INSTRUCTIONS FOR USE

Pathogens xB_instructions for use_E_2025_07_01

IVD CE

July 2025.

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

IPC	*Basic UDI-DI 912012773PathoxBK8*	*REF / UDI-DI 09120127730169*
PCR-Box IPC		*REF / UDI-DI 09120127730114*
GINA 500	*Basic UDI-DI 912012773GINA7H*	*REF / UDI-DI 09120127730244*
GINA 500 + DNA Purification		*REF / UDI-DI 09120127730145*
Kit for enriching bacterial and fungal DNA from human blood (+ DNA purification) including an Internal Process Control (IPC)		
LINA	*Basic UDI-DI 912012773LINA8L*	*REF / UDI-DI 09120127730152*
A modulation buffer for extraction-free testing of Bronchoalveolar Lavage (BAL) and Blood Culture (BC)		

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

PCR-Box Bacteria	*Basic UDI-DI 912012773PathoxBK8*	*REF / UDI-DI 09120127730084*
PCR-Box Bacteria 48rx-s		*REF / UDI-DI 09120127730725*
PCR-Box Resistance		*REF / UDI-DI 09120127730763*
PCR-Box Bacteria Fungi		*REF / UDI-DI 09120127730091*
PCR-Box Fungi 48rx-s		*REF / UDI-DI 09120127730732*
hybcell Bacteria DNA xB		*REF / UDI-DI 09120127730053*
hybcell Fungi DNA xB		*REF / UDI-DI 09120127730060*
hybcell Pathogens DNA xB		*REF / UDI-DI 09120127730077*
Multiplex DNA tests for detection of bacterial 16S DNA and bacterial antibiotic resistance marker genes from human samples with an indication of homologies to known bacterial type strains and detection of fungal 28S DNA from human samples with an indication of homologies to known fungal type strains.		

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Content

Version information

These instructions for use have been revised in some parts. Especially the intended uses (2.) have been adapted to the rules and recommendations set out in the IVDR regulations (EU 2017/746 and its supplements and recommendations). For better readability and clarification, the technical description and the description of the application have been merged (3.). Changes in the testing of antibiotic resistance genes and the provision of product variants of the PCR-mixes are incorporated. The description of the workflow using the geneLEAD VIII (of PSS) was added. Minor changes and corrections have been made.

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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 variant *GINA 500* 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.

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-Boxes

PCR-Box Bacteria 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 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 is a ready-to-use Polymerase-Chain-Reaction (PCR)-mix to amplify and detect different antibiotic resistance genes in eluates from suitable RNA/DNA extraction processes.

The product delivers qualitative results that are used 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.

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hybcells Pathogens xB

hybcell Bacteria DNA xB is a cartridge-based test to identify bacterial species, genera or groups of species and antibiotic resistance genes using Cube Dx' proprietary *compact sequencing* and the *hyborg Dx RED2/3* instrument. *hybcell Fungi DNA xB is a* cartridge-based test to identify fungal species, genera, or groups of species using Cube Dx' proprietary *compact sequencing* and the *hyborg Dx RED2/3* instrument.

hybcell Pathogens DNA xB is a cartridge-based test to identify bacterial and fungal species, genera, or groups of species and antibiotic resistance genes using Cube Dx' proprietary *compact sequencing* and the *hyborg Dx RED2/3* instrument.

Amplicons produced by either *PCR-Box Bacteria xB* and*/or PCR-Box Resistance xB* and/or *PCR-Box Fungi xB* are introduced into the cartridges and serve as samples (see above).

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

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3. General Device Description

General Information

Identifying the causative microorganism directly from whole blood in case of suspected blood stream infections (BSI) enables an early and targeted antimicrobial therapy. The latter being a precondition for successful treatment of the infection and the limitation of often severe adverse effects, which might ultimately lead to the death of the patient. Conventional culturing methods might fail to deliver early results, especially if the causative microorganism is a fastidious bacterium (e.g., *Bordetella pertussis*) that requires unique growth conditions, or a slow-growing bacteria (e.g. *Helicobacter pylori* which needs up to 7 days to grow in culture) or fungi (e.g. *Candida glabrata*). Especially in such cases, Cube Dx's early identification of microorganisms has the potential to be beneficial for patients, as the chances of survival and recovery after suffering from sepsis and other severe infections may be increased by early identification and targeted treatment of the causing pathogen(s).

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 suspected of sepsis.

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 also result in discrepant results. We strongly recommend collecting EDTA samples at the same time as blood culture samples are collected, if still possible before the administration of antimicrobials.

This test is to be carried out as suggested in this instructions for use; interruptions of the workflow may alter results as well.

IPC, GINA 500, GINA 500 + DNA Purification

The kit *GINA 500* (for 500µl of sample volume, with or without DNA purification) is designed for clinical routine application to enrich pathogenic (bacterial, fungal) DNA. After enrichment, the solution is purified, and the eluate may be used in different PCR reactions (e.g., bacterial DNA, fungal DNA, resistance marker genes). In case of using Cube Dx' PCR-mixes, the respective pathogen can be identified straightforwardly by Cube Dx's *compact sequencing* after amplification.

GINA pathogen enrichment kits remove the vast majority of human cells and cellular debris from whole blood. 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.

As a consequence, only microorganisms that can still harm 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, microorganisms are not removed, as they remain intact. These microorganisms pose a risk to the patient, once the antimicrobial treatment stops.

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

The kit is based on the following process steps:

- Lysis and removal of human cells: Sample is added to the *LE Solution*, and most human (and compromised microbial) cells are lysed and removed after centrifugation.
- Lysis of pathogen cells: *NA Solution* is added and incubated. Pelleted pathogen cells are lysed.
- Neutralization: The lysate is transferred into the *T Solution* to stop the process of lysis and neutralize the resulting solution.
- *Including DNA purification*: spin column technology is used to purify DNA from the *GINA* lysate.

The result 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.

The following circumstances deteriorate the results for a sample:

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

Quality assurance concepts for such highly sensitive molecular pathogen identification from human samples must ensure that negative results are only caused by negative samples - and not by any flaws during the processing of the sample. Therefore, stringent process control has to undergo the same procedures as the sample itself – without setting off sensitivities of the tests. Cube Dx's Internal Process Control (IPC) consists of frozen biological material dissolved within the human sample before the enrichment process starts. The *IPC* undergoes the same extraction procedures as the sample itself. Both the follow-up PCR and the *hybcell* test (in case that the amplicon of IPC-PCR is transferred) confirm the presence of *IPC* and therefore the validity of the results.

