# INSTRUCTIONS FOR USE

Pathogens xB IFU E 2022_04_27


IVD CE

April 2022.

| | | |
|---|---|---|
| ***IPC*** | REF | *HC0471-25* |
| ***GINA 500*** | REF | *HC0400-50* |
| ***GINA 500 + DNA Purification*** | REF | *HC0404-50* |

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

| | | |
|---|---|---|
| ***LINA*** | REF | *HC0405-50* |

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

| | | |
|---|---|---|
| ***PCR-Box Bacteria / Resistance / Fungi / IPC*** | REF | *HC0410-12* |
| | REF | *HC0460-12* |
| | REF | *HC0420-12* |
| | REF | *HC0470-12* |
| ***hybcell Bacteria / Fungi / Pathogens DNA xB*** | REF | *HC0412-24* |
| | REF | *HC0422-24* |
| | REF | *HC0431-24* |

*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.*

| | | |
|---|---|---|
| ***EPC S.aureus 10000*** | REF | *HC0473-10* |
| ***EPC C.albicans 10000*** | REF | *HC0475-10* |

*External Process Controls (EPC) for quality assurance of molecular infectious disease testing products.*



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# Content





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# List of abbreviations

*BAL……...Bronchoalveolar Lavage*
*°C.....Celsius degree*
*µL.....Microliter*
*Cq…….. Cycle of quantification*
*CE.....Conformité Européenne (European Conformity)*
*CV.....Coefficient of variation*
*DNA.....Desoxyribonucleic acid*
*DNase .....Deoxyribonuclease*
*Dx .....Diagnostics*
*EDTA.....Ethylenediaminetetraacetic acid*
*EG .....European Community*
*.....(Europäische Gemeinschaft)*
*g.....Gravity*
*ID .....Identification*
*IVD .....In-vitro Diagnostic*
*LOD.....Limit of Detection*
*M.....Molar*
*min.....Minute*
*Min.....Minimum*
*mL .....Millilitre*
*NTC…… No-template control*
*PCR.....Polymerase chain reaction*
*PE.....Primer Extension*
*pg.....Picogram*
*qPCR.....Quantitative Polymerase Chain Reaction*
*s.....Second*
*sp.....Species*
*UV .....Ultraviolet*



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# 1. Explanation of symbols

| Symbol | Explanation |
|---|---|
| CE IVD | CE mark.<br>In vitro diagnostic medical device. |
|  | Manufacturer. |
|  | Date of manufacture. |
| LOT | Lot/batch number. |
| REF | Catalog number. |
| SN | Serial number. |
|  | Keep away from rain/humidity. |
|  | Keep away from sunlight. |
|  | Only use once. Do not re-use. |
|  | Don’t use it if the package is damaged. |
|  | Do not eat or drink. |
|  | Use by date. |
|  | Temperature limit for storage. |
|  | Sufficient for <n> tests. |
| R 22 | Harmful if swallowed. |
| S 1 / 2 | Store in a secure location and away from children. |
| S 18 | Open and handle the container with caution. |
| S 20 | Do not eat or drink while handling. |
| S 24 / 25 | Prevent contact with eyes and skin. |
| S 36 / 37 | Wear appropriate protective gloves and clothing while handling. |



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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 processing 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' Internal Process Control (IPC) consists of frozen biological material, which is dissolved within the human sample before the enrichment process starts. 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 Cube Dx' products for the identification of pathogens.***

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

Identification of pathogens and antibiotic resistance genes from positive human blood cultures and bronchoalveolar lavages (BAL) containing an abundance of microorganisms 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.

Together with Cube Dx' PCR products (Bacteria, Fungi, Resistance) and pathogen Identification hybcells microorganisms and resistance genes can be determined in less than 2 hours from such samples.

