INSTRUCTIONS FOR USE

Pathogens xC instructions for use E 2025_06_02

RUO / EVAL

June 2025.

GINA 500 + DNA Purification	**REF** **UDI-DI**	*09120127730145*
GINA 500	**REF** **UDI-DI**	*09120127730244*
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	**REF /** **UDI-DI**	*09120127730152*

Modulation buffer for extraction-free testing of positive Blood Culture (BC) media

PCR-Box Bacteria xC 48rx-b / PCR-Box Bacteria xC 48rx-s	**REF** **UDI-DI**	*09120127730510 /* *09120127730701*
PCR-Box Fungi xC 48rx-b / PCR-Box Fungi xC 48rx-s	**REF** **UDI-DI**	*09120127730473 /* *9120127730633*
hybcell Bacteria DNA xC	**REF** **UDI-DI**	*09120127730336*
hybcell Fungi DNA xC	**REF** **UDI-DI**	*09120127730404*
hybcell FungiPlus 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-b / PCR-Box Res g+ xC 24rx-s	**REF** **UDI-DI**	*09120127730459 /* *09120127730640*
PCR-Box Res g- AB xC 24rx-b / PCR-Box Res g- AB xC 24rx-s	**REF** **UDI-DI**	*09120127730718 /* *09120127730657*
PCR-Box Res g- CD xC 24rx-b / PCR-Box Res g- CD xC 24rx-s	**REF** **UDI-DI**	*09120127730688 /* *09120127730695*

Multiplex PCR tests for detection of bacterial antibiotic resistance marker genes from human samples, including internal control.

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Content

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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 uses

GINA

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 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 the screening of suspected bacterial and/or fungal infections in different clinical contexts, including bloodstream, respiratory and tissue 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 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-Boxes xC

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

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

Both products deliver qualitative results that are used to screen and diagnose bacterial and fungal infections in different clinical contexts.

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 – provided in different fillings – to amplify and detect different antibiotic resistance genes in eluates from suitable RNA/DNA extraction processes.

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

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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.

hybcells Pathogens xC

hybcell Bacteria DNA xC is a cartridge base test to identify bacterial species, genera or groups of species using Cube Dx' proprietary *compact sequencing* and the *hyborg Dx RED2* or *hyborg Dx RED3* instrument. *hybcell Fungi DNA xC* and *hybcell FungiPlus DNA xC* are cartridge based tests to identify 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 either *PCR-Box Bacteria xC* or *PCR-Box Fungi xC* are introduced into the cartridges and serve as samples (see above).

Both products deliver qualitative results that are used to diagnose bacterial and fungal infections in different clinical contexts.

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.

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3. Product background and technical description

General

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 a result of a very low number of microorganisms in the patient's blood and a lack of sensitivity of the test. Another reason might be the occurrence of type strains that have not been considered during the test design. Yet another reason might be 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 500µl or less of ETDA whole blood, with or without DNA purification) and *GINA 1000* (for 1000µl 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, 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 500µl (or less) or 1000µl 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**
ETDA 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 / …)
Pleura punctation	Infections of the pleura (diagnosing pleurisy / …)

* for these samples no sample preparation with GINA components is necessary

(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 ETDA 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 follow-up 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 2mL tubes or / and 5mL Eppendorf tubes is necessary. The centrifuge should be able to apply 11.000g (e.g., Eppendorf, Hermle, etc.).

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

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(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 remaining 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 2mL tubes or / and 5mL Eppendorf tubes) is needed.

If including DNA purification based on spin columns:

Neutralization:

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

After the lysis of microbial cells the resulting RNA/DNA is purified. This purification is either done with spin columns from Machery Nagel (included in the *GINA 500 + DNA purification* kit) or with automated RNA/DNA extractors. Currently protocols for Promega's Maxwell series (with the Maxwell®CSC Pathogen Kit (AS1860)) and 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 of dry storage between 4°C and 8°C.

LINA

LINA is 8mL of modulation buffer filled in a single ready-to-use tube. 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 reduces the time to result drastically.

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 DNA and fungal 28S DNA. During amplification, single DNA strands are labeled with a fluorescent dye that is used later in the *compact sequencing* process (during the hybcell read-out).

The PCR 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 a tube with negative control (NTC.. Non Template Control) is added.

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 the amplification curves and Ct-values of different fluorescence channels (see below).

PCR-Box Bacteria xC:

Target	Fluorophore
Bacteria	FAM
IC	CAL.RED

PCR-Box Fungi xC:

Target	Fluorophore
Fungi	JOE
IC	CAL.RED

The PCR mixes are provided either as single reactions, pre-filled in 0.2ml PCR tubes (-s, 20µl each) or in bulk filled in 0.5mL tubes (-b, 500µl 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 and a Ct below threshold as defined by different intended uses – are singled out for the identification of the bacterial or fungal species (or genus).

For differentiation and identification of bacterial and fungal species, the resulting DNA amplicons can be further processed by *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 *Required Equipment* (examples of such instruments are CFX96 from Biorad, Quantstudio 3/5 from Thermo Fisher or the geneLEAD VIII instrument of PSS).

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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		*Bacteroides fragilis*
	Bartonella	*Bartonella bacilliformis / quintana*
		Bordetella pertussis / parapertussis
	Borrelia	
	Borreliella	
	Brucella	
		Burkholderia cepacia complex
		Burkholderia pseudomallei
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/lenticulare* *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.

As the tests are used for general identification of bacteria and / or fungi from eluates of different human samples, some of these bacterial and fungal targets might not always be of high relevance for the clinical diagnosis, as they might not be considered as pathogenic if found in the respective sample. 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 instrument (hyborg Dx RED2 - 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 *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 there is suspicion that a result is incorrect or deteriorated, the 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.

The *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 PCR 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 a tube with negative control (NTC.. Non Template Control) is added.

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

The PCR mixes are provided either as single reactions, pre-filled in 0.2ml PCR tubes (-s, 20µl each) or in bulk filled in 2.0mL tubes (-b, 250µl each).

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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 *Required Equipment* (examples of such instruments are CFX96 from Biorad, Quantstudio 3/5 from Thermo Fisher or the geneLEAD VIII instrument of PSS).

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4. Product components, shelf life

GINA 500, GINA 500 + DNA purification, GINA 1000, GINA Lysis 200 (shelf life 24 months):

- *GINA 500* (REF / UDI-DI 09120127730244): store at **room temperature (8 to 25°C)**
  - 2 x 25 *LE solution* (1400µl); (2 x 25 x 2mL tubes with yellow cap)
  - 1 x 12mL *NA solution* (red mark on bottle and cap)
  - 1 x 25mL *T solution* (green mark on bottle and cap)
- *GINA 500 + DNA Purification* (REF / UDI-DI 09120127730145): store at **room temperature (8 to 25°C)**
  - 2 x 25 *LE solution* (1400µl); (2 x 25 x 2mL tubes with yellow cap)
  - 1 x 12mL *NA solution* (red mark on the bottle and cap)
  - 1 x 25mL *T solution* (green mark on the bottle and cap)
  - 1 x 30mL *Wash Buffer BW* (bottle)
  - 1 x 60mL *Wash Buffer B5* (bottle)
  - 1 x 13mL *Elution Buffer BE* (bottle)
  - 50 x *Column*
  - 50 x *Collection Tube*
  - 50 x *Elution Tube*
- *GINA 1000* (REF / UDI-DI 09120127730480): store at **room temperature (8 to 25°C)**
  - 48 x *LE solution* (2800µl); (48 x 5mL tubes with grey cap)
  - 48 x *NA solution* (200µl); (48 x 2mL tubes with red cap)
- *GINA Lysis 200* (REF / UDI-DI 09120127730480): store at room temperature (8 to 25°C)
  - 50 x Sterile *Forensic Swab* (Sarstedt 80.629)
  - 50 x 400µL *NA solution* (50x 2mL tubes with red cap)