For processing *GINA* kits, a table-top centrifuge with a rotor for 2 mL microtubes that can apply 11.000g (e.g., Eppendorf, Hermle, etc.) and a conventional heating block (e.g., Analytic Jena, Coyote Bioscience) capable to heat to 100°C are needed.

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 (Bacteria, Fungi, Resistance) and *hybcells* for pathogen identification, microorganisms, and resistance genes can be determined 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 the results for a sample:

- Use of a larger sample volume than specified (increases inhibitors).

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PCR-Box Bacteria / Resistance / Fungi / IPC
hybcell Bacteria / Fungi / Pathogens DNA xB

The tests *hybcell Bacteria DNA xB, hybcell Fungi DNA xB,* and *hybcell Pathogens DNA xB* and their related PCR reaction mix – *PCR-Box Bacteria, PCR-Box Resistance, PCR-Box Fungi,* and *PCR-Box IPC* – are designed for clinical routine application to detect and identify pathogenic bacteria and their antibiotic resistance genes as well as pathogenic fungi by using DNA extracted from samples like whole blood, BAL, or positive blood cultures, based on homologies to known DNA sequences.

PCR-Box IPC amplifies DNA from the *IPC* to confirm the validity of the test procedure by a positive IPC result on the hybcell. If using the geneLEAD VIII from PSS, an alternative concept for the internal control is used in all PCR-mixes (*PCR-Box Bacteria 48rx-s, PCR-Box Fungi 48rx-s*). For those mixes, a synthetic long DNA oligo serves as an internal control (IC) to confirm the validity of results (especially of negative results).

The tests are especially useful for patients in need of immediate and specific antimicrobial treatment (e.g., sepsis, pneumonia), for patients having already undergone treatment with antibiotics/antimycotics (as culturing might then be inhibited), or if the causative pathogens are difficult to culture.

The test is based on the following process steps/test principles:

- Amplification of DNA – detection of bacteria/fungi/resistance genes: Isolated DNA is amplified by polymerase chain reaction (PCR). Target regions are 16S rDNA for bacteria, 28S rDNA for fungi, and respective resistance genes. During amplification, single DNA strands are labeled with fluorescent dye. If using a qPCR device, the presence of bacteria, fungi, or resistance marker might be derived from the resulting amplification and melting curves.
- Identification: Qualitative analysis is performed by applying *compact sequencing*. Amplicons bind to their complementary, immobilized probes, which 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. The *hyborg* (an instrument for analysis) scans and analyzes the specific fluorescent signals.

Bacteria and antibiotic resistance genes potentially presented by *hybcell Bacteria DNA xB* and by *hybcell Pathogens DNA xB*:

Blood Culture Sepsis Pneumonia

Genus	Species	Profile
Abiothrophia	*Abiotrophia defectiva*	
Acinetobacter	*Acinetobacter baumannii*	
	Acinetobacter calcoaceticus complex	
Actinobacillus	*Actinobacillus pleuropneumoniae*	
Anaerococcus		
Bacteriodes	*Bacteroides fragilis*	
Bordetella	*Bordetella pertussis*	
Borreliella		
	Borreliella burgdorferi	
Brucella		
Burkholderia	*Burkholderia cepacia complex*	
	Burkholderia pseudomallei	

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Campylobacter				
Citrobacter	*Citrobacter koseri*			
	Citrobacter freundii complex			
Corynebacterium				
	Corynebacterium diphtheriae			
	Corynebacterium jeikeium			
	Corynebacterium ulcerans			
Enterobacter	*Enterobacter cloacae*			
	Enterobacter cloacae complex			
Enterococcus	*Enterococcus faecalis*			
	Enterococcus faecium			
Escherichia	*Escherichia coli*			
Finegoldia	*Finegoldia magna*			
Fusobacterium				
	Fusobacterium nucleatum			
	Fusobacterium necrophorum			
Granulicatella	*Granulicatella adiacens*			
Haemophilus	*Haemophilus haemolyticus*			
	Haemophilus influenzae			
Helicobacter	*Helicobacter pylori*			
Klebsiella	*Klebsiella aerogenes*			
	Klebsiella oxytoca			
	Klebsiella pneumoniae			
Legionella	*Legionella pneumophila*			
Listeria				
Moraxella	*Moraxella catarrhalis*			
Morganella	*Morganella morganii*			
Neisseria	*Neisseria meningitidis*			
Pasteurella	*Pasteurella multocida*			
Prevotella	*Prevotella buccae*			
	Prevotella intermedia			
Propionibacterium				
	Propionibacterium acnes			
Proteus				
	Proteus mirabilis			
Providencia	*Providencia stuartii*			
Pseudomonas	*Pseudomonas aeruginosa*			
	Pseudomonas non-aeruginosa			
Salmonella	*Salmonella enterica*			
Serratia	*Serratia marcescens*			
Staphylococcus				
	Staphylococcus aureus			
	Staphylococcus non-aureus			
Stenotrophomonas	*Stenotrophomonas maltophilia group*			
Streptococcus				

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	Streptococcus anginosus group	
	Streptococcus agalactiae	
	Streptococcus dysgalactiae	
	Streptococcus gordonii	
	Streptococcus mitis group	
	Streptococcus pneumoniae	
	Streptococcus pyogenes	
	Streptococcus salivarius group	
Yersinia	*Yersinia enterocolitica*	
	Yersinia pseudotuberculosis complex	

Resistance	Resistance genes	Profile
Vancomycin resistances	*vanA*	
	vanB	
Methicillin resistances	*mecA*	
	mecC	

Fungi potentially presented by *hybcell Fungi DNA xB* and by *hybcell Pathogens DNA xB*:

Genus	Species	Profile
Aspergillus		
	Aspergillus clavatus	
	Aspergillus flavus	
	Aspergillus fumigatus	
	Aspergillus niger	
	Aspergillus terreus	
Candida		
	Candida albicans	
	Candida dubliniensis	
	Candida parapsilosis	
	Candida tropicalis	
Nakaseomyces	*Candida glabrata*	
Clavispora	*Candida auris*	
Cladosporium		
Filobasidiella	*Cryptococcus neoformans*	
	Cryptococcus gattii	
Fusarium	*Fusarium oxysporum species complex*	
	Fusarium solani species complex	
Pichia	*Pichia kudriavzevii*	
Pneumocystis	*Pneumocystis jirovecii*	
	Pneumocystis murina	
Saccharomyces		
	Saccharomyces cerevisiae	
Scedosporium		

The test may be used for different diagnostic applications, but not all bacterial and fungal targets are relevant for all uses. Therefore, it is possible to narrow the scope of results of a report within the *hyborg software* by defining a profile (by selecting the targets which should be considered for the report). Cube Dx provides three profiles: Blood Culture (for positive blood cultures), Sepsis (for whole blood), Pneumonia (for BAL).

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The result may be falsified due to errors during sample preparation (low amount of DNA, contamination with environmental pathogens / DNA) or other influences during preparation (degraded DNA, contamination with chemicals), technical errors, or errors during amplification or identification. If there is suspicion that a result is incorrect or deteriorated, the results should not be considered for diagnostic decisions and the sample should be tested again. Even if internal controls should single out most of the erroneous results, some of these results may remain uncovered.

The necessary equipment includes a freezer (-15°C to -25°C) as well as a DNA workbench. The sample materials are solutions containing DNA (=eluates) that were extracted with an appropriate DNA extraction product/procedure, for example, geneLEAD VIII from PSS.

For processing *PCR-Box Bacteria, PCR-Box Resistances, PCR-Box Fungi,* and *PCR-Box IPC,* either a qPCR device (either CFX96 from Biorad or Quantstudio from Thermo Fisher Scientific) or a thermal cycler (Biometra TOne / TPersonal from Analytic Jena) or the geneLEAD VIII from PSS (combining DNA extraction and PCR) is needed.

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

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4. Product Components

IPC:

- *IPC* (REF / UDI-DI 09120127730169)
  - 25 x 20 µL *IPC* (1 box with 25 microtubes (0.5 mL) each filled with 20 µL *IPC*)

GINA 500, GINA 500 + DNA Purification:

- *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*)

LINA:

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

PCR-Boxes xB:

- *PCR-Box Bacteria* (REF / UDI-DI 09120127730084)
  - 12 x 20 µL PCR-mix Bacteria (1 bag with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix Bacteria)
- *PCR-Box Bacteria 48rx-s* (REF / UDI-DI 09120127730725)
  - 48 x 20 µL PCR-mix Bacteria (4 bags with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix Bacteria)
  - 1 x 1000 µL NTC (1 tube (2 mL) filled with 1000 µL *NTC* (no template control))
- *PCR-Box Fungi* (REF / UDI-DI 09120127730091)
  - 12 x 20 µL PCR-mix Fungi (1 bag with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix Fungi)
- *PCR-Box Fungi 48rx-s* (REF / UDI-DI 09120127730732)
  - 48 x 20 µL PCR-mix Fungi (4 bags with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix Fungi)
  - 1 x 1000 µL NTC (1 tube (2 mL) filled with 1000 µL *NTC* (no template control))
- *PCR-Box Resistance* (REF / UDI-DI 09120127730763)
  - 12 x 20 µL PCR-mix Resistance (1 bag with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix Resistance)
- *PCR-Box IPC* (REF / UDI-DI 09120127730114)
  - 12 x 20 µL PCR-mixes IPC (1 bag with 12 PCR tubes (0.2 mL) each filled with 20 µL PCR-mix IPC)

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hybcell DNA xB

- *hybcell Bacteria DNA xB* (REF / UDI-DI 09120127730053)
  - 24 x *hybcell Bacteria DNA xB* Rev.2 (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 xB* (REF / UDI-DI 09120127730060)
  - 24 x hybcell Fungi DNA xB Rev.2 (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 Pathogens DNA xB* (REF / UDI-DI 09120127730077)
  - 24 x hybcell Pathogens DNA xB Rev.2 (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*	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 xB *hybcell Fungi DNA xB* *hybcell Pathogens DNA xB*	24	8 to 25°C	4 to 40°C	Residual waste
IPC	24	-15 to -25°C	< 0°C	Residual waste
PCR-Box Bacteria *PCR-Box Fungi* *PCR-Box Resistance* *PCR-Box IPC*	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 or the IPC after thawing destroys the product and the product must not be used. Thawed PCR reactions and IPC 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* and LE-solution tubes (*GINA 500* Kit) 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*!

BAL and Positive Blood Culture

- 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
PE-Buffer	Cube Dx: 1 L, sufficient for 96 *hybcells*	09120127730138	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 DNA extractors, 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 *PE-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*)
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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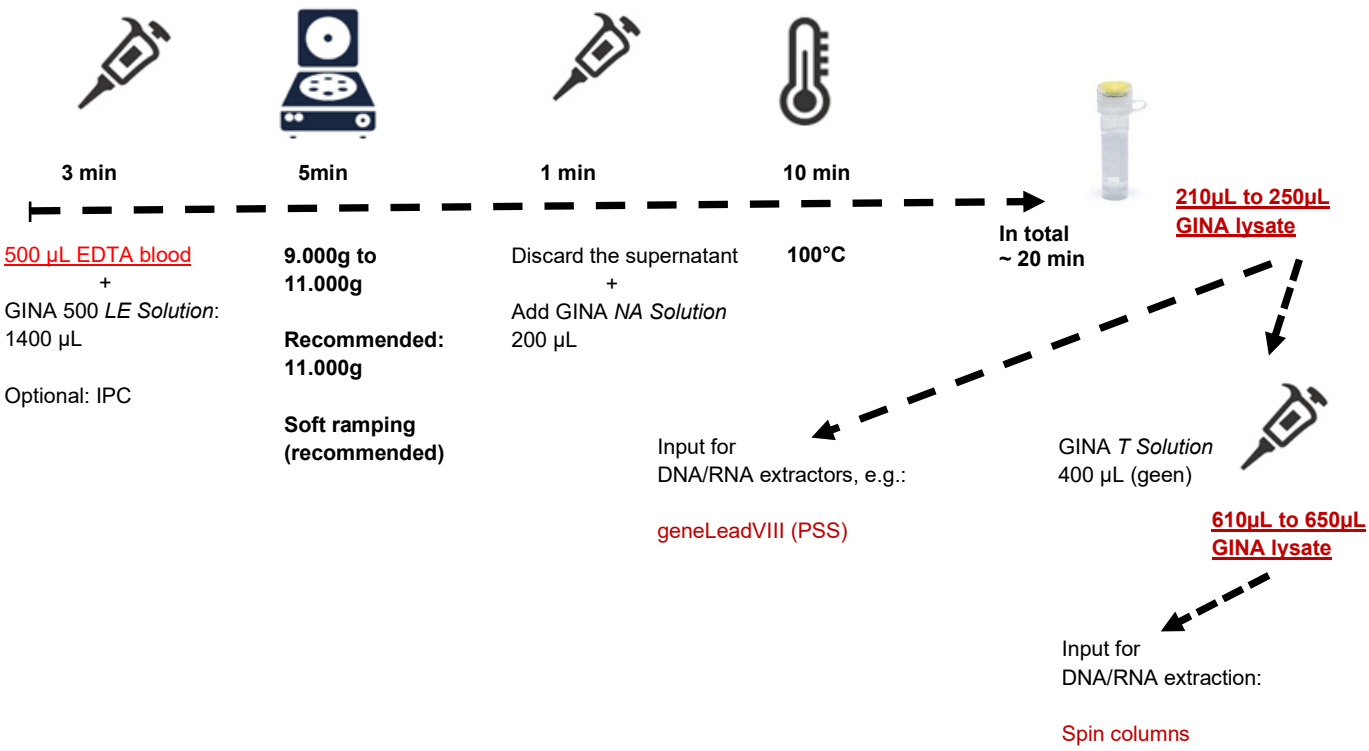
1 Sample Preparation

EDTA whole blood – enrichment of microbes: GINA 500

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). 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, IPC*

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. *EPC*) 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.*

   Check if the recommended g-force of 11,000g 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)!

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) of EDTA blood into the LE Solution and pipette up and down to mix.

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2. Optional: Pipette 500 µL of the blood sample into the *IPC* (and/or spike material like Cube Dx' *EPC S.aureus 10000* and *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 9.000g 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 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****.*

BAL or positive blood cultures: LINA

Required products: *LINA, IPC*

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. Optional: Pipette 20 µL IPC (one reaction) into the *LINA* tube.
3. Pipette the sample into the *LINA* tube:
   - **2 µL** of **positive blood culture**
   - **10-20 µL** of **BAL**
4. Close the *LINA* tube and invert several times or vortex firmly.
5. No further DNA extraction is needed. Use the solution directly for PCR (20 µL of the solution for each PCR reaction).

   Remark:

   Different institutes have varying methods of collecting BAL samples. Therefore, the amount of BAL used may have to be adapted. However, too high volumes of BAL may result in inhibition. So, using IPC is recommended to indicate inhibition of PCR.

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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, *GINA* lysate). 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 48rx-s / PCR-Box Fungi 48rx-s

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

EDTA whole blood

If working with *GINA* and EDTA whole blood: Remove the lid from the LE Solution-tube after completion of the GINA process (see above) and use this tube as the sample container for the geneLEAD VIII instrument.

BAL or positive blood cultures

If working with *LINA* and BAL or positive blood culture: use an elution tube from the geneLEAD VIII Consumable Set (F8900) and pipette 50 µL of the *LINA*-sample-mix into this tube

Loading and starting the instrument

1. Unpack and thaw the necessary PCR-mixes (*PCR-Box Bacteria 48rx-s* / *PCR-Box Fungi 48rx-s*).
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 DNA purification and follow-up PCR. Most of the consumables for the geneLEAD VIII (except sample and eluation tube) are shaped like right-angled bars. All consumables 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 for EDTA whole blood processed with *GINA* (see above): 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):

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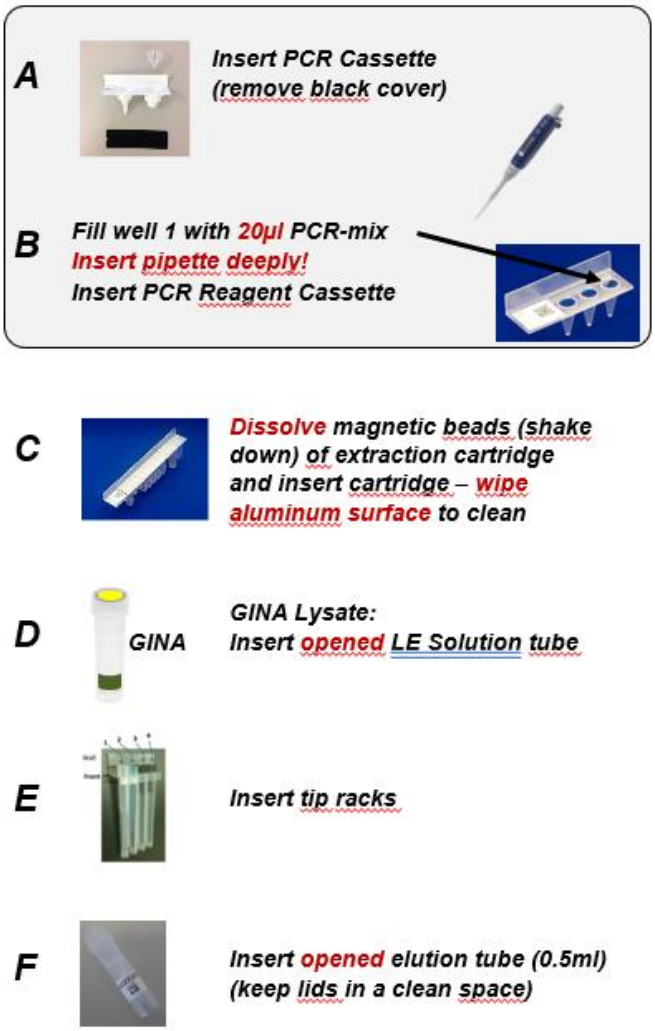
D. Only for EDTA whole blood processed with *GINA* (see above): Load the sample (*LE Solution*-tube, see above) into the instrument. The sample volume must be at least 200 µL.

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 tubes which are as well provided in the kit). Open the used elution tubes.

- EDTA whole blood (*GINA*): use a new (and empty) elution tube
- BAL and positive blood culture (*LINA*): elution tube with *LINA*-sample-mix (see above)

- Put Tray 2 into the instrument



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 - 200 µL, Extracted Elute Volume - 100 µL.
6. Choose the corresponding protocol named *CDX Bacteria_xB_gLVIII_xx* or *CDX_Fungi_xB_gLVIII_xx* or *CDX_Resistance_xB_gLVIII_xx* and select *Extraction + PCR* (xx stands for the current version of the protocol) for EDTA whole blood (*GINA*) or *PCR Only* for BAL and positive blood cultures (*LINA*). If the protocols are not available on the instrument, it 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".

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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

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 / PCR-Box Fungi / PCR-Box Resistance

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.

EDTA whole blood

If working with *GINA* and EDTA whole blood: Continue with the *GINA* lysate (including the 400 µL T Solution, see workflow above) at step 1. below.

BAL or positive blood cultures

If working with *LINA* and BAL or positive blood culture: Continue with step 9. below. Instead of eluate, fill 20 µL of the LINA-Sample-mixture into the PCR tubes.

1. For each *GINA* lysate, place one *Column* into a *Collection Tube* and mark the *Collection Tube* with the sample ID. Transfer the whole *GINA* lysate (600 to 650 µL) into the *Column*. Discard the tube for the *LE 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.

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

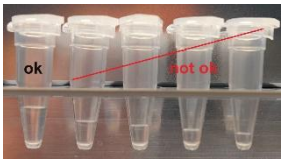
10. 1	95°C for 2:00
2	95°C for 0:10
3	56°C for 0:10
4	72°C for 0:30
	+Plate Read
	GO TO 2, 44 more times
5	75°C for 1:00
6	Melt curve 75°C to 95°C in increments of 0,3°C for 0:10
	+Plate Read
7	25°C for hold

Fluorophore / channel: SYBR Green / FAM

Remark!

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

10. Unpack and thaw single 0,2 mL tubes of the required PCR-mixes for IPC, bacteria, fungi, and resistance genes. Homogenize (vortex) and spin down the liquid of each tube briefly.
11. 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.



12. Add 20 µL DNA eluate from the sample / 20 µL from the LINA-sample-mixture (or 20 µL of NTC or 20 µL of Control DNA SA/CA – available from Cube Dx) to the PCR-mixes.
13. Close PCR tubes, homogenize, and spin down liquids before starting PCR.
14. Start (q)PCR program *Patho_xB* (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").

15. 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 or more amplicons resulting from (q)PCR (see above).

Required products: *hybcell Bacteria DNA xB / hybcell Fungi DNA xB / hybcell Pathogens 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. Pipette a maximum of 3 amplicons from the same sample into one *hybcell.* Use one of the PCR tubes to make the mix first and add the *PPE-Additive*. For example, pipette 40 µL each of *PCR-Box Bacteria*, *PCR-Box Fungi, or PCR-Box Resistance* into one of the PCR tubes (e.g., *PCR-Box Bacteria).* Thereafter, pipette 30 µL of the *PPE-Additive* (within the *hybcell* kit box) into the tube with the amplicon mixture.

   Attention!

   Using of only 1 or 2 PCR amplicons for the hybcell test results in the best performance due to lower signal background. Up to 3 PCR amplicons can be used for a single hybcell. By using all 4 amplicons for the same hybcell test the risk of increased signal background rises and this may lead to reduced specificity and sensitivity of the test.

   Remember that the validity of the Internal Process Control (IPC) can already be derived from the PCR amplification curves (compare results in Chapter 9).

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

Examples of amplicon combinations suitable for the loading of the hybcell

Results of PCR				hybcell…	Transferred amplicons
Bacteria	Fungi	Resist.	IPC		
pos.	pos.	pos.	pos.	*...Pathogens DNA xB*	Bacteria + Fungi + Resistance
pos.	pos.	neg.	pos.		Bacteria + Fungi
pos.	neg.	pos.	pos.	*…Bacteria DNA xB*	Bacteria + Resistance
pos.	--	pos.	pos.		Bacteria + Resistance
pos.	--	neg.	pos.		Bacteria + IPC
--	pos.	--	pos.	*…Fungi DNA xB*	Fungi + IPC
neg.	pos.	neg.	pos.		Fungi + IPC

4. Pipette up and down to mix all the constituents in the tube (**a pH indicator is present in the additive; the color of the solution may therefore change. This does not influence the performance of the product**). Avoid bubbles!
5. Pipette the entire volume from the tube (~ 150 µL maximum) into the *hybcell* (through the central channel) at once. The final volume is dependent on number of amplicons used (see above).

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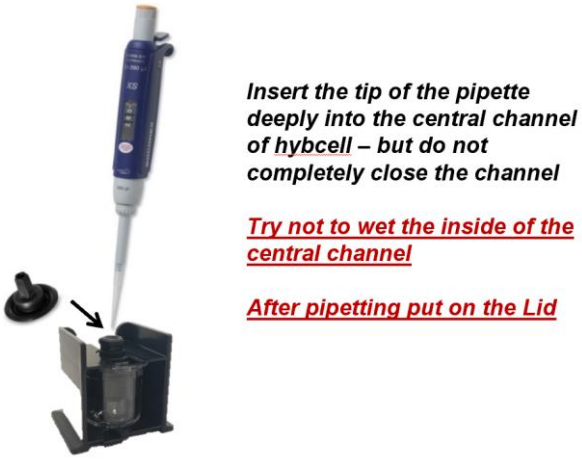
Use a 200 µL pipette with appropriate filter tips! Do not block the hybcell central channel (sample inflow) with the pipette tip while introducing the amplicon mix! Only insert the tip as deep as needed into the hybcell central channel, make sure to allow a loose fit.

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



7. 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.

   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.

All PCR-mixes use the same fluorescent dye *SYBR Green*.

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*.

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.
- The *PCR-Box Resistance* comprise several primers (resistance genes). Therefore, primer dimers are more likely to occur than with the single-plex PCR such as *PCR-Box Bacteria* or *Fungi*.
- 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 appearance of 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 other samples (not negative) for further testing with *hybcell*.

Option 1: geneLEAD VIII

PCR-Box Bacteria 48rx-s and *PCR-Box Fungi 48rx-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

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

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Option 2: Manual extraction + PCR instrument

PCR-Box Bacteria, PCR-Box Fungi and *PCR-Box Resistance* only amplify the respective target (bacteria, fungi or resistance genes for gram+-associated resistances) Hence, when testing negative samples (no target in the sample), the PCR test does not show any amplification and no melting peak.

Therefore, an *Internal Process Control (IPC*) confirms the validity of negative results in a parallel PCR. It is a positive process control that allows 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 *IPC* is either confirmed by the PCR result (when using qPCR instruments, see above) or with the *hybcell* test (see below).

IPC and therefore the test is valid if the following criteria for the *PCR-Box IPC* are met. In that case, adding the amplicon to the *hybcell* test is not necessary (see above, limitation of PCR amplicons).

Attention!

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

The table below shows the cut-offs to differentiate between negative samples and samples that need a follow-up*hybcell* analysis.

		Amplification	Melting curve
		Ct	Tm1
IPC	Valid	< 34	85°C ± 1°C
	Invalid	> 34	--
Bacteria	Negative	> 36	--
	Test with hybcell	< 36	80°C to 90°C
Fungi	Negative	> 36	--
	Test with hybcell	< 36	80°C to 94°C
Resistance	Negative	> 36	--
	Test with hybcell	< 36	80°C to 90°C

Grey Boxes indicate mandatory criteria.

Ct ... Cycle threshold (cycle number)

Tm ... Melting temperature (°C)

Below is an example of a blood sample spiked with an External Process Control (EPC) of *Candida albicans* and Internal Process Control (IPC).

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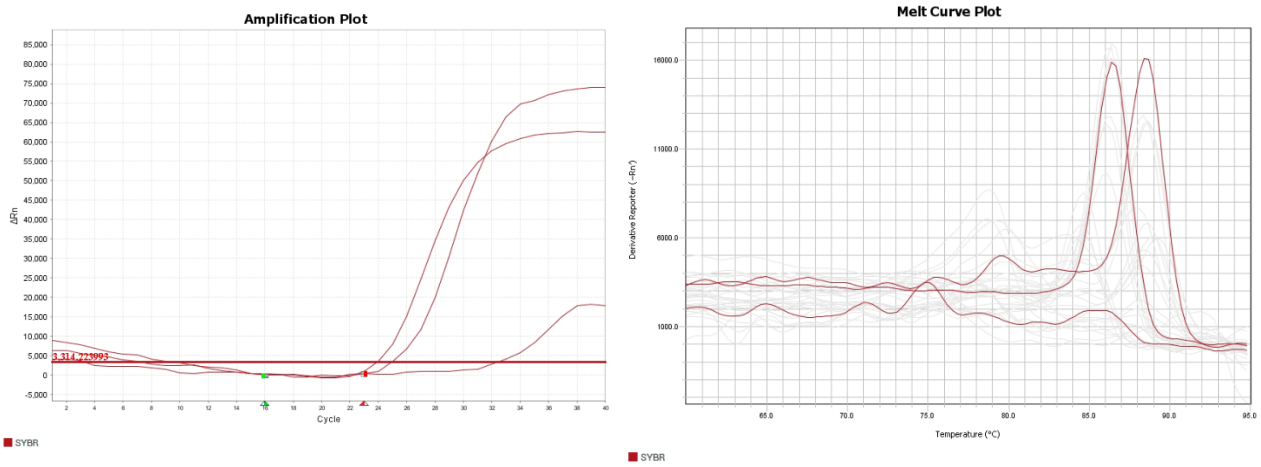
	Ct-Value	Melting Temperature	Derived Result
IPC	24	86.51°C	**Valid**
Bacteria	32.8	74.92°C	**Negative**
Fungi	25	88.54°C	**Positive**



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.

Check for PCR-mixes

As the user chooses to use all or just selected PCR-mixes, the usage of PCR-mixes is indicated with probes on the *hybcell* surface. If a PCR-mix is added, the result on the report for this mix is 'ADDED'; otherwise it is 'MISSING'.

- **Bacteria PCR Mix**: Checks if the *PCR-Box Bacteria* was used.
- **Fungi PCR Mix**: Checks if the *PCR-Box Fungi* was used.

Specificity Control

The tests feature a test-specific control. If the control is passed, the result is 'PASSED'. Otherwise, the result is 'FAILED'. Even if it fails, the test is analyzed and results are presented. However, these controls help to judge the plausibility of the results.

- **Specificity Control**: Checks if *compact sequencing* was successful.

Internal Process Control (IPC)

The Internal Process Control (*IPC*)is a test-specific control that is examined with every *hybcell* test. Only if the (positive) PCR-amplicon of *IPC* is added into the *hybcell* and corresponding probes on the *hybcell* surpass the

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signal threshold, the result is ‘DETECTED’. Otherwise – if either no positive amplicon was added or the signal threshold was not passed – the result is ‘NOT DETECTED’.

- **Internal Process Control**: Checks if the amplicon of *IPC* was added and the threshold for *IPC* was surpassed.

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.
- **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.

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hyborg Reports

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cube dx
hybCell technology

Sample	SAE3 CAE2	**Test**	hybcell Pathogens DNA xB (1)
Date	21.01.2026 09:38	**Profile**	Sepsis (22.08.2025)
Remark		**hybcell**	2733A510277
Liquids	1: PE-Buffer (138283001) / S: System Liquid (2840010028)		

Controls		
Controls	PASSED	
Bacteria PCR Mix	ADDED	
Fungi PCR Mix	ADDED	

Parameters	Result	Representation
Specificity Control	PASSED	
Internal Process Control	NOT DETECTED	
BACTERIA	Not Detected	
FUNGI	Detected	
Candida sp.	Detected	100 99999
Candida albicans	Detected	100 99999

Off-profile parameters	Result	Representation
Bacteria Pan	Detected	100 99999
Fungi Pan	Detected	100 99999

Negative Parameters

Abiotrophia defectiva, Acinetobacter baumannii, Actinobacillus pleuropneumoniae, Anaerococcus sp., Aspergillus clavatus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus sp., Aspergillus terreus, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Borrelia sp., Brucella sp., Burkholderia cepacia complex, Burkholderia pseudomallei, Campylobacter sp., Candida dubliniensis, Candida parapsilosis, Candida tropicalis, Citrobacter freundii complex, Citrobacter koseri, Corynebacterium diphtheriae, Corynebacterium jeikeium, Corynebacterium sp., Corynebacterium ulcerans, Cryptococcus gattii, Cryptococcus neoformans, Enterobacter cloacae, Enterobacter cloacae complex, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Finegoldia magna, Fusarium oxysporum species complex, Fusarium solani species complex, Fusobacterium necrophorum, Fusobacterium nucleatum, Fusobacterium sp., Granulicatella adiacens, Haemophilus haemolyticus, Haemophilus influenzae, Helicobacter pylori, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Legionella pneumophila, Listeria sp., Moraxella catarrhalis, Morganella morganii, Nakaseomyces glabratus, Neisseria meningitidis, Pasteurella multocida, Pichia kudriavzevii, Pneumocystis jirovecii, Pneumocystis murina, Prevotella buccae, Prevotella intermedia, Proteus mirabilis, Proteus sp., Providencia stuartii, Pseudomonas aeruginosa, Salmonella enterica, Scedosporium, Serratia marcescens, Staphylococcus aureus, Staphylococcus sp., Stenotrophomonas maltophilia group, Streptococcus agalactiae, Streptococcus anginosus group, Streptococcus dysgalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus salivarius group, Yersinia enterocolitica

Example of a PDF report for a sample tested positive for Candida albicans and unspecific bacteria.