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.***

***The kit 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<br>hybcell Bacteria / Fungi / Pathogens DNA xB

The *PCR-Box Bacteria*, *PCR-Box Fungi*, *PCR-Box Resistance,* and the qualitative tests *hybcell Bacteria DNA xB*, *hybcell Fungi DNA xB,* and *hybcell Pathogens DNA xB* are in-vitro tests for detection and identification of bacteria, antibiotic resistance mechanisms and fungi from human samples based on homologies of bacterial 16S DNA,



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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 or/and 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*:

| | Genus | Species | LINA + BC | LINA + BAL | GINA + Blood |
|---|---|---|---|---|---|
| Bacteria | Abiothrophia | *Abiotrophia defectiva* | • | | • |
| | Acinetobacter | *Acinetobacter baumannii*<br>*Acinetobacter calcoaceticus complex* | •<br>• | •<br>• | • |
| | Actinobacillus | *Actinobacillus pleuropneumoniae* | • | • | • |
| | **Anaerococcus** | | • | | • |
| | Bacteriodes | *Bacteroides fragilis* | • | | • |
| | Bordetella | *Bordetella pertussis* | • | | • |
| | **Borreliella** | <br>*Borreliella burgdorferi* | •<br>• | | •<br>• |
| | **Brucella** | | • | | • |
| | Burkholderia | *Burkholderia cepacia complex*<br>*Burkholderia pseudomallei* | •<br>• | | •<br>• |
| | **Campylobacter** | | • | | • |
| | Citrobacter | *Citrobacter koseri*<br>*Citrobacter freundii complex* | •<br>• | •<br>• | •<br>• |
| | **Corynebacterium** | <br>*Corynebacterium diphtheriae*<br>*Corynebacterium jeikeium*<br>*Corynebacterium ulcerans* | •<br>•<br>•<br>• | | •<br>•<br>•<br>• |
| | Enterobacter | *Enterobacter cloacae*<br>*Enterobacter cloacae complex* | •<br>• | •<br>• | •<br>• |
| | Enterococcus | *Enterococcus faecalis*<br>*Enterococcus faecium* | •<br>• | | •<br>• |
| | Escherichia | *Escherichia coli* | • | • | • |
| | Finegoldia | *Finegoldia magna* | • | | • |
| | **Fusobacterium** | <br>*Fusobacterium nucleatum*<br>*Fusobacterium necrophorum* | •<br>•<br>• | | •<br>•<br>• |
| | Granulicatella | *Granulicatella adiacens* | • | | • |
| | Haemophilus | *Haemophilus haemolyticus*<br>*Haemophilus influenzae* | •<br>• | •<br>• | •<br>• |
| | Helicobacter | *Helicobacter pylori* | • | | • |



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| | Genus | Species | LINA + BC | LINA + BAL | GINA + Blood |
|---|---|---|---|---|---|
| Bacteria | 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** | | • | | |
| | | *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* | • | | |

Bacterial type strains of hybcell Bacteria DNA xB and hybcell Pathogens DNA xB. Grey: To support a better understanding of taxonomy (not shown in the report). All denotations are taken from the NCBI database. No dot: Not included in the corresponding profile (shown as an off-profile result, if using the profile).



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| Resistance | gram+ Resistance | Resistance gene | LINA + BC | LINA + BAL | GINA + Blood |
|---|---|---|---|---|---|
| | Vancomycin resistances | *vanA* | • | | • |
| | | *vanB* | • | | • |
| | Methicillin resistances | *mecA* | • | | • |
| | | *mecC* | • | | • |
| | **gram- Resistance** | **Resistance gene** | **LINA + BC** | **LINA + BAL** | **GINA + Blood** |
| | Betalactamase/ Carpabenemase | *CTX m1/m3* | • | | • |
| | | *IMP* | • | | • |
| | | *KPC* | • | | • |
| | | *NDM* | • | | • |
| | | *OXA48* | • | | • |

Resistance marker genes of hybcell Bacteria DNA xB and hybcell Pathogens DNA xB. Grey: To support a better understanding of taxonomy (not shown in the report). All denotations took from the NCBI database.

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

| Fungi | Genus | Species | LINA + BC | LINA + BAL | GINA + Blood |
|---|---|---|---|---|---|
| | **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** | | • | | • |

Fungal type strains of hybcell Fungi DNA xB and hybcell Pathogens DNA xB. Grey: To support a better understanding of taxonomy (not shown in the report). All denotations are taken from the NCBI database. No dot: Not included in the corresponding profile (shown as an off-profile result, if using the profile).



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The test might be used for different diagnostic uses and 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 de-contamination. ***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 equipment has to include a freezer (-15 to -25°C) as well as a DNA workbench. The sample materials are solutions containing DNA which were 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 needed.

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

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

## EPC S. aureus 10000, EPC C. albicans 10000

Quality assurance concepts for (highly sensitive) molecular pathogen identification from human samples must ensure stable results over time. Therefore, well-defined control material should be tested periodically to check and document the constant outcome.

EPCs are quantified, inactivated bacterial or fungal cells in different concentrations (frozen). They are intended to safeguard the stability of the molecular pathogen ID processes of human samples over time. Periodic usage of EPCs documents the laboratory's ability to detect defined bacterial and fungal DNA levels.

EPCs come as single-use controls in different concentrations to fit different diagnostic applications.

Running tests with EPCs should be carried out in an environment suitable for molecular biological testing. Dependent on the required sensitivities of the test, this might include DNA-and DNase-free pipets, separated rooms for DNA isolation and amplification/detection, and the possibility of UV decontamination. ***Running tests with the EPCs should exclusively be performed by qualified personnel, which have been trained in the use of the used products.***



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# 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 are higher after an 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 the enrichment procedure. This biological material is similar to pathogenic microorganisms causing sepsis or other severe infections.

The kits *GINA 500 (for 500µl of sample liquid, with or without DNA purification) a*re designed for clinical routine application to enrich pathogenic (bacterial, fungal) DNA. After enrichment, the solution is purified and the eluate is for example 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' *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 the majority of 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 outcome may be corrupted by the nature of the sample or errors during the procedure (low amount of DNA, contamination with environmental microorganisms / DNA), other influences (degraded DNA, contamination with chemicals), or technical errors.***

Following circumstances deteriorate results for a sample:

- Time between drawing the (blood) sample and the start of sample preparation is more than 4 hours.
- Storage of sample between drawing and start of sample preparation is not according to specification (specified: store dry and between 4°C and 8°C).

## LINA

LINA is 8mL of buffer filled in a single ready-to-use tube. The buffer dilutes any PCR inhibitors in the sample so that these are not effective anymore. 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 outcome may be corrupted by the nature of the sample or errors during the procedure (e.g. low number of microorganisms in the sample or technical errors.***

Following circumstances deteriorate results for a sample:

- Higher volume of sample than indicated taken (this 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 its 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 its antibiotic resistance marker genes as well as pathogenic fungi 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 causing 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 reactions (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 specific fluorescence is scanned and analyzed by the hyborg (device) and its software.

***Test results may be corrupted by 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 most erroneous results, some of these results remain uncovered.***

Following circumstances deteriorate the results of a sample:

- Time between drawing the sample and the start of sample preparation is more than 4 hours
- Storage of sample between drawing the sample and start of sample preparation not according to specification (specified: store dry and between 4°C and 8°C).

## EPC S. aureus 10000, EPC C. albicans 10000

EPCs are quantified, inactivated bacterial or fungal cells in different concentrations (frozen).

The cells are intact but blocked in growth. Therefore, the DNA extraction process can be tested with these “sample-like” EPCs, the same as with still growing organisms.

Every single tube contains 20 µL of the solution with the inactivated cells. The 20 µL in the tube is intended to be one sample for the periodic testing of the molecular diagnostic test (single-use reagent).

***Spoilt products may result in negative results for the test.***



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# 4. Product components

Internal Process Control (IPC):

- *IPC* (order number HC0471-25): store 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): 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) sample, following specific products are required:

- *GINA 500* + DNA Purification (order number HC0404-50): 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)
  - 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): 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): store 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 mastermixes Bacteria (each 20µL))

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

- *PCR-Box Resistance* (order number HC0460-12): store 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 mastermixes Resistance (each 20µL))



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To detect fungi, the following specific products are required:

- *PCR-Box Fungi* (order number HC0420-12): store 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 mastermixes Fungi (each 20µL))

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

- *PCR-Box IPC* (order number HC0470-12): store 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 mastermixes 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): 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

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): 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

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): 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

To assure the quality of bacterial testing, the following specific products are required:

- *EPC S. aureus 10000* (order number HC0473-10): store at **-15 to -25°C**
  - 10 x 20 µL EPC S. aureus 10000
    (10 separately packed 0,5mL micro tubes with ~ 10.000 CFU each (in 20µL solution))

To assure the quality of fungal testing, the following specific products are required:

- EPC *C. albicans* 10000 (order number HC0473-10): store at **-15 to -25°C**
  - 10 x 20 µL EPC *C. albicans* 10000
    (10 separately packed 0,5mL micro tubes with ~ 10.000 CFU each (in 20µL solution))

***Pay attention not to mix up components of different lots!***



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# 5. Storage and shelf life

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 products' label.

- *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 **18°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 are stored at **18°C to 25°C**.
- *EPCs* are delivered frozen and must be stored at **-15 to -25°C**.

***If the protective sealing of hybcells or any other packaging (e.g., any tubes) is damaged / or the minimum shelf life has expired, the product/component must not be used. hybcells have to be used immediately after opening the protective sealing. Repeated freezing-and unfreezing cycles (> 2x) of PCR-Boxes should be avoided. Thawing and freezing again destroy IPC and EPC and are strictly forbidden. IPC / EPC has to be used immediately after opening the tube.***



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# 6. Required equipment

The following equipment is required for conducting the test:

| **Required Accessories / Infrastructure** | | REF |
|---|---|---|
| Mini-centrifuge (0,2 mLrotor) | Thermo[1]: MySpin | |
| Mini Vortex Mixer | Fisher Scientific[2] | |
| Freezer (-20°C) | | |
| DNA workbench | Starlab[3] (example):<br>Laminar Flow PCR workbench with UV-light<br>PEQLAB[4] (example):<br>PCR-working station | N2530-8200<br>90-UV / PCR2 |
| Pipettes:<br>▪ 20 – 200 µL<br>▪ 100 – 1000µl | GILSON[5]:<br>PIPETMAN P200N<br>PIPETMAN P1000N | <br>F144565<br>F144566 |
| Standard table centrifuge<br>(with rotor for 2 mL tubes) | Eppendorf[6]:<br>Centrifuge 5430 | |
| ▪ Standard heating block | Coyote Bioscience[7]<br>H2O3-H | |
| qPCR device or thermal cycler | Qiagen[8]:<br>Rotor-Gene<br>Biorad[9]:<br>CFX96<br>Thermo[10]<br>Quantstudio 3 / 5<br>Analytic Jena[11]:<br>TPersonal Thermocycler (Biometra) | |
| System Liquid | Cube Dx:<br>1l, sufficient for 8 weeks | HC0003-1 |
| PE-Buffer | Cube Dx:<br>1l, sufficient for 96 hybcells | HC0006-1 |
| hyborg | Cube Dx:<br>hyborg Dx RED2 | HB0102-1 |

Required accessories.

1 https: / / www.thermofisher.com / order / catalog / product / 75004081
2 https: / / www.fishersci.com / shop / products / variable-speed-mini-vortex-mix / 14955163
3 http: / / www.starlab.de
4 http: / / www.peqlab.de
5 http: / / www.gilson.com
6 http: / / www.eppendorf.com
7 http: / / www.coyotebio.com
8 http: / / www.qiagen.com
9 http: / / www.bio-rad.com
10 https://www.thermofisher.com
11 http: / / www.biometra.com



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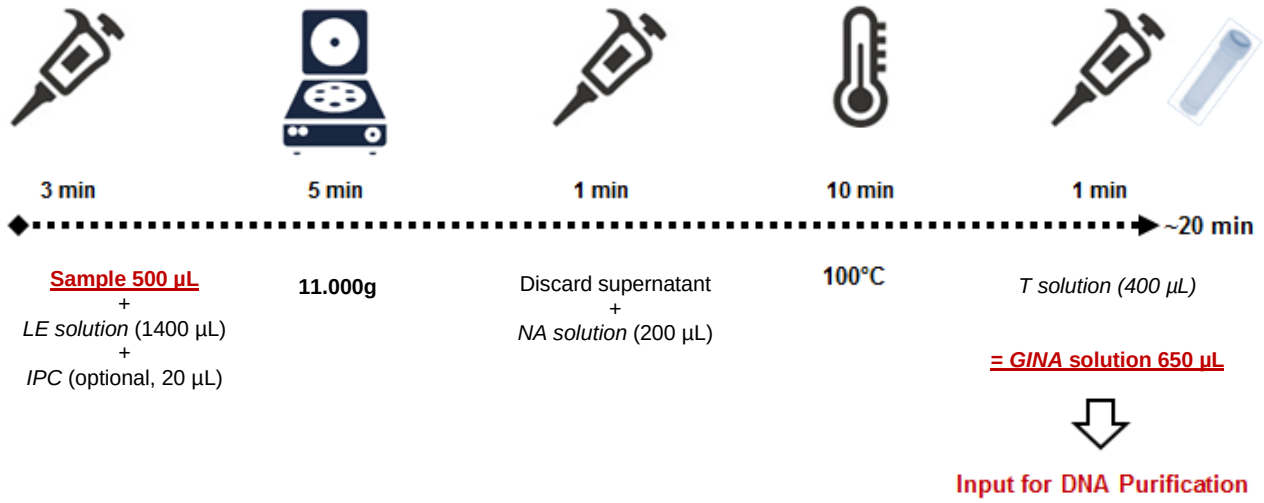
# 7. Test procedure

## GINA: Enrichment (and Purification) procedure

***The procedure starts with a native sample of EDTA-whole blood.***




***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.***

1. Make sure that all components of the kit and the equipment are ready for use. Briefly spin down the needed tubes with *LE solution* to avoid carry-over of liquids potentially present in the screw caps, when opening the vials. Heat 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: Add 20 µL *IPC* to the *LE solution/blood* mixture (add *IPC* after EDTA blood!).
4. Close tube, mark it, and vortex vigorously for 5 seconds or invert tubes several times. Incubate ~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 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.



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

10. Purify DNA, using common DNA extraction products (in case of *GINA 500 + DNA purification*: Machery Nagel Nucleo Spin reagents are included in the kit. Otherwise: follow the manufacturer's instructions, 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 *Column*, decant the flow-through liquid and insert *Column* again.
13. Add 500µl *Wash Buffer BW* and centrifuge for 1 minute at between 9.000 and 11.000g. Remove *Column*, decant the flow-through liquid and insert *Column* again.
14. Add 600µl *Wash Buffer B5* and centrifuge for 1 minute at between 9.000 and 11.000g. Remove *Column*, decant the flow-through liquid and insert *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 150µl *Elution Buffer BE*. Incubate at room temperature for 1min. 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 that all components of the kit and the equipment are ready for use.
2. Pipette the sample into the LINA tube:
    - (Positive) blood culture: 2µL
    - BAL: 20µL
3. Close the tube and shake or vortex firmly.

## 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.***



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***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 that all components of the kit and the equipment are ready for use.
2. (q)PCR reaction:

- Program qPCR device or PCR-thermocycler and store under “Patho_1”:
  - 1 minute 94°C
  - All together 41 cycles: 5 seconds 94°C, 10 seconds 56°C, and 30 seconds 72°C
  - 1 minute 72°C
  - (if applicable – using a qPCR device – do a melting curve analysis from 75°C to 94°C in 0,5°C steps, 10 seconds each step)
  - 25°C forhold
- Unpack and thaw single 0,2 ml tubes with the needed master mixes 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. Assure that the hyborg is ready for operation:

- Is the hyborg switched on and the software active (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).

4. Open the packaging of the hybcell (rip the sealing at the notch), and place the hybcell into the rack (positions A-H).
5. Pipette at least 30 µL of *PCR-Box Bacteria*, *PCR-Box Fungi,* and *PCR-Box IPC* of the same sample into the hybcell (through the central channel). Pipette 10 µL of *PCR-Box Resistance* of the same sample into the hybcell (through the central channel).

   ***Use a 200 µL pipette with appropriate filter tips! Insert the tip of the pipette deeply into the hybcell and try not to wet the hybcell’s inside margins.***
6. Put a Lid onto the hybcell.



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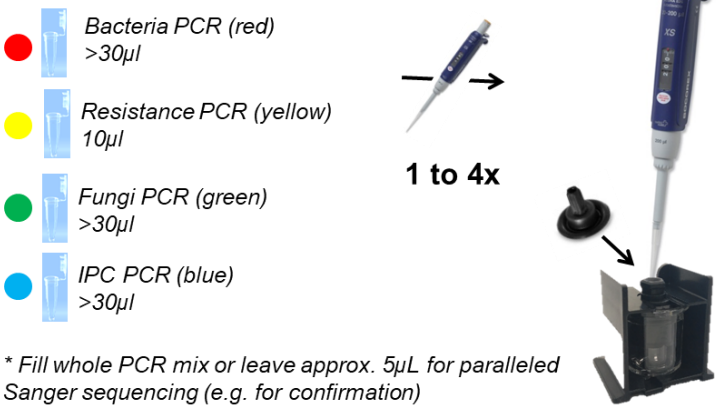


| | Bac. PCR | Res. PCR | Fun. PCR | IPC PCR |
|---|---|---|---|---|
| *hybcell Bacteria* | • | • | | • |
| *hybcell Fungi* | | | • | • |
| *hybcell Pathogens* | • | • | • | • |

** Fill whole PCR mix or leave approx. 5µL for paralleled Sanger sequencing (e.g. for confirmation)*

7. Start processing the samples after entering the sample and hybcell ID with help of the hyborg software (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 incorrect position.***

## EPC S. aureus 10000, EPC C. albicans 10000

***Note, that using the EPC requires the thawing of reagents. As these may be 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 should be worn to avoid contamination of the test reagents. Preparation should be done under a DNA workbench.***

1. Pipette 20 µL of the EPC (one vial) into the container of a negative patient sample.
2. Follow the instruction of your test procedure as you would do for a patient sample.
3. Compare the results of the test with the expected result and document the outcome.



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# 8. 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) control(s) 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.

- **Background CV**: Checks background deviations, sufficient washing, and unspecific binding.
- **Background Mean** Checks background intensity, sufficient washing, and unspecific binding.
- **Grid**: Checks hybcell type, hyborg protocol, and some 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.



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## Report

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Westbahnstr. 55
4300 St. Valentin
Austria

cube dx
hybCell technology

| | | | |
|---|---|---|---|
| **Sample** | John Doe | **Test** | hybcell Patho xB (2) |
| **Date** | 13.02.2020 00:00 | **Profile** | Sepsis (25.02.2020) |
| **Remark** | | **hybcell** | 2204A510330 |
| **Liquids** | | | |

| Controls | | |
|---|---|---|
| Controls | PASSED | |
| Bac_PCR | ADDED | 1000 10000 |
| Res_PCR | ADDED | 1000 10000 |
| Fun_PCR | ADDED | 1000 10000 |
| IPC_PCR | ADDED | 1000 10000 |

| Parameters | Result | Representation |
|---|---|---|
| Specificity Control | PASSED | |
| Internal Process Control | PASSED | |
| BACTERIA | | |
| Bacteria Pan | Positive | 50 10000 |
| Gram neg | Positive | 50 10000 |
| Pseudomonas aeruginosa | Positive | 50 10000 |
| Gram pos | Positive | 50 10000 |
| Staphylococcus aureus | Positive | 50 10000 |

| Off-profile parameters | Result | Representation |
|---|---|---|
| Propionibacterium sp. | Positive | 50 10000 |
| Propionibacterium acnes | Positive | 50 10000 |

***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 its specified ranges (e.g., liquid delivery, heating, laser power, etc.).

A lot of specific protocols might have to be imported into the hyborg software before the first use of a new lot. Up-to-date protocols are either updated automatically or provided on the Cube Dx homepage (Support → Protocols) or by your local distributor.



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## Off-profile parameters

Each intended use limits the possible results to those that are clinically relevant. The definition of clinically relevant bacteria, resistance gene markers and fungis is made within the protocol for the lot (and fixed for the CE-IVD testkits). Positive results outside this definitions are shown as “off-profile parameters” as such results can be relevant for the infectious disease specialist as well.



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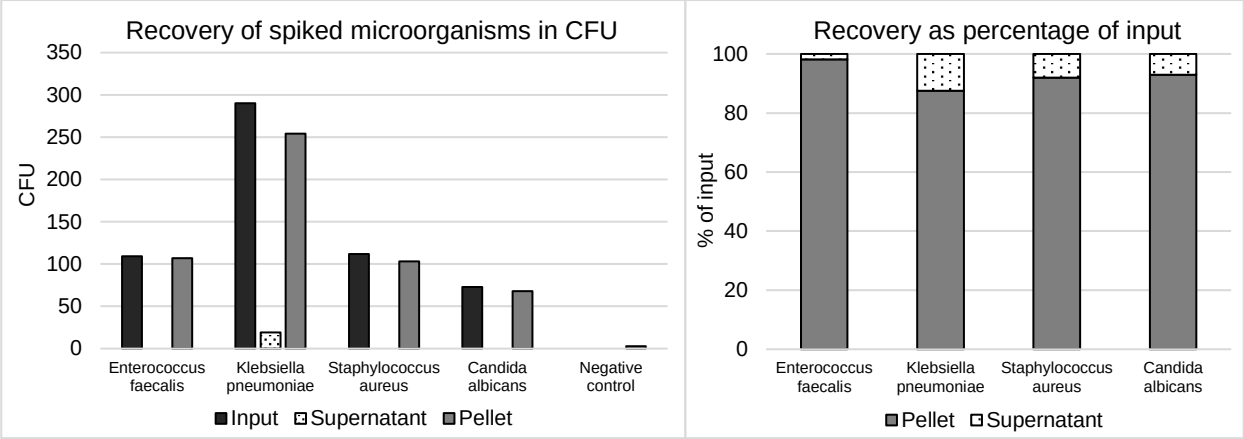
# 9. Performance data

## 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 volunteers. 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 plated out as well, to determine the number of living microorganisms, which 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 aeroginosa* 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).

| | | | |
|---|---|---|---|
| *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 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 for 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 it 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):

| | | | |
|---|---|---|---|
| *Aspergillus clavatus* | ATCC 1007D-2 | *Aspergillus flavus* | ATCC (strain?) |
| *Aspergillus fumigatus* | ATCC 1022 | *Aspergillus niger* | DSM1957 |
| *Candida albicans* | ATCC 11006 | *Candida dubliniensis* | DSM28723 |
| *Candida glabrata* | ATCC (strain?) | *Candida parapsilosis* | ATCC 22019D-5 |
| *Candida tropicalis* | ATCC MYA-3404D-5 | *Cladosporium sp.* | DSM19653 |
| *Cryptococcus neoformans* | ATCC MAY-565 | *Pichia kudriavzevii* | ATCC (strain?) |
| *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 hybcell report.

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 for 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.



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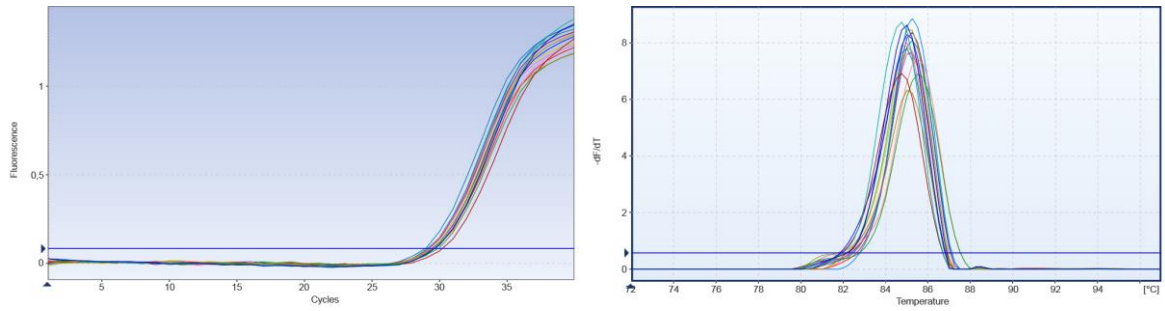


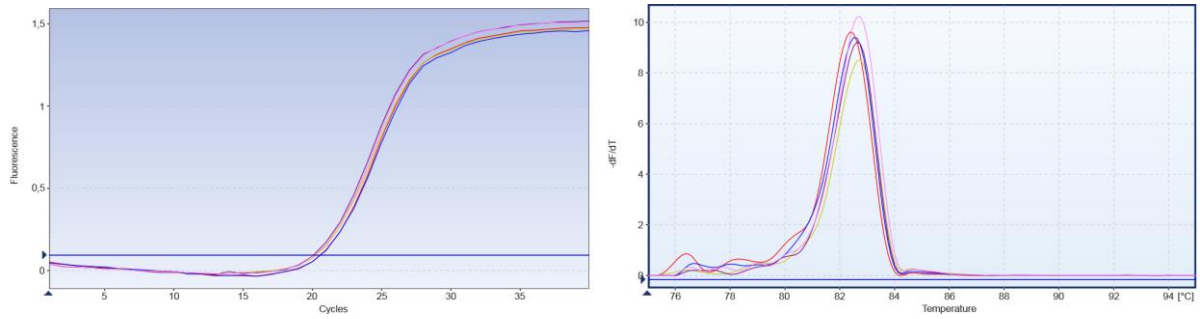

The analysis of quantification cycles (Cq) resulted in (all values rounded):

| | |
|---|---|
| Average: | 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.

## EPC S. aureus 10000, EPC C. albicans 10000

**Repeatability** was tested with 5 different EDTA whole blood samples for each EPC. EPC (20 µL) was added, and the samples were processed according to the *GINA 500* protocol (including DNA purification). *PCR-Box Bacteria* or *PCR-Box Fungi* (see example graphs for EPC *S.aureus* 10.000 below) was run and the results were verified by running *hybcell Pathogens DNA xB*.




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

| | EPC S.aureus 10000 | EPC C.albicans 10000 |
|---|---|---|
| Average: | 20,3 | 26,2 |
| Standard Deviation: | 0,2 | 0,7 |
| Coefficient of Variation (CV): | 1,1% | 2,6% |



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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%. In total, 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 | | | |
| LAB | 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.

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



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## 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 traditional blood culture methods, the Unyvero system, and Cube Dx's *LINA and compact sequencing technology*. The 'true' result was assumed as the result that at least two of the three methods provided. For one sample no agreement between two of the three methods could be found, so the number of samples for the analysis 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 | | | |
| Cube Dx | Positive | 31 | 9 | 81% | 84% | 78% |
| | Negative | 6 | 32 | | | |
| Total | | 78 | | | | |



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# 11. Changes in analytical performance and disposal

For verification of the functionality of the test and the implementation, weekly examination with a reference standard (e.g., Cube Dx's External Process Controls (EPCs)) is stipulated.

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

For verification of the functionality of the EPCs, run several tests and compare outcomes with the expected outcome. If the outcome is not as expected, use EPCs from another lot and repeat the tests.

All single-use materials (PCR tubes, hybcells, pipette tips, etc.) can be disposed of without 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) as well as EPCs are potentially containing infectious material and have to be disposed of according to your organization's rules for disposal of infectious material.



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# 12. Troubleshooting

| Problem | Possible causes | Measures |
|---|---|---|
| Background CV<br><br>Backgroundnd Mean | ▪ Liquids are empty.<br>▪ Liquid handling of the device is erroneous.<br>▪ Insufficient washing procedure.<br>▪ Using expired/spoilt hybcell. | ▪ Check filling levels of all liquids.<br>▪ If necessary, refill liquids.<br>▪ Check functionality of the hyborg, by using *hybcell Control xC*.<br>▪ Repeat the test. |
| Grid | ▪ Using “wrong” hybcell.<br>▪ Using “wrong” protocol.<br>▪ Using expired/spoilt products (for example due to damaged package, etc.)<br>▪ Software error.<br>▪ Device error. | ▪ Check hybcell type and used protocol.<br>▪ Check expiry dates of products.<br>▪ Repeat the test. |
| Specificity Control | ▪ Using expired products.<br>▪ Insufficient / no PCR-product pipetted into hybcell.<br>▪ Spoilt PCR.<br>▪ No or insufficient PE-Buffer used. | ▪ Check filling levels of all liquids.<br>▪ If necessary, refill liquids.<br>▪ Check the functionality of the hyborg.<br>▪ Repeat the test. |

***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.



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