

INSTRUCTIONS FOR USE



Pathogens xB instructions for use E 2023-07-17
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IVD **CE**
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IPC	REF	HC0471-25
	GTIN	9120127730169
GINA 500	REF	HC0400-50
	GTIN	9120127730244
GINA 500 + DNA Purification	REF	HC0404-50
	GTIN	9120127730145

Kit for enriching bacterial and fungal DNA from human blood (+ DNA purification) including an Internal Process Control (IPC)

LINA	REF	HC0405-50
	GTIN	9120127730152

A modulation buffer for extraction-free testing of Bronchoalveolar Lavage (BAL) and Blood Culture (BC)

PCR-Box Bacteria / Resistance / Fungi / IPC	REF	HC0410-12
	GTIN	9120127730084
	REF	HC0460-12
	GTIN	9120127730107
	REF	HC0420-12
	GTIN	9120127730091
	REF	HC0470-12
	GTIN	9120127730114
hybcell Bacteria / Fungi / Pathogens DNA xB	REF	HC0412-24
	GTIN	9120127730053
	REF	HC0422-24
	GTIN	9120127730060
	REF	HC0431-24
	GTIN	9120127730077

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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1. Explanation of symbols

Symbol	Explanation
	CE mark. In vitro diagnostic medical device.
	Manufacturer.
	Date of manufacture.
	Lot/batch number.
	Catalog number.
	Serial number.
	Keep away from rain/humidity.
	Keep away from sunlight.
	Only use it once. Do not reuse.
	Do not use it if the package is damaged.
	Do not eat or drink.
	Use by date.
	Temperature limit for storage.
	Sufficient for <n> tests.
H301	Toxic if swallowed.
H315	Causes skin irritation.
H318	Causes serious eye damage.
H371	May cause damage to organs.
H412	Harmful to aquatic life with long lasting effects.
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.



P501	Dispose of contents/container in accordance with local/regional/national/international regulations.
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2. Introduction and intended use

IPC, GINA 500, GINA 500 + DNA Purification

GINA pathogen enrichment (and DNA purification) kits remove the vast majority of human (blood) cells and cellular debris from human whole blood and other human samples. The procedure is intended to drastically increase the percentage of pathogenic (bacterial and fungal) DNA of intact pathogens relative to human DNA in the resulting solution and to provide better conditions for downstream PCR reactions.

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. The follow-up PCR and hybcell test confirm the presence of IPC DNA and therefore the validity of the results.

The procedure must be carried out in an environment suitable for molecular biological testing. This includes DNA- and DNase-free pipets, separated rooms for DNA isolation and amplification/detection, and the possibility of UV decontamination.

The test should exclusively be performed by qualified personnel, which have been trained in the use of CubeDx products for the identification of pathogens.

For processing *GINA* kits, a table-top centrifuge with a rotor for 2mL tubes 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.

The kit is not intended for follow-up quantitative determination of pathogens (in terms of colony-forming units) present in the sample.

LINA

The identification of pathogens and antibiotic-resistance genes should be simple and fast. The LINA transfer and modulation buffer shorten the time for molecular identification as it eliminates the RNA/DNA extraction processes and enables direct 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 and for positive human blood cultures.

Together with Cube Dx's PCR products (Bacteria, Fungi, Resistance), pathogen identification hybcells, microorganisms, and resistance genes can be determined in less than 2 hours!

The procedure must be carried out in an environment suitable for molecular biological testing. This includes DNA- and DNase-free pipets, separated rooms for DNA isolation and amplification/detection, and the possibility of UV decontamination.

The test should exclusively be performed by qualified personnel, which have been trained in the use of Cube Dx products for the identification of pathogens.

This buffer is not intended for follow-up quantitative determination of pathogens (in terms of colony-forming units) present in the sample.



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

The *PCR-Box Bacteria*, *PCR-Box Fungi*, *PCR-Box Resistance*, and qualitative tests *hybcell Bacteria DNA xB*, *hybcell Fungi DNA xB*, and *hybcell Pathogens DNA xB* are in-vitro tests for the detection and identification of bacteria, antibiotic resistance mechanisms and fungi from human samples based on homologies of bacterial 16S DNA, resistance genes and fungal 28S DNA. The *PCR-Box IPC* amplifies the DNA of the IPC (Internal Process Control) added to the initial EDTA blood sample processed with *GINA*. The test might support therapeutic decisions for suspected (severe) bacterial and/or fungal infections in combination with other clinical information.

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		
Abiotrophia	<i>Abiotrophia defectiva</i>	■	■	■
Acinetobacter	<i>Acinetobacter baumannii</i>	■	■	■
	<i>Acinetobacter calcoaceticus complex</i>	■	■	■
Actinobacillus	<i>Actinobacillus pleuropneumoniae</i>	■	■	■
Anaerococcus		■	■	■
Bacteroides	<i>Bacteroides fragilis</i>	■	■	■
Bordetella	<i>Bordetella pertussis</i>	■	■	■
Borrelia		■	■	■
	<i>Borrelia burgdorferi</i>	■	■	■
Brucella		■	■	■
Burkholderia	<i>Burkholderia cepacia complex</i>	■	■	■
	<i>Burkholderia pseudomallei</i>	■	■	■
Campylobacter		■	■	■
Citrobacter	<i>Citrobacter koseri</i>	■	■	■
	<i>Citrobacter freundii complex</i>	■	■	■
Corynebacterium	<i>Corynebacterium diphtheriae</i>	■	■	■
	<i>Corynebacterium jeikeium</i>	■	■	■
	<i>Corynebacterium ulcerans</i>	■	■	■
Enterobacter	<i>Enterobacter cloacae</i>	■	■	■
	<i>Enterobacter cloacae complex</i>	■	■	■
Enterococcus	<i>Enterococcus faecalis</i>	■	■	■
	<i>Enterococcus faecium</i>	■	■	■
Escherichia	<i>Escherichia coli</i>	■	■	■
Fingoldia	<i>Fingoldia magna</i>	■	■	■
Fusobacterium	<i>Fusobacterium nucleatum</i>	■	■	■
	<i>Fusobacterium necrophorum</i>	■	■	■
Granulicatella	<i>Granulicatella adiacens</i>	■	■	■
Haemophilus	<i>Haemophilus haemolyticus</i>	■	■	■
	<i>Haemophilus influenzae</i>	■	■	■
Helicobacter	<i>Helicobacter pylori</i>	■	■	■



Klebsiella	<i>Klebsiella aerogenes</i> <i>Klebsiella oxytoca</i> <i>Klebsiella pneumoniae</i>			
Legionella	<i>Legionella pneumophila</i>			
Listeria				
Moraxella	<i>Moraxella catarrhalis</i>			
Morganella	<i>Morganella morganii</i>			
Neisseria	<i>Neisseria meningitidis</i>			
Pasteurella	<i>Pasteurella multocida</i>			
Prevotella	<i>Prevotella buccae</i> <i>Prevotella intermedia</i>			
Propionibacterium	<i>Propionibacterium acnes</i>			
Proteus	<i>Proteus mirabilis</i>			
Providencia	<i>Providencia stuartii</i>			
Pseudomonas	<i>Pseudomonas aeruginosa</i> <i>Pseudomonas non-aeruginosa</i>			
Salmonella	<i>Salmonella enterica</i>			
Serratia	<i>Serratia marcescens</i>			
Staphylococcus	<i>Staphylococcus aureus</i> <i>Staphylococcus non-aureus</i>			
Stenotrophomonas	<i>Stenotrophomonas maltophilia group</i>			
Streptococcus	<i>Streptococcus anginosus group</i> <i>Streptococcus agalactiae</i> <i>Streptococcus dysgalactiae</i> <i>Streptococcus gordonii</i> <i>Streptococcus mitis group</i> <i>Streptococcus pneumoniae</i> <i>Streptococcus pyogenes</i> <i>Streptococcus salivarius group</i>			
Yersinia	<i>Yersinia enterocolitica</i> <i>Yersinia pseudotuberculosis complex</i>			



Gram	Resistance	Resistance genes	Profile
Positive	Vancomycin resistances	<i>vanA</i> <i>vanB</i>	
	Methicillin resistances	<i>mecA</i> <i>mecC</i>	
Negative	Betalactamase/ Carbapenemase	<i>CTX m1/m3</i>	
		<i>IMP</i>	
		<i>KPC</i>	
		<i>NDM</i>	
		<i>OXA48</i>	

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

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

The test must be carried out in an environment suitable for molecular biological testing. This includes DNA- and DNase-free pipets, separated rooms for DNA isolation and amplification/detection, and the possibility of UV decontamination.

The test should exclusively be performed by qualified personnel, which have been trained in the use of Cube Dx products for the identification of pathogens.

The necessary equipment includes a freezer (-15 to -25°C) as well as a DNA workbench. The sample materials are solutions containing DNA that was extracted with an appropriate DNA extraction product/procedure.



For processing *PCR-Box Bacteria*, *PCR-Box Resistances*, *PCR-Box Fungi*, and *PCR-Box IPC* either a qPCR device (either Rotor-Gene from Qiagen; CFX96 from Biorad or Quantstudio from Thermo) or a thermal cycler (TPersonal from Analytic Jena) is needed.

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

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

These tests are not intended for the quantitative determination of pathogens (in terms of colony-forming units) present in the sample. These tests do not substitute or replace conventional microbiological/culturing procedures.



3. Technical description

The course of sepsis or other severe infections and especially the chances of recovery and survival are dependent on early identification of the causing pathogen(s).

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).

IPC, GINA 500, GINA 500 + DNA Purification

Cube Dx' Internal Process Control (IPC) consists of frozen biological material, which is dissolved in the sample before enrichment. This biological material is similar to pathogenic microorganisms causing sepsis or other severe infections.

The kit *GINA 500 (for 500µl of sample liquid, with or without DNA purification)* is designed for clinical routine application for enriching pathogenic (bacterial, fungal) DNA. After enrichment, the solution is purified and the eluate may be used in PCR reactions (e.g., bacterial DNA, fungal DNA, resistance marker genes). In case PCR products have been amplified in a sample, the respective pathogen can be identified straight-forward by Cube Dx's *compact sequencing*.

The kit is based on the following process steps:

- Lysis and removal of human cells: LE solution is added to the sample, and most human (and compromised pathogen) 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* solution.

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 results for a sample:

- 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 (specified: store dry and between 4°C and 8°C, refer to the storage and shelf-life chapter).

LINA

LINA is 8mL of 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 (increases inhibitors).



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 mixes – *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 marker genes as well as pathogenic fungi by using DNA extracted from samples like whole blood or positive blood cultures. *PCR-Box IPC* amplifies DNA from the *IPC* to confirm the validity of the test procedure by a positive *IPC* result on the *hybcell*.

The test is especially useful for patients in need of immediate and specific antimicrobial treatment (e.g., sepsis), 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:

- Sample preparation: See Pathogen Enrichment *GINA* (Cube Dx) including follow-up DNA purification and the *LINA* manual.
- Amplification of DNA – detection of bacteria/fungi/resistance marker genes: Isolated DNA is amplified by polymerase chain reaction (PCR). Target regions are 16S rDNA of bacteria, 28S rDNA of fungi, and respective resistance marker genes. During amplification, single DNA strands are labeled with a fluorescent dye. If using a qPCR device, the presence of bacteria, fungi, or resistance marker genes might be derived from the resulting amplification 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 fluorescence signals.

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 taken into account. Even if internal controls should single out the most erroneous results, some of these results may remain uncovered.

The following circumstances deteriorate the results of a sample:

- The time between drawing the sample and the start of sample preparation is more than 4 hours
- Storage of the sample between drawing the sample and the start of sample preparation is not according to the specifications (specified: store dry and between 4°C and 8°C, refer to the storage and shelf-life chapter).



4. Product components

Internal Process Control (IPC):

- *IPC* (order number HC0471-25, GTIN 9120127730169): store **frozen at -15 to -25°C**
 - 25 x 20 µL IPC
 - (25 x separately packed 0,5mL microtubes with biological material (IPC, each 20µL))

To enrich pathogens (bacteria and fungi) from a 500µl (or less) sample, the following specific products are required:

- *GINA 500* (order number HC0400-50, GTIN 9120127730244): 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)

To enrich pathogens (bacteria and fungi) and purify RNA/DNA from 500µl (or less) of the sample, the following specific products are required:

GINA 500 + DNA Purification (order number HC0404-50, GTIN 9120127730145): 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*

To directly test samples with an abundance of microorganisms (positive blood cultures, BAL), the following specific product is required:

- *LINA* (order number HC0405-50, GTIN 9120127730152): store at **room temperature (8 to 25°C)**
 - 50 x *LINA* (8ml)

To detect bacteria, the following specific products are required:

- *PCR-Box Bacteria* (order number HC0410-12, GTIN 9120127730084): store **frozen at -15 to -25°C**
 - 12 x 20 µL PCR master mixes Bacteria Rev.2
 - (12 x separately packed 0,2mL PCR tubes with PCR master mixes Bacteria (each 20µL))

To detect resistance marker genes, the following specific products are required:

- *PCR-Box Resistance* (order number HC0460-12, GTIN 9120127730107): store **frozen at -15 to -25°C**
 - 12 x 20 µL PCR master mixes Resistance Rev.2



(12 x separately packed 0,2mL PCR tubes with PCR master mixes Resistance (each 20µL))

To detect fungi, the following specific products are required:

