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Instructions for Use Molecular Culture ID

catalogue number: 150001, 24 tests

For Research Use Only. Not for use in diagnostic procedures.

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Instructions for use revision 1 October 2026

For Research Use Only. Not for use in diagnostic procedures.
This is the initial release of the U.S. Research Use Only (RUO) Instructions for Use for Molecular Culture ID.
See Revision History.

Safety Data Sheet	https://inbiome.com/sds
Software	https://antoni-research.inbiome.com
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Complaints and improvements	https://forms.monday.com/forms/fb08dbf03b2cb724a16b74282b16b276?r=use1

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1. Intended purpose

Molecular Culture ID is a semi-automated and semi-quantitative research assay for the detection and identification of bacterial DNA isolated from the sample types listed below (see Sample types). Molecular Culture ID is intended for research applications only. It is not intended for use in diagnostic procedures, and results must not be used for the diagnosis, prevention, monitoring or treatment of disease, or for any other clinical or patient-management decision.

For Research Use Only. Not for use in diagnostic procedures.

The following species of the phyla *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Bacteroidetes* and *Proteobacteria* are included in the Molecular Culture ID identification database (see Annex I).

Detected bacteria of the phyla *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Bacteroidetes* and *Proteobacteria*, outside this list will be reported as unknown species belonging to one of the three groups: FAFV (*Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia*), *Bacteroidetes* and *Proteobacteria*.

Sample types

- Effusions: a) Synovial, b) Peritoneal, c) Pleural d) Pericardial
- Cerebrospinal fluid
- Tissue biopsies: a) Bone biopsies b) Soft tissue biopsies
- Drain fluids and pus
- EDTA anticoagulated whole blood

2. Product description

Technical background

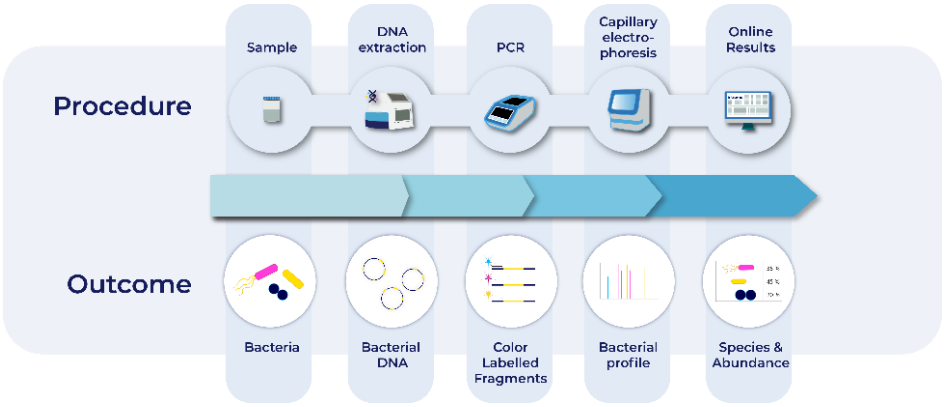
Molecular Culture ID is based on the InterSpace-profiling (IS-Pro™) technology. IS-pro™ is a bacterial profiling technique based on species-specific length polymorphisms of the IS (InterSpace) region and phylum-specific sequence polymorphisms of 16S rDNA. Molecular Culture ID consists of two multiplex PCRs. The first PCR is specific for *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia,* and *Bacteroidetes*. The second PCR is specific for *Proteobacteria*. An internal PCR amplification control, present in the second PCR is used to monitor PCR inhibition and fragment length calling.

The IS region is amplified with fluorescently labelled phylum-specific forward primers targeting the 16S rDNA and unlabelled reverse primers targeting the 23S rDNA. After PCR, the fluorescently labelled amplicons are added to eMix containing a length reference marker. The length and fluorescent colour of the amplicons are determined by high-resolution capillary electrophoresis. The resulting Molecular Culture ID peak profiles provide two levels of information: the colour shows bacterial presence at phylum level and amplicon lengths can be used to further identify bacteria to the genus or species level.

Quantification normalisation is performed, which is based on the fluorescence intensity of the length reference marker.

Workflow

Molecular Culture ID is a semi-automated test, that consists of laboratory processing and software analysis. The main steps are shown in the figure below.



Sample collection and preparation

The process starts with a sample suspected of containing bacteria. The methodology to obtain the sample is the responsibility of the customer, as sample collection devices are not provided with Molecular Culture ID.
The sample is treated with Bacterial Shock Buffer 1 (IBB23000, inbiome) and Bacterial Shock Buffer 2 (IBB24000, inbiome). For EDTA anticoagulated blood, the sample is treated with Host Depletion Buffer 1 (IBB250002, inbiome), Host Depletion Buffer 2(IBB250003, inbiome), Neutralisation Buffer (IBB250004, inbiome), Wash Buffer (IBB250005, inbiome) and Bacterial Shock Buffer 1 (IBB230001, inbiome) and Bacterial Shock Buffer 2 (IBB240001).

DNA extraction/isolation

Bacterial DNA is isolated from the sample.
Molecular Culture ID can be used with standard guanidine iso-thiocyanate-based lysis reagents and magnetic beads capture devices for DNA isolation. Molecular Culture ID is validated for:

- bioMérieux NucliSENS® easyMAG™ / bioMérieux EMAG,
- Promega Maxwell CSC in combination with Maxwell® Genomic DNA Kit (AS1850) and
- ThermoFisher KingFisher™ Apex in combination with Maxwell® HT Genomic DNA Kit reagents (A6050).

Validated sample types:

bioMérieux NucliSENS® easyMAG™ / bioMérieux EMAG®	ThermoFisher KingFisher™ Apex in combination with Maxwell® HT Genomic DNA Kit reagents (A6050)	Promega Maxwell CSC in combination with Maxwell® Genomic DNA Kit (AS1850)
Effusions	Effusions	Effusions
Cerebrospinal fluid	Cerebrospinal fluid	Cerebrospinal fluid
Tissue biopsies	Tissue biopsies	Tissue biopsies
Drain fluids and pus	Drain fluids and pus	Drain fluids and pus
EDTA anticoagulated whole blood	EDTA anticoagulated whole blood	EDTA anticoagulated whole blood

PCR

PCR machines validated are the Applied Biosystems™ Veriti from Thermo Fisher Scientific, the Applied Biosystems™ SimpliAmp™ Thermal Cycler from Thermo Fisher Scientific, CFX Opus Real-Time PCR Systems from Bio-Rad, Applied Biosystems™ MiniAmp™ Thermal Cycler from Thermo Fisher Scientific and Applied Biosystems™ VeritiPro™ Thermal Cycler from Thermo Fisher Scientific

The Molecular Culture Starter is used for PCR machine verification.

Capillary electrophoresis
PCR amplicons are sorted by length and colour, using capillary electrophoresis.
ABI Genetic Analyzer 3500, 3500XL and SeqStudio Flex are validated for capillary electrophoresis with the G5 (DS-33) dyeset, a 50cm capillary and POP7 polymer).

The Molecular Culture Starter is used for Capillary Electrophoresis machine verification.

Online results
inbiome offers online software 'antoni' for analysing the data files resulting from capillary electrophoresis to identify and semi-quantify bacteria. Please see Section 9.3: Data Analysis.

3. Molecular Culture ID components

3.1 Included in Molecular Culture ID

Mastermix FIRBAC
Mastermix FIRBAC consists of 2 vials, labelled "Mastermix FIRBAC" containing 180 µL ready to use PCR mix. This mixture contains eight primers for amplification of the bacterial DNA of *Firmicutes/Actinobacteria/ Fusobacteria/Verrucomicrobia* and *Bacteroidetes*. The mastermix contains all ingredients for PCR amplification including DNA polymerase.

Mastermix PROTEO
Mastermix PROTEO consists of 2 vials labelled "Mastermix PROTEO" containing 180 µL ready to use PCR mix. This mixture contains eight primers for amplification of the bacterial DNA of *Proteobacteria* and contains DNA and primers for amplification of the internal control. The mastermix contains all ingredients for PCR amplification including DNA polymerase.

Positive Control FIRBAC
Positive Control FIRBAC consists of 2 vials labelled "Positive Control FIRBAC" containing 30 µL positive control. The positive control contains the bacterial DNA of *Firmicutes/Bacteroidetes (S.cristatus* and *B. fragilis*).

Positive Control PROTEO
Positive Control PROTEO consists of 2 vials labelled "Positive Control PROTEO" containing 30 µL positive control. The positive control contains the bacterial DNA of *Proteobacteria*. (*P.aeruginosa*).

eMix
eMix consists of 1 vial labelled "eMix" containing 480 µL eMix.
eMix contains Hi-Di Formamide, and the labelled sizemarker RadiantDy 632 (BioVentures, Murfreesboro, USA).

Note: Use all components of the same kit lot number.

