EDTA whole blood samples and efficiently lyse remaining microbial cells.

GINA Lyse 200 is used for manual sample preparation of viscous or solid human samples (or liquid human samples containing solid components) like BAL, sputum, aspirates, saliva and soft tissue. The kit provides a swab for safe transfer of viscous samples into the lysis buffer and an efficient lysis of microbial and human cells.

After sample preparation, the microbial DNA/RNA must be purified/extracted. Different suitable DNA/RNA purification/extraction products can be used.

The products are used in conjunction with DNA-based tests, for example, PCR tests or DNA sequencing, to deliver qualitative results and aid in the diagnosis or in screening of suspected bacterial and/or fungal infections in the clinical contexts of bloodstream infections.

The usage of the products is independent of the patient's age, gender, genotype, or any demographic aspect. No specific patient group is excluded from testing. The attending physician decides if the sample-taking procedure is justifiable for any individual patient.

The products are intended for professional use as part of comprehensive diagnostic workflows.

LINA

LINA is a sample preparation kit designed for manual preparation of (positive) blood cultures and bronchoalveolar lavages (BAL) for follow-up DNA testing of bacteria and fungi. The kit is a reagent that modulates the sample for the direct usage – without DNA extraction – for DNA-based testing.

The product is used in conjunction with DNA-based tests, for example, PCR tests or DNA sequencing, delivers qualitative results and aids the diagnosis of bacterial and/or fungal respiratory (BAL) or other infections (blood culture).

The usage of the product is independent of the patient's age, gender, genotype, or any demographic aspect. No specific patient group is excluded from testing. The attending physician decides if the sample-taking procedure is justifiable for any individual patient.

The product is intended for professional use as part of comprehensive diagnostic workflows.

PCR-Box Bacteria xC

PCR-Box Bacteria xC is a ready-to-use Polymerase-Chain-Reaction (PCR)-mix to amplify and detect bacterial 16S-rDNA in eluates from suitable RNA/DNA extraction processes.

The product delivers qualitative results and is used to screen and aid to diagnose unspecific bacterial infections in different clinical contexts.

The usage of the product is independent of the patient's age, gender, genotype, or any demographic aspect. No specific patient group is excluded from testing.

The product is intended for professional use as part of comprehensive diagnostic workflows.

PCR-Box Fungi xC

PCR-Box Fungi xC is a ready-to-use Polymerase-Chain-Reaction (PCR)-mix to amplify and detect fungal 28S-rDNA in eluates from suitable RNA/DNA extraction processes.

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The product delivers qualitative results and is used to screen and aid to diagnose unspecific fungal infections in different clinical contexts.

The usage of the product is independent of the patient's age, gender, genotype, or any demographic aspect. No specific patient group is excluded from testing.

The product is intended for professional use as part of comprehensive diagnostic workflows.

PCR-Box Resistance g+ / g- AB / g-CD xC

PCR-Box Resistance g+ xC, PCR-Box Resistance g- AB xC and *PCR-Box Resistance g- CD xC* are ready-to-use Polymerase-Chain-Reaction (PCR)-mixes to amplify and detect different antibiotic resistance genes in eluates from suitable RNA/DNA extraction processes.

The products deliver qualitative results and are used to aid to diagnose possible antibiotic resistances in different clinical contexts based on genotypic associations of mutations to observed phenotypic resistance.

The usage of the products is independent of the patient's age, gender, genotype, or any demographic aspect. No specific patient group is excluded from testing.

The products are intended for professional use as part of comprehensive diagnostic workflows.

hybcell Bacteria DNA xC

hybcell Bacteria DNA xC is a cartridge base test to identify a defined panel of bacterial species, genera or groups of species using Cube Dx' proprietary *compact sequencing* and the *hyborg Dx RED2* or *hyborg Dx RED3* instrument.

Amplicons produced by *PCR-Box Bacteria xC* are introduced into the hybcell cartridges and serve as samples.

The product delivers qualitative results and is used to aid to diagnose bacterial infections in different clinical contexts.

The usage of the product is independent of the patient's age, gender, genotype, or any demographic aspect. No specific patient group is excluded from testing.

The product is intended for professional use as part of comprehensive diagnostic workflows.

hybcell Fungi DNA xC / hybcell FungiPlus DNA xC

hybcell Fungi DNA xC and *hybcell FungiPlus DNA xC* are cartridge based tests to identify defined fungal species, genera or groups of species using Cube Dx' proprietary *compact sequencing* and the *hyborg Dx RED2* or *hyborg Dx RED3* instrument

Amplicons produced by *PCR-Box Fungi xC* are introduced into the hybcell cartridges and serve as samples.

Both products deliver qualitative results and are used to aid to diagnose fungal infections in different clinical contexts. The products differ in the number of tested fungal species, genera or groups of species: *hybcell FungiPlus DNA xC* offers an extended panel over *hybcell Fungi DNA xC*.

The usage of the products is independent of the patient's age, gender, genotype, or any demographic aspect. No specific patient group is excluded from testing.

The products are intended for professional use as part of comprehensive diagnostic workflows.

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3. General Device Description

General Information

The early identification of the causative microorganism of a bacterial or fungal infection enables early targeted antimicrobial therapy. Especially severe infections require such targeted antimicrobial therapy as a precondition for successful treatment and the limitation of often severe adverse effects, that might ultimately lead to the death of the patient. Especially sepsis as a syndrome triggered by an infection or pneumonia, meningitis or endocarditis and tissue infections claim many deaths.

Conventional culturing methods often fail to deliver early results, especially if the causative microorganism is a fastidious bacterium (e.g., *Bordetella pertussis, Bartonella* or *Rickettsia)* that requires unique growth conditions, a slow-growing bacteria (e.g. *Pseudomonas aeruginosa* or *Streptococcus pyogenes*) or fungi (e.g. *Candida glabrata*). Especially in such cases, Cube Dx's DNA-based early identification of microorganisms has the potential to be beneficial for patients.

However, the products are designed for complementary use with blood culturing. It is by no means intended to replace blood culture techniques. Results obtained from the direct blood test should be interpreted in conjunction with other relevant clinical and laboratory findings, to aid in the provision of targeted therapy for patients suffering from microbial infections.

Contradictory results to blood culture may occasionally occur: for example, a negative result may be presented by the products while a blood culture result is positive, and vice versa. Such discrepancies may be result of a very low number of microorganisms in the patient's blood, as only 0.5 mL sample volume is taken for the test (in comparison to 2x10 mL for blood culturing). Another reason might be the occurrence of rare type strains that have not been considered during the test design or the fundamental differences in the underlying technologies for the read-out of results (genetic information based on selected type strains vs. protein patterns used by MALDI-TOF).

Different points in time when the samples are taken may result in discrepant results as well. We strongly recommend collecting samples for the Cube Dx test at the same time when blood culture samples are collected – if still possible before the administration of antimicrobials.

This test has to be carried out as described in this instructions for use. Interruptions of the workflow, for example by freezing the eluate for some days, may alter results as well.

The test results should be evaluated in the context of the patient's medical record, his/her clinical status, and other findings.

Attention! The test must be carried out in an environment suitable for PCR testing. As the test is very sensitive and amplifies even few copies of bacterial or fungal DNA much attention has to be put in avoiding any microbial contamination.

Contaminations can result from sample taking, improper personal protective equipment, improper handling, surfaces or contaminated consumables.

So, apart from reagents and consumables provided by Cube Dx, suitable equipment and material should be chosen accordingly (e.g. DNA- and DNase-free pipet tips) and some infrastructure for PCR testing must be available (e.g. separated rooms for sample preparation and DNA isolation, PCR and Post-PCR (= hybcell test)).

The sample preparation and PCR set-up should be done under a laminar flow or at least PCR hood.

GINA 500 (+ DNA Purification), GINA 1000, GINA Lyse 200

The kits *GINA 500* (for 500 µL or less of ETDA whole blood, with or without DNA purification) and *GINA 1000* (for 1000 µL of ETDA whole blood) are designed for enriching microbial (bacterial, fungal) DNA of whole blood samples. The kit *GINA Lyse 200* is a variant of the GINA kit and used for other than whole blood sample to provide a homogenisation of the sample and a harsh microbial lysis.

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The kits are intended to efficiently prepare clinical samples for follow-up extraction of microbial RNA/DNA. This includes homogenisation of the samples, normalisation of the input, efficient lysis of the microbes and in case of EDTA whole blood samples the removal of the vast majority of human blood cells and DNA.

The products and process facilitate downstream diagnostic applications like Cube Dx' PCR products and *hybcell* tests (*compact sequencing*), as well as third-party RNA/DNA based diagnostic molecular tests for microbial identification – including sequencing.

A key feature of the *GINA* sample preparation is its ability to efficiently isolate microbial cells from 500 µL (or less) or 1000 µL of ETDA whole blood. Combined with the high lysis efficiency of the *GINA* products the kits' relatively low sample input are particularly beneficial for detecting (bloodstream) infections in neonates or infants and elderly or immune-compromised patients.

However, low sample volumes and efficient lysis and release of microbial RNA/DNA are critical and a major challenge for most state-of-the-art procedures for pathogen identification, not only for bloodstream infections. The *GINA* products – or components and parts thereof – are therefore intended to be used for different clinical samples. Dependent on the sample type the whole kit or just parts are applied and protocols and workflows are adapted to these sample types. The sample type defines the intended clinical application. Below table defines the intended uses of different sample types:

Sample type	**Intended clinical use**
EDTA whole blood	Blood stream infections (diagnosing sepsis / bacteremia / fungemia)
BAL / saliva / sputum	Respiratory tract infections (diagnosing pneumonia / bronchitis / …)
Tissue / synovial fluid*	Tissue, joint and bone infections (diagnosing endocarditis / fasciitis / implant infections / …)
Urine* / ejaculate / vaginal swab	Urinary and genital tract infections (diagnosing urosepsis / prostatitis / …)
Cerebrospinal fluid*	Infections of the nervous system (diagnosing meningitis / …)
Pleural fluid*	Infections of the pleura (diagnosing pleurisy / …)
Cultivated blood culture bottles*	Identification of cultivated bacterial / fungal species

* for these samples no sample preparation with *GINA* components is necessary (except they contain solid particles as well)

(Soft) lysis and removal of human cells (from EDTA whole blood):

In a first step of the *GINA* kit the vast majority of human (blood) cells and cellular debris from EDTA whole blood are removed. The whole blood is added to the ready-to-use *LE Solution*, human cells are lysed and the remaining microbes are transferred into a pellet by the following centrifugation step. The supernatant with the debris of human cells is discarded.

The procedure is intended to drastically increase the percentage of pathogenic (bacterial and fungal) DNA of intact microorganisms relative to human DNA in the resulting solution and to provide better conditions for downstream PCR reactions. Intact microorganisms are those that are still viable (active or attenuated (= inhibited in their growth for example by administration of certain antibiotics)). On the contrary, damaged microorganisms and free DNA are removed during the procedure. The procedure is not biased towards any characteristic of different microorganisms.

As a consequence, only microorganisms that can still do harm to the patient are relevant for the follow-up processes (*compact sequencing*). Some antimicrobials focus on preventing growth but do not neutralize microbes. In such cases, the microorganisms are not removed, as they are still intact. These microorganisms pose a risk to the patient, once the antimicrobial treatment stops.

For the depletion of human cells from whole blood a table-top centrifuge with a rotor for 2 mL tubes or / and 5 mL Eppendorf tubes is necessary. The centrifuge should be able to apply 11.000g (e.g., Eppendorf, Hermle, etc.).