LINA (shelf life 24 months):

- *LINA* (REF / UDI-DI 09120127730152): store at **room temperature (8 to 25°C)**
  - 50 x *LINA* (8ml)

PCR-Boxes (shelf life 24 months):

- *PCR-Box Bacteria xC 48rx-b* (REF / UDI-DI 09120127730510): store **frozen** at **-15 to -25°C**
  - 2 x 500µL PCR-mix Bacteria xC (2 x 2mL tube with red cap)
  - 1 x 1000µL NTC (no template control) (1 x 2mL tube with transparent cap)
  - 1 x 100µL PCT (positive control, eluated DNA of S.aureus) (1 x 0,5mL tube)
- PCR-Box Bacteria xC 48rx-s (REF / UDI-DI 09120127730701): store **frozen** at **-15 to -25°C**
  - 48 x 20µl PCR-mix Bacteria xC (24 x 0.2ml tubes with red mark)
  - 1 x 1000µL NTC (no template control) (1 x 2mL tube with transparent cap)
  - 1 x 100µL PCT (positive control, eluated DNA of S.aureus) (1 x 0,5mL tube)
- *PCR-Box Fungi xC 48rx-b* (REF / UDI-DI 09120127730473): store **frozen** at **-15 to -25°C**
  - 2 x 500µL PCR-mix Fungi xC (2 x 2mL tube with green cap)
  - 1 x 1000µL NTC (no template control) (1 x 2mL tube with transparent cap)

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- 1 x 100µL PCT (positive control, eluated DNA of C.albicans) (1 x 0,5mL tube)

- *PCR-Box Fungi xC 48rx-s* (REF / UDI-DI 9120127730633): store **frozen** at **-15 to -25°C**
  - 48 x 20µl PCR-mix Fungi xC (24 x 0,2ml tubes with green mark)
  - 1 x 1000µL NTC (no template control) (1 x 2mL tube with transparent cap)
  - 1 x 100µL PCT (positive control, eluated DNA of C.albicans) (1 x 0,5mL tube)
- *PCR-Box Res g+ xC 24rx-b* (REF / UDI-DI 09120127730459): store **frozen** at **-15 to -25°C**
  - 2 x 250µL PCR-mix Res g+ xC (2 x 2mL tube with purple cap)
  - 1 x 1000µL NTC (no template control) (1 x 2mL tube with transparent cap)
  - 1 x 100µL PCT (positive control, eluated DNA of mecA) (1 x 0,5mL tube)
- *PCR-Box Res g+ xC 24rx-s* (REF / UDI-DI 09120127730640): store **frozen** at **-15 to -25°C**
  - 24 x 20µl PCR-mix *Res g+ xC* (24 x 0,2ml tubes with purple mark)
  - 1 x 1000µL NTC (no template control) (1 x 2mL tube with transparent cap)
  - 1 x 100µL PCT (positive control, eluated DNA of mecA) (1 x 0,5mL tube)
- *PCR-Box Res g- AB xC 24rx-b* (REF / UDI-DI 09120127730718): store **frozen** at **-15 to -25°C**
  - 2 x 250 µL PCR-mix Res g- AB xC (2 x 2mL tube with black cap)
  - 1 x 1000µL NTC (no template control) (1 x 2mL tube with transparent cap)
  - 1 x 100µL PCT (positive control, eluated DNA of KPC) (1 x 0,5mL tube)
- *PCR-Box Res g- AB xC 24rx-s* (REF / UDI-DI 09120127730657): store **frozen** at **-15 to -25°C**
  - 24 x 20 µl PCR-mix *Resistance g- AB xC* (24 x 0,2ml tubes with black mark)
  - 1 x 1000µL NTC (no template control) (1 x 2mL tube with transparent cap)
  - 1 x 100µL PCT (positive control, eluated DNA of KPC) (1 x 0,5mL tube)
- *PCR-Box Res g- CD xC 24rx-b* (REF / UDI-DI 09120127730688): store **frozen** at **-15 to -25°C**
  - 2 x 250 µL PCR-mix Res g- CD xC (2 x 2mL tube with black cap)
  - 1 x 1000µL NTC (no template control) (1 x 2mL tube with transparent cap)
  - 1 x 100µL PCT (positive control, eluated DNA of OXA48) (1 x 0,5mL tube)
- *PCR-Box Res g- CD xC 24rx-s* (REF / UDI-DI 09120127730695): store **frozen** at **-15 to -25°C**
  - 24 x 20 µl PCR-mix *Resistance g- CD xC* (24 x 0,2ml tubes with black mark)
  - 1 x 1000µL NTC (no template control) (1 x 2mL tube with transparent cap)
  - 1 x 100µL PCT (positive control, eluated DNA of OXA48) (1 x 0,5mL tube)

hybcells (shelf life 24 months):

- *hybcell Bacteria DNA xC* Kit (REF / UDI-DI 09120127730336): store at **room temperature (8 to 25°C)**
  - 24 x hybcell Bacteria DNA xC Rev.2

    (24 x separately packed hybcells Bacteria DNA xC)
  - 24 x Lid
  - 1x PPE-Additive (900µl)
- *hybcell Fungi DNA xC* Kit (REF / UDI-DI 09120127730404): store at **room temperature (8 to 25°C)**
  - 24 x hybcell Fungi DNA xC Rev.2

    (24 x separately packed hybcells Fungi DNA xC)
  - 24 x Lid

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- 1x PPE-Additive (900µl)

- *hybcell FungiPlus DNA xC* Kit (REF / UDI-DI 09120127730367): store at **room temperature (8 to 25°C)**
  - 24 x hybcell Pathogens DNA xC Rev.2

    (24 x separately packed hybcells Pathogens DNA xC)
  - 24 x Lid
  - 1x PPE-Additive (900µl)

Pay attention not to mix up components of different lots!

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5. Storage and transportation

Products

The minimum shelf life of the products is only guaranteed if the required temperature and humidity conditions are safeguarded during transportation and storage. The expiry date of the products is printed on the product labels.

- *GINA 500, GINA 500 + DNA Purification, GINA 1000, LINA, hybcells (Bacteria DNA xC / Fungi DNA xC / FungiPlus DNA xC,) and their PPE-Additive* are delivered at room temperature (<40°C) and must be stored at **8 to 25°C**.
- *PCR-Box Bacteria xC, PCR-Box Fungi xC*, *PCR-Box Res g+ xC, PCR-Box Res g- AB xC* are *PCR-Box Res g- CD xC* delivered frozen (< 0°C) and must be stored at **-15 to -25°C**.

If the protective sealing of hybcells or any other packaging (e.g., any tubes) is damaged / or the minimum shelf life has expired, the product/component must not be used. hybcells have to be used immediately after opening the protective sealing.

More then 15 freezing-and unfreezing cycles of PCR-Boxes should be avoided. Once opened, put the PCR mixes back in the freezer (-15°C to -25°C). IC can be thawed and refrozen up to 24 times.

Samples

Blood

- Store cool (between 4°C and 8°C) and dry for a maximum of 4 hours for optimal results. Storing samples under these conditions is accepted for a maximum of 48 hours.
- Do not freeze blood!

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6. Required equipment

The following equipment is required for conducting the test:

Required Accessories / Infrastructure	Example or REF / UDI-DI
Mini centrifuge (0,2 mL rotor) If using 0,2 mL PCR tubes, the mini centrifuge is used to spin-down the PCR-mix.	MySpin from Thermo[1]
Workbench (laminar flow) for sample preparation including DNA extraction The workbench should be equipped with following tools and consumables: • Vortex mixer - to homogenize sample-buffer-mixtures • Pipette 20-200µl + corresponding filter tips • Pipette 100 – 1000µl + corresponding filter tips • Rack for GINA tubes: 2mL (LE, NA), rack for (EDTA) samples • Holder and trash bag for discarding the supernatant of the centrifugation process	Laminar Flow or PCR work-benches with UV-light from Starlab[2] Vortex mixer Fisherbrand Classic Vortex mixer from Fisher Scientific[3] Pipettes PIPETMAN P200 and PIPETMAN P1000 from GILSON[4]
Workbench for PCR setup • Rack for PCR tubes (dependent on the used PCR machine) • Pipette 10 – 100µl + corresponding filter tips	PCR-working station from PEQLAB[5] Pipette PIPETMAN P100 from GILSON[6]
Standard table centrifuge for pellet formation (and DNA purification with spin columns if using *GINA 500 + DNA purification*) With rotor for 2mL tubes and 5mL Eppendorf tubes, capable to deliver at least 5,000g, ideally up to 11,000g. Note: operating the centrifuge inside the workbench for sample preparation helps to reduce contamination.	Centrifuge 5430 and Rotor FA-45-16-17 (using adaptors for 2mL) from Eppendorf[7]
Standard heating block to heat 2mL tubes and 5mL Eppendorf tubes (if using GINA 1000) Cube Dx provides a tailor-made aluminum block for perfect heat transmission. Note: operating the heating block inside the workbench for sample preparation helps to reduce contamination.	Heating block H203-H from Coyote Bioscience[8] Aluminium block from Cube Dx (REF 09120127730619)
Automated RNA/DNA extraction technology as an alternative to DNA purification with spin columns Several products are eligible, as long as following criteria are met: ▪ RNA/DNA extraction based on magnetic beads	Maxwell series + Maxwell®CSC Pathogen Kit (AS1860) kit from Promega[9]

1 www.thermofisher.com / order / catalog / product / 75004081
2 www.starlab.de
3 www.fishersci.com / shop / products / variable-speed-mini-vortex-mix / 14955163
4 www.gilson.com
5 www.peqlab.de
6 www.gilson.com
7 www.eppendorf.com
8 www.coyotebio.com
9 www.promega.com

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▪ Single use cartridges offered ▪ Input volume 200 µL ▪ Volume of eluate 100µL ▪ Yield >90%	geneLeadVIII from PSS[10]
(q)PCR instrument to amplify DNA and optionally evaluate positive/negative samples Several products are eligible, as long as following criteria are met: ▪ PCR volume of 40µl ▪ Heating / cooling rate of 1.6°C / sec. or faster ▪ Optional: fluorescence channels for FAM, JOE, CAL.RED, Cy5 If working with geneLEAD VIII from PSS, a rack to hold PCR cartridges and custom pliers to open these cartridges are recommended.	Rotor-Gene from Qiagen[11] CFX96 from Biorad[12] Quantstudio 3 / 5 from Thermo[13] geneLEAD VIII from PSS[14] PCR Rack (REF 09120127730541) PCR Pliers (REF 09120127730534)
Mandatory if using hybcell Bacteria / Fungi / FungiPlus DNA: hyborg instrument to process and evaluate hybcell tests The operation of the instrument and processing of hybcells requires buffers and accessories (for details, refer to the hyborg instructions for use): ▪ System Liquid (1l, sufficient for 8 weeks) ▪ CS-Buffer (1l, sufficient for 96 hybcells)	hyborg Dx RED2 from Cube Dx (REF 09120127730015) System Liquid from Cube Dx (REF 09120127730022) CS-Buffer from Cube Dx (REF 09120127730503)

Direct (=DNA based) testing of microorganisms from whole blood requires an environment/infrastructure and tools that are free of any microbial contamination – be it living cells or microbial RNA/DNA. If possible, a room for sample preparation and DNA extraction, a room for PCR and a post-PCR room for identification with hybcell should be provided.

Attention!: Never put the hyborg Dx RED2 and the PCR instrument in the same room.

Do not forget to clean all instruments and tools regularly. Use a cleaning agent that destroys DNA as well (remember that alcohol does not destroy DNA, solutions containing hypochlorite destroy DNA).

10 www.pss.co.jp
11 www.qiagen.com
12 www.bio-rad.com
13 www.thermofisher.com
14 www.pss.co.jp

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7. Test procedure

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

- Check readiness of all used lab instruments (e.g. geneLeadVIII, Promega's Maxwell extractor, PCR instruments, etc.)
- Check if the hyborg is switched on (check the screen of the device – refer to the hyborg Dx IFU 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 (position W).
- Check if the necessary hybcell protocol is available (if not, load the protocol, 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, latex 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 laminar flow box (or a similar area protected against bacterial or fungal contamination). Steps that should be done under these conditions are written in red.

For more details read our recommendations how to prevent from contaminations: *Contanmination Prevention Instructions for use_E_xxxx_xx_xx (xxxx_xx_xx = year_month_day)*.

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

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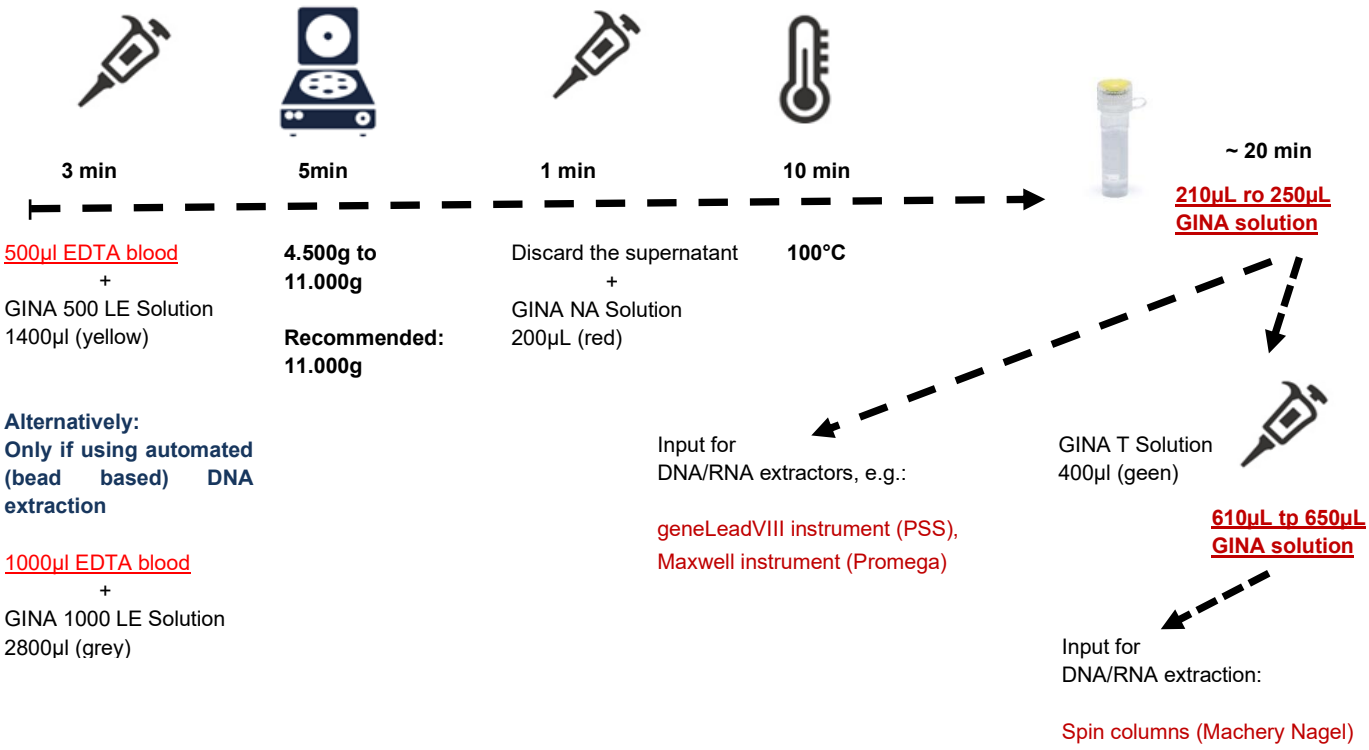
1 Sample Preparation

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

Whole blood samples can be collected in K3E K3EDTA or K2E 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 to sensitize medical staff taking the samples on the importance of this issue. If necessary, train the medical staff accordingly.

The procedure starts with a native sample of EDTA-whole blood of either 500µl (or less) or 1ml. 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. When opening the vials, briefly spin or shake down the needed tubes like LE solution or any other material (e.g. EPC) to avoid carry-over of liquids potentially present in the screw caps. Put on and set the heating block to 100°C. Make sure the adapters of the heating block are suitable for the tubes you will use (the LE tubes and if using spin column the elution tubes as well).

   Remark!

   The centrifuge requires ***different adapters*** *(for 5mL and 2mL tubes) when working with the larger LE tubes of GINA 1000 (gray cap).*

   Check if the required g-force of at least 4,500g (recommended 11,000g) is provided by the centrifuge. Don't mix up rotational speed (rpm) and g-force!

2. Prepare the *LE solution* and the sample. The LE solution is provided in a tube with yellow cap when working with 500µL of the blood sample. When using 1000µL of the sample, the provided LE tube has a gray cap.

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Do not shake or agitate the *LE solution* tube (yellow cap/gray cap) to avoid the build-up of foam! Transfer 500µl (or less) / 1000µl of EDTA blood (or other diluted samples) into the *LE solution* and pipet up and down to mix.

3. Close the tube, mark it, and vortex vigorously for 5 seconds or invert the tubes several times. Incubate for around 2 minutes at room temperature (18°C to 25°C).
4. Centrifuge for 5 minutes with a g-force of at least 4,500 (preferably with 11,000g). If available, use soft ramping of the centrifugation speed.
5. Remove the supernatant carefully by **decanting** and add 200 µL *NA solution* (red cap) into the LE tube. Close the screw cap tightly.

   Remark!

   Some sample liquid (~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 (+ / - 1 minute), using a heating block.
8. If you are using GINA 1000: Transfer the whole volume (~ 250µl) to the NA tube (compare 5.).
9. If continuing with the spin columns provided in the *GINA 500 + DNA purification* kit, add 400µl of T-Solution (green cap) to the tube.

Samples from the respiratory tract, swabs and other viscous samples: BAL, saliva, sputum, nasopharyngeal swabs, vaginal swabs and ejaculate

Required products: *GINA Lysis 200*

1. Use the swab of the GINA Lysis 200 kit to take up the sample by putting the swab into the sample tube. The swab transfers around 150µL to 200µL of sample solution. Twist the swab and let it rest in the sample for around 10 seconds. If taking a nasopharyngeal swab, do not use the provided forensic swab but a more suitable swab for sample taking.
2. Put the swab into a tube with *NA Solution* (400µL, red cap) 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 (+ / - 1 minute), using a heating block.
6. If you use RNA/DNA extraction based on spin columns, add 400µL *T Solution*.

Tissue samples

Required products: *GINA Lysis 200*

1. Open the tube with the *NA Solution* (400µL, red cap) 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 (+ / - 1 minute), using a heating block.
4. If you use RNA/DNA extraction based on spin columns, add 400µL *T Solution* before starting extraction.

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Sterile samples: CSF, synovial fluid, urine, liquid from pleura punctation

Required products: *None or*
GINA Lysis 200

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

However, if you suspect a fungal infection or an infection with bacteria that are hard to lyse, we recommend following procedure:

1. Use the swab of the GINA Lysis 200 kit to take up the sample by putting the swab into the sample tube. The swab transfers around 150µL to 200µL 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* (400µL, red cap) 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 (+ / - 1 minute), using a heating block.
6. If you use RNA/DNA extraction based on spin columns, add 400µL *T Solution* before starting extraction.

Positive blood cultures – direct testing with LINA

Required products: *LINA*

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

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2 RNA/DNA extraction and detection by PCR/qPCR

The test procedure starts with the solution resulting from GINA pathogen enrichment (see above). The three available options differ in the used instrumentation and hence in the workflow.

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

Required products: *Reagents for RNA/DNA extraction (dependent on the selected method)*
PCR-Box Bacteria xC 48rx-b / PCR-Box Bacteria xC 24rx-s
PCR-Box Fungi xC 48rx-b / PCR-Box Fungi xC 24rx-s

Option 1: *geneLEAD VIII (PSS)*
Option 2: *Manual extraction: GINA 500 + DNA Purification + PCR instrument*
Option 3: *Automated Extraction: Maxwell (Promega) + PCR instrument*

Option 1: geneLEAD VIII

1. If working with GINA and EDTA whole blood: Remove the lid from the LE-tube or the NA tube (if using GINA 1000) and use this tube as the sample container for the geneLEAD VIII instrument.

   For other samples, pipette 230µl into the sample container provided with the geneLEAD VIII Consumable Set (F8900 – pointed tube). If the sample is not sufficient, reduce the volume. Minimum volume to use is 100µl.

2. Unpack and thaw the RT-PCR master mixes.

3. Homogenize the RT-PCR master-mix by inverting the tube several times. Pipette 23µl of the PCR-mix deeply into the first cavity (orientation from the back of the instrument) of each PCR reagent cartridge of the instrument (geneLEAD VIII PCR Reagent Cassette Set / F8820). **Pay attention that the pipetted PCR-mix is really covering the bottom of the well/cavity!**

4. Once you have thawed the RT-PCR mix you must store the remaining rest in the freezer again (-15°C to -25°C). Do not thaw-freeze more often than 15 times.

5. Load the instrument for DNA purification and following PCR.

- Tray 1:
  - A. Load the PCR cartridges into the instrument: geneLEAD VIII PCR Cassette (F8840)
  - B. Load the PCR-mix into the instrument: geneLEAD VIII PCR Reagent Cassette Set (F8820)
  - C. Load the extraction cartridge into the instrument: MagDEA Dx SV (E1300) – **Shake firmly so that magnetic beads are dissolved!**
  - ○ Put Tray 1 into the instrument
- Tray 2:
  - D. Load the sample (see 1.) into the instrument. The sample volume must be at least 200µl.
  - E. Load the tips into the instrument: geneLEAD VIII Consumable Set (F8900)
  - F. Load the elution tube into the instrument: geneLEAD VIII Consumable Set (F8900)
  - ○ Put Tray 2 into the instrument

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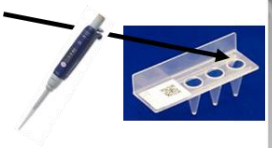
A  ***Insert PCR cassette***

B ***Fill well 1 with 23µl PCR-mix***
Insert pipette deeply!
Insert PCR mix cassette



C  ***Shake down magnetic beads of extraction cartridge and insert cartridge - wipe surface softly***

D  ***GINA***

Insert GINA tube without lid or the sample tube from the geneLEAD VIII Consumable Set

E  ***Insert tip racks***

F  ***Insert elution tube (0.5ml) without lid (you can as well remove the lid after you inserted the elution tube)***

Check if all reagents are „in line“ before starting

6. Start the instrument run according the instructions for use of the instrument. Main menu → Perform Run → Select: Extraction Input Volume - 200µl Extracted Elute Volume - 100µl.
7. Chose the corresponding protocol named *CDX_Bacteria_xC_xx* or *CDX_Fungi_xC_xx* and select *Extraction + PCR* (xx stands for the current version of the protocol). If the protocols are not available on the instrument, it has to be uploaded first (get the file from the local distributor or Cube Dx and import it following the instructions of the geneLEAD VIII software).

   The PCR protocol follows below steps:

   - 2 minutes 95°C
   - All together 45 Cycles:
     - 15 seconds 95°C
     - 60 seconds 60°C
     - Scan (FAM/JOE/CAL.RED)
8. Analyse results and check the amplification curves of all channels.

- If you have added IC to the sample, check the validity of results first by checking the Ct-value of the IC channel (CAL.RED). **The IC is only relevant, if no amplification is visible in the channel(s) for the target(s) (FAM or JOE)!** The Ct-value has to be below 37 to represent a valid internal control. Discard all results where neither any target nor the IC shows any amplification.
- Check if the target (bacteria / FAM or fungi / JOE) is positive. Refer to section *Results* for the interpretation of results and positivity.

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9. Remove and close the elution tubes from the instrument and put them in the fridge (4°C to 8°C). Remove and discard all consumables expect the PCR cartridges of the positive samples from the instrument as these will be further analyzed (see 3. Identification). Take these to the post-PCR room (where *hyborg Dx RED2* is operated).
10. Start the UV sterilization after usage of the instrument: Main menu → UV Irradiation

Attention! Do not open PCR cartridges close to the geneLEAD VIII or any other PCR instrument!

Option 2: Manual extraction + PCR instrument

This option does not require an automated extractor and uses the centrifuge which is necessary for sample preparation instead. The method is more laborious but fast and efficient if only a low number of samples is expected.

The manual extraction is based on spin columns technology provided by Machery Nagel. The necessary material comes with the product *GINA 500 + DNA Purification*.

1. Purify DNA, using common DNA extraction products (in the case of *GINA 500 + DNA purification*: Machery Nagel Nucleo Spin reagents are included in the kit. Otherwise: follow the manufacturer's instructions, and skip steps 11-17).
2. For each sample, place one *Column* into a *Collection Tube* and mark the *Collection Tube* with the sample ID. Transfer the whole *GINA* solution (600 to 650 µL) to the column. Discard the tube with the yellow cap.
3. Centrifuge for 1 min between 9.000 and 11.000g. Remove the *Column*, decant the flow-through liquid, and insert the *Column* again.
4. Add 500µl *Wash Buffer BW* and centrifuge for 1 minute at between 9.000 and 11.000g. Remove the *Column*, decant the flow-through liquid, and insert the *Column* again.
5. Add 600µl *Wash Buffer B5* and centrifuge for 1 minute at between 9.000 and 11.000g. Remove the *Column*, decant the flow-through liquid, and insert the *Column* again.
6. Centrifuge for 1 minute at between 9.000 and 11.000g to dry the silica membrane. Check if some liquid remains at the bottom of the *Column*. If yes, repeat this step.
7. Place the *Column* into an *Elution Tube* and mark the *Elution Tube* with the sample ID. Add 100 to 150µl *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*.
8. Open the *Elution Tube* and incubate at 100°C for 3 minutes in the heating block.
9. The collected liquid containing the DNA (eluate) might be used for PCR-based applications or stored at -20°C for later processing. Before using the eluate, **vortex** the *Elution Tube* firmly.
10. Program the qPCR device or PCR-thermocycler and save the program as *Pathogens_xC*

    The PCR protocol follows below steps:

    - 2 minutes 95°C
    - All together 45 Cycles:
      - 15 seconds 95°C
      - 60 seconds 60°C
      - Scan (FAM/JOE/CAL.RED)

    Remark!

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

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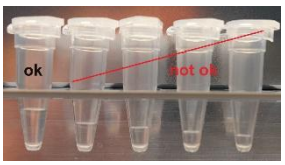
11. Unpack and thaw single 0,2 ml tubes (product versions 24rx-s) of the needed PCR-mixes for Bacteria and Fungi. Homogenize (vortex) and spin down briefly the solution in each tube.
12. Check, if the volume of the PCR-mix is approximately 20µl (see picture, the left tube is filled with 20µl). Do not use PCR-mixes that have been improperly filled.



13. Add 20 µL DNA eluate from the sample (or 20 µL of NTC or 20µL of Control DNA SA/CA – available from Cube Dx) to the PCR master mixes.
14. Close PCR tubes (if you don't use a rotating thermocycler, e.g. RotorGene from Qiagen, homogenize and spin down liquids before starting PCR).
15. Start (q)PCR program Pathogens xC (see above).

The amplified DNA is either used immediately for the identification / compact sequencing reaction (see below) or stored at 4°C to 8°C for maximum 18 hours or stored frozen at -15°C to -25°C for longer periods.

16. Analyse results and check the amplification curves of the relevant channels.

- Check the validity of results first by checking the Ct-value of the IC channel (CAL.RED). **The IC is only relevant, if no amplification is visible in the channel(s) for the target(s) (FAM or JOE)!** The Ct-value has to be below 37 to represent a valid internal control. Discard all results where neither any target nor the IC shows any amplification.
- Check if the target (bacteria / FAM or fungi / JOE) is positive. Refer to section *Results* for the interpretation of results and positivity.

17. Remove and close the elution tubes from the instrument and put them in the fridge (4°C to 8°C). Remove and discard all consumables expect the PCR cartridges of the positive samples from the instrument as these will be further analyzed (see 3. Identification). Take these to the post-PCR room (where *hyborg Dx RED2* is operated).

Attention! Do not open PCR cartridges close to the geneLEAD VIII or any other PCR instrument!

Option 3: Promega Maxwell (or other) + PCR instrument

1. Pipette 300µl of the Promega Lysis Buffer (MC110) into the LE tube (yellow cap).
2. Transfer the whole volume from the LE tube (~ 550µl) into the cavity for the sample (no. 1) of the extraction cartridge for Promega's Maxwell (using the Maxwell ® CSC Pathogen Kit (AS1860).
3. Follow the instruction of Promega's Maxwell, 100µl eluate is required (use the elution buffer from the Maxwell ® CSC Pathogen Kit).
4. Continue as with the eluate from the spin columns → **No. 10. and following above.**

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3 Identification: using hybcell and compact sequencing

The test procedure starts with the amplicon resulting from Detection by PCR/qPCR (see above).

Required products: *hybcell Bacteria DNA xC*
hybcell Fungi DNA xC
hybcell FungiPlus DNA xC

1. Assure that the hyborg is ready for operation.
2. Open the packaging of the associated hybcell (rip the sealing at the notch), and place the hybcell into the rack (positions A-H).
3. Thereafter pipette 30µl of the PPE-additive (found in the hybcell box) 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 central channel (sample inflow) with the pipette tip while introducing the amplicon mix! Only insert the tip as deep as needed into the hybcell central channel, make sure to allow a loose fit.
5. Cover the hybcell using the provided lid.

6. Start processing the samples after entering the sample and hybcell ID (see hyborg Dx RED2 manual for further details). Load the device with the prepared rack.

   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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4 Testing resistance genes with different multiplex PCR mixes

Once a bacterial ID is confirmed, a number of resistance genes can be tested from the already existing eluate of the sample. Once again different instrument options are available.

Required products: *PCR-Box Res g+ xC 24rx-b / PCR-Box Res g+ xC 24rx -s*
PCR-Box Res g- AB xC 24rx-b / PCR-Box Res g- AB xC 24rx-s
PCR-Box Res g- CD xC 24rx-b / PCR-Box Res g- CD xC 24rx-s

Option 1: *geneLEAD VIII (PSS)*
Option 2: *Conventional PCR instrument*

Option 1: geneLEAD VIII

1. Take the eluates of the corresponding samples from the fridge.
2. Unpack and thaw the RT-PCR master mixes.
3. Homogenize the RT-PCR master-mixes by inverting the tube several times. Put 23µl of the PCR-mix into each PCR reagent cartridge of the instrument (geneLEAD VIII PCR Reagent Cassette Set / F8820) into the first cavity (orientation from the back of the instrument).
4. Once you have thawed the RT-PCR mix you must store the remaining rest in the freezer again (-15°C to -25°C). Do not thaw-freeze more often than 15 times.
5. Load the instrument for DNA purification and following PCR.

- Tray 1:
    G. Load the PCR cartridges into the instrument: geneLEAD VIII PCR Cassette (F8840)
    H. Load the PCR-mix into the instrument: geneLEAD VIII PCR Reagent Cassette Set (F8820)
    - Put Tray 1 into the instrument
- Tray 2:
    I. Load the tips into the instrument: geneLEAD VIII Consumable Set (F8900)
    J. Load the elution tube into the instrument: geneLEAD VIII Consumable Set (F8900)
    - Put Tray 2 into the instrument
- A picture of the trays and the positions for loading is above.

6. Start the instrument run according the instructions for use of the instrument. Chose the corresponding protocol named *CDX_Res g+_xC_xx, CDX_Res g-_AB_xC_xx* or *CDX_Res g-_CD_xC_xx* and select *PCR only* (xx stands for the current version of the protocol). If the protocols are not available on the instrument, it has to be uploaded first (get the file from the local distributor or Cube Dx and import it following the instructions of the geneLEAD VIII software).

   The PCR protocol follows below steps:
    - 2 minutes 95°C
    - All together 45 Cycles:
        - 15 seconds 95°C
        - 60 seconds 58°C
        - Scan (FAM/JOE/CAL.RED/Cy5)
7. Analyse results and check the amplification curves of all channels.

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- If you have added IC to the sample, check the validity of results first by checking the Ct-value of the IC channel (CAL.RED). **The IC is only relevant, if no amplification is visible in the channel(s) for the target(s)!** The Ct-value has to be below 37 to represent a valid internal control. Discard all results where neither any target nor the IC shows any amplification.
- Check if any target is positive. Refer to section *Results* for the interpretation of results and positivity.

8. Remove and discard all consumables.

Option 2: **Conventional PCR instrument**

1. Program the qPCR device or PCR-thermocycler and save the program as *Resistance_xC*

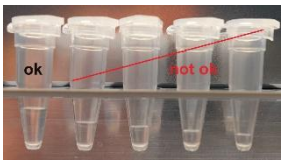
   The PCR protocol follows below steps:

   - 2 minutes 95°C
   - All together 45 Cycles:
     - 15 seconds 95°C
     - 60 seconds 58°C
     - Scan (FAM/JOE/CAL.RED/Cy5)

   Remark!

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

2. Unpack and thaw single 0,2 ml tubes (product versions 24rx-s) of the needed PCR-mixes for different resistance genes. Homogenize (vortex) and spin down briefly the solution in each tube.
3. Check, if the volume of the PCR-mix is approximately 20µl (see picture, the left tube is filled with 20µl). Do not use PCR-mixes that have been improperly filled.



4. Add 20 µL DNA eluate from the sample (or 20 µL of NTC or 20µL of Control DNA SA/CA – available from Cube Dx) to the PCR master mixes.
5. Close PCR tubes (if you don't use a rotating thermocycler, e.g. RotorGene from Qiagen, homogenize and spin down liquids before starting PCR).
6. Start (q)PCR program *Resistance_xC* (see above).
7. Analyse results and check the amplification curves of the relevant channels.

- If you have added IC to the sample, check the validity of results first by checking the Ct-value of the IC channel (CAL.RED). **The IC is only relevant, if no amplification is visible in any channel for the target(s)!** The Ct-value has to be below 37 to represent a valid internal control. Discard all results where neither any target nor the IC shows any amplification.
- Check if the targets are positive. Refer to section *Results* for the interpretation of results and positivity.

8. Remove and discard all consumables.

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8. Results

General

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.

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 document.

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.

PCR Analysis

PCR results are the key determinant of whether a sample is positive or negative for bacterial, fungal or IC DNA. A positive PCR result directs the user into the next step which is the identification of the microorganism using hybcell. A negative PCR result does not require further identification – the test result is negative.

To determine if a result 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.

Attention!

Be aware that every PCR instrument slightly differs in its characteristics and that Ct-values might vary from site to site. Therefore, the below parameters are recommendations based on experience how to set thresholds for Ct-values.

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 run hybcell tests for all samples where there is the slightest doubt that the PCR is negative. Be more generous in the inclusion of hybcell tests in the early phase of usage.

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PCR-Box Bacteria:

	Positive PCR	Negative PCR	Invalid PCR
Bacteria	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
IC	irrelevant	**< 40**	No Ct or > 40

PCR-Box Fungi:

	Positive PCR	Negative PCR	Invalid PCR
Fungi	**Ct < 37**	No Ct or Ct > 37	No Ct or > 37
IC	irrelevant	**< 40**	No Ct or > 40

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

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Some reasons for variations in Ct-values:

- The threshold for the Ct-Value calculation is set by the user
- Different PCR instruments offer different software with different characteristics. For example, auto-scale, threshold settings, and so forth which influence the Ct values and the visual presentation of the curves.
- Bacterial or fungal contaminations introduced during sample taking or the test procedure lower the Ct value.
- 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.

Internal Control (IC)

The Internal Control (IC) confirms the validity of negative results. It is a positive process control that enables the user to differentiate between negative results and invalid results – results that most probably have been hampered by flaws in the process.

The validity of IC is either confirmed by the PCR result (when using Realtime PCR instruments, see above) or with the hybcell test (see below).

Attention!

The Internal Control (IC) is designed to confirm negative results. In case of a positive amplification of the PCR-mixes for the targets, the amplification results for IC can be ignored and the hybcell test should be performed. Analogous to that, the result for IC can be ignored in case of any identification of bacteria or fungi with hybcell, if the IC result of the test is "NOT DETECTED" where it is expected to be "DETECTED".

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 failed, 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 the processing of the hybcell.
- **Surface Control:** Checks the hybcell type, sufficient fluorescence, and the scanning process.
- **Background Noise Control**: Checks unspecific binding, and basic features of the hyborg software.

Test specific

The tests feature a test-specific check for an internal control. If added, the check should be passed ('**Detected**'). The IC helps to judge the plausibility of the results.

- **Internal Control (IC)**: The IC might be (and should be) added to the DNA extraction process (see above). If added, 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 positive if a species 16S rDNA was amplified and corresponding primer extension took place (e.g., *Staphylococcus aureus*).
- **Bacteria genus** is positive if a species 16S rDNA was amplified and if the primer extension pattern matches a genus (e.g., *Staphylococcus*), but not necessarily a specific species of the tested panel.

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- **Bacteria pan** is positive if amplified bacterial DNA is present.
- **Gram pos / Gram neg** is positive if a gram-positive or gram-negative bacteria is detected.
- **Fungal species** is positive if a species 28S rDNA was amplified and corresponding primer extension took place (e.g., *Candida albicans*).
- **Fungal genus** is positive if a species 28S rDNA was amplified and if the primer extension pattern matches a genus (e.g., *Candida*), but not necessarily a specific species of the tested panel.
- **Fungi pan** is positive if amplified fungal 28S rDNA is present.

Report

CubeDx
Westbahnstr. 55
4300 St. Valentin
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 report.

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Protocol (.hyb)

Calibration curves and pattern recognition were done for all microorganisms (identified bacterial 16S rDNA / identified fungal 28S rDNA) 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 within its specifications and environmental conditions (e.g. liquid delivery, heating, laser power, etc.).

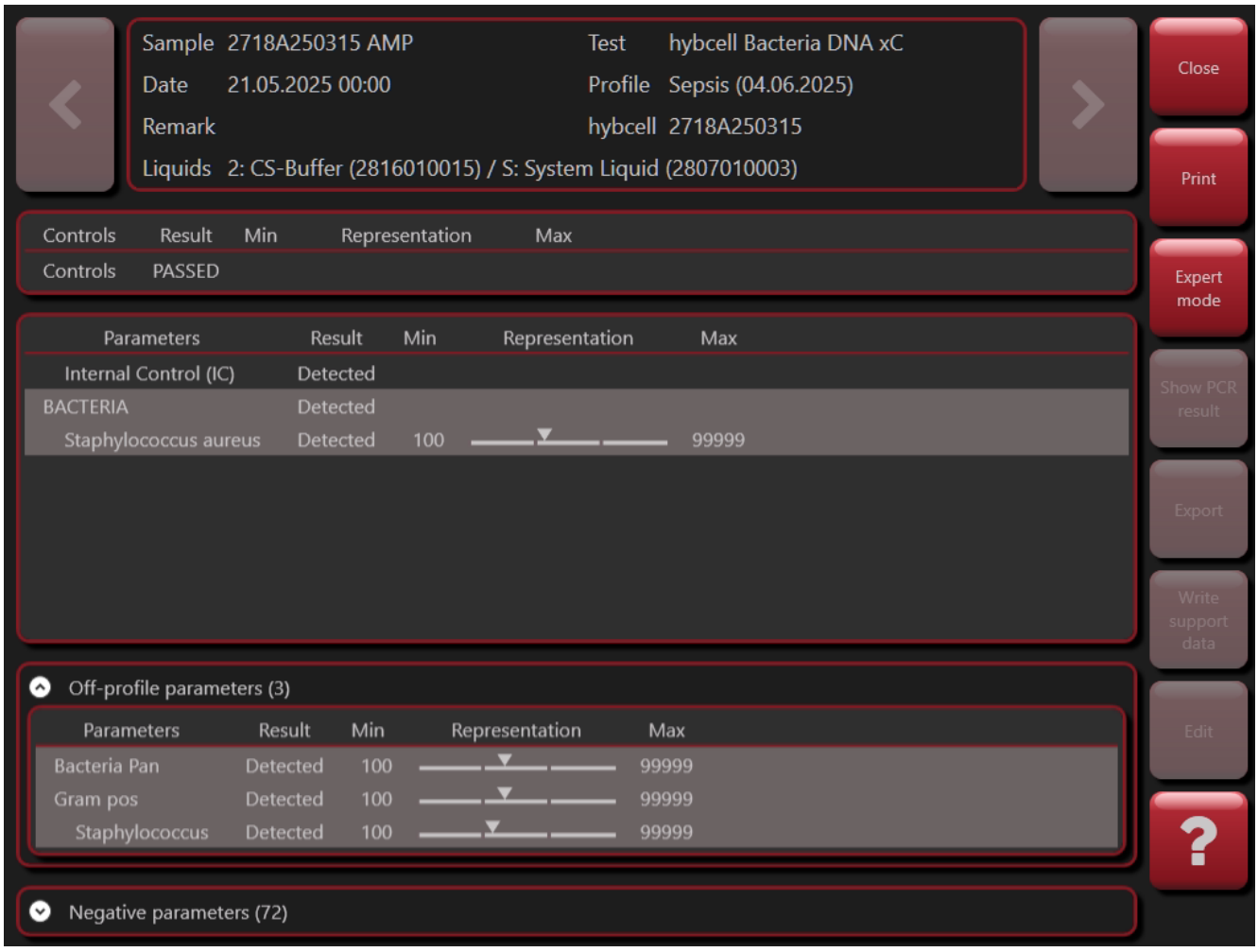
Specific protocols are imported into the hyborg software before the first use of a new lot. Up-to-date protocols are provided on the Cube Dx homepage (https://www.cubedx.com/protocols) or by your local distributor. Protocols are updated automatically by the instrument, if it is connected to the internet.

Off-profile parameters

According to the intended purpose, clinically relevant results are indicated. The protocol for the different lots contain definitions of clinically relevant bacteria and fungi for such applications like diagnosing blood stream infections, pneumonia or a general application covering all targets. The results outside this scope are labelled as “off-profile parameters”. Such results may be interpreted by infectious disease specialists.

Screen report

The result provided on the screen provides the same information as the printed report. Below an example (for another sample) of a report on the screen.



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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.

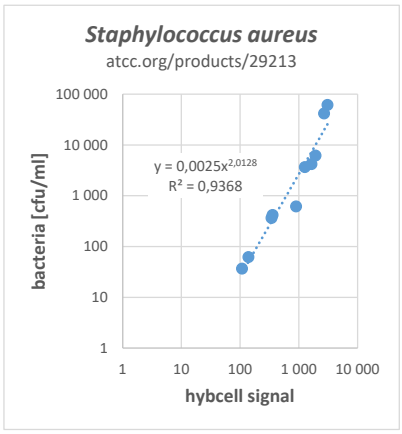
All samples have been confirm with the respective hybcells. Several bacterial and fungal targets have been analysed.

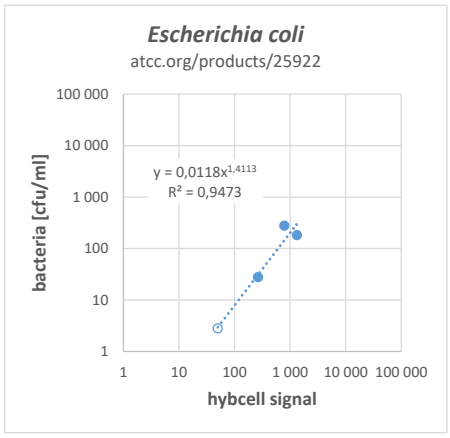
Limit of Detection (LOD) Bacteria

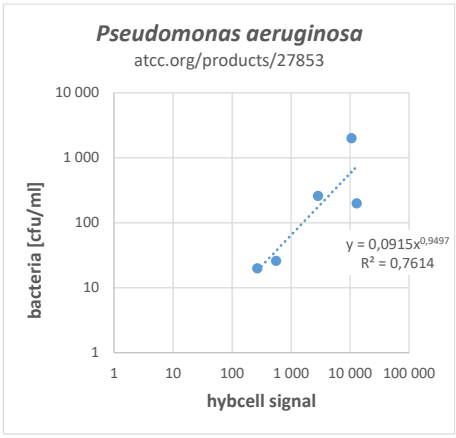
For each target, 2 replicates have been tested (for Staphylococcus aureus 4 replicates (2x2)). 3 dilutions have been prepared (for Staphylococcus aureus 5 dilutions).

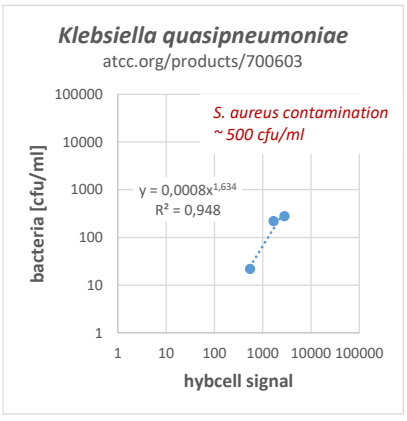
The LOD has been calculated for one ml of ETDA whole blood, by estimating the intersection of the interpolated line with the signal threshold of the hybcell (which is a signal count of 100).

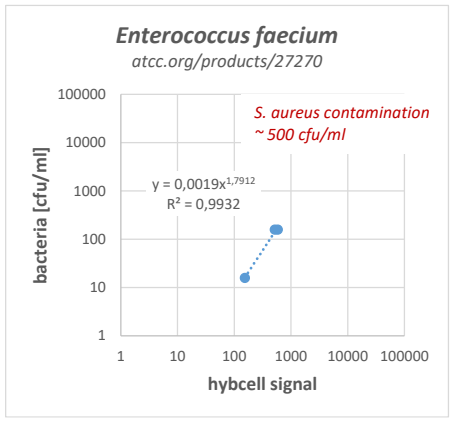
For some targets a contamination with Staph. aureus (caused by the lab technician) was observed. Even with that contamination, the LOD was below 10/ml.

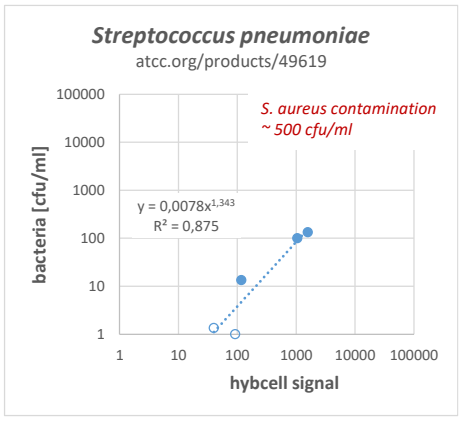












The calculated LOD for bacteria is summarized below:

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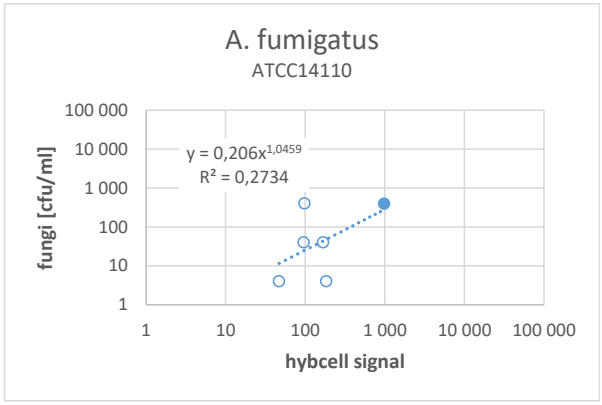
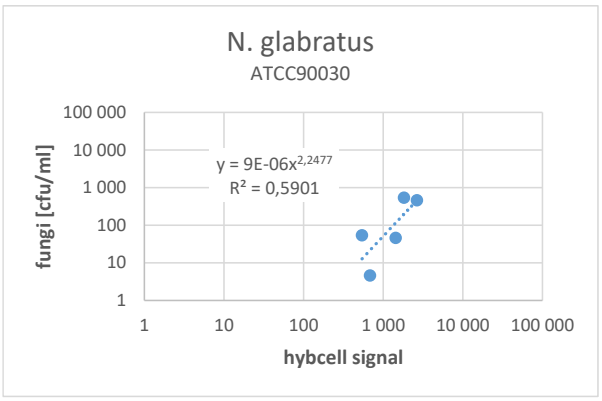
	Calculated LoD cfu/ml
Staphylococcus aureus	27
Escherichia coli	8
Pseudomonas aeruginosa	7
Klebsiella quasipneumoniae	1
Enterococcus faecium	7
Streptococcus pneumoniae	4

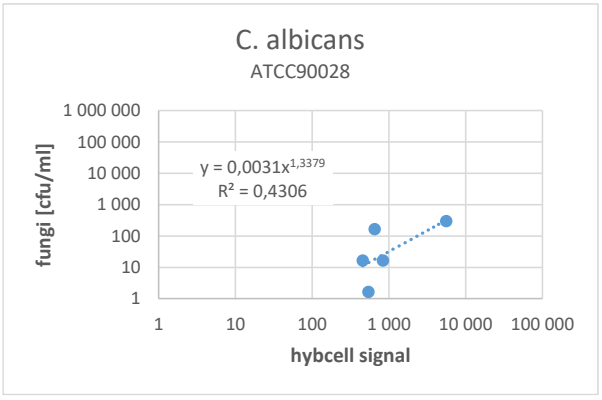
Limit of Detection (LOD) Fungi

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 and disposal

Changes in analytical performance

To verify the functionality of the test and implementation, a weekly examination with a reference standard (e.g., Cube Dx's External Process Controls (EPCs)) 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* (below) of this manual.

In an event that the shortcomings cannot be resolved, please contact Cube Dx or respective distribution partners.

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) and LE-solution tubes (GINA 500 Kit, yellow cap) are potentially containing infectious material and have to be disposed of according to your organization's rules for disposal of infectious material.

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12. Troubleshooting

Sample Preparation

Problem	Possible causes	Measure / Precaution
Loss of the pellet	▪ Pipetted away	▪ Start with decanting the supernatant and thereafter pipette away the remaining solution ▪ Repeat the extraction step
Contamination	▪ Contamination during the sample preparation step	▪ Use the recommended safety gear ▪ Clean surfaces with 1% hypochlorite, followed by 80% EtOH

Detection by PCR

Problem	Possible causes	Measure / Precaution
PCR inhibition	▪ Dilution of the PCR mix ▪ Using too high sample volumes, especially with BAL samples	▪ Use the recommended eluate amount for the PCR reaction ▪ use a dilution series when unsure what volume of BAL is suitable

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Identification by the hybcell

Problem	Possible causes	Measure / Precaution
Unspecific hybcell signals	▪ unprocessed hybcells (containing the amplicons) are not processed for too long (1-2 days) ▪ 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 procedure. • Using expired/spoilt hybcell	▪ Transfer the amplicons into the hybcell only when they can be processed immediately; IF NOT; store the amplicons as instructed in the manual. ▪ Check the lifetime of the buffers after opening the bottles ▪ Gently introduce the pipette tip into the hybcell without sealing its central channel ▪ Check the 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*. Repeat the test.
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. ▪ Repeat the test. ▪ Check the filling levels of all liquids. If necessary, refill liquids.

Troubleshooting

Cube Dx GmbH, Westbahnstraße 55, A-4300 St. Valentin / Austria, info@cubedx.com, www.cubedx.com

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 manual.

Serious Incidents / Vigilance

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