3.2 Materials required but not provided in Molecular Culture ID

3.2.1 General laboratory equipment and materials

The following equipment and materials are required for the Molecular Culture ID workflow:

General laboratory equipment

- Appropriate sample collection devices
- Heating shaker capable of operating at 95 °C and 800 rpm
- Tabletop centrifuge capable of reaching 3,000 × g
- Computer with internet access
- Appropriate personal protective equipment for handling eMix, as described in Section 5.2

PCR instruments
One of the following thermal cyclers is required:

- Applied Biosystems™ Veriti™ Thermal Cycler (Thermo Fisher Scientific)
- Applied Biosystems™ VeritiPro™ Thermal Cycler (Thermo Fisher Scientific)
- Applied Biosystems™ SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific)
- Applied Biosystems™ MiniAmp™ Thermal Cycler (Thermo Fisher Scientific)
- CFX Opus Real-Time PCR System (Bio-Rad)

Capillary electrophoresis instruments
One of the following instruments is required:

- Applied Biosystems™ 3500 Genetic Analyzer (Thermo Fisher Scientific)
- Applied Biosystems™ 3500xL Genetic Analyzer (Thermo Fisher Scientific)
- Applied Biosystems™ SeqStudio™ Flex Genetic Analyzer (Thermo Fisher Scientific)

The capillary electrophoresis instrument must be configured with the G5 (DS-33) dye set, a 50 cm capillary array and POP-7™ polymer.

Sample pretreatment reagents

- Bacterial Shock Buffer 1 (IBB230001)
- Bacterial Shock Buffer 2 (IBB240001)

3.2.2 Additional materials for specific sample types

Biopsy samples
The following additional materials are required for processing biopsy samples:

- Mechanical bead-beating device capable of operating at 4.5 m/s
- Zirconia/silica beads, 0.1 mm (11079101Z, BioSpec)
- UltraPure™ DNase/RNase-Free Distilled Water (10977035, Invitrogen)

EDTA-anticoagulated whole blood
The following additional materials are required for processing EDTA-anticoagulated whole blood:

- Centrifuge with swing-out rotor capable of reaching 2,791 × g
- Tabletop centrifuge capable of reaching 16,000 × g
- Host Depletion Buffer 1 (IBB250002)
- Host Depletion Buffer 2 (IBB250003)
- Neutralization Buffer (IBB250004)
- Wash Buffer (IBB250005)

3.2.3 DNA extraction equipment and reagents

An appropriate DNA extraction platform and the corresponding extraction reagents are required. The equipment and reagents listed below depend on the selected extraction method.
bioMérieux NucliSENS® easyMAG™ or EMAG®

- bioMérieux NucliSENS® easyMAG™ or EMAG® DNA extraction system
- Corresponding bioMérieux extraction reagents
- Buffer AL (19075, QIAGEN)

Promega Maxwell® CSC with Maxwell® Genomic DNA Kit (AS1850)

- Maxwell® CSC instrument
- Maxwell® Genomic DNA Kit (AS1850, Promega)

Thermo Fisher Scientific KingFisher™ Apex with Maxwell® HT Genomic DNA Kit reagents (A6050)

- KingFisher™ Apex instrument
- Lysis Buffer (MC5018, Promega)
- Maxwell® HT Resin C (A7751, Promega)
- Binding Buffer (A3121, Promega)
- Wash Buffer (MC5041, Promega)
- Elution Buffer (A828, Promega)
- Absolute ethanol
- UltraPure™ DNase/RNase-Free Distilled Water (10977035, Invitrogen)

Only the reagents and equipment applicable to the selected extraction platform are required. Follow the corresponding DNA extraction procedure described in this IFU.

3.2.4 Requirements for software use

To make use of 'antoni', the user must meet the following system and network requirements:

- An up-to-date browser, either Firefox or Chromium based (Chrome or Edge).
- A screen of at least 1024x1240, 1920x1080 or 2560x1440 px, other screen resolutions or scaling might result in visual artifacts.
- 20Mbps or greater upload and download speeds. Lower speeds will function, but uploading and viewing of results will be impacted negatively.
- Network access via HTTPS over port 443 outbound access to https://antoni-research.inbiome.com and https://fa-api.inbiome.com (no specific IPv4 or IPv6 address as the system is proxied via CloudFlare).
- If HTTP redirect is desired, network access via HTTP over port 80 outbound access to http://antoni.inbiome.com is required. This will always redirect to the HTTPS version and thus is not required to use the system.
- As antoni is operated by inBiome, there are no additional software requirements for data analysis.

4. Storage

- Molecular Culture ID is shipped on dry ice. All kit components should arrive frozen. If one or more components are not frozen upon receipt, or if the kit is damaged, please contact your local distributor and/or inbiome.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Mastermixes (more than two times), positive control (more than two times), and eMix (more than two times) should be avoided, as this might affect the performance of the assay.
- Refreeze within half an hour after thawing.
- Protect all components from light.
- Alteration in the physical appearance of test kit materials may indicate instability or deterioration. Please contact techsupport@inbiome.com when there are concerns about the physical appearance of the test kit materials. Expiry dates shown on component labels indicate the date beyond which components should not be used.
- Mastermix and control vials should be stored in a ***Sample Preparation area*** or ***Pre-Amplification area***
- eMix can be stored in the ***Amplification area***

5. Warnings and Precautions

5.1 General Precautions

- **For Research Use Only. Not for use in diagnostic procedures.**
- Results generated with Molecular Culture ID are intended for research purposes only and must not be used for diagnosis, patient management or treatment decisions.
- A unidirectional workflow in the laboratory is strongly recommended:
  Sample Preparation area: Dedicated area to prepare the samples. All materials (equipment, supplies, protection, gloves, etc.) must be dedicated to this area. Materials from this area may not be moved to the Pre-Amplification area.
  Pre-Amplification area: Dedicated area to prepare the reagents. All materials (equipment, supplies, protection, gloves, etc.) must be dedicated to this area.
  Amplification area: Dedicated area for amplification. All materials (equipment, supplies, protection, gloves, etc.) must be dedicated to this area. Materials from this area, may not be moved to the Pre-Amplification area, and may not be moved to the Sample Preparation Area.
- Use PCR grade (sterile, nuclease-free), aerosol-resistant pipette tips, PCR grade consumables, and wear protective gloves.
- Protect kit contents or generated PCR products from direct sunlight.
- User is responsible for maintenance and calibration of machines, pipets and equipment used in conjunction with Molecular Culture ID.
- The user is responsible for reading and following the safety and precautions stated by the manufacturer of the machines, that are used in combination with Molecular Culture ID.
- Sample handling is the responsibility of the customer. To avoid sample mix-up leading to unreliable results, please handle samples with proper lab practices.
- Do not use Molecular Culture ID after its expiration date.

- Careful analytical techniques and strict adherence to the directions in the test instructions are essential to obtain reliable results.

5.2 Safety Precautions

- Wear proper Personal Protective Equipment (PPE), such as disposable, clean, powder-free gloves, protective lab coats, and appropriate eye protection. Refer to the applicable Safety Data Sheets (SDSs) for reagent-specific PPE requirements.
- Protect skin, eyes, and mucosal surfaces from potential hazards.
- Frequently change gloves when in contact with reagents or samples to prevent cross-contamination.
- Comply with your organization's specific biosafety procedures.
- Dispose of all assay-related materials, from reagents to bio-waste, in compliance with local and national environmental and health regulations.



CAUTION: Handle all human-derived samples as biohazardous material.
Handle samples as if capable of transmitting an infectious agent.

All human-derived samples should be handled as if they are capable of transmitting an infectious agent. Use safe laboratory procedures as outlined in publications such as Centers for Disease Control and Prevention/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories".

The DNA extraction/isolation step for use with this kit requires an external extraction procedure that may contain hazardous substances, including **guanidine salts**.

- Users are responsible for following the safety instructions and handling precautions provided by the manufacturer of the extraction reagents.
- **Refer to the Safety Data Sheet (SDS) provided by the manufacturer of the extraction kit for detailed information on chemical hazards, safe handling, and disposal requirements.**
- Use in accordance with institutional safety protocols and applicable local regulations.