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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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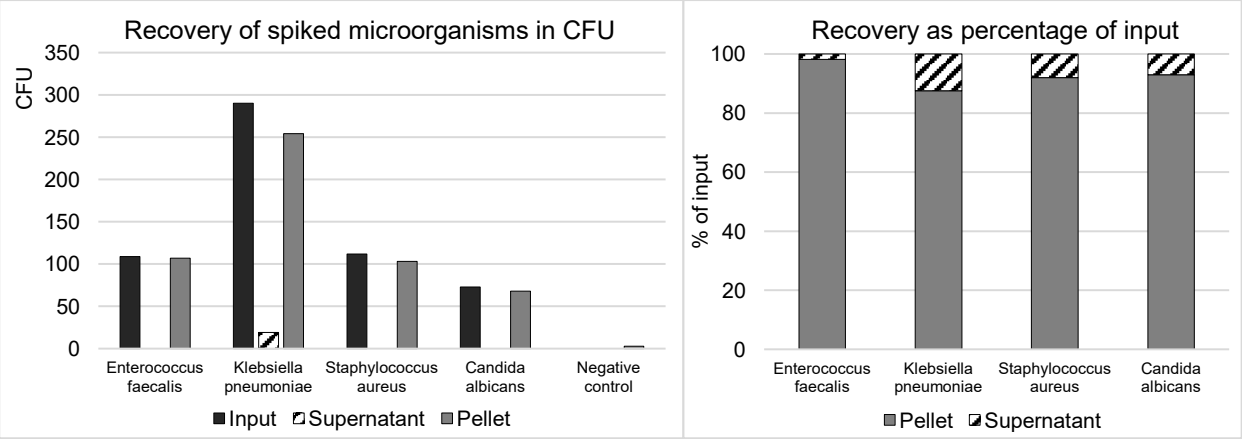
9. Analytical Performance

GINA 500, GINA 500 + DNA Purification

Recovery of pathogens: Living microorganisms (*Staphylococcus aureus, Candida albicans*, *Enterococcus faecalis, Klebsiella pneumoniae*) were spiked into EDTA whole blood samples of healthy probands. These samples were homogenized (vortexed). Empty growth medium was spiked as a negative control. The first step of the *GINA 500* protocol was executed (LE Solution + centrifugation). The resulting pellets were resuspended in 100 µL EDTA whole blood and plated out on LB agar. After centrifugation, 100 µL of the supernatant were plated out to determine the number of living microorganisms that were not bound in the pellet (= loss). Colonies were counted and documented after 24 to 48 hours of incubation.



The rate of recovery lies between 88% (*Klebsiella pneumoniae*) and 98% (*Enterococcus faecalis*).

PCR-Box Bacteria / hybcell Bacteria DNA xB

The limit of detection (LOD) was determined by diluting cultures of *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Pseudomonas aeroginosa* and processing these with the *GINA 500 + DNA purification* product and protocol. To determine the corresponding colony forming units (CFUs), aliquots of the dilutions were plated out and colonies were counted after 24 / 48 hours of incubation.

For all three targets, the **LOD was determined between 10 to 20 CFU / mL**.

Selectivity was tested with referenced DNA samples from ATCC (American Type Culture Collection) and DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen).

Acinetobacter baumannii c.	DSM30007	*Actinobacter pleuropneumoniae*	DSM13472
Borreliella burgdorferi	DSM4680	*Burkholderia cepacia complex*	DSM7288
Brucella sp.	DSM103976	*Campylobacter jejunii*	DSM4688
Citrobacter freundii compl.	DSM30039	*Citrobacter koseri*	DSM4596
Corynebacterium diphtheriae	ATCC 700971D-5	*Corynebacterium jeikeium*	DSM7113
Corynebacterium ulcerans	DSM46325	*Enterobacter aerogenes*	DSM30053
Enterobacter cloacae compl.	DSM30054	*Enterococcus faecium*	DSM20477
Enterococcus faecalis	DSM20478	*Escherichia coli*	DSM30083
Finegoldia magna	DSM20470	*Fusobacterium necrophorum*	DSM20698
Fusobacterium nucleatum	DSM15643	*Haemophilus influenzae*	DSM4690
Helicobacter pylori	DSM21031	*Klebsiella oxytoca*	DSM5175
Klebsiella pneumoniae	DSM30104	*Legionella pneumophila*	DSM25213
Listeria monocytogenes	DSM15675	*Moraxella catarrhalis*	DSM9143
Morganella morganii	DSM30117	*Neisseria meningitidis*	DSM10036
Prevotella intermedia	DSM20706	*Propionibacterium granulosum*	ATCC 25746D-5

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Proteus mirabilis	DSM4479	*Pseudomonas aeroginosa*	DSM50070
Pseudomonas syringae	DSM50274	*Salmonella enterica*	DSM554
Serratia marcescens	DSM30121	*Staphylococcus aureus*	DSM20774
Staphylococcus epidermidis	DSM20044	*Staphylococcus haemolyticus*	DSM20263
Stenotrophomonas maltophilia	DSM21257	*Streptococcus agalactiae*	DSM2134
Streptococcus anginosus gr.	DSM20563	*Streptococcus dysgalactiae*	DSM20662
Streptococcus pneumoniae	DSM20566	*Streptococcus pyogenes*	DSM20565
Yersinia enterocolitica	DSM11067	*Yersinia pseudotuberculosis*	DSM8992

For each experiment DNA of two different species were mixed and tested.

Each tested bacterial DNA did show the **expected result** on the *hyborg* report.

No unspecific results or cross-reactivities have been observed.

Repeatability was determined by amplifying different dilutions of *Staphylococcus aureus* DNA several times.

- **PCR-Box Bacteria**, calculated CV at a mean Cq-value of 23,4: CV = 1,3 %.

PCR-Box Fungi / hybcell Fungi DNA xB

The limit of detection (LOD) was determined by diluting cultures of *Candida albicans* and processing them with the *GINA 500 + DNA purification* product and protocol. To determine the corresponding CFUs, aliquots of the dilutions were plated out and colonies were counted after 24 / 48 hours of incubation.

The **LOD** for *Candida albicans* **is ~ 2 CFU / mL**.

Selectivity was mainly tested with referenced DNA samples from ATCC (American Type Culture Collection) and DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen):

Aspergillus clavatus	ATCC 1007D-2	*Aspergillus flavus*	ATCC
Aspergillus fumigatus	ATCC 1022	*Aspergillus niger*	DSM1957
Candida albicans	ATCC 11006	*Candida dubliniensis*	DSM28723
Candida glabrata	ATCC	*Candida parapsilosis*	ATCC 22019D-5
Candida tropicalis	ATCC MYA-3404D-5	*Cladosporium sp.*	DSM19653
Cryptococcus neoformans	ATCC MAY-565	*Pichia kudriavzevii*	ATCC
Saccharomyces cerevisiae	*Molzym P1*		

For each experiment, DNA of a bacterial species and a fungal species was mixed and tested.

Each tested fungal DNA did show the **expected result** on the *hyborg* report.

The following unspecific results could be observed:

Testing ***Aspergillus clavatus*** showed positive results for *Aspergillus* ***clavatus*** + *Aspergillus* ***fumigatus***.

Repeatability was determined by amplifying different dilutions of *Candida albicans* DNA several times.

- **PCR-Box Fungi**, calculated CV at a mean Cq-value of 35,4: CV = 2,4 %.

IPC / PCR-Box IPC

Repeatability was tested with 16 different EDTA whole blood samples: *IPC* (20 µL) was added, and the samples were processed according to the *GINA 500* protocol (including DNA purification). The IPC-PCR (see graphs below) was run, and the results were verified by running *hybcell Pathogens DNA xB* as well as by sequencing (Sanger) the PCR products.

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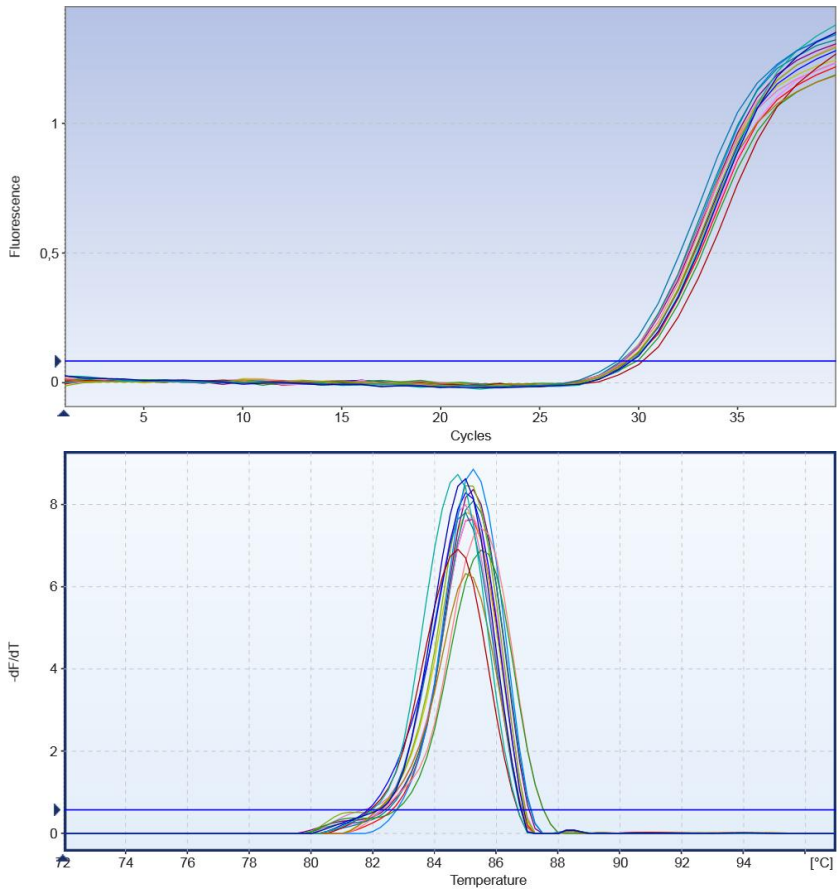

The analysis of quantification cycles (Cq) resulted in (all values rounded):

Average:	Cq 29,6
Standard Deviation:	+/- 0,3
Coefficient of Variation (CV):	1,1%

The **threshold for the Cq** of the IPC is set to **30 +/- 4** (26 to 34). This threshold can slightly vary between different PCR instruments.

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10. Clinical Performance

GINA – whole blood

Performance evaluation presented during ECCMID 2021 (01833 SMARTDIAGNOS – next-generation molecular sepsis diagnosis):

Results / Conclusions

In total 352 samples were tested with *GINA and compact sequencing* and compared to blood culturing with MALDI-TOF identification or clinical evaluation. Sensitivity was 74% and specificity 98%. 96% of the samples were correctly classified by the *GINA and compact sequencing* system. The system performs well in detecting pathogens directly in blood and covers at least 80-85% of the microorganisms causing severe infections in Europe. The system is easy to use with a 3-4h response time for a single sample.

		Blood Culture		Overall Correctness	Sensitivity	Specificity
		Positive	Negative			
Cube Dx	Positive	28	5	96%	74%	98%
	Negative	10	309			
Total		352				

LINA – (positive) blood culture

Performance evaluation presented during ECCMID 2020 (Abstract 6917 – Molecular pathogen identification and resistance gene detection from positive blood culture):

Results / Conclusion

In total 277 samples were tested with *LINA and compact sequencing* and compared to blood culturing with MALDI-TOF identification or clinical evaluation. Results for positive BC samples with *LINA compact sequencing* were obtained within 2-3 hours. *LINA* detected almost all positive blood cultures concordantly with currently established methods, resulting in a sensitivity of 98%. In addition, several mixed infections and slow-growing bacteria were identified that were missed by culturing and MALDI-TOF identification, including *Acinetobacter species,* which are highly relevant carriers of antibiotic resistance genes.

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		Blood Culture		Overall Correctness	Sensitivity	Specificity
		Positive	Negative			
Cube Dx	Positive	166	13	94%	98%	88%
	Negative	4	94			
	Total	277				

LINA – BAL

Performance evaluation performed in cooperation with a German University hospital (unpublished):

Results / Conclusion

BAL samples from 79 patients (Institute for Medical Microbiology, University Hospital Essen / Germany) were analyzed by the state-of-the-art blood culture method, the Unyvero system (DNA based), and Cube Dx's *LINA and compact sequencing technology*. The matching results of at least two of the reference methods were considered "true". One sample was excluded from further analysis because the reference methods could not determine a consistent result. Therefore, the total number of samples was reduced to 78. For 31 samples the result was correctly classified as positive, for 32 samples the result was correctly classified as negative. Of the 9 false-positive results, 5 showed *Haemophilus influenzae*. Of 6 false-negative results, 3 did not indicate *Staphylococcus aureus*.

		Concession-Results		Overall Correctness	Sensitivity	Specificity
		Positive	Negative			
Cube Dx	Positive	31	9	81%	84%	78%
	Negative	6	32			
	Total	78				

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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* (below) 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, especially with BAL samples ▪ Ethanol residues present in the eluate	▪ Use the recommended eluate amount for the PCR reaction ▪ Use a dilution series when unsure what volume of BAL 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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