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For other than whole blood samples, the depletion of human cells and DNA is not relevant.

(Harsh) lysis of microorgansims:

The second fundamental feature of the *GINA* kit is its highly effective and efficient lysis of bacterial, fungal and remaining human cells (after enrichment).

NA Solution is added to the resulting pellet (if whole blood has been used) or different human samples and incubated at high temperature. Microbial cells and human tissue are lysed and DNA is released into the solution.

For the effective lysis of microorganisms a conventional heating block (e.g., Analytic Jena, Coyote Bioscience) capable to heat up to 100°C (for 2 mL tubes or / and 5 mL Eppendorf tubes) is needed.

If the workflow includes DNA purification based on spin columns:

Neutralization:

The lysate is transferred into the *T Solution* to stop the process of lysis and to neutralize the resulting solution.

After the lysis of microbial cells the resulting RNA/DNA is purified. This purification is either done with spin columns (included in the *GINA 500 + DNA purification* kit) or with automated RNA/DNA extractors. Currently protocols for PSS' geneLEAD VIII (with the MagDEA® Dx SV) are validated.

Other products can be used, but have to be validated. We strongly recommend cartridge based systems using a bead based extraction.

Results may be falsified due to the nature of the sample, errors during the procedure (low amount of DNA, contamination with environmental microorganisms / DNA), other influences (degraded DNA, contamination with chemicals), or technical errors.

If working with whole blood samples following circumstances deteriorate results for a sample:

- Time between drawing the (blood) sample and the start of sample preparation is longer than 4 hours.
- The storage between sample drawing and the start of sample preparation is not according to the specifications (refer to the Storage, Transportation, Shelf Life and Disposal)

LINA

The identification of pathogens and antibiotic-resistance genes should be simple and fast. The *LINA* transfer and modulation buffer shortens the time for molecular identification as it eliminates the RNA/DNA extraction processes and enables direct transfer into PCR.

This buffer is designed for use with samples containing an abundance of microorganisms, for example, Broncho Alveolar Lavage (BAL) in the diagnosis of pneumonia, or positive blood cultures.

Together with Cube Dx's PCR products and *hybcells* for pathogen identification, microorganisms can be identified in less than 2 hours.

LINA consists of 8 mL of buffer filled in single ready-to-use tubes. The buffer dilutes any PCR inhibitors in the sample, so these are no longer effective. The sample buffer mixture is directly transferred into the PCR reactions (without any further extraction process). The short and simple protocol drastically reduces the time to result.

The result may be falsified due to the nature of the sample or errors during the procedure (e.g., a low number of microorganisms in the sample or technical errors).

The following circumstances deteriorate results for a sample:

- Use of a larger sample volume than specified, as this might increase the concentration of PCR-inhibitors.

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PCR-Box Bacteria xC / PCR-Box Fungi xC

PCR-Box Bacteria xC and *PCR-Box Fungi xC* are in-vitro diagnostic tests for the detection of either bacterial or fungal DNA from suitable eluates based on homologies to bacterial 16S rDNA and fungal 28S rDNA. During amplification, single DNA strands are labelled with a fluorescent dye that is used later in the *compact sequencing* process (for the *hybcell* read-out).

The products are provided as ready-to-use mixes which contain all buffers, enzymes, DNA primers, internal control (IC) in a single mix for a single reaction (20 µL).

A synthetic long DNA oligo serves as internal control (IC) to confirm the validity of results (especially of negative results) by showing an additional peak in the melting curve.

The mixes are equipped with an Uracil-N-Glykosylase (UNG) enzyme to prevent carryover contaminations. The built-in hot-start functionality of the PCR mix allows working without cooling beds.

In each PCR kit one tube of positive control (PTC.. Positive Template Control, solution containing synthetic oligos) and one tube with negative control (NTC.. Non Template Control, empty elution buffer) are added. The synthetic oligo of the PTC for *PCR-Box Bacteria xC* mimics *Bacterioides fragilis*, the one for *PCR-Box Fungi xC* mimics *Pichia spp.*.

The tests indicate if any bacterial or fungal DNA is present in the eluate and – as a conclusion – if any bacteria or fungi are present in the sample. Therefore the PCR mixes can be used for the screening of any bacterial or fungal infection.

The result is derived by analysing Ct-values and melting peaks of the amplification and melting curves for the SYBR Green channel (see below).

PCR-Box Bacteria xC:

Target	Fluorophore
Bacteria / IC	FAM

PCR-Box Fungi xC:

Target	Fluorophore
Fungi / IC	FAM

The PCR-mixes are provided as single reactions, pre-filled in 0.2 mL PCR tubes (20 µL each).

Based on the result of the test with *PCR-Box Bacteria xC* and *PCR-Box Fungi xC* positive samples – the ones that show amplification (Ct below threshold) and a melting peak in a certain temperature range as defined by different intended uses – are singled out for following identification of the bacterial or fungal species (or genus):

For differentiation and identification of bacterial and fungal species, the resulting DNA amplicons are processed with *hybcell Bacteria xC*, *hybcell Fungi xC* or *hybcell FungiPlus xC* microarray cartridges using a *hyborg Dx RED2/3* instrument. Alternatively, the amplicons can be sequenced. If no amplification is visible for the targets (bacteria / fungi), a negative result is assumed (e.g. bacteria not verified). In such cases, the internal control (IC) must show amplification – to prove that the PCR process was flawless.

For processing *PCR-Box Bacteria xC* and *PCR-Box Fungi xC* a thermocycler or a qPCR instrument is necessary. The requirements and necessary technical features are described in Accessories and Device Combinations.

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hybcell Bacteria DNA xC/ hybcell Fungi DNA xC/ hybcell FungiPlus DNA xC

Dependent on the PCR result different *hybcell* tests for identification are offered. *hybcell Bacteria DNA xC* is used when the identification of bacteria is intended (PCR for bacteria shows amplification) and *hybcell Fungi DNA xC* or *hybcell FungiPlus DNA xC* are used when identification of fungi is intended (PCR for fungi shows amplification).

The qualitative analysis provided by *hybcell* is performed by applying *compact sequencing:* The amplicons resulting from *PCR-Box Bacteria xC* or *PCR-Box Fungi xC* bind to their complementary, immobilized probes on the *hybcell*. The probes are elongated by a highly-specific DNA polymerase in case of a perfect match (primer extension). Unspecific amplicons and non-elongated primers are removed during stringent washing steps. As a last step, the *hyborg Dx RED2/3* instrument scans and analyzes the specific fluorescence signals and provides a comprehensive report.

hybcell Bacteria DNA xC:

Tested bacterial species and genera for *hybcell Bacteria DNA xC:*

PCR-Box Bacteria xC + hybcell Bacteria DNA xC:

A		*Abiotrophia defectiva / Granulicatella elegans*
		Acinetobacter baumannii *Acinetobacter calcoaceticus / baumannii complex*
		Actinobacillus pleuropneumoniae
		Aerococcus urinae *Aerococcus viridans*
		Aggregatibacter actinomycetemcomitans *Aggregatibacter aphrophilus*
	Alcaligenes	
	Anaerococcus	
B		*Bacillus cereus*
		Bacteroides fragilis
	Bartonella	*Bartonella bacilliformis / quintana*
		Bordetella pertussis / parapertussis
	Borrelia	
	Borreliella	
	Brucella	
		Burkholderia cepacia complex *Burkholderia pseudomallei group*
C	**Campylobacter**	
	Capnocytophaga	
		Cardiobacterium hominis *Cardiobacterium valvarum*
	Chlamydia	
		Citrobacter freundii *Citrobacter koseri*
		Clostridium perfringens
		Corynebacterium diphtheriae *Corynebacterium jeikeium* *Corynebacterium ulcerans*
		Coxiella burnetti
		Cutibacterium acnes *Cutibacterium avidum*
E	**Ehrlichia**	
		Eikenella corrodens
		Enterobacter asburiae/ cancerogenus *Enterobacter cloacae* *Enterobacter cloacae complex* *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**	*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 lugdunensis*
		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* *Yersinia pseudotuberculosis complex*

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hybcell Fungi DNA xC:

Tested fungal species and genera for *hybcell Fungi DNA xC:*

PCR-Box Fungi xC + hybcell Fungi DNA 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*
N		*Nakaseomyces bracarensis* *Nakaseomyces glabratus* *Nakaseomyces nivariensis*
P	**Pichia**	*Pichia kudriavzevii*
		Pneumocystis jirovecii
S	**Sacharomyces**	*Saccharomyces cerevisiae*
	Scedosporium	

hybcell FungiPlus DNA xC:

Tested fungal species and genera for *hybcell FungiPlus DNA xC:*

A		*Arthroderma insingulare/lenti-culare* *Arthroderma quadrifidum* *Arthroderma uncinatum*
	Aspergillus	*Aspergillus clavatus* *Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus niger* *Aspergillus terreus*
B		*Blastomyces dermatitidis*
C	**Candida**	*Candida albicans* *Candida dubliniensis* *Candida parapsilosis* *Candida tropicalis*
		Candidozyma auris *Candidozyma duobushaemulonii* *Candidozyma haemulonii*
	Cladosporium	
		Coccidioides immitis/posadasii
	Cryptococcus	*Cryptococcus gattii* *Cryptococcus neoformans*
	Curvularia	
D		*Debaryomyces hansenii*
E		*Epidermophyton floccosum*
F		*Fusarium dimerum* *Fusarium fujikuroi* *Fusarium oxysporum* *Fusarium solani*
H		*Histoplasma capsulatum*
M	**Malassezia**	
		Meyerozyma guilliermondii
	Microsporum	
	Mucorales	*Mucor circinelloides/ramosissimus* *Mucor irregularis* *Mucor racemosus*
N	**Nakaseomyces**	*Nakaseomyces bracaransis* *Nakaseomyces glabratus* *Nakaseomyces nivariensis*
	Nannizzia / Lophophyton gallinae	
P		*Paracoccidioides brasiliensis/lutzii*
		Paraphyton cookei/cutaneum
	Pichia	*Pichia kudriavzevii*
		Pneumocystis jirovecii
	Pseudogymnoascus	
R	**Rhizopus**	
S	**Saccharomyces**	*Saccharomyces cerevisiae*
	Scedosporium	
		Scopulariopsis brevicaulis
	Sporothrix	
T		*Talaromyces marneffei*
		Trichophyton benhamiae *Trichophyton interdigitale/* *Paraphyton cookiellum* *Trichophyton rubrum* *Trichophyton soudanense* *Trichophyton tonsurans* *Trichophyton violaceum*
Z		*Zygosaccharomyces rouxii*

For processing *hybcell Bacteria DNA xC, hybcell Fungi DNA xC,* or *hybcell FungiPlus DNA xC,* a *hyborg Dx RED2*/3 instrument with preinstalled hyborg Software (Cube Dx) is required.

The tests are used for the identification of bacteria and / or fungi from different human samples. Therefore, results for some bacterial or / and fungal targets might not always be relevant for the clinical diagnosis, as they might not be considered as pathogenic in the given circumstances. Some of the tested bacteria and fungi can even be considered as contaminants in certain circumstances (e.g. *Cutibacterium acnes* is rather considered as a contaminant when testing whole blood samples).

Therefore the scope of the results can be narrowed by defining profiles within the software of the *hyborg Dx RED2/3* - by selecting the targets which should be considered for the report. Learn more about how results are interpreted and possible limitations regarding the results in section Interpretation of Results.

Result may be falsified due to technical errors (e.g. not filling in PPE Additive, pipetting wrong volumes, etc.) or errors during amplification (e.g. inhibition) or identification. If incorrect or deteriorated results are suspected, these results should not be taken into account. Even if several controls should single out the most erroneous results, some of these results may remain uncovered.

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PCR-Box Res g+ xC / PCR-Box Res g- AB xC / PCR-Box Res g- CD xC

In case of a positive bacterial amplification and/or identification one or two multiplex PCR tests can be started to determine the presence of any antimicrobial resistance genes.

PCR-Box Res g+ xC, PCR-Box Res g- AB xC and *PCR-Box Res g- CD xC* are in-vitro diagnostic tests for the detection of bacterial antibiotic resistance genes. The tests also amplify DNA of the IC (Internal Control) inside the PCR-mixes.

The products are provided as ready-to-use mixes which contain all buffers, enzymes, DNA primers, internal control (IC) and Taqman probes in a single mix .

A synthetic long DNA oligo serves as internal control (IC) to confirm the validity of results (especially of negative results) in a separate PCR channel.

The mixes are equipped with an Uracil-N-Glykosylase (UNG) enzyme to prevent carryover contaminations. The built-in hot-start functionality of the PCR mix allows working without cooling beds.

In each PCR kit one tube of positive control (PTC.. Positive Template Control, solution containing synthetic oligos) and one tube with negative control (NTC.. Non Template Control, empty elution buffer) are added. The synthetic oligo of the PTC for *PCR-Box Res g+ xC* mimics *Van B*, the one for *PCR-Box Res g- AB xC* mimics *IMP* and *PCR-Box Res g- CD xC* mimics *Oxa48*.

The tests indicate if certain bacterial resistance genes associated with either gram-positive or gram-negative bacteria are present in the sample. If yes, certain antibiotic resistances can be expected with high probability. The result is derived by analysing the amplification curves and Ct-values of different fluorescence channels (see below).

Attention! The tests do not provide phenotypic information on antibiotic resistances.

PCR-Box Res g+ xC:

Target	Fluorophore
mecA, mecC	FAM
vanA	JOE
vanB	Cy5
IC	CAL.RED

PCR-Box Res g- AB xC:

Target	Fluorophore
NDM, VIM, IMP	FAM
KPC	JOE
CTX-M (Group 1: CTX-M-1, -3, -10, -15, -32, -37, -55, -57, -71, -82, -101, -182)	Cy5
IC	CAL.RED

PCR-Box Res g- CD xC:

Target	Fluorophore
OXA48	FAM
AmpC	JOE
mcr-1	Cy5
IC	CAL.RED

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The PCR-mixes are provided as single reactions, pre-filled in 0.2 mL PCR tubes (20 µL each).

For processing *PCR-Box Res g+ xC, PCR-Box Res g- AB xC* and *PCR-Box Res g-CD* a thermocycler or a qPCR instrument is necessary. The requirements and necessary technical features are described in Accessories and Device Combinations.

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4. Product Components

GINA 500, GINA 500 + DNA purification, GINA 1000, GINA Lysis 200:

- *GINA 500* (REF / UDI-DI 09120127730244)
  - 100 x 1400 µL *LE Solution* (4 boxes with 25 microtubes (2 mL) each filled with 1400 µL *LE Solution*)
  - 100 x 200 µL *NA Solution* (4 boxes with 25 microtubes (2 mL) each filled with 200 µL *NA Solution*)
- *GINA 500 + DNA Purification* (REF / UDI-DI 09120127730145)
  - 50 x 1400 µL *LE Solution* (2 boxes with 25 microtubes (2 mL) each filled with 1400 µL *LE Solution*)
  - 50 x 200 µL *NA Solution* (2 boxes with 25 microtubes (2 mL) each filled with 200 µL *NA Solution*)
  - 50 x 400 µL *T Solution* (2 boxes with 25 microtubes (2 mL) each filled with 400 µL *T Solution*)
  - 1 x 30 mL *Wash Buffer BW* (1 bottle filled with 30 mL *Wash Buffer*)
  - 1 x 60 mL *Wash Buffer B5* (1 bottle filled with 60 mL *Wash Buffer B5*)
  - 1 x 13 mL *Elution Buffer BE* (1 bottle filled with 13 mL *Elution Buffer BE*)
  - 50 x *Column* (1 bag with 50 *Columns*)
  - 50 x *Collection Tube* (1 bag with 50 *Collection Tubes*)
  - 50 x *Elution Tube* (1 bag with 50 *Elution Tubes*)
- *GINA 1000* (REF / UDI-DI 09120127730480)
  - 48 x 2800 µL *LE Solution* (48 pieces Eppendorf tubes (5mL) each filled with 2800 µL *LE Solution*)
  - 48 x 200 µL *NA Solution* (48 pieces microtubes (2 mL) each filled with 200 µL *NA Solution*)
- *GINA Lysis 200* (REF / UDI-DI 09120127730664)
  - 50 x Sterile *Forensic Swab* (50 pieces Sarstedt 80.629)
  - 50 x 400µL *NA Solution* (50 pieces microtubes (2 mL) each filled with 400 µL *NA Solution*)

LINA:

- *LINA* (REF / UDI-DI 09120127730152)
  - 50 x 8 mL *LINA* (1 bag with 50 tubes filled with 8 mL *LINA*)

PCR-Boxes xC:

- *PCR-Box Bacteria xC 24rx-s* (REF / UDI-DI 09120127730701)
  - 24 x 20 µL PCR-mix Bacteria xC
    (2 bags with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix Bacteria xC)
  - 1x 100 µL PTC
    (1 tube (0.5 mL) filled with 100 µL PTC (positive template control - synthetic oligo mimicking B.fragilis DNA))
  - 1 x 1000 µL NTC
    (1 tube (2 mL) filled with 1000 µL NTC (no template control))
- *PCR-Box Fungi xC 24rx-s* (REF / UDI-DI 09120127730633)
  - 24 x 20 µL PCR-mix Fungi
    (2 bags with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix Fungi xC)
  - 1x 100 µL PCT
    (1 tube (0.5 mL) filled with 100 µL PTC (positive template control - synthetic oligo mimicking Pichia spp. DNA))
  - 1 x 1000 µL NTC

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(1 tube (2 mL) filled with 1000 µL NTC (no template control))

- *PCR-Box Res g+ xC 24rx-s* (REF / UDI-DI 09120127730640)
  - 24 x 20µl PCR-mix *Res g+ xC*

    (2 bags with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix Res g+ xC)
  - 1 x 100µL PCT (1 tube (0.5 mL) filled with 100 µL PTC

    (1 tube (0.5 mL) filled with 100 µL PTC (positive template control - synthetic oligo mimicking VanB DNA))
  - 1 x 1000 µL NTC

    (1 tube (2 mL) filled with 1000 µL NTC (no template control))
- *PCR-Box Res g- AB xC 24rx-s* (REF / UDI-DI 09120127730657)
  - 24 x 20 µl PCR-mix Res g- AB xC

    (2 bags with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix Res g- AB xC)
  - 1 x 100µL PCT

    (1 tube (0.5 mL) filled with 100 µL PTC (positive template control - synthetic oligo mimicking IMP DNA))
  - 1 x 1000 µL NTC

    (1 tube (2 mL) filled with 1000 µL NTC (no template control))
- *PCR-Box Res g- CD xC 24rx-s* (REF / UDI-DI 09120127730695)
  - 24 x 20 µl PCR-mix Resistance g- CD xC

    (2 bags with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix Res g- CD xC)
  - 1 x 100µL PCT

    (1 tube (0.5 mL) filled with 100 µL PTC (positive template control - synthetic oligo mimicking OXA48 DNA))
  - 1 x 1000 µL NTC

    (1 tube (2 mL) filled with 1000 µL NTC (no template control))

hybcell DNA xC

- *hybcell Bacteria DNA xC* (REF / UDI-DI 09120127730336)
  - 24 x *hybcell Bacteria DNA xC* (24 *hybcells* separately sealed in plastic foil)
  - 24 x *Lid* (1 bag with 24 *Lids*)
  - 1x 900 µL *PPE-Additive* (1 tube (2 mL) filled with 900 µL *PPE-Additive*)
- *hybcell Fungi DNA xC* (REF / UDI-DI 09120127730404)
  - 24 x hybcell Fungi DNA xC (24 *hybcells* separately sealed in plastic foil)
  - 24 x *Lid* (1 bag with 24 *Lids*)
  - 1x 900 µL *PPE-Additive* (1 tube (2 mL) filled with 900 µL *PPE-Additive*)
- *hybcell FungiPlus DNA xC* (REF / UDI-DI 09120127730367)
  - 24 x hybcell FungiPlus DNA xC (24 *hybcells* separately sealed in plastic foil)
  - 24 x *Lid* (1 bag with 24 *Lids*)
  - 1x 900 µL *PPE-Additive* (1 tube (2 mL) filled with 900 µL *PPE-Additive*)

Pay attention not to mix up components of different lots!

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5. Storage, Transportation, Shelf Life and Disposal

All products have to be kept dry and should be protected from sunlight. The maximum shelf life of products is only guaranteed if the products are kept at required temperatures during transportation and storage. The expiry date of the products is printed on the product labels.

Products	Shelf life [months]	Temperatures [°C]		Disposal
		Storage	Transport	
GINA 500 *GINA 500 + DNA Purification* *GINA 1000* *GINA Lyse 200*	24	8 to 25°C	4 to 40°C	LE-solution: potentially infectious
LINA	24	8 to 25°C	4 to 40°C	Potentially infectious
hybcell Bacteria DNA xC *hybcell Fungi DNA xC* *hybcell FungiPlus DNA xC*	24	8 to 25°C	4 to 40°C	Residual waste
IPC	24	-15 to -25°C	< 0°C	Residual waste
PCR-Box Bacteria xC *PCR-Box Fungi xC* *PCR-Box Res g+ xC* *PCR-Box Res g- AB xC* *PCR-Box Res g- CD xC*	24	-15 to -25°C	< 0°C	Residual waste

If the protective sealing of hybcells or any other packaging (e.g. tubes) is damaged / the minimum shelf life has expired / the storage conditions could not be met, the product must not be used.

hybcells have to be used immediately after opening the protective sealing.

Freezing of PCR reactions after thawing destroys the product and the product must not be used. Thawed PCR reactions have to be used immediately after thawing.

Disposal

All single-use materials (PCR tubes, *hybcells*, pipette tips, etc.) can be disposed of without any special procedures. The usual precautions for potentially infectious material have to be applied.

Patient sample containers (e.g., EDTA tubes), *LINA, LE Solution* tubes (*GINA 500/1000*) and *NA Solution* tubes from *GINA Lyse 200* potentially contain infectious material due to their direct contact with the sample and have to be disposed complying with your organization's rules for the disposal of infectious material.

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Storage of Samples

EDTA Blood

- Processing of a fresh sample should start within 4 hours after sample taking. Keep the sample at room temperature (between 8°C and 25°C) or in the fridge (between 4°C and 8°C) before starting the test.
- Store in the fridge (between 4°C and 8°C) for a maximum of 48 hours.
- Do not process previously frozen EDTA whole blood samples if working with *GINA*!
- If working with geneLEAD VIII and not using GINA for sample preparation (using EDTA blood directly), samples can be stored in the freezer (-15°C and -25°C) for a maximum of 1 month.

Other samples (Respiratory Samples, Soft Tissue, Sterile Fluids, Blood Culture, etc.)

- Processing of a fresh sample should start within 4 hours after sample taking. Keep the sample at room temperature (between 8°C and 25°C) or in the fridge (between 4°C and 8°C) before starting the test.
- Store in the fridge (between 4°C and 8°C) for a maximum of 48 hours.
- Freezing samples should be avoided. If necessary store in the freezer (-15°C and -25°C) for a maximum of 1 month.

Storage of Lysates, Eluates and Amplicons

Processing of samples may be interrupted for example due to time constraints. However, processing must be carried on until either a lysate (result of the *GINA* process), an eluate (result of the DNA purification) or an amplicon (result of the PCR process) is available.

- Keep at room temperature (between 8°C and 25°C) for a maximum of 4 hours.
- Store in the fridge (between 4°C and 8°C) for a maximum of 18 hours.
- Store in the freezer (between -15°C and -25°C) for a maximum of 1 month.

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6. Accessories and Device Combinations

The following accessories and equipment are required for conducting the test procedure:

Required Accessories / Infrastructure		REF / UDI-DI	Alternative products acceptable?
Mini-centrifuge (0,2 mL rotor)	Thermo[1]: MySpin		yes
Mini Vortex mixer	Fisher Scientific[2]		yes
Freezer (-20°C)	--		--
DNA workbench	Starlab[3]: GuardOne Laminar Flow workbench		yes
Pipettes: ▪ 20 – 200 µL ▪ 100 – 1000µl	GILSON[4]: PIPETMAN P200N PIPETMAN P1000N		yes
Standard table centrifuge (With rotor for 2 mL tubes)	Eppendorf[5]: Centrifuge 5430		yes, with 11.000g
Standard heating block	Coyote Bioscience[6]: H2O3-H		yes
DNA extractor and qPCR device	PSS[7]: geneLEAD VIII		no
qPCR device or thermal cycler	Biorad[8]: CFX96 Thermo Fisher Scientific[9]: Quantstudio 3/5 Analytic Jena[10]: Biometra TOne Thermocycler		no
System Liquid	Cube Dx: 1 L, sufficient for 8 weeks	09120127730022	no
CS Buffer	Cube Dx: 1 L, sufficient for 96 *hybcells*	09120127730503	no
hyborg	Cube Dx: hyborg Dx RED2/3	09120127730015	no

Device Combinations

For DNA extraction / purification and follow-up PCR, different options regarding instrumentation are possible. The corresponding device combinations are:

1 www.thermofisher.com / order / catalog / product / 75004081
2 www.fishersci.com / shop / products / variable-speed-mini-vortex-mix / 14955163
3 www.starlab.de
4 www.gilson.com
5 www.eppendorf.com
6 www.coyotebio.com
7 www.pss.co.jp/english/
8 www.bio-rad.com
9 www.thermofisher.com
10 www.biometra.com

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Option 1: Using geneLEAD VIII from PSS for DNA extraction and PCR

DNA Extraction + PCR: geneLEAD VIII (PSS)

Microbial ID: hyborg Dx RED2/3 (Cube Dx)

Option 2: Manual DNA extraction and PCR with conventional PCR instruments

DNA Extraction: Centrifuge 5430 (Eppendorf) - or equivalent

PCR: CFX 96 (Biorad) or Quantstudio 3/5 (Thermo) or Biometra TOne (Analytic Jena)

Microbial ID: hyborg Dx RED2/3 (Cube Dx)

The kits have been validated for use with the instruments/equipment listed above. The use of equipment not explicitly listed may affect the performance characteristics. If the user chooses to employ alternative equipment (e.g. another PCR instrument or a DNA extractor for DNA purification), it is the responsibility of the user to perform a performance evaluation for the new device combination to ensure results are equivalent and reliable.

The performance evaluation has to comply to IVDR regulations. Cube Dx provides a guideline document in English with suggestions how to structure such evaluation: Pathogens xB+xC_guidline device combination evaluation (download under www.cubedx.com/documents).

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7. Test Procedure

!Before beginning the test procedure. Assure that the used instruments (for example geneLEAD VIII, PCR instrument, hyborg Dx RED2/3) are ready for operation!

- Check readiness of all used lab instruments (e.g. geneLeadVIII, PCR instruments, etc.)
- Check if the *hyborg* is switched on (check the screen of the device – refer to the latest *hyborg Dx* IFU, available under www.cubedx.com/documents, for further details).
- Check if the *hyborg* is equipped with sufficient *System Liquid* and *CS Buffer*. If not, refill these liquids.
- Empty the waste container if necessary.
- Check if the necessary *hybcell* protocol is available (if not, load the protocol, available under www.cubedx.com/protocols, refer to the *hyborg Dx* IFU for further details).

Note that some steps of the procedure require the preparation of equipment or reagents or the thawing of reagents. As these tasks may be associated with waiting times, read the entire chapter of the procedure before starting.

During processing the samples, a laboratory coat, disposable gloves, sleeve guards, a surgical mask and if reasonable a hair and beard net must be worn to avoid contamination of the test reagents. Pathogen enrichment (*GINA* process) and PCR preparation must be done under a PCR workbench or laminar flow box (or a similar area protected against microbial contamination). Steps that should be done under these conditions are written in red in the following.

For more details read our recommendations and guidelines on how to prevent from contaminations: Pathogens xB+xC_guidline contamination prevention (download under www.cubedx.com/documents).

In the following sections, the workflow is described based on the following 3 steps;

1. Sample Preparation of different sample types (e.g. enrichment of whole blood with *GINA 500 / GINA 1000*)
2. DNA/RNA Extraction and Detection with PCR/qPCR (different configurations of instruments)
3. Identification: compact sequencing on *hyborg Dx RED 2/3*

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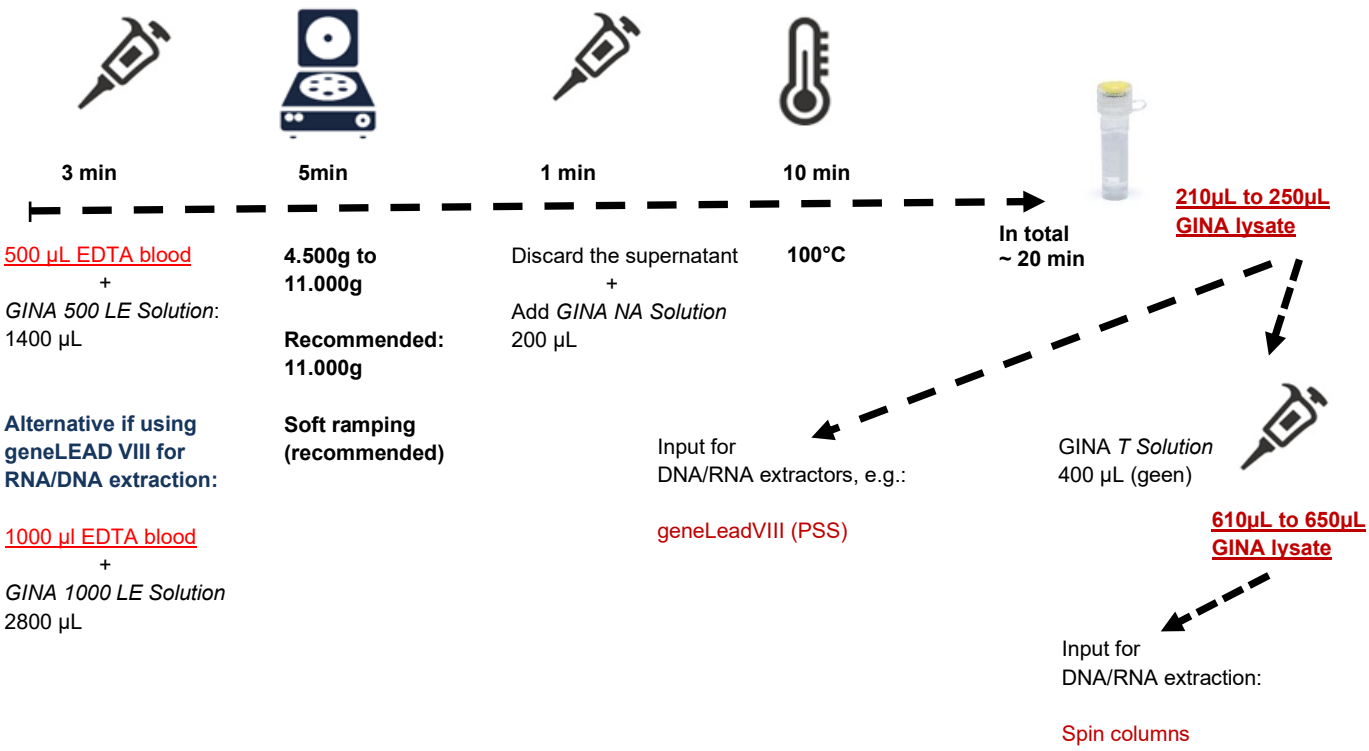
1 Sample Preparation

EDTA whole blood – enrichment of microbes: GINA 500 / GINA 1000

Whole blood samples can be collected in K3EDTA or K2EDTA Vacuette tubes (Greiner BioOne) or similar products from other manufacturers.

Any microbial contamination during the sampling process will falsify the later result. Therefore, we recommend sensitizing medical staff taking the samples regarding this issue. If necessary, train the medical staff accordingly.

The procedure starts with a native sample of EDTA-whole blood of either 500 µL (or less) or 1000 µL. Vortex the sample before use! The enrichment uses 2 different buffers and requires a centrifugation and heating step.



Required products: *GINA 500 / GINA 500 + DNA purification / GINA 1000*

1. Ensure that the equipment and all kit components are ready for use. Before opening the tubes like *LE Solution* or any other material (e.g. spike material like *EPC S.aureus 10000*) briefly spin or shake down to avoid carry-over of liquids potentially present in the screw caps. Start the heating block and set temperature to 100°C. Make sure the used tubes fit into the cavities of the heating block (*LE Solution* and if using spin column the *Elution Tubes* as well).

   Remark:

   The centrifuge requires ***a rotor*** *for 2 mL tubes. When working with the larger LE tubes of GINA 1000* ***different rotors (or adapters)*** *(for 5 mL and 2 mL tubes) are required.*

   Check if the recommended g-force of 11,000g (or at least 4,500) is provided by the centrifuge. The centrifuge should as well provide the option for soft ramping. Don't mix up rotational speed (rpm) and centrifugal force (g)!

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1. Prepare *LE Solution* and the whole blood sample. **Do not shake or agitate the *LE Solution* tube to avoid the build-up of foam**! Transfer 500 µL (or less) or 1000µL for GINA 1000 of EDTA blood into the *LE Solution* and pipette up and down to mix.

2. Optional: Pipette 500 µL / 1000 µL of the blood sample into prepared spike material like Cube Dx' *EPC S.aureus 10000* or *EPC C.albicans 10000* and thereafter transfer the mixture into the LE Solution.

3. Close the tube, mark it, and vortex vigorously for 5 seconds or invert several times till the liquid appears homogeneous. Incubate for ~2 min at room temperature (18°C to 25°C).

4. Centrifuge for 5 minutes between 4.500g and 11.000g (preferably 11.000g). If available, use soft ramping for the centrifugation to prevent loosing the pellet.

5. Remove the supernatant carefully by **decanting** and add 200 µL *NA Solution* into the *LE Solution* tube. Close the screw cap tightly.

   Remark:

   Some supernatant (~50 µL) may stay on top of the pellet after decanting. Whole blood samples should ***turn greenish*** *at this point.*

6. Vortex vigorously for 5 seconds. Make sure that the tubes are still tightly closed.

7. Incubate at 100°C for 10 minutes, using a heating block.

8. If you are using GINA 1000 and processing 1000 µL blood: Transfer the whole volume (~ 250 µL) to the *NA Solution* tube.

9. If continuing with the spin columns provided in the *GINA 500 + DNA purification* kit, add 400 µL of *T Solution* to the tube.

   Remark:

   Whole blood samples should turn ***from greenish to dark reddish****.*

Positive blood cultures: LINA

Required products: *LINA*

1. If testing positive blood cultures: Invert the blood culture bottle several times and use a sterile syringe to extract 20-50 µL through the septum of the blood culture bottles. Deposit the sample in a sterile and DNA-free tube (e.g. 2 mL tube).
2. Pipette the sample into the *LINA* tube:
   - **2 µL** of **positive blood culture**
3. Close the *LINA* tube and invert several times or vortex firmly.
4. No further DNA extraction is needed. Use the solution directly for PCR (20 µL of the solution for each PCR reaction).

Samples from respiratory tract, swabs and other viscous samples: GINA Lyse 200

Required products: *GINA Lyse 200*

1. Use the swab of the *GINA Lyse 200* kit to take up the sample by putting the swab into the sample tube. The swab transfers around 150 µL to 200 µL of sample solution. Twist the swab and let it rest in the sample for around 10 seconds. If taking a nasopharyngeal swab (or any other swab taking a sample from a patient), do not use the provided forensic swab but a more suitable swab for sample taking.

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2. Put the swab into a tube with *NA Solution* (400 µL) and twist the swab in the *NA Solution* for around 10 seconds.
3. Discard the swab and close the tube with the *NA Solution* and sample.
4. Vortex vigorously for 5 seconds.
5. Incubate at 100°C for 10 minutes, using a heating block.
6. If you use RNA/DNA extraction based on spin columns, add 400 µL *T Solution*.

Soft Tissue samples: GINA Lyse 200

Required products: *GINA Lyse 200*

1. Open the tube with the *NA Solution* (400 µL) and put a piece of tissue into the tube. Close the tube.
2. Vortex vigorously for 5 seconds.
3. Incubate at 100°C for 10 minutes, using a heating block.
4. If you use RNA/DNA extraction based on spin columns, add 400 µL *T Solution* before starting extraction.

Sterile samples (e.g. EDTA blood, CSF, synovial fluid, pleura fluid…): GINA Lyse 200

Required products: *None or*
GINA Lyse 200

Sterile fluids do usually not require a special sample preparation and the sample can be used directly for RNA/DNA extraction.

However, under following circumstances we recommend the procedure below:

- if you suspect a **fungal infection**,
- if you suspect an infection with **bacteria** that are **hard to lyse** or
- if the sterile fluid comes **with solid constituents** (e.g. synovial fluid) or is **viscous and hard to pipette**.

1. Use the swab of the *GINA Lyse 200* kit to take up the sample by putting the swab into the sample tube. The swab transfers around 150 µL to 200 µL of sample solution. Twist the swab and let it rest in the sample for around 10 seconds.
2. Put the swab into a tube with *NA Solution* (400 µL) and twist the swab in the *NA Solution* for around 10 seconds.
3. Discard the swab and close the tube with the *NA Solution* and sample.
4. Vortex vigorously for 5 seconds.
5. Incubate at 100°C for 10 minutes, using a heating block.
6. If you use RNA/DNA extraction based on spin columns, add 400 µL *T Solution* before starting extraction.

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2 RNA/DNA extraction and detection by PCR/qPCR (including resistance genes)

The test procedure starts with the solutions resulting from the procedures described above (1 Sample Preparation) for example with *GINA* lysate, mixture of *LINA* and sample, mixture of *NA Solution* and sample or with the sample itself. Two available options differ in the used instrumentation and hence in the workflow.

Option 1: Using geneLEAD VIII from PSS for DNA extraction and PCR

Option 2: Manual DNA extraction and PCR with conventional PCR instruments (see Accessories and Device Combinations for details).

The **elution volume should be 100 µL** – regardless of the option you have chosen.

Option 1: geneLEAD VIII

Required products:
Kits for RNA/DNA extraction for the geneLEAD VIII (PSS)
PCR-Box Bacteria xC 24rx-s / PCR-Box Fungi xC 24rx-s
PCR-Box Res g+ xC 24rx-s
PCR-Box Res g- AB xC 24rx-b / PCR-Box Res g- CD xC 24rx-s

Dependent on the sample type, the loading of the instrument needs to be adjusted.

Samples processed with GINA 500 / GINA 1000 or GINA Lyse 200

Remove the lid from the *LE Solution* tube or *NA Solution* tube after completion of the *GINA* process and use this tube as the sample container for the geneLEAD VIII instrument.

Positive blood cultures processed with LINA

Use an *Elution Tube* from the *geneLEAD VIII Consumable Set* (F8900) and pipette 50 µL of the *LINA*-sample-mix into this tube.

Already available eluates

Use an *Elution Tube* from the *geneLEAD VIII Consumable Set* (F8900) and pipette at least 20 µL of the eluate into the *Elution Tube*. If the eluate is already collected in the *Elution Tube*, use this tube directly.

Different samples without sample preparation

Use a *Sample Tube* from the *geneLEAD VIII Consumable Set* (F8900) and pipette the sample into the *Sample Tube* (the higher the sample volume, the higher the potential sensitivity):

- EDTA whole blood: 60 µL to 450 µL
- All other samples: 60 µL to 230 µL

Loading and starting the instrument

1. Unpack and thaw the necessary PCR-mixes (see above).
2. Once you have taken out the required PCR-mixes, freeze the kit immediately again (-15°C to -25°C).
3. Spin down PCR-mixes and pipette 20 µL each (=volume of a single PCR-mix tube) deeply into the first well (orientation from the back of the instrument) of each *PCR Reagent Cassette* of the instrument (geneLEAD VIII PCR Reagent Cassette Set / F8820). **Pay attention that the pipetted PCR-mix covers the well and no air bubbles are trapped on the bottom!**
4. Load the instrument for RNA/DNA extraction and follow-up PCR. Most of the consumables for the geneLEAD VIII (except *Sample Tube* and *Elution Tube*) are shaped like right-angled bars. All consumables

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have to be put into the instrument, so that the angle pointing upwards appears on the left side when looking into the instrument from the front (door side).

- Tray 1 (closer to the back of the instrument):
    A. Load the *PCR Cassettes* into the instrument (geneLEAD VIII PCR Cassette / F8840) and remove the cover of the cartridges
    B. Load the filled *PCR Reagent Cassettes* (3.) into the instrument (geneLEAD VIII PCR Reagent Cassette Set / F8820)
    C. Only if RNA/DNA extraction is required (*GINA*, *GINA Lyse*, direct sample): Load the extraction cartridge into the instrument (MagDEA Dx SV / E1300) – **Shake down firmly before loading so that magnetic beads are dissolved!**
    - Put Tray 1 into the instrument
- Tray 2 (closer to the front of the instrument):
    D. Only if RNA/DNA extraction is required (*GINA*, *GINA Lyse*, direct sample): Load the sample (*LE Solution* tube or *NA Solution* tube or *Sample Tube*, see above) into the instrument.
    E. Load the tip racks into the instrument (geneLEAD VIII Consumable Set / F8900)
    F. Load the *Elution Tube* into the instrument (geneLEAD VIII Consumable Set / F8900). The *Elution Tube* is conical towards its end (do not mix-up with the *Sample Tube* which is as well provided in the kit). Open the *Elution Tubes*.
        - LINA and positive blood culture + available eluates: already filled / prepared *Elution Tube*
        - All other samples: use a new (and empty) *Elution Tube*
    - Put Tray 2 into the instrument

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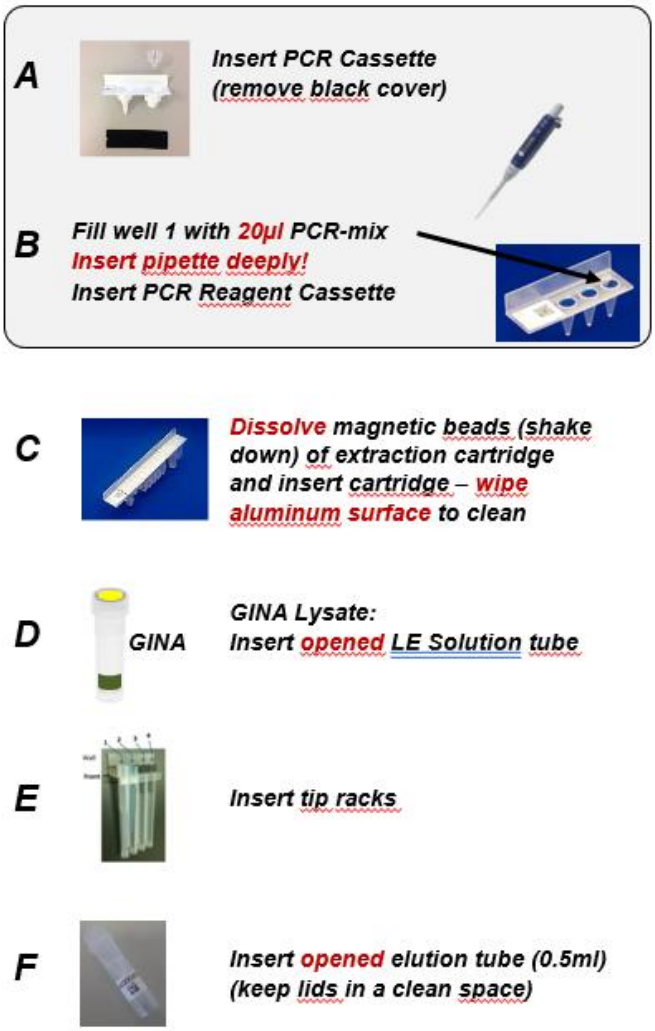

Check if all reagents are „in line" before starting

5. Start the instrument and configure the run according to the instructions for use of the geneLEAD VIII. Main menu → Perform Run → Select: Extraction Input Volume - 400 µL, Extracted Elute Volume - 100 µL.
6. Choose the corresponding protocol named

   CDX_Bacteria_xC_gLVIII_xx or

   CDX_Fungi_xC_gLVIII_xx or

   CDX_Res_g+_xC_gLVIII_xx or

   CDX_Res_g-_AB_xC_gLVIII_xx or

   CDX_Res_g-_CD_xC_gLVIII_xx

   (xx stands for the current version of the protocol) and select ***Extraction + PCR*** for all samples for which RNA/DNA extraction is required and ***PCR Only*** for all tests where an eluate (or LINA-sample-mix) is already available. If the protocols are not available on the instrument, they must be uploaded first (download the file from www.cubedx.com/pcrprotocols, import the file following the instructions for use of the geneLEAD VIII).
7. Analyse results and check the amplification and melting curves of all tracks (samples). Refer to "Interpretation of Results".
8. Remove and close the *Elution Tubes* from the instrument. Remove and discard all consumables except the *PCR Cassettes* of those samples that will be further processed with *hybcell* (see 3. Identification) from the instrument. Take the *PCR Cassettes* to the post-PCR area (where *hyborg Dx RED2/3* is operated).

   The amplified DNA is either used immediately for the identification / compact sequencing reaction using a corresponding hybcell (see below), or stored. The remaining eluate should be stored until the results are approved as well (see Storage, Transportation, Shelf Life and Disposal).
9. Start the UV sterilization after usage of the instrument: Main menu → UV Irradiation

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Attention! Do not open *PCR Cassettes* close to the geneLEAD VIII or any other PCR instrument or in the area for sample preparation!

Option 2: Manual extraction + PCR instrument

Required products: *GINA 500 + DNA Purification kit*
PCR-Box Bacteria xC 24rx-s / PCR-Box Fungi xC 24rx-s
PCR-Box Res g+ xC 24rx-s
PCR-Box Res g- AB xC 24rx-b / PCR-Box Res g- CD xC 24rx-s

DNA purification for this option is accomplished by spin columns. The same centrifuge as for processing whole blood with *GINA* is used during the process. The method requires more hands-on time compared to automated extraction, but is very efficient if only a low number of samples is expected.

The necessary material for DNA purification is provided in the *GINA 500 + DNA Purification* kit.

Samples processed with GINA 500 or GINA Lyse 200

Continue with the *GINA* lysate (including the 400 µL T Solution, see workflow above) at step 1. below.

Already available eluates or positive blood cultures processed with LINA

Continue with step 9. below. Fill 20 µL of eluate or 20 µL of the LINA-sample-mixture into the PCR tubes.

DNA Purification + PCR

1. Place one *Column* into a *Collection Tube* and mark the *Collection Tube* with the sample ID for each sample (= *GINA* lysate + 400 µL *T Solution*). Transfer 650 µL of the sample into the *Column*. Discard the tube for the *LE Solution* or *NA Solution*.

2. Centrifuge for 1 min between 9.000g and 11.000g. Remove the *Column*, decant the liquid in the *Collection Tube* and insert the *Column* again.

3. Add 500 µL *Wash Buffer BW* to the *Column* and centrifuge for 1 minute at between 9.000g and 11.000g. Remove the *Column*, decant the liquid in the *Collection Tube* and insert the *Column* again.

4. Add 600 µL *Wash Buffer B5* to the *Column* and centrifuge for 1 minute at between 9.000g and 11.000g. Remove the *Column*, decant the liquid in the *Collection Tube* and insert the *Column* again.

5. Centrifuge for 3 minutes at between 9.000g and 11.000g to dry the silica membrane. Check if any liquid remains at the bottom of the *Column*. If yes, repeat this step.

6. Place the *Column* into an *Elution Tube* and mark the *Elution Tube* with the sample ID. Add 100 µL *Elution Buffer BE*. Incubate at room temperature for 1 min. Centrifuge for 1 minute at between 9.000g to 11.000g. Check the elution volume. If the volume appears to be too low, repeat centrifugation. Discard the *Column*.

7. Open the *Elution Tube* and incubate with open lid at 100°C for 3 minutes in the heating block.

8. The eluate (collected liquid containing the DNA) should be used immediately or stored for later usage (see Storage, Transportation, Shelf Life and Disposal). Before using the eluate, vortex the *Elution Tube* firmly.

9. Program the (q)PCR device and save the program as *Patho_xC* or download file from www.cubedx.com/pcrprotocols and import into device.

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1	95°C for 2:00
2	95°C for 0:10
3	56°C for 0:10
4	72°C for 0:30
	+Plate Read (FAM)
	GO TO 2, 44 times
5	72°C for 1:00
6	Melt curve 75°C to 95°C in increments of 0.3°C for 0:10
	+Plate Read
7	25°C for hold

10. Program the (q)PCR device and save the program as *Resi_xC* or download file from www.cubedx.com/pcrprotocols and import into device.

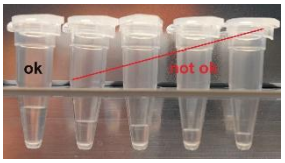
1	95°C for 2:00
2	95°C for 0:15
3	58°C for 1:00
	+Plate Read (FAM/JOE/CAL.RED/Cy5)
	GO TO 2, 44 times
4	25°C for hold

Remark!

Individual PCR devices may differ in their thermal characteristics. Therefore, the ***optimization of the temperatures is recommended*** *when unexpected results are observed.*

As the both PCR protocols for bacteria/fungi and for resistance genes differ, the corresponding PCRs must be separated in different runs!

11. Unpack and thaw single 0.2 mL tubes of the required PCR-mixes for bacteria, fungi, and resistance genes. Homogenize (vortex) and spin down the liquid of each tube briefly.
12. Check if the volume of the PCR-mix is approximately 20 µL (see picture, the left tube is filled with 20 µL). Do not use PCR-mixes that have been improperly filled.
13. Add 20 µL DNA eluate from the sample / 20 µL from the LINA-sample-mixture (or 20 µL of NTC/PTC – included in the kits) to the PCR-mixes.



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14. Close PCR tubes, homogenize, and spin down liquids before starting PCR.
15. Start one of the (q)PCR programs: *Patho_xC* or *Resi_xC* (see above).

    The amplified DNA is either used immediately for the identification / compact sequencing reaction using a corresponding hybcell (see below), or stored (see "5. Storage, Transportation, Shelf Life and Disposal").

16. Analyse results and check the amplification and melting curves of all wells / samples (refer to Interpretation of Results).

Attention! Do not open PCR tubes close to the PCR instrument or in the area for sample preparation!

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3 Identification: using hybcell and compact sequencing

The test procedure starts with one amplicon resulting from (q)PCR (see above).

Required products: *hybcell Bacteria DNA xC*
hybcell Fungi DNA xC / hybcell Fungi DNA xB

1. Assure that the *hyborg* is ready for operation.
2. Open the packaging of the *hybcell* (rip the packaging at the notch) and place the *hybcell* into the rack (positions A-H).
3. Thereafter pipette 30µL of the PPE-additive (comes with the hybcell kit) into the tube with the amplicon.
4. Pipette up and down to mix (**a pH indicator is present in the additive; the color of the solution may therefore change upon introducing the amplicons. This does not influence the performance of the product**). Avoid bubbles!

   Pipette the entire volume from the tube (~ 70 µL) into the hybcell (through the central channel) at once.

   Use a 200 µL pipette with appropriate filter tips! Do not block the hybcell's central channel (sample inflow) with the pipette tip while introducing the amplicon mix! Insert the tip deeply (but not too deep).

   Different *hybcells* require different amplicons (from different *PCR-Boxes*).

Used PCR-Box	Required hybcell
PCR-Box Bacteria xC	*hybcell Bacteria DNA xC*
PCR-Box Fungi xC	*hybcell Fungi DNA xC*
PCR-Box Fungi xC	*hybcell FungiPlus DNA xC*

5. Cover the *hybcell* using the provided *Lid*.

Insert the tip of the pipette deeply into the central channel of hybcell – but do not completely close the channel

Try not to wet the inside of the central channel

After pipetting put on the Lid

6. Start processing the samples after entering the sample and *hybcell* ID (see *hyborg Dx RED2/3* instructions for use (download under www.cubedx.com/documents) for further details). Load the device with the prepared rack (*hyborg Dx RED2*) or the prepared hybcells (*hyborg Dx RED3*).

   Insert rack correctly (hybcell barcodes/labels have to face the inside of the device)! Pay attention that all hybcells are in the correct position.

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8. Interpretation of Results

PCR Analysis

PCR results like amplification and melting curves are key determinants of whether processing of a sample can be considered successful (e.g. no PCR inhibition suspected). Furthermore the presence of bacterial or fungal DNA or resistance genes is derived from the results.

Attention!

Be aware that every PCR device is slightly different in its characteristics and that Ct-values and melting temperatures vary. Therefore, all suggested cut-off values might vary as well and should be seen as recommendations.

Every lab must verify these recommendations and, if necessary, adjust to its own thresholds. The final positive result is established by the hybcell test. So, consider including all samples in your hybcell runs where there might be the slightest doubt that the PCR is negative. Especially during setting up the test in your lab and in the early phase of using it, be more generous with including samples in your hybcell testing.

Some reasons for variations in Ct-values and melting temperatures:

- The threshold for the Ct-value calculation is set differently by the user
- Different PCR devices offer different software with different characteristics. For example, auto-scale, threshold settings, and so forth, which might influence the Ct-values and the visual presentation of the curves.
- *PCR-Box Res g+ xC / PCR-Box Res g- AB xC / PCR-Box Res g- CD xC* contain several primer pairs (testing different resistance genes). Therefore, primer dimers are more likely to occur than with the singleplex PCR such as *PCR-Box Bacteria xC* or PCR-Box *Fungi xC*.
- Bacterial or fungal contaminations acquired during sample taking or the test procedure lower the Ct-value. Possible reasons for contamination are described in our brief guidelines for contamination prevention. The guidelines provide information on necessary infrastructure, sample processing, required protective gear, disinfection of surfaces, etc.
- The salt concentration and other conditions of the eluates might vary due to variances in the composition of samples and usage of different sample collection products.
- Finally, the amplified microorganism itself may influence the Ct-value and even more the melting temperature.

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General workflow

1. Check the amplification and melting curve.
    a. Assess the appearance and shape of the amplification and melting curves
    b. Verify the calculated Ct-values and melting points (approximation)
2. Evaluate if any inhibition might have taken place. This is indicated if the shape of the curves is not as expected (e.g. flat curves) or if, for example, no Ct and/or no melting curve peaks could be calculated for the internal control.
3. Evaluate Ct-values (and melting peaks) according to the rules and examples below. Detailed rules and examples are available in separate guidelines.
4. Determine which samples have tested negative / not detectable for the target.
5. Select all samples (not negative for bacteria or fungi) for further testing with *hybcell*.

PCR-Box Bacteria xC / PCR-Box Fungi xC

All samples that are not clearly tested negative for the targets via PCR have to be further tested with a *hybcell* (identification of the pathogen). A negative result means there is no pathogen DNA or resistance gene present, therefore, there is no need for identification by the *hybcell*.

PCR-Box Bacteria xC 24rx-s and *PCR-Box Fungi xC 24rx-s* not only amplify the respective target (bacteria or fungi), they also have an internal control (IC) included in the PCR-mix as well. These PCR tests should always show amplification and a melting peak of the internal control. In case a target is amplified as well, earlier amplification (lower Ct) and a second melting peak should be present.

The table below shows the cut-offs to differentiate between negative samples and samples that have to be tested with *hybcell*.

	Amplification	**Melting curve**		
	Ct	**Tm1 (background)**	**Tm2 (IC)**	**Tm3 (target)**
Bacteria				
Test with *hybcell*	**< 37**	(< 79.5 °C)	(79.5 – 81.5 °C)	> 83 °C
Negative	**> 37**	(< 79.5 °C)	79.5 – 81.5 °C	--
Fungi				
Test with *hybcell*	< 37	(< 80.0 °C)	(80.0 – 81.8 °C)	> 83 °C
Negative	> 37	(< 80.0 °C)	80.0 – 81.8 °C	--

Grey Boxes indicate mandatory criteria.

Ct ... Cycle threshold (cycle number) IC … Internal control
Tm ... Melting temperature (°C) ( ) ... could be missing at low Ct

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A separate guideline document describes in more detail PCR result interpretation for Option 1 (download under www.cubedx.com/documents).

PCR-Bos Res g+ xC / PCR-Box Res g- AB xC / PCR-box Res g- CD xC

To determine if a target (resistance gene) is positive or negative the amplification curves of the targets and the IC have to be analysed. Whenever the Ct-value for the target is below a certain threshold, the amplification of IC is irrelevant (see below). So, check the result of the IC amplification first: if there is no amplification for the IC and no amplification for the target, the PCR is invalid. If there is an amplification for one of the targets below a certain threshold, the result is positive.

PCR-Box Res g+ xC:

	Positive PCR	Negative PCR	Invalid PCR
mec A/C	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
Van A	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
Van B	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
IC	irrelevant	**< 40**	No Ct or > 40

PCR-Box Res g- AB xC:

	Positive PCR	Negative PCR	Invalid PCR
NDM, VIM, IMP	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
KPC	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
CTX-M	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
IC	irrelevant	**< 40**	No Ct or > 40

PCR-Box Res g- CD xC:

	Positive PCR	Negative PCR	Invalid PCR
OXA48	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
AmpC	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
mcr-1	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
IC	irrelevant	**< 40**	No Ct or > 40

hybcell tests

The test design is based on genetic information derived from the *National Center for Biotechnology Information* (NCBI - www.ncbi.nlm.nih.gov). Genetic information, taxonomy and naming are subject to change. The design of the test has been revised over the last years and the data for the current test design has been last updated in April 2025. Even if Cube Dx is trying to keep the underlying records updated, discrepancies to NCBI are possible.

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For most of the microorganisms more then a single record of genetic information is available - and the single records are sometimes contradictory. In such cases, the sequence data with the highest occurrence was taken into consideration. In some cases the genetic information of certain type strains was considered.

Naming of the targets is derived from the taxonomy provided by NCBI and follows the rules of LOINC (an international standard aiming to standardize medical terms).

Some targets describe single species, some whole genera and others groups of species. The test tries to reflect the targets, as they are described in NCBI. Deviations from that principle are described in a separate guideline (download under www.cubedx.com/documents).

The same is true for possible cross-reactivities. The test is designed to reflect the occurrences of microorganisms in human samples. Microorganisms that are associated with other milieus (e.g. environmental microorganisms, organisms used in biotechnology) are not considered.

hybcell Controls

hybcell tests feature several internal controls to ensure proper results. If all internal controls are passed, the result for 'Controls' is 'PASSED' (and shown as such on the report). If one or more controls fail, the controls are marked as 'FAILED' on the report. If any control fails, the results are invalid, and the test has to be repeated.

- **Process Control**: Checks proper processing of the *hybcell*.
- **Surface Control:** Checks the *hybcell* type and the fluorescence read-out (scanning process).
- **Background Noise Control**: Checks for unspecific binding and basic requirements from *hyborg* software.

Test Specific Control(s)

The tests feature a test-specific check for an internal control. If added, the check should be passed and 'DETECTED' is displayed. Otherwise the result is 'NOT DETECTED'. The IC helps to judge the plausibility of the results.

- **Internal Control (IC):** The IC comes with the PCR-mix. A passed IC indicates that the whole process has not experienced major flaws. Especially negative results are confirmed by the IC**.**

General nomenclature

- **Bacteria species** are dected if 16S rDNA of a bacterial species was amplified and a corresponding signal pattern for that species matches (e.g., *Staphylococcus aureus*).
- **Bacteria genera** are detected if 16S rDNA of a bacterial species was amplified and a corresponding signal pattern for a bacterial genus matches (e.g., *Staphylococcus*).
- **Fungal species** are detected if 28S rDNA of a fungal species was amplified and a corresponding signal pattern for that species matches (e.g., *Candida albicans*).
- **Fungal genera** are detected if 28S rDNA of a fungal species was amplified and a corresponding signal pattern for a fungal genus matches (e.g., *Candida*).

Off-profile Parameters

According to the intended purpose, clinically relevant results are indicated. The protocol for the lot (specified for the CE-IVD test kits) defines clinically relevant bacteria, resistance genes and fungi. The results outside this scope are labelled as 'Off-profile parameters'. Such results may help infectious disease specialists to interpret results.

Following results are always displayed "off-profile":

- **Bacteria pan** is detected if amplified bacterial 16S rDNA is present.

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- **Gram pos / Gram neg** is detected if amplified gram+ / gram- bacterial DNA is present.
- **Fungi pan** is detected if amplified fungal 28S rDNA is present.

hyborg Reports

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Austria

cube dx
hybCell technology

Sample	2718A250315 AMP	**Test**	hybcell Bacteria DNA xC (1)
Date	21.05.2025 00:00	**Profile**	Sepsis (04.06.2025)
Remark		**hybcell**	2718A250315
Liquids	2: CS-Buffer (2816010015) / S: System Liquid (2807010003)		

Controls		
Controls	PASSED	

Parameters	Result	Representation
Internal Control (IC)	Detected	
BACTERIA	Detected	
Staphylococcus aureus	Detected	100 99999

Negative Parameters

Acinetobacter baumannii, Aerococcus urinae, Aerococcus viridans, Alcaligenes, Anaerococcus, Bacteroides fragilis, Bordetella pertussis/parapertussis, Borreliella, Burkholderia cepacia complex, Burkholderia pseudomallei, Capnocytophaga, Cardiobacterium hominis, Cardiobacterium valvarum, Citrobacter freundii, Citrobacter koseri, Clostridium perfringens, Corynebacterium diphtheriae, Corynebacterium jeikeium, Corynebacterium ulcerans, Cutibacterium avidum, Eikenella corrodens, Enterobacter asburiae/cancerogenus, Enterobacter cloacae, Enterobacter cloacae complex, Enterobacter hormaechei, Enterobacter kobei/ludwigii, Enterobacter roggenkampii, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Finegoldia magna, Francisella tularensis, Fusobacterium, Fusobacterium necrophorum, Fusobacterium nucleatum, Granulicatella adiacens, Haemophilus influenzae, Haemophilus parainfluenzae, Helicobacter pylori, Kingella kingae, Klebsiella aerogenes, Klebsiella michiganensis, Klebsiella oxytoca, Klebsiella pneumoniae complex, Legionella pneumophila, Leptospira, Listeria, Moraxella catarrhalis, Morganella morganii, Mycoplasmoides pneumoniae, Neisseria meningitidis, Nocardia, Pantoea agglomerans, Parvimonas, Pasteurella multocida, Prevotella intermedia, Proteus, Proteus mirabilis, Providencia stuartii, Pseudomonas aeruginosa group, Salmonella enterica, Serratia marcescens, Staphylococcus lugdunensis, Stenotrophomonas maltophilia group, Streptococcus agalactiae, Streptococcus anginosus group, Streptococcus dysgalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus salivarius, Vibrio vulnificus, Yersinia enterocolitica

Example of a PDF report for a sample tested positive for S. aureus.

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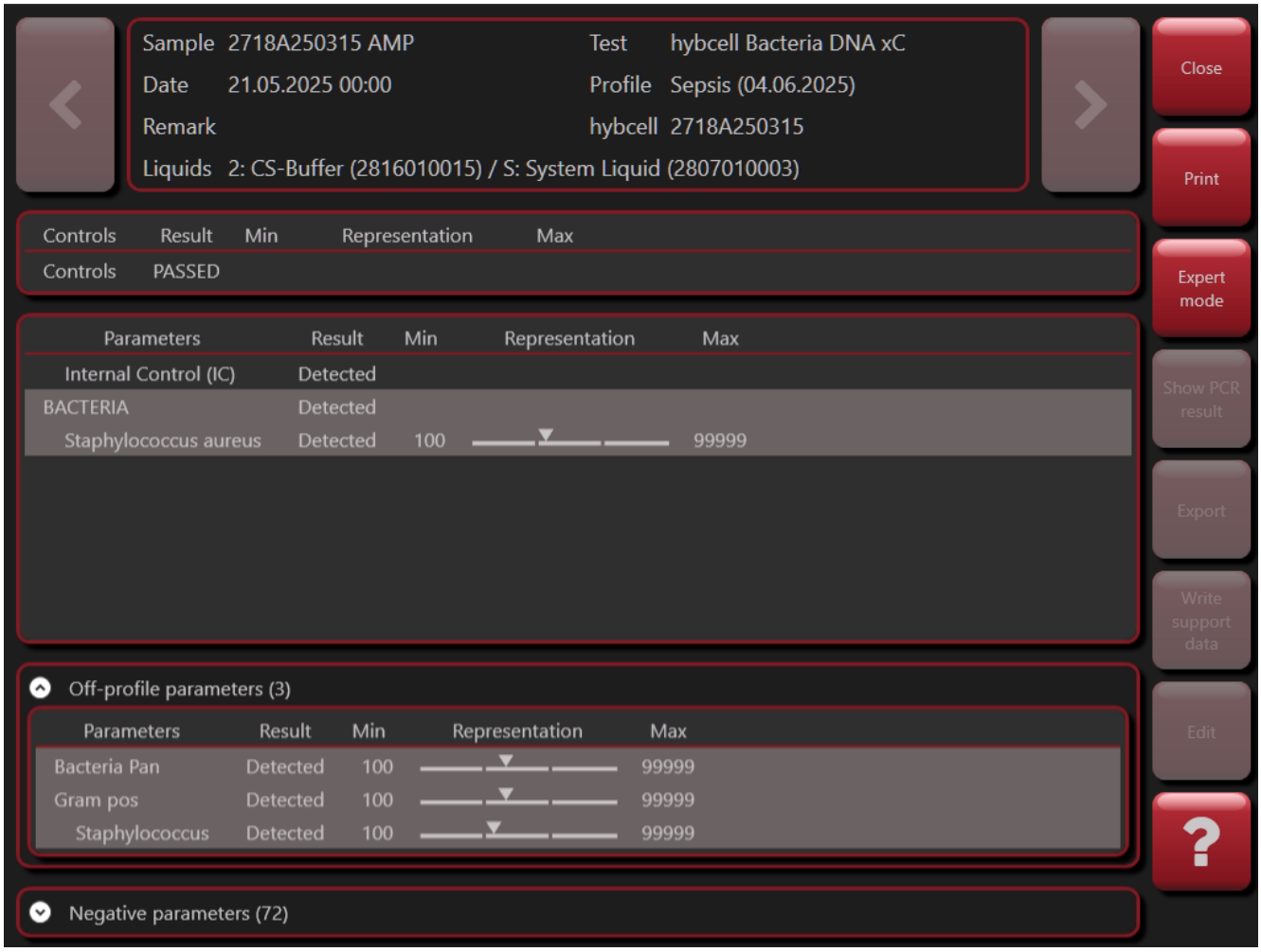
Screen report

The result provided on the screen provides the same information as the printed report. Below an example of a report on the screen.



Example of screen report for a sample tested positive for S. aureus.

Protocol (.hyb)

Calibration curves and pattern recognition were set for all microorganisms and genes (identified bacterial 16S rDNA / identified fungal 28S rDNA / identified resistance genes) and are part of the *hyborg* protocol (XML-file with the extension .hyb). Calibration is independent of the *hyborg* device (unit use). However, it is a precondition that the *hyborg* operates in the specified environmental conditions (e.g., liquid delivery, heating, laser power, etc.).

Specific protocols are imported into the *hyborg* software before the first use of a new lot. Up-to-date protocols are provided on the Cube Dx homepage (www.cubedx.com/protocols). Protocols may also be updated automatically, if the *hyborg* is permanently connected to the internet.

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9. Analytical Performance

The Limit of Detection (LOD) of the products has been evaluated externally (a hospital) using 500µl of ETDA whole blood, the GINA 500 sample preparation and the geneLEAD VIII instrument of PSS for RNA/DNA extraction and amplification.

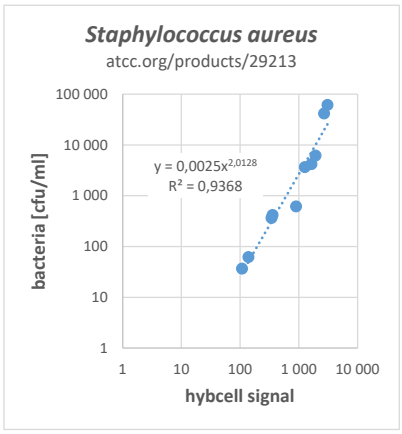
All samples have been confirm with the respective hybcells. Several bacterial and fungal targets have been analysed.

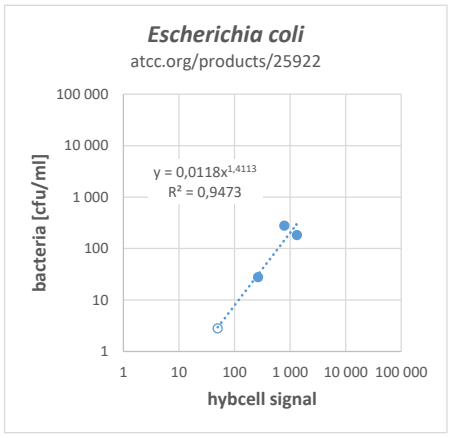
Limit of Detection (LOD) Bacteria

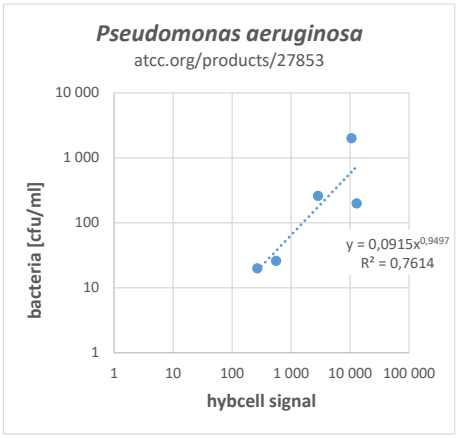
For each target, 2 replicates have been tested (for Staphylococcus aureus 4 replicates (2x2)). 3 dilutions have been prepared (for Staphylococcus aureus 5 dilutions).

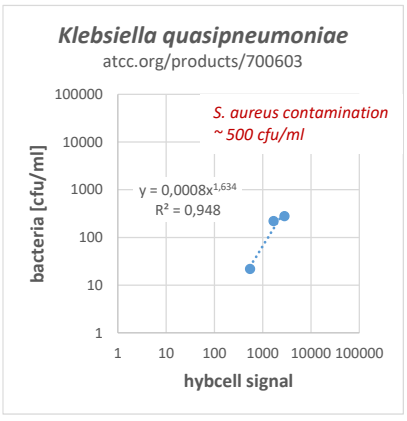
The LOD has been calculated for one ml of ETDA whole blood, by estimating the intersection of the interpolated line with the signal threshold of the hybcell (which is a signal count of 100).

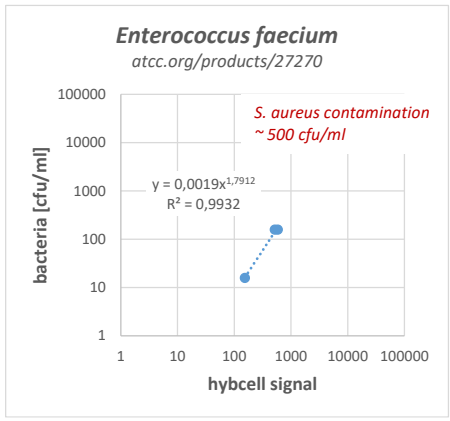
For some targets a contamination with Staph. aureus (caused by the lab technician) was observed. Even with that contamination, the LOD was below 10/ml.

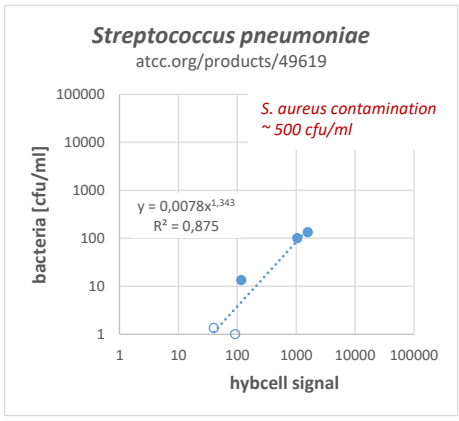












The calculated LOD for bacteria is summarized below:

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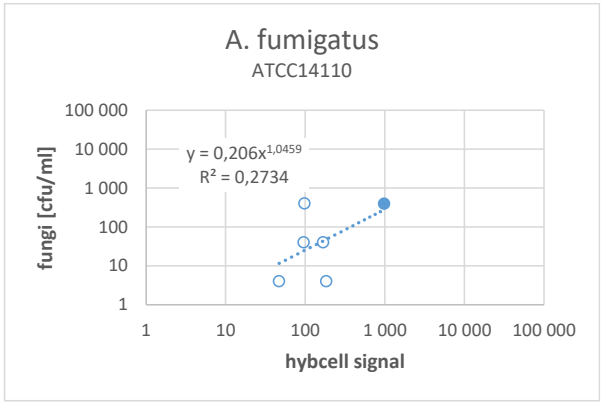
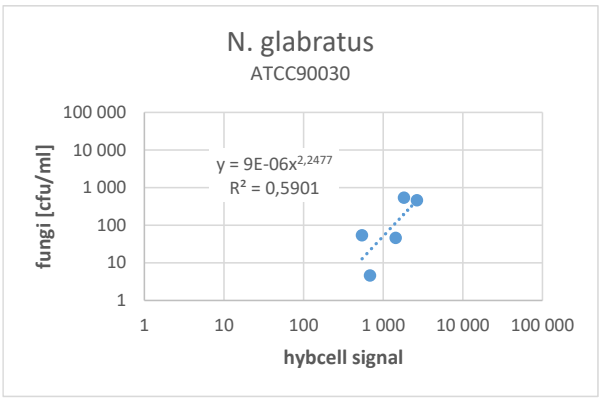
	Calculated LoD cfu/ml
Staphylococcus aureus	27
Escherichia coli	8
Pseudomonas aeruginosa	7
Klebsiella quasipneumoniae	1
Enterococcus faecium	7
Streptococcus pneumoniae	4

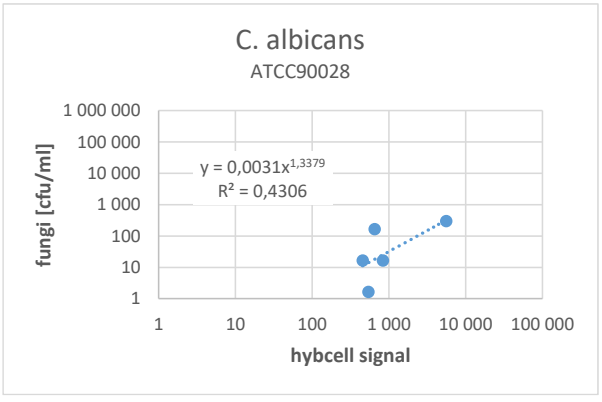
Limit of Detection (LOD) Fungi

For each target, 2 replicates have been tested. 3 dilutions have been prepared.

The LOD has been calculated for one ml of ETDA whole blood, by estimating the intersection of the interpolated line with the signal threshold of the hybcell (which is a signal count of 100).







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The calculated LOD for bacteria is summarized below:

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

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10. Clinical Performance

The products are currently subject of several clinical validations. Clinical performance data will follow.

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11. Changes in Analytical Performance

Changes in analytical performance

To verify the functionality of the test and implementation, a monthly examination with a reference standard (e.g., Cube Dx's External Process Controls (*EPC S.aureus 10000* and *EPC C.albicans 10000*)) is recommended.

To verify the functionality of the EPCs, run several tests and check the outcome. If the outcome is not as expected, use EPCs from another lot and repeat the tests.

In case of changing analytical performance refer to the section *Troubleshooting* of this instructions for use.

In case the shortcomings cannot be resolved, please contact Cube Dx or the respective distribution partners for assistance.

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12. Troubleshooting

Sample Preparation

Problem	Possible causes	Measure / Precaution
Loss of the pellet	▪ Pipetted away / decanted	▪ Start with decanting the supernatant and thereafter pipette away the remaining solution ▪ Repeat the extraction step
Contamination	▪ Contamination during the sample preparation step	▪ Follow the recommendation set out in Cube Dx' guidelines to prevent contamination (www.cubedx.com/documents)

Detection by PCR

Problem	Possible causes	Measure / Precaution
Odd-looking amplification curves	▪ Spread out of the eluate in the PCR tube ▪ Uneven distribution of the eluate-PCR-mix solution ▪ Bubbles at the bottom of the PCR tube	▪ Spin down the PCR tubes before introducing them into the device ▪ Check the filling of PCR tubes
PCR inhibition	▪ Dilution of the PCR-mix ▪ Using too high sample volumes ▪ Ethanol residues present in the eluate	▪ Use the recommended eluate amount for the PCR reaction ▪ Use a dilution series when unsure what volume of sample is suitable ▪ Check the column for EtOH residue before elution, and follow the protocol's 3-minute heating step after elution.

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Identification by the hybcell

Problem	Possible causes	Measure / Precaution
Unspecific *hybcell* signals	▪ Prepared *hybcells* (containing amplicons) are not processed immediately ▪ Expiration of opened buffers ▪ Forceful introduction of the pipette tip into the *hybcell* ▪ Liquids are empty or the liquid handling of the device is erroneous ▪ Insufficient washing • Using expired/spoilt *hybcells*	▪ Transfer the amplicons into the *hybcell* only if they are processed immediately; IF NOT; store the amplicons as described ▪ Check the lifetime of buffers after opening the bottles ▪ Gently introduce the pipette tip into the *hybcell* without sealing its central channel ▪ Check filling levels of all liquids. If necessary, refill liquids
Grid	▪ Using the wrong *hybcell* ▪ Using the “wrong” protocol. ▪ Using expired/spoilt products (for example due to damaged package, etc.) ▪ Software error ▪ Device error	▪ Check the hybcell type and used protocol ▪ Check the expiry dates of products ▪ Check the functionality of the *hyborg*, by using *hybcell Control xC*
Specificity Control	▪ Using expired products ▪ Insufficient / no PCR-product pipetted into *hybcell* ▪ Spoilt PCR ▪ No or insufficient *PE Buffer* used	▪ Check the functionality of the *hyborg* by using *hybcell Control xC* ▪ Repeat the test ▪ Check the filling levels of all liquids. If necessary, refill liquids

In case of problems with the device or the test, please contact:

▪ Cube Dx GmbH
Westbahnstraße 55, 4300 St. Valentin, Austria
Contact information: www.cubedx.com

For additional information about device and software usage see the *hyborg Dx RED2/3* instructions for use. Download under www.cubedx.com/documents.

Serious Incidents / Vigilance

Make sure to immediately report serious incidents related to the use of the test or the device to Cube Dx or respective distribution partners and the national competent authority. Please follow your national legislation about reporting serious incidents!

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