Reagent Hazard Statements

(OSHA Hazard Communication Standard, 29 CFR 1910.1200)

Reagent	Section	Hazard Class	Category	Hazard class and category	Hazard statement Code	Hazard statement
eMix [MolCul 150006]	3.6	Carcinogenicity	2	Carc. 2	H351	Suspected of causing cancer
	3.7	Reproductive toxicity	1B	Repr. 1B	H360F	May damage fertility, may damage the unborn child
	3.9	Specific target organ toxicity, repeated exposure	2	STOT RE 2	H373	May cause damage to organs (blood, cardiovascular system) through prolonged or repeated exposure
Host Depletion Buffer 1 [IBB250002]	A.3	Serious eye damage/eye irritation	2	Eye Irrit. 2	H319	Causes serious eye damage
Host Depletion Buffer 2 [IBB250003]	A.3	Serious eye damage/eye irritation	1	Eye Dam. 1	H318	Causes serious eye damage

Reagent Hazard Statements

(OSHA Hazard Communication Standard, 29 CFR 1910.1200)

Reagent	Section	Hazard Class	Category	Hazard class and category	Hazard statement Code	Hazard statement
Bacterial Shock Buffer 1 [IBB230001]	A.2	skin corrosion/irritation	1	Skin Corr. 1	H314	Causes severe skin burns and eye damage
	A.3	serious eye damage/eye irritation	1	Eye Dam. 1	H318	Causes serious eye damage
	B.6	flammable liquid	4	Flam. Liq. 4	H227	Combustible liquid
	B.16	substance or mixture corrosive to metals	1	Met. Corr. 1	H290	May be corrosive to metals

Reagent Precautionary Statements

(OSHA Hazard Communication Standard, 29 CFR 1910.1200)

Reagent	Precautionary Statement
eMix [MolCul 150006]	P308 + P313 - If exposed or concerned: Get medical advice/attention P202 - Do not handle until all safety precautions have been read and understood P260 - Do not breathe dust/fume/gas/mist/vapours/spray P201 - Obtain special instructions before use P280 - Wear protective gloves/protective clothing/eye protection/face protection. P314 - Get medical advice/attention if you feel unwell
Host Depletion Buffer 1 [IBB250002]	P280 - Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 - If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 - If eye irritation persists: Get medical advice/attention.
Host Depletion Buffer 2 [IBB250003]	P280 - Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 - If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 - Immediately call a poison center/doctor

Reagent Precautionary Statements (OSHA Hazard Communication Standard, 29 CFR 1910.1200)	
Reagent	**Precautionary Statement**
Bacterial Shock Buffer 1 [IBB230001]	P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P234 Keep only in original packaging. P260 Do not breathe dusts or mists. P280 Wear protective gloves/protective clothing/eye protection/face protection. P301+P330+P331 If swallowed: Rinse mouth. Do NOT induce vomiting. P303+P361+P353 If on skin (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower. P304+P340 If inhaled: Remove person to fresh air and keep comfortable for breathing. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a poison center/doctor. P321 Specific treatment (see on this label). P363 Wash contaminated clothing before reuse. P370+P378 In case of fire: Use carbon dioxide, powder extinguisher, water spray or alcohol resistant foam to extinguish. P390 Absorb spillage to prevent material damage. P403 Store in a well-ventilated place. P405 Store locked up. P406 Store in corrosion resistant container with a resistant inner liner. P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Reagent-Specific Handling

The reagents listed in the hazard classification table may require additional precautions. Refer to the applicable Safety Data Sheets (SDSs) for detailed handling, exposure response, and disposal instructions. **Do not dispose of these reagents down the drain.**

- **eMix (MolCul 150006):** Contains formamide. Handle in a well-ventilated area. Avoid inhalation and skin contact. Refer to the SDS for additional precautions related to suspected carcinogenicity, reproductive toxicity, and prolonged or repeated exposure.
- **Host Depletion Buffer 1 (IBB250002):** Causes serious eye damage. In case of eye contact, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a poison center or doctor.
- **Host Depletion Buffer 2 (IBB250003):** Contains Triton X-100 (Poly(oxy-1,2-ethanediyl), .alpha.-[4-(1,1,3,3-tetramethylbutyl)phenyl]-.omega.-hydroxy-). Causes serious eye damage. In case of eye contact, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a poison center or doctor.
- **Bacterial Shock Buffer 1 (IBB230001):** Contains sodium dodecyl sulfate. Causes severe skin burns and eye damage. Handle in a well-ventilated area and keep away from heat, sparks, and open flames. Wear protective gloves, protective clothing, and appropriate eye/face protection. In case of skin contact, immediately remove contaminated clothing and rinse skin with water. In case of eye contact, rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Immediately call a poison center or doctor.

NOTICE: Please report any product complaint, malfunction or safety concern related to Molecular Culture ID to inbiome (see Technical Assistance).

6. Sample conditions and storage

Non-EDTA-anticoagulated whole blood samples should be processed as soon as possible. If storage or transport are required, non-EDTA-anticoagulated whole blood samples should be stored at -80°C. Based on inbiome data, samples may be stored for a maximum of 7 years at -80°C.
For the sample type EDTA-anticoagulated whole blood, samples should also be processed as soon as possible. EDTA-anticoagulated whole blood may be stored at 2–8 °C for up to 3 days. Frozen storage of EDTA-anticoagulated whole blood at −20 °C or −80 °C is validated for up to 6 days. More than one freeze–thaw cycle is not recommended for this sample type.

7. Sample collection and preparation

Collect samples in sterile PCR grade sample collection devices that can be used for standard DNA isolation procedures.

Sample collection should be performed with utmost caution to avoid bacterial contamination from the environment.

8. Sample preparation

The following procedure must be performed in the Sample Preparation Area in a class 2 Biological Safety Cabinet (protection to user and material).

Molecular Culture ID targets a broad range of bacteria. Therefore, a blank control (negative control) must be included throughout the entire workflow, starting from sample preparation, to monitor for potential bacterial contamination.

The blank control consists of the buffers used in the sample preparation and DNA extraction procedures, without the addition of sample material. It must undergo the same processing steps as the clinical samples.

Sample preparation validated for

- bioMérieux NucliSENS® easyMAG™and bioMérieux EMAG®:
- Promega Maxwell CSC in combination with Maxwell® Genomic DNA Kit (AS1850)
- ThermoFisher KingFisher™ Apex in combination with Maxwell® HT Genomic DNA Kit (A6050)
  - KingFisher™ Apex protocol (Maxwell HT Genomic DNA_InBiome_modified_version_01) available via techsupport@inbiome.com

8.1 Pretreatment Procedure

The pretreatment procedure depends on the sample type.
Follow the corresponding pretreatment procedure described below.

Sample input and buffer volumes for pretreatment

Tissue biopsy	Approximately 1.5 mm diameter piece of tissue + 100 µL DNase/RNase-free water	250 µL	25 µL
Synovial effusion, pus	50 µL	250 µL	25 µL
Drain fluid, peritoneal, pleural or pericardial effusion, cerebrospinal fluid (CSF)	200 µL	250 µL	25 µL
EDTA-anticoagulated whole blood	1–10 mL (prior to host depletion)	250 µL	25 µL

NOTE: Tissue biopsies require mechanical disruption by bead beating before BSB treatment. EDTA-anticoagulated whole blood requires host depletion and washing before BSB treatment. Follow the sample-specific instructions below.

Tissue biopsies

1. Add several small, finely cut pieces of biopsy material from different areas of the biopsy to a PCR-grade tube (capacity 1.5 mL) containing 400 mg of 0.1 mm Zirconia/Silica beads.

NOTE: The combined amount of biopsy material should be approximately equivalent to a 1.5 mm diameter piece of tissue.

2. Add 100 µL DNase/RNase-free water.
3. Bead beat the sample for 180 seconds at 4.5 m/s at room temperature.
4. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds to collect liquid at the bottom of the tube.

Bacterial lysis with BSB buffers

5. Add 250 µL Bacterial Shock Buffer 1.
6. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds to collect liquid at the bottom of the tube.
7. Incubate for 10 minutes at 95 °C and 800 rpm in a heater shaker device.
8. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds.
9. Add 25 µL of Bacterial Shock Buffer 2 and vortex.
10. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds.
11. Carefully collect the supernatant for DNA extraction, avoiding transfer of beads and tissue debris.

Synovial effusion and pus

1. Add 50 µL sample to the PCR grade tube

Bacterial lysis with BSB buffers

2. Add 250 µL Bacterial Shock Buffer 1.
3. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds to collect liquid at the bottom of the tube.
4. Incubate for 10 minutes at 95 °C and 800 rpm in a heater shaker device..
5. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds.
6. Add 25 µL of Bacterial Shock Buffer 2 and vortex.
7. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds.
8. Use the complete volume for DNA extraction

Drain fluids, peritoneal (ascites) effusion, pleural effusion, pericardial effusion, cerebrospinal fluid

1. Add 200 µL sample to the PCR grade tube.

Bacterial lysis with BSB buffers

2. Add 250 µL Bacterial Shock Buffer 1.
3. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds to collect liquid at the bottom of the tube.
4. Incubate for 10 minutes at 95 °C and 800 rpm in a heater shaker device..
5. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds.
6. Add 25 µL of Bacterial Shock Buffer 2 and vortex.
7. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds.
8. Use the complete volume for DNA extraction

EDTA anticoagulated whole blood

Host depletion

1. Prepare Host Depletion Buffer by combining Host Depletion Buffer 1 and Host Depletion Buffer 2 in a 9:1 ratio in a PCR-grade container (e.g. 45 mL HDB1 with 5 mL HDB2).

NOTE: The prepared Host Depletion Buffer should appear milky and non-transparent.

2. Add Host Depletion Buffer in a 1:1 (v/v) ratio to 1–10 mL of EDTA-anticoagulated blood.
3. Mix thoroughly by vortexing for 10 seconds or by gently inverting the tube three times.
4. Incubate with Host Depletion Buffer on a roller bank for no longer than 3 minutes.