- *PCR-Box Fungi* (order number HC0420-12, GTIN 91201277300921): store **frozen** at **-15 to -25°C**
 - 12 x 20 µL PCR master mixes Fungi Rev.2
- (12 x separately packed 0,2mL PCR tubes with PCR master mixes Fungi (each 20µL))

To detect IPC DNA, the following specific products are required:

- *PCR-Box IPC* (order number HC0470-12, GTIN 9120127730114): store **frozen** at **-15 to -25°C**
 - 12 x 20 µL PCR master mixes IPC Rev.2
- (12 x separately packed 0,2mL PCR tubes with PCR master mixes IPC (each 20µL))

To identify bacteria and resistance marker genes, the following specific products are required (apart from general buffers for the hyborg device):

- *hybcell Bacteria DNA xB Kit* (order number HC0412-24, GTIN 9120127730053): store at **room temperature (8 to 25°C)**
 - 24 x hybcell Bacteria DNA xB Rev.2

(24 x separately packed hybcells Bacteria DNA xB)

- 24 x Lid
- 1x PPE-Additive (900µl)

To identify fungi, the following specific products are required (apart from general buffers for the hyborg device):

- *hybcell Fungi DNA xB Kit* (order number HC0422-24, GTIN 9120127730060): store at **room temperature (8 to 25°C)**
 - 24 x hybcell Fungi DNA xB Rev.2

(24 x separately packed hybcells Fungi DNA xB)

- 24 x Lid
- 1x PPE-Additive (900µl)

To identify bacteria, fungi, and resistance marker genes, the following specific products are required (apart from general buffers for the hyborg device):

- *hybcell Pathogens DNA xB Kit* (order number HC0431-24, GTIN 9120127730077): store at **room temperature (8 to 25°C)**
 - 24 x hybcell Pathogens DNA xB Rev.2

(24 x separately packed hybcells Pathogens DNA xB)

- 24 x Lid
- 1x PPE-Additive (900µl)

Pay attention not to mix up components of different lots!



5. Storage and shelf life

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.

- *IPC* is delivered frozen and must be stored at **-15 to -25°C**.
- *GINA 500* and *GINA 500 + DNA Purification* are delivered at room temperature and must be stored at **room temperature (8 to 25°C)**.
- *LINA* is stored at **8°C to 25°C**.
- *PCR-Box Bacteria*, *PCR-Box Resistance*, *PCR-Box Fungi*, and *PCR-Box IPC* are delivered frozen and must be stored at **-15 to -25°C**.
- *hybcells* and their PPE-Additive are stored at **8°C 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. Repeated freezing-and unfreezing cycles (> 2x) of PCR-Boxes should be avoided. Repeated thawing and freezing destroy IPC and have to be avoided. IPC has to be used immediately after opening the tube.

Samples

Blood

- store cool and dry between 4°C to 8°C for a maximum of 4 hours for the best results, and up to 48 hours when needed.
- Do not freeze blood!

BAL

- store cool and dry between 4°C to 8°C for a maximum of 4 hours for the best results, and up to 48 hours when needed.
- avoid freezing, if possible, if needed store frozen between -15°C to -25°C

Blood Culture

- store cool and dry between 4°C to 8°C for up to 48 hours
- store frozen between -15°C to -25°C



6. Required equipment

The following equipment is required for conducting the test:

Required Accessories / Infrastructure		REF / GTIN
Mini-centrifuge (0,2 mL rotor)	Thermo ¹ : MySpin	
Mini Vortex Mixer	Fisher Scientific ²	
Freezer (-20°C)		
DNA workbench	Starlab ³ (example): Laminar Flow PCR workbench with UV-light PEQLAB ⁴ (example): PCR-working station	
Pipettes: ▪ 20 – 200 µL ▪ 100 – 1000µl	GILSON ⁵ : PIPETMAN P200N PIPETMAN P1000N	
Standard table centrifuge (With rotor for 2 mL tubes)	Eppendorf ⁶ : Centrifuge 5430	
▪ Standard heating block	Coyote Bioscience ⁷ H2O3-H	
qPCR device or thermal cycler	Qiagen ⁸ : Rotor-Gene Biorad ⁹ : CFX96 Thermo ¹⁰ Quantstudio 3 / 5 Analytic Jena ¹¹ : TPersonal Thermocycler (Biometra)	
System Liquid	Cube Dx: 1l, sufficient for 8 weeks	HC0003-1/ 9120127730022
PE-Buffer	Cube Dx: 1l, sufficient for 96 hybcells	HC0006-1/ 9120127730138
Hyborg	Cube Dx: hyborg Dx RED2	HB0102-1/ 9120127730015

Required accessories.

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.peqlab.de

5 www.gilson.com

6 www.eppendorf.com

7 www.coyotebio.com

8 www.qiagen.com

9 www.bio-rad.com

10 www.thermofisher.com

11 www.biometra.com



7. Test procedure

! Before beginning the test procedure. Assure that the hyborg is ready for operation!

- Check if the hyborg is switched on (check the screen of the device – refer to the hyborg Dx manual 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 (position W).
- Check if the necessary protocol is available (if not, load the protocol, refer to the hyborg Dx manual for further details).

Note, that some steps of the procedure require the preparation of equipment or 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, hair (and beard) net, and a surgical mask must be worn to avoid contamination of the test reagents. Pathogen enrichment (see steps 2.-8. below, in red) must be done under a DNA workbench.

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

1. Sample Preparation: with GINA/LINA
2. Detection: PCR/qPCR
3. Identification: compact sequencing

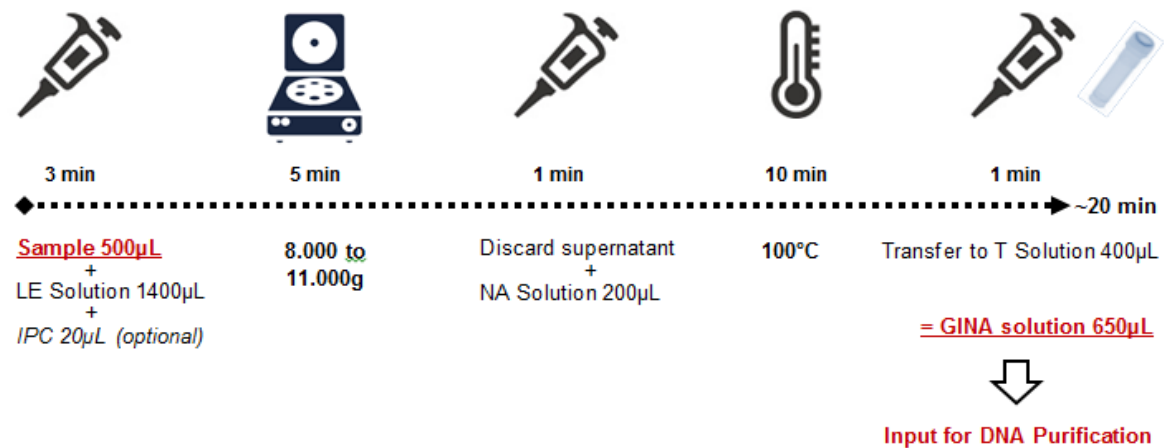


1 Sample prep: GINA/LINA

IPC + GINA: Enrichment (and Purification) procedure

Whole blood samples can be collected in K3E K3EDTA or K2E K2EDTA Vacuette Tubes.

The procedure starts with a native sample of EDTA-whole blood. Vortex the sample before use! If you are using IPC, pipette 500µl of the blood sample into the IPC first.



1. Make sure the equipment and all kit components are ready for use. Briefly spin down the needed tubes with *LE solution*, *IPC*, or *EPC* to avoid carry-over of liquids potentially present in the screw caps, when opening the vials. Turn on the heating block to 100°C.
2. Prepare *LE solution* and sample. **Do not shake or agitate the *LE solution* tube (yellow cap) to avoid the build-up of foam!** Transfer 500µl (or less) of EDTA blood (or other diluted samples) into the *LE solution* (yellow cap) and pipet up and down to mix.
3. Optional: Pipette 500µl of the blood sample into the IPC and thereafter pipette the mixture into the LE buffer.
4. Close the tube, mark it, and vortex vigorously for 5 seconds or invert the tubes several times. Incubate for ~2 min at room temperature (18°C to 25°C).
5. Centrifuge for 5 minutes between 9.000 and 11.000g (preferably with 11.000g). If available, use a soft ramping of the centrifugation speed.
6. Remove the supernatant carefully by **decanting** and add 200 µL *NA solution* (red cap) into the tube with the yellow cap. 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.**
7. Vortex vigorously for 5 seconds. Make sure that the tubes are still tightly closed.
8. Incubate at 100°C for 10 minutes (+ / - 1 minute), using a heating block.
9. Add 400µl *T solution* (green cap) into the tube with the yellow cap to neutralize.
 - Remark: **Whole blood samples should turn from greenish to dark reddish.**



10. 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).
11. 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.
12. Centrifuge for 1 min between 9.000 and 11.000g. Remove the *Column*, decant the flow-through liquid and insert the *Column* again.
13. 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.
14. 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.
15. 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.
16. 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*.
17. Open the *Elution Tube* and incubate at 100°C for 3 minutes in the heating block.
18. 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.

LINA: Modulation procedure

The procedure starts with either a (positive) blood culture or a BAL sample.

Note, that some steps of the procedure require the preparation of equipment or 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, hair (and beard) net, and a surgical mask must be worn to avoid contamination of the test reagents.

1. Make sure the equipment and all kit components are ready for use.
2. Optional: Pipette 20µl IPC (one reaction) into the LINA tube.
3. Pipette the sample into the LINA tube:
 - (Positive) blood culture: 2µL
 - BAL: 20µL

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.

4. Close the tube and shake or vortex firmly.



2 Detection: PCR/qPCR

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

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

During test preparation and processing a laboratory coat, latex gloves, sleeve guards, hair (and beard) net, and a surgical mask must be worn to avoid contamination of the test reagents. Preparation of PCR (see step 2. below, in red) must be done under a DNA workbench.

The test procedure starts with the solution resulting from GINA pathogen enrichment and DNA purification or the LINA modulation buffer (e.g., with positive blood culture).

1. Make sure the equipment and all kit components are ready for use.
2. (q)PCR reaction:
 - Program the qPCR device or PCR-thermocycler and save the program as "Patho_1":

1	95°C for 2:00
2	95°C for 0:10
3	56°C for 0:10
5	72°C for 0:30
	+Plate Read
	GO TO 2, 44 more times
6	75°C for 1:00
7	Melt curve 75°C to 95°C in increments of 0,3°C for 0:10
	+Plate Read
8	25°C for hold

Fluor: SYBR Green

Remark:

Thermal cyclers may differ in their thermal characteristics, therefore the optimization of the temperatures stated in the protocol may be recommended (for validated devices only) if the results are undesirable.



- Unpack and thaw single 0,2 ml tubes with the needed master mixes of Bacteria (red dot), master mixes Resistance (yellow dot), Fungi (green dot), and IPC (blue dot). Homogenize (vortex) and spin down briefly the solution in each tube.
- Add 20 µL sample DNA solution (or 20 µL DNA-free water as NTC) to the PCR master mixes.
- Close PCR tubes (if you don't use a rotating thermocycler homogenize and spin down liquids before starting PCR).
- Start (q)PCR program "Patho_1" (programmed before).

The amplified DNA is either used immediately for the compact sequencing reaction or it can be stored at 4°C to 8°C overnight or stored frozen at -15°C to -25°C for longer periods.

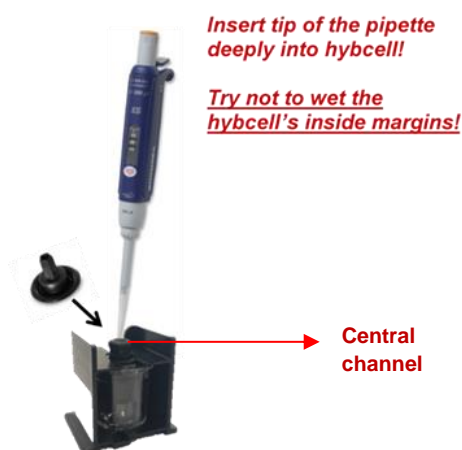


3 Identification: compact sequencing

1. Assure that the hyborg is ready for operation.
2. Open the packaging of the hybcell (rip the sealing at the notch), and place the hybcell into the rack (positions A-H).
3. Combine all the desired amplicons from the same sample – or at least 30µl thereof (*PCR-Box Bacteria*, *PCR-Box Fungi*, *PCR-Box IPC*, or *PCR-Box Resistance*) – into one of the amplicon tubes (e.g., *PCR-Box Bacteria*). Thereafter pipette 30µl of the PPE-additive (found in the hybcell box) into the tube with the amplicon mixture.
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 upon introducing the amplicons. This does not influence the performance of the product**). Avoid bubbles!
5. Pipette the entire volume from the tube (~ 150 µL) into the hybcell (through the central channel) at once. The final volume is dependent on the presence of suitable amplicons after the qPCR or on the used PCR products.

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 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.



4. Results

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.

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'.

- **Bac_PCR:** Checks if the *PCR-Box Bacteria* was used.
- **Res_PCR:** Checks if the *PCR-Box Resistance* was used.
- **Fun_PCR:** Checks if the *PCR-Box Fungi* was used.
- **IPC_PCR:** Checks if the *PCR-Box IPC* was used.

Test specific Controls

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

- **Specificity Control:** Checks if the process of compact sequencing suffered major flaws.
- **Internal Process Control:** The IPC might be added to the whole blood sample. If added, a passed IPC indicates that the whole process has not experienced major flaws. Especially negative results are confirmed by the IPC.

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.
- **Bacteria pan** is positive if amplified bacterial DNA is present.
- **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 GmbH
Westbahnstr. 55
4300 St. Valentin
Austria



Sample # John Doe
Date 13.02.2020 00:00
Remark
Liquids

Test hybcell Patho xB (2)
Profile Sepsis (25.02.2020)
hybcell 2204A510330

Controls		
Controls	PASSED	
Bac_PCR	ADDED	1000 100000
Res_PCR	ADDED	1000 100000
Fun_PCR	ADDED	1000 100000
IPC_PCR	ADDED	1000 100000

Parameters	Result	Representation
Specificity Control	PASSED	
Internal Process Control	PASSED	
BACTERIA		
Bacteria Pan	Positive	50 100000
Gram neg	Positive	50 100000
Pseudomonas aeruginosa	Positive	50 100000
Gram pos	Positive	50 100000
Staphylococcus aureus	Positive	50 100000

Off-profile parameters	Result	Representation
Propionibacterium sp.	Positive	50 100000
Propionibacterium acnes	Positive	50 100000

An example of a report.

Protocol (.hyb)

Calibration curves and pattern recognition were done for all microorganisms and genes (identified bacterial 16S rDNA / identified fungal 28S rDNA / identified resistance marker DNA) 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 (<https://www.cubedx.com/support/protocols>) or by your local distributor. Protocols may also be updated automatically.



Off-profile parameters

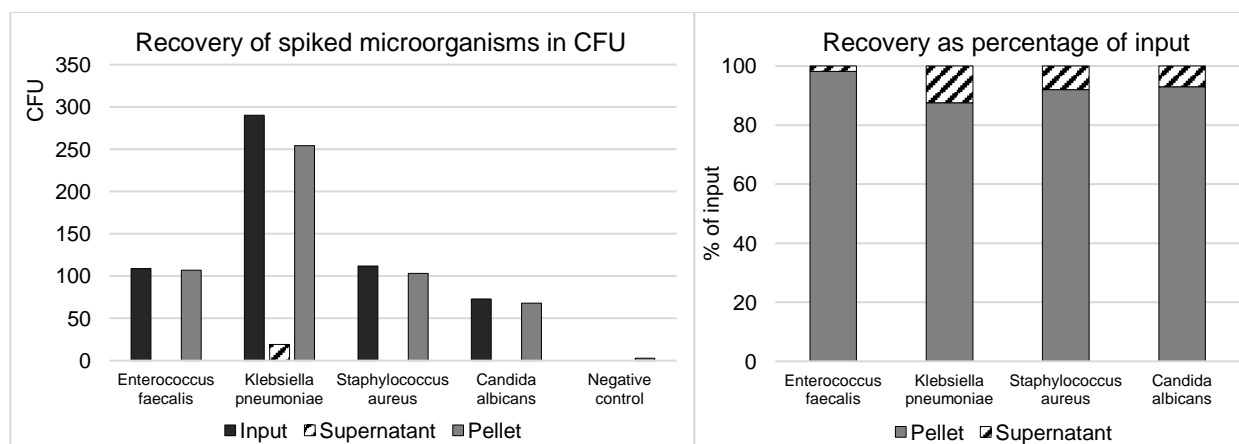
According to the intended purpose, clinically relevant results are indicated. The protocol for the lot (fixed for the CE-IVD test kits) defines clinically relevant bacteria, resistance gene markers, and fungi. The results outside this scope are labelled as “off-profile parameters”. Such results may be interpreted by infectious disease specialists.



5. Analytical Performance

IPC, 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). The 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 100µl EDTA whole blood and plated out on LB agar. After centrifugation, 100µl of the supernatant was also 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*).

Bacteria

The limit of detection (LOD) was determined by diluting cultures of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* and processing these 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.

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).

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



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

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

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

No unspecific results or cross-reactivities have been observed.

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

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

Fungi

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 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):

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

The following unspecific results could be observed:

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

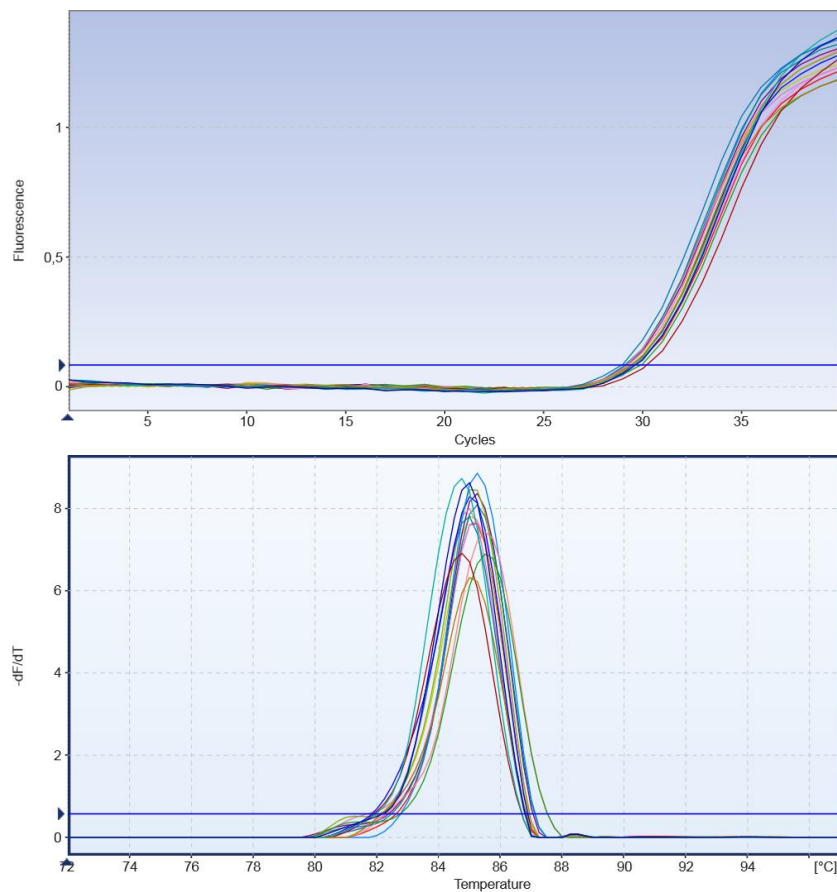
Repeatability was determined by amplifying different dilutions of *Candida albicans* DNA several times each.

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

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.





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 +/- 2** (28 to 32). This threshold can slightly vary between different PCR machines.



6. 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	96%	74%	98%
Cube Dx	Positive	28	5			
	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.



		Blood Culture		Overall Correctness	Sensitivity	Specificity
		Positive	Negative	94%	98%	88%
Cube Dx	Positive	166	13			
	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, 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. From 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	81%	84%	78%
Cube Dx	Positive	31	9			
	Negative	6	32			
Total		78				



7. 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.

Additionally, run a control hybcell once every month to control the basic functionalities of hyborg Dx RED2. These functionalities are liquid transport, optics and scanning, temperature control, and hybcell handling.

In case of changing analytical performance refer to the section *Troubleshooting* (below) of this manual.

In the 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.



8. Troubleshooting

Sample Preparation

Problem	Possible causes	Measure / Precaution
Loss of the pellet	<ul style="list-style-type: none"> Pipetted away 	<ul style="list-style-type: none"> Start with decanting the supernatant and thereafter pipette away the remaining solution Repeat the extraction step
Contamination	<ul style="list-style-type: none"> Contamination during the sample preparation step 	<ul style="list-style-type: none"> Use the recommended safety gear Clean surfaces with 1% hypochlorite, followed by 80% EtOH

Detection by PCR

Problem	Possible causes	Measure / Precaution
Odd-looking amplification curves	<ul style="list-style-type: none"> Spread out of the eluate in the PCR tube Uneven distribution of the sample-PCR mix solution Bubbles at the bottom of the PCR tube 	<ul style="list-style-type: none"> Spin down the PCR tubes before introducing them into the device
PCR inhibition	<ul style="list-style-type: none"> Dilution of the PCR mix Using too high sample volumes, especially with BAL samples Ethanol residues present in the eluate 	<ul style="list-style-type: none"> 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.



Identification by the hybcell

Problem	Possible causes	Measure / Precaution
Unspecific hybcell signals	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Using the wrong hybcell Using the “wrong” protocol. Using expired/spoilt products (for example due to damaged package, etc.) Software error. Device error. 	<ul style="list-style-type: none"> Check the hybcell type and used protocol. Check the expiry dates of products. Check the functionality of the hyborg, by using <i>hybcell Control xC</i>. Repeat the test.
Specificity Control	<ul style="list-style-type: none"> Using expired products. Insufficient / no PCR-product pipetted into hybcell. Spoilt PCR. No or insufficient PE-Buffer used. 	<ul style="list-style-type: none"> Check the functionality of the hyborg. Repeat the test. Check the filling levels of all liquids. If necessary, refill liquids.

Troubleshooting



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!