WARNING: Do not exceed an incubation time of 3 minutes during Host Depletion Buffer treatment. Prolonged incubation may cause lysis of sensitive bacterial species (e.g. *Pseudomonas* spp.), which can adversely affect test performance.

5. Add Neutralisation Buffer in a 1:1 ratio relative to the Host Depletion Buffer volume used in step 2 to the blood–Host Depletion Buffer mixture.
6. Mix thoroughly by vortexing for 15 seconds or by gently inverting the tube three times.

Wash steps

7. Centrifuge for 10 minutes at 2,791 × g using a swing-out rotor.
8. Carefully decant the supernatant into a suitable waste container.

WARNING: Do not disturb or discard the pellet.

9. Keep the tube inverted and remove residual liquid from the rim using a 100 µL pipette.
10. Add 1 mL Wash Buffer (IBB25004, inbiome) to the pellet.
11. Resuspend the pellet by pipetting up and down three times.
12. Transfer the suspension to a 1.5 mL PCR-grade tube (capacity 1.5 mL).
13. Centrifuge at 16,000 × g in a tabletop centrifuge for 10 minutes.
14. Carefully decant the supernatant into a waste container.

WARNING: Do not disturb or discard the pellet.

15. Keep the tube inverted and remove residual liquid from the rim using a 100 µL pipette.

NOTE: A small reddish pellet may be visible. If no pellet is visible, continue with the next step as described.

Bacterial lysis with BSB buffers

16. Add 250 µL Bacterial Shock Buffer 1.
17. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds to collect liquid at the bottom of the tube.
18. Incubate for 10 minutes at 95 °C and 800 rpm in a heater shaker device..
19. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds.

20. Add 25 µL of Bacterial Shock Buffer 2 and vortex.
21. Centrifuge briefly at approximately 3,000 × g for 5–10 seconds.
22. Use the complete volume for DNA extraction

8.2 DNA extraction using the bioMérieux NucliSENS® easyMAG™ or EMAG®

Refer to the manufacturer's instructions for use for information on instrument operation, system features, extraction protocols, and general operating procedures.
Perform the following procedure for each sample, including the blank extraction control.

A. Sample preparation and lysis

1. Add 1 mL of bioMérieux Lysis Buffer and 1 mL of QIAGEN Buffer AL to the extraction vessel.

NOTE: See section 3.2 for reagent specifications and catalogue numbers.

2. Transfer the entire volume of the pretreated sample (Section 8.1) to the extraction vessel.
3. Mix the contents by pipetting up and down three times.
4. Incubate for at least 10 minutes at room temperature.
5. Remove any visible debris that could clog the instrument's pipette tips using a sterile pipette.
6. Add 70 µL of bioMérieux magnetic silica to the extraction vessel.
7. Mix thoroughly by pipetting. Use a new pipette tip for each sample to prevent cross-contamination.

B. DNA extraction

8. Start the extraction run according to the manufacturer's instructions, using the applicable settings:

- Extraction protocol: Specific A
- Lysis incubation: Off-board
- Elution volume: 70 µL

C. Post-extraction handling

9. Upon completion of the extraction run, transfer the eluted DNA to a PCR-grade tube.
10. Store the extracted DNA at 2–8 °C until further processing

8.3 DNA extraction using the ThermoFisher KingFisher™ Apex in combination with Maxwell® HT Genomic DNA Kit (A6050)

The following procedure describes DNA extraction using the Promega Maxwell® HT Genomic DNA Kit in combination with the KingFisher™ Apex instrument, the machine protocol:
(Maxwell HT Genomic DNA_InBiome_modified_version_01) is available via techsupport@inbiome.com
Perform the procedure for each sample, including the blank extraction control.

A. Preparation of reagents

1. Prepare fresh 80% (v/v) ethanol using absolute ethanol and DNase/RNase-free water. Approximately 1,150 µL is required per sample, including the blank extraction control. Prepare an additional 5% to compensate for pipetting losses.
2. Prepare the Binding Mix by combining the following reagents per sample:

Reagent	Volume per sample
Binding Buffer (A3121, Promega)	334.8 µL
Maxwell® HT Resin C (A7751, Promega)	15.2 µL
Total	**350 µL**

Prepare an additional 5% Binding Mix to compensate for pipetting losses. Mix thoroughly before dispensing.

B. Preparation of extraction plates

3. Prepare the wash and elution plates according to the following table:

Plate	Reagent	Volume per well	Plate type
Wash Plate 1	80% ethanol	900 µL	Deep-well
Wash Plate 2	Wash Buffer (MC5041, Promega)	350 µL	Deep-well
Wash Plate 3	Wash Buffer (MC5041, Promega)	300 µL	Deep-well
Wash Plate 4	80% ethanol	250 µL	Deep-well

Elution Plate	Elution Buffer (A828, Promega)	70 µL	Low-well

4. Prepare the sample plate (deep-well plate) by adding the following reagents to each well:

Reagent	Volume per sample
Lysis Buffer (MC5018, Promega)	180 µL
Binding Mix	350 µL
Pretreated sample (Section 8.1)	Complete available volume

NOTE: The maximum expected total volume per well is 1,005 µL.

C. DNA extraction procedure

5. Switch on the KingFisher™ Apex instrument.
6. Select 'Open Protocol' and navigate to the protocol provided by inbiome.
7. Select the protocol.
8. Press 'Play' to initiate the extraction procedure.
9. Load the prepared plates and tip comb into the instrument as prompted. The instrument automatically rotates the plate carousel to the appropriate loading position.
10. Load the plates according to the following configuration:

Position	Plate
1	Tip comb in low-well plate
2	Sample plate
3	Wash Plate 1
4	Wash Plate 2
5	Wash Plate 3
6	Wash Plate 4
7	Elution Plate

11. Insert each plate into the illuminated loading position. Ensure that each plate is correctly oriented, with well A1 aligned with the A1 position indicated on the plate carousel
12. Once all plates have been loaded, close the instrument door. The extraction run will start automatically.
13. The extraction procedure takes approximately 47 minutes.

D. Post-extraction handling

14. Upon completion of the extraction run, remove the elution plate from the instrument.
15. Seal the elution plate and store the extracted DNA at 2–8 °C until further processing.

8.4 DNA extraction using the Promega Maxwell® CSC Instrument and Maxwell® CSC Genomic DNA Kit (AS1850)

Refer to the manufacturer's instructions for use (Maxwell® CSC Genomic DNA Kit Technical Manual, TM677) for information on instrument operation, cartridge preparation, extraction procedures, and general operating instructions.

Perform the following procedure for each sample, including the blank extraction control.

A. Sample preparation and lysis

1. Add an equal volume (1:1, v/v) of Promega Lysis Buffer (MC110A), supplied with the Maxwell® CSC Genomic DNA Kit, to the collected lysate after Bacterial Shock Buffer pretreatment (section 8.1).
2. Vortex for 10 seconds to mix thoroughly.
3. Transfer the entire volume of the lysate to well #1 (the largest well) of a Maxwell® CSC cartridge.
4. Mix the lysate with the binding solution in well #1 by pipetting up and down to obtain a homogeneous mixture. Use a new pipette tip for each sample to prevent cross-contamination.

B. Cartridge preparation and DNA extraction

5. Prepare the Maxwell® CSC cartridges and 0.5 mL elution tubes according to Section 5.G, *Preparing the Maxwell® CSC Genomic DNA Cartridge*, of the manufacturer's technical manual (TM677).

NOTE: The RNase A Solution supplied with the Maxwell® CSC Genomic DNA Kit is not required when using the Molecular Culture ID workflow in combination with Inbiome BSB pretreatment. Omit the RNase A addition described in the manufacturer's protocol.

6. Add 70 µL of Elution Buffer, supplied with the Maxwell® CSC Genomic DNA Kit, to each 0.5 mL elution tube.
7. Start the DNA extraction run using the Maxwell® CSC Genomic DNA purification method, following the instructions in Section 6, *Maxwell® Instrument Run*, of the manufacturer's technical manual (TM677).

C. Post-extraction handling

8. Upon completion of the extraction run, remove the elution tubes containing the purified DNA and close the tubes securely.
9. Store the extracted DNA at 2–8 °C until further processing.

9. Molecular Culture ID Procedure

This procedure must be performed in the Pre-Amplification Preparation Area. Use PCR grade, aerosol barrier tips during the whole test procedure. Thaw only the components that are going to be used. Mix and spin down reaction tubes briefly (three seconds) before use.

9.1 Procedure PCR

1. Prepare the required number of reaction tubes or wells for the number of samples to be measured. Two reactions per sample (and controls) should be prepared, accounting for one FIRBAC and one PROTEO reaction. NOTE: The first run of each kit should contain a Positive Control FIRBAC and Positive Control PROTEO sample. Handle positive controls as DNA.

2. Thaw and vortex Mastermix FIRBAC and Mastermix PROTEO. Per sample, add 15 µl of Mastermix FIRBAC to the first reaction tube/well and 15 µl of Mastermix PROTEO to the second reaction tube/well.

3. Vortex and spin down the DNA sample. Add 10 µl DNA of each sample to the reaction tube/well containing Mastermix FIRBAC and add 10 µl DNA of each sample to the reaction tube/well containing Mastermix PROTEO.

4. Close the reaction tubes or seal the plate and spin down for 30 seconds at approximately 1000 rpm.

5. Place the reaction tubes/plate into the verified Molecular Culture ID thermocycler and program the system as listed below.

6. The PCR products can be stored for 12 hours at 2-8 ˚C in the dark.

PCR Protocol	
Hotstart	10 min 95 ˚C
10 cycles	30 sec 95 ˚C
	45 sec 67 ˚C, reduce 1 ˚C per cycle
	1 min 72 ˚C
25 cycles	20 sec 95 ˚C
	30 sec 57 ˚C
	30 sec 72 ˚C
Final elongation and cooldown	2 min 72 ˚C
	∞ 4 ˚C

9.2 Procedure Capillary Electrophoresis

This procedure must be performed in the Amplification area. Thaw only the components that are going to be used. Mix and spin down reaction tubes briefly (three seconds) before use.

1. Thaw and vortex eMix.

2. Per sample, two PCR reactions were previously performed (FIRBAC and PROTEO). For every sample, fill one well of the ABI plate with 20 µl eMix (MolCul 150006). Add 2.5 µl of the FIRBAC PCR product and 2.5 µl of the PROTEO PCR product. Note: The Positive Control FIRBAC and Positive Control PROTEO can be pipetted together as one sample.

3. Spin down the plate for 30 seconds at approximately 1000 rpm.

4. Place the plate in the thermocycler at 94°C for 3 minutes followed by a cooling step to 4 °C. Use the appropriate cover for the plate so no evaporation occurs.

5. Replace the plate-cover with an ABI septa.

6. Store the plate at 2-8°C until capillary electrophoresis. The plate can be stored for five days at 2-8°C in the dark.

7. Run the capillary electrophoresis according to the settings shown below:

Settings ABI 3500

Instrument protocol ABI 3500				
Application type:	Capillary Length	Polymer	Dye Set	Advanced Options
Fragment	50 cm	POP7	G5	unchanged from default settings

Instrument Protocol Properties								
Run Module	Oven Temperature	Run Time	Run voltage	PreRun Time	PreRun Voltage	Injection Time	Injection Voltage	Data Delay
FragmentAnalysis50_POP7	60°C	4500 sec	11.0 kVolts	180 sec	15 kVolt	15 sec	3 kVolts	200 sec

Dye Set protocol ABI 3500				
Dye Set Name	Chemistry	Dye Selection	Reduced Selection	Calibration Peak Order
G5	Matrix Standard	Blue Green Yellow Red Orange	Blue Green Yellow Red Orange	Blue=5, Green=4, Yellow=3, Red=2, Orange=1
Parameters				
Matrix Condition Number Upper Limit			13.5	
Locate Start Point			After Scan 1000, Before Scan 5000, Limit Scans To 3250, Sensitivity 0.4, Minimum Quality Score 0.95	

Sizecalling protocol ABI 3500 *										
Protocol name	Fragment Analysis PA_Protocol									
Size Standard	GS600LIZ (60-600) + Normalisation									
Sizecaller	SizeCaller v1.1.0									
Analysis Settings										
Analysis Range	Sizing Range	Size Calling Method	Primer Peak	Minimum Peak Height	Use Smoothing	Use Baselining (Baseline window (Pts))	Minimum Peak Half Width	Peak Window Size	Polynomial Degree	Slope Threshold Peak Start
Full	Full	Local Southern	Present	All colors 175	None	51	2	15	3	0.0
QC Settings										
Fail if Value	Suspect Range		Pass if Value	Assume Linearity		Pull Up				
0.25	0.25-0.75		≥ 0.75	0 bp To 800 bp		Actuate Pull-Up flag if Pull-Up Ratio ≤0.05 and Pull-Up Scans≤ 1				

* The online software 'antoni' does not require a sizecalling protocol because it uses raw data from the .fsa. Since sizecalling is required in the instrument software, please use the sizecalling protocol above.

Settings ABI 3500XL

Instrument protocol ABI 3500XL				
Application type:	Capillary Length	Polymer	Dye Set	Advanced Options
Fragment	50 cm	POP7	G5	unchanged from default settings

Instrument Protocol Properties								
Run Module	Oven Temperature	Run Time	Run voltage	PreRun Time	PreRun Voltage	Injection Time	Injection Voltage	Data Delay
Fragment Analysis50_POP7	60°C	4500 sec	11.0 kVolts	180 sec	15 kVolt	45 sec	3 kVolts	200 sec

Dye Set protocol ABI 3500XL				
Dye Set Name	Chemistry	Dye Selection	Reduced Selection	Calibration Peak Order
G5	Matrix Standard	Blue Green Yellow Red Orange	Blue Green Yellow Red Orange	Blue=5, Green=4, Yellow=3, Red=2, Orange=1
Parameters				
Matrix Condition Number Upper Limit			13.5	
Locate Start Point			After Scan 1000, Before Scan 5000, Limit Scans To 3250, Sensitivity 0.4, Minimum Quality Score 0.95	

Sizecalling protocol ABI 3500XL *										
Protocol name	Fragment Analysis PA_Protocol									
Size Standard	GS600LIZ (60-600) + Normalisation									
Sizecaller	SizeCaller v1.1.0									
Analysis Settings										
Analysis Range	Sizing Range	Size Calling Method	Primer Peak	Minimum Peak Height	Use Smoothing	Use Baselining (Baseline window (Pts))	Minimum Peak Half Width	Peak Window Size	Polynomial Degree	Slope Threshold Peak Start
Full	Full	Local Southern	Present	All colors 175	None	51	2	15	3	0.0

Fail if Value	Suspect Range	Pass if Value	Assume Linearity	Pull Up
0.25	0.25-0.75	≥ 0.75	0 bp To 800 bp	Actuate Pull-Up flag if Pull-Up Ratio ≤0.05 and Pull-Up Scans≤ 1

* The online software 'antoni' does not require a sizecalling protocol because it uses raw data from the .fsa. Since sizecalling is required in the instrument software, please use the sizecalling protocol above.

Settings SeqStudio Flex

Instrument protocol SeqStudio Flex			
Application type	Capillary Length	Polymer	Dye Set
Fragment analysis	50 cm	POP7	G5

Instrument Protocol Properties								
Run Module	Oven Temperature	Run Time	Run voltage	PreRun Time	PreRun Voltage	Injection Time	Injection Voltage	Data Delay
adjusted from FragmentAnalysis50_POP7	60°C	4500 sec	11 kVolts	180 sec	15 kVolt	24 caps: 45 sec 8 caps: 22 sec	3 kVolts	350 sec

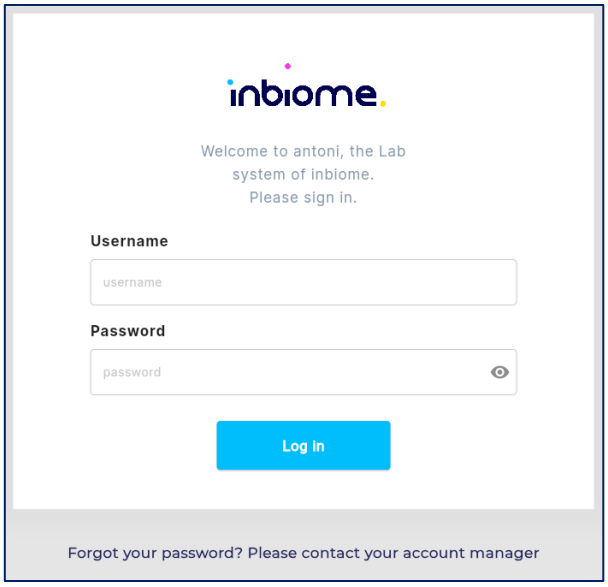
Note: The online software 'antoni' does not require a sizecalling protocol because it uses raw data from the .fsa. Since sizecalling is required in the instrument software, please select a LIZ sizemarker.

9.3 Data analysis

inbiome offers the software platform antoni for analysing data. The system consists of several components. The main internal pages are:

1. Login
2. Dashboard
3. Upload
4. Onboarding
5. Groups (Results & Interpretations)

Login



To access the login page, open your web browser and enter the provided URL: https://antoni-research.inbiome.com. Ensure that your system meets the minimum technical requirements. Technical requirements are described in paragraph 3.2.2 Requirements for software use.

Your unique username and password have been provided to you upon account creation. If you do not have this information or need assistance, please contact the account manager/customer support.

Once you navigate to the login page, you can:

1. Enter your username and password in the designated fields
2. Press the “Log in” button to access the system

If you forget your credentials or encounter any issues during the login process, please contact your account manager/customer support.

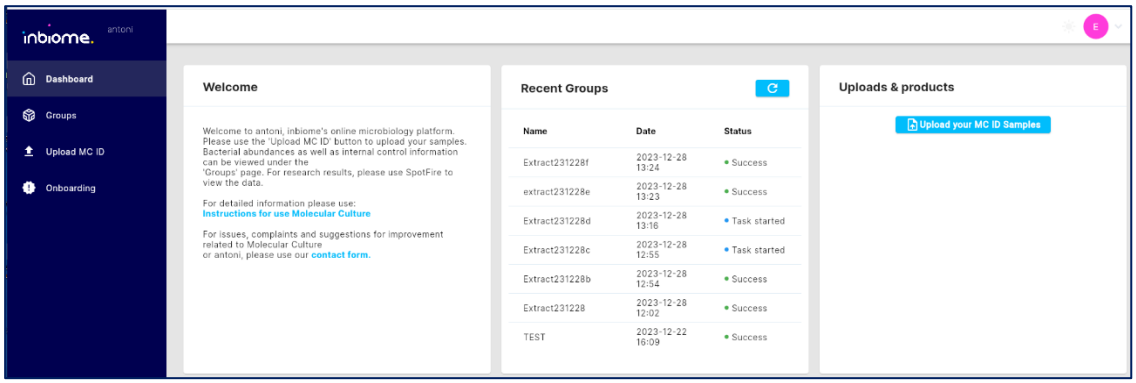
Note: If multiple antoni users need to share information across departments, studies or institutes, you have the option to request ‘Group studies’. The request for 'Group studies’ is possible via techsupport@inbiome.com. Please provide us with the department name or official acronym and the full name of the study for which users need access.

Onboarding

Customers are introduced to Molecular Culture ID as part of the onboarding process facilitated by antoni. This onboarding process provides information on the Molecular Culture ID and aids in setting up laboratory equipment.

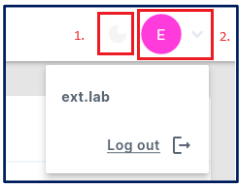
Dashboard

After a successful login, you are redirected to the dashboard page.



The dashboard serves as the central hub for information and tools. The left side of the screen contains a sidebar, which allows users to access different sections and features of the website.

The top right corner contains two icons:

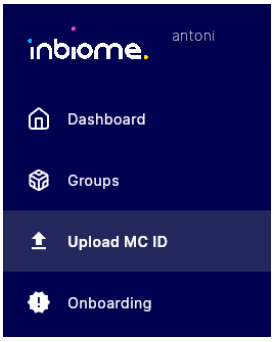


1. An icon that switches the theme of the website from “Light” to “Dark” and vice versa.
2. An icon that represents the user account. By clicking the icon, you can see the full username, and you gain the option of logging out of the system by pressing the “Log out” button.

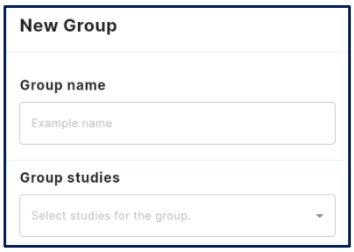
The main body of the dashboard contains three parts:

1. The left section contains a brief welcome message, describes the features accessible from the sidebar and contains the link to a contact form used for issues, complaints and other suggestions. And a link to the Instructions for Use
2. The middle section of the dashboard shows a selection of the most recently uploaded groups, showing the groups’ name, date and time of upload and the status of the group. Clicking on a group will redirect you to the group page for that specific group. The top right corner of this part of the dashboard also contains a “Reload” button, which will manually refresh the “Recent Groups” list.
3. The right part of the dashboard contains a button which will redirect you directly to the “Upload” page, where you can commence uploading samples to the system.

Upload

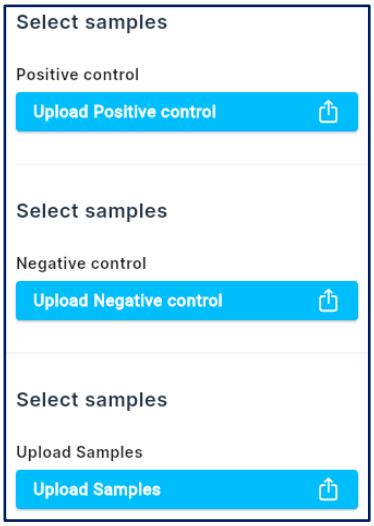


Uploading files for analysis is done through the “Upload MC ID” tab.
On on this page, users can upload multiple fsa files from the capillary electrophoresis run, corresponding to samples to be analyzed within a single group. After analysis in antoni, the group can be viewed on the Groups page.



A **Group name** must be provided. This can be any distinct name of your choice, facilitating easy identification of the specific batch of fsa files chosen for uploading.

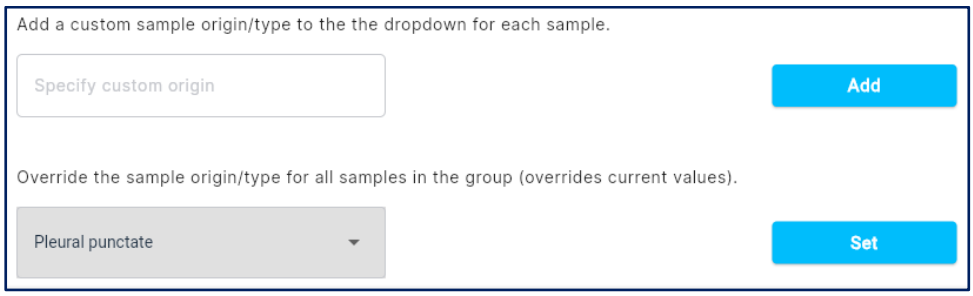
The **Group project** is an optional tag that allows the user to easily find multiple groups uploaded with that same tag. A specific 'Group project' is only visible when requested for an antoni user account. This option also allows for sharing uploads between antoni users.



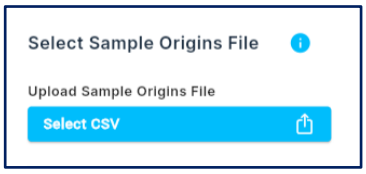
The samples and corresponding positive and negative controls are uploaded using the corresponding buttons. Multiple samples can be selected through the file selection interface of the user's operating system.



Once a file is selected, the sample type or study can be edited for individual samples. Wrongly selected samples can also be removed here using the red trashcan icon.



All samples in the group can be changed to the same sample type by using the sample type dropdown and pressing the **Set** button.



If multiple samples are selected with different sample types, an optional “Sample Origins File” can be used to automatically set the sample origin for these samples. The format of this file is described when pressing the info “**i**” button.

The group can be uploaded once it has been named and the desired samples, including positive and negative controls, have been added.

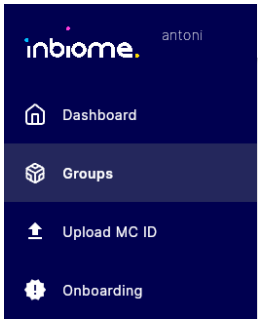


If any requirements are not met, the user will not be able to upload, and the unmet requirements are shown next to the upload button.

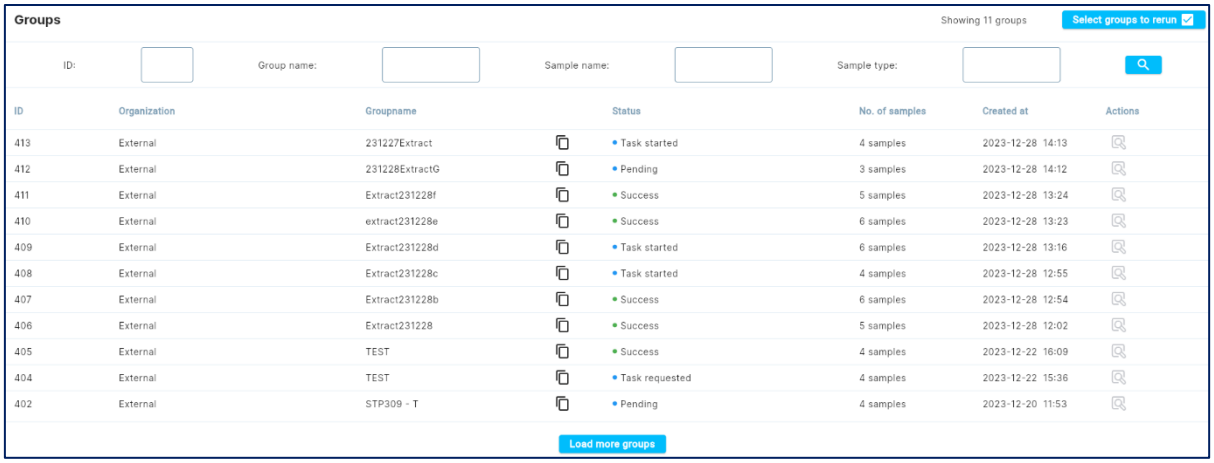


Groups

The Groups page can be accessed from the sidebar by pressing the “Groups” button. The Groups page displays the results and interpretations of the assay after uploading.



This page shows the user a list of all uploaded groups of samples that the user has access to.



For every group, the following information is displayed:

- **ID**: the internal group ID, a successive number created and used by the system.
- **Organization**: the name of the organization which uploaded the group
- **Groupname**: name of the group given by the user This column also includes an icon that allows the user to copy the groupname
- **Status**: the status of the processing of the group. In following order, the processing starts with ‘Created’, ‘Task requested’, ‘Task started’, ‘Pending’ (processing is ongoing). After processing, the status is ‘Succes' when all samples of a group have been successfully processed by the system, ‘Partial Success’ when a part of the samples have been successfully processed or failed where no samples of a group have been successfully processed.
- **No. of samples**: number of individual samples present in the group
- **Created at**: date and time at which the group was uploaded
- **Actions**: selection of icons which represent possible actions that can be taken on that group

Clicking on the “View” button on the “Actions” column redirects the user to the group page for that specific group.

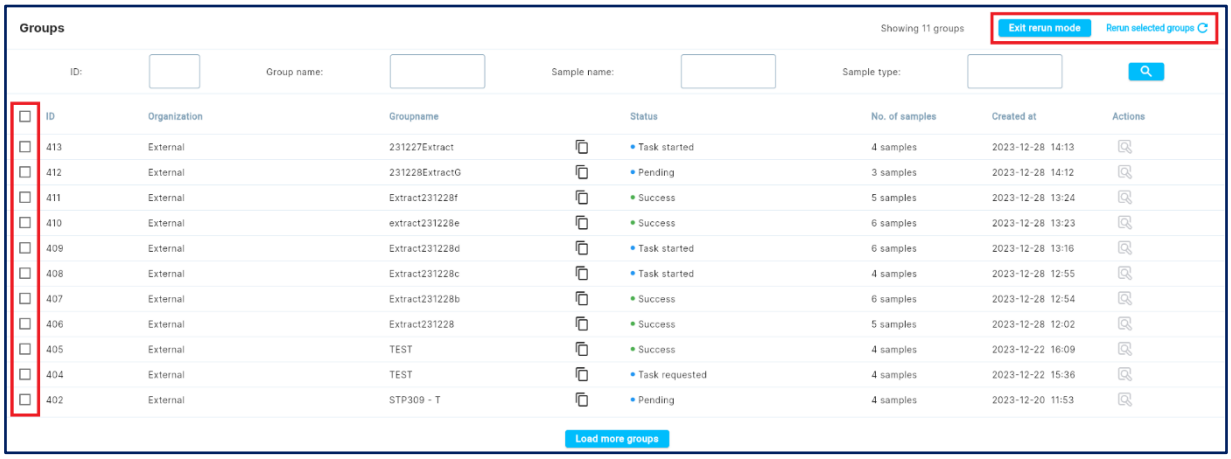


The bar located directly above the list allows the user to filter the groups based on: ID, group name, sample name or sample type. The filter is applied once the button on the right side of the filter bar is pressed.



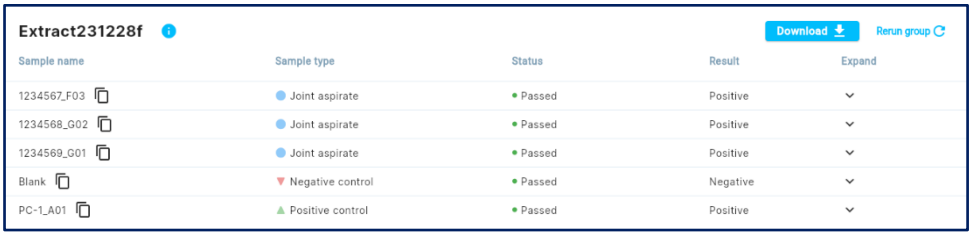
The top right corner of the page shows the number of groups currently displayed on the page. More groups can be loaded by pressing the “Load more groups” button on the bottom of the page.

The upper right corner of the groups page contains the “Select groups to rerun” button. This button enables “Rerun mode”. The “rerun mode” is primarily used by antoni users working with samples from a longitudinal study, using the same version of the system for all samples from this longitudinal study. New versions (updates) of the system are communicated via email to all antoni users.



“Rerun mode” allows the user to manually select groups they wish to rerun using the checkboxes that appear on the left side of the screen. By pressing the ‘Rerun selected groups’ button in the upper right corner, these groups will then be rerun by the latest update of the system. The user can also exit ‘Rerun mode’ by pressing the ‘Exit rerun mode’ button in the upper right corner. Rerunning allows re-analysing of a group of samples without having to manually upload the samples as a group again. The system recreates the group, giving it a new ID and starting the data processing again.

Results and their interpretation
When a group is selected, a list of samples in that group is shown.

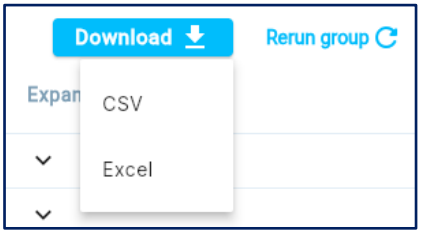


The top of the group page contains the name of the group, as well as an “i” icon which, when clicked, reveals additional metadata about the group.

The group page for a specific group contains a list of all samples in that group. Each row represents a sample, and gives the following information:

- **Sample name**: This column also includes an icon that allows the user the copy the sample name
- **Sample type:** Type of sample uploaded. For example, “Negative control”, “Positive control”, or a specific type, such as “Tissue biopsy” or “Joint aspirate”.
- **Status:** Indicates if the sample passed, is rejected, pending, or has an internal control warning.
- **Result:** Shows whether the sample is positive or negative.
- **Expand**: Contains a button that unfolds the selected sample, showing additional information about that sample.

The top right corner of the page contains the “Download” button, which allows the user to download the data shown in the group page in either Excel or CSV format, and a “Rerun group” button, which reruns all the samples of that group.



Clicking on one of the samples will unfold the results for that sample. This looks as follows:



The unfolded information contains all bacteria detected in a sample, as well as their abundance and confidence score. The bacteria are sorted by abundance.

Bacterial species shows the bacteria detected in the sample. Note: Sometimes it is not possible to distinguish two or more species due to genetic similarities and/or to identical InterSpace lengths. When this happens, Bacterial species displays an equivalent set. For example: *Escherichia coli/Shigella spp.* The Bacterial species and equivalence sets is to be found in Annex I.
When the IS-fragment detected in the sample has no match the bacterial database, the Phylum of the IS-fragment detected is shown. For example, unknown Bacteroidetes bacteria.

Note: While Molecular Culture ID can detect and identify many bacterial species, there is always a possibility of misidentification, in particular with rare or underrepresented bacteria due to limitations in the reference library. The reference database is updated regularly to minimize this risk. For questions or assistance, you can contact our technical support at techsupport@inbiome.com.

Next to the names is the **Abundance**, shown as a single-word term (Low/Medium/High) and as a bar chart. The abundance is semiquantitative and corresponds to the following number of genome copies (gcp) per PCR reaction with a 90% confidence: <32 gcp (low), 16-512 gcp (medium), >512 gcp (high).

The Confidence Score indicates how closely the Molecular Culture profile of a sample matches the reference profile of a given bacterial species. The score is calculated by evaluating both (i) the intensity pattern of detected peaks and (ii) the similarity of the sample profile to reference profiles from other bacterial species. It is calculated in two steps:

- Statistical matching: A model compares the sample’s fragment pattern to a database of known bacterial

profiles and assigns an initial likelihood per species (or equivalence set).
- Calibration: A secondary machine learning model refines these likelihoods using manually validated samples and sample-specific features to produce a final confidence score.

Confidence Scores are categorized as follows:

- **High confidence** (≥ 0.90): Very strong match; Species identification is considered highly reliable.
- **Moderate confidence** (0.70–0.90): Likely match; Species identification is considered reliable but should be interpreted in the context of the sample type and other available laboratory or research data.
- **Low confidence** (0.50–0.70): Possible match; Species identification should be interpreted with caution, as the sample profile may also be consistent with other species, particularly within the same phylum.

For Confidence Scores < 0.50, species-level identification is not reported. In these cases, only phylum-level identification is displayed to reduce the risk of overinterpretation of uncertain results.

In the section '**Quality Controls**' the status of the internal control is described. This can be uninhibited, partially inhibited or fully inhibited. When a sample is inhibited, a recommendation is visible about how to proceed with a dilution of the DNA in the 'Comments' section.
The 'Comments' section also provides a message when a bacterial peak has been identified only at the phylum level and not yet at the species level. In such cases, the message will indicate where you can optionally send the PCR product for sequencing, allowing the result to be added to the bacterial reference database in the future.

Finally, next to the optional comment, is the date of processing.

Note: For positive and negative controls, the online software 'antoni' only performs a technical quality control, ensuring that internal control peaks and bacterial peaks, if any, are accurately shown. It is up to the user to decide when the positive and negative controls are rejected or approved for a run according to the Quality control section.

For valid runs (Valid Positive and Negative Control – See Quality Control), interpret the specimen results as follows:

Quality Control
In each product, positive controls for the FIRBAC and PROTEO reaction are provided. Additional controls may be analyzed in addition to those provided. Established statistical methods for analysing control values and trends should be employed.

If the controls do not comply with the established limits and repetition excludes a technical issue, check the following areas:

1. The expiration date on the reagent package and prepared reagents
2. The temperature of the reagents
3. Settings of the PCR system
4. Settings of the Capillary Electrophoresis System
5. Contamination

If controls are still invalid, please contact techsupport@inbiome.com. **Note:** The following criteria are obtained with antoni.

Positive Control
The positive control should contain three bacterial species

1. Either: *Haemophilus influenzae / Pseudomonas aeruginosa, Haemophilus influenzae*, or *Pseudomonas aeruginosa*
2. *Bacteroides fragilis* group
3. *Streptococcus cristatus/criceti*

The three bacteria all should have a Medium or High abundance.
If these criteria are not met, the complete run is invalid, and the test procedure must be repeated.

Internal control
Internal control should be uninhibited when no bacterial peaks are found. If these criteria are not met, the sample is invalid, and the test procedure must be repeated.

When no internal control peaks are found in a sample without bacterial peaks, this sample is automatically rejected in antoni with a notification to dilute DNA before repeating the procedure in the comment section. With

any bacterial abundance, inhibited samples are shown, but it is still recommended to dilute the DNA according to the inhibition status.

Negative Control
No bacterial peaks should be detected. If these criteria are not met, the negative control or the mastermix is contaminated.

10. Limitations of the procedure

1. Use only specimens described in the intended use. Other specimen types have not been validated and may result in false positive or false negative results.

2. Specimen collection, transport and storage may affect the number of organisms and their associated DNA present in the specimen, affecting the outcome of the result (causing a false positive or a false negative result). It is crucial to interpret results alongside additional experimental or laboratory information for a comprehensive understanding.

3. Bacteria belonging to phyla other than Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia, Bacteroidetes, and Proteobacteria are not detected by this method. For instance, Chlamydia spp., Mycoplasma spp., and Treponema spp. fall outside the detection range and require alternative methods for their detection.

4. Species in which the 16S and 23S rRNA genes are not adjacent, such as *Candidatus Neoehrlichia mikurensis*, cannot be detected by this technique because of the absence of an intergenic spacer region. This represents a limitation of the method.

5. Bacteria not listed in Annex I but from the phyla Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia, Bacteroidetes, and Proteobacteria are identified only at the phylum level and not at the species level.

6. Good laboratory practices and strict adherence to these test instructions are indispensable to avoid contamination of reagents and/or specimens.

7. Molecular Culture ID is for Research Use Only. Results must not be used for diagnosis, patient management or treatment decisions.

8. The performance of Molecular Culture ID has not been established for pooled samples.

9. The likelihood of inaccurate results arising from interference by substances or competing microorganisms has only been assessed for those specified in section 11.6, cross-reactivity with non-bacterial organisms and section 11.7, interfering substances. Erroneous results may occur if there is interference or inhibition from substances or concentrations not covered in the mentioned sections.

10. The quantification results of the Molecular Culture ID kit output the number of genome copies detected. These numbers indicate the number of genomic copies per bacterial species present in the PCR tube. The exact number of bacteria that were present in the sample depends on many factors, including sample prep (centrifugation, dilution of the sample, etc.), the input volume of sample versus the elution volume, as well as the efficiency of DNA extraction. These factors should be considered when making statements about the original bacterial count of the sample and the Molecular Culture ID quantification output should not be considered as CFU/mL

11. While Molecular Culture ID can detect and identify a wide range of bacterial species, the possibility of misidentification cannot be fully excluded—particularly for rare or underrepresented organisms due to limitations in the reference database. The reference database is updated regularly to reduce this risk. For questions or assistance, please contact our technical support at techsupport@inbiome.com.

12. Samples with bacterial loads below the Limit of Detection of Molecular Culture ID (5 CFU/10µl) are highly likely to result in undetected bacteria.

13. Certain bacterial species may be detected due to background DNA or contamination introduced during specimen collection, transport, storage, or laboratory processing. Such detections should be evaluated in the context of sample type, organism abundance, and other available research data.

Examples of organisms that may be associated with background DNA or contamination include:

Species	Context
Cutibacterium acnes	Frequently detected as background DNA in molecular assays. A higher Limit of Blank is applied for this species.
Enterococcus cecorum	Not a recognized human commensal. Low-abundance detections are typically interpreted as incidental background DNA unless supported by additional evidence.
Haemophilus parainfluenzae	Upper respiratory tract commensal. May be detected as background.
Lactococcus lactis	Environmental and food-associated species. Low-level detections are commonly interpreted as background DNA.
Micrococcus luteus	Common, typically harmless skin-associated bacteria. Low-level detections are usually interpreted as background microbiome DNA.

11. Performance characteristics

11.1 Limit of Detection

The limit of detection (LoD) of the Molecular Culture ID kit was determined using quantified stock cultures. The LoD is defined as the lowest concentration at which the analyte is detected with ≥95% confidence. The results of the LoD determination are presented in the table below.

Phylum	Bacteria	LoD
Firmicutes	*Streptococcus cristatus* (DSM 8249T / ATCC 13637)	5 CFU/10µL
Firmicutes	*Streptococcus bovis* (IS-I, clinical sample, no strain known)	5 CFU/10µL
Bacteroidetes	*Bacteroides fragilis* (NCTC 9343 / DSM 2151)	1 CFU/10µL
Bacteroidetes	*Bacteroides thetaiotaomicron* (VPI 5483/DSM 2079)	5 CFU/10µL
Proteobacteria	*Stenotrophomas maltophilia* (DSM 50170 / ATCC 13637)	5 CFU/10µL
Proteobacteria	*Pseudomonas aeruginosa* (ATCC 27853)	5 CFU/10µL

11.2 Limit of Blank

The Limit of Blank is 4000 RFU with an exception for *Cutibacterium acnes* (maximal intensity of 10 000 RFU). The Limit of Blank is verified based on the ISO 2859-4 with a DQL of 0.4% (LQR level III). The probability of falsely contradicting this DQL is 4.0%.

11.3 Quantification

Molecular Culture ID outputs three different semi-quantified classifications with 90% confidence. Low corresponds to <32 genomic copies; Medium corresponds to 16-512 genomic copies; High corresponds to >512 genomic copies.

11.4 Cross-reactivity between the three different phyla *(FAFV group, Bacteroidetes, and Proteobacteria)*

All bacteria described in Annex I were analyzed and only cross-reactivity for the following species or equivalence* sets were observed when the bacterial load was very high. Crossreactivity occurs with the following species: *Amygdalobacter indicium, Anaerococcus prevotii, Anaerococcus vaginalis, Bifidobacterium breve, Cereibacter sphaeroides, Clostridium tetani, Dialister pneumosintes, Fannhesia vaginae, Escherichia coli, Faecalibacterium*

prausnitzii, Fusobacterium mortiferum, Fusobacterium varium, Megasphaera statonii, Staphylococcus aureus, Streptococcus constellatus, Streptococcus pneumoniae/mitis group, and Odoribacter splanchnicus. Only cross-reactivity between *Firmicutes/Bacteroidetes* and *Proteobacteria primers* could be observed. The Proteobacteria peaks caused by cross-reactivity only manifest in the presence of FAFV or Bacteroidetes peaks. No bacterial signal corresponding to Proteobacteria was detected in any other channel except for *E. coli* where a Bacteroidetes peak can appear with loads of ~1.2 x 10^9 CFU/mL. All other reference profiles in the database don't show cross-reactivity.

Cross-reactivity information was added to the bacterial reference database to optimize bacterial identification.

* Bacteria assigned to an equivalence set are those bacterial species whose IS-profiles are either consistently or conditionally indistinguishable, owing to various factors like low load or the presence or genetic similarities.

11.5 Cross-reactivity with human DNA

Cross-reactivity with human DNA can occur when the human DNA load is high and bacterial DNA is absent or in very low concentrations relative to the human DNA. Human cross-reactivity peaks may occur alone or in various combinations. antoni automatically detects and deletes such fragments.

11.6 Cross-reactivity with non-bacterial organisms

The analytical specificity of the Molecular Culture ID was tested against cultured *Candida albicans, Cryptococcus neoformans/neoformans, Malassezia furfur, Absidia corymbifera, Rhizomucor pusillus, Rhizopus oryzae, Aspergillus fumigatus, Fusarium oxysporum, Saccharomyces cerevisiae, Trichosporon asashii, Blastocystis hominus,* and human *Cytomegalovirus*. All tested organisms gave negative results, indicating no cross-reactivity with DNA from the tested cultured organism.

11.7 Interfering substances

The presence of PCR inhibitors may cause false negative results. The tables below show the effect of possible interfering compounds.

Possible interfering compound	Interference with FIRBAC PCR	Interference with PROTEO PCR
Metronidazole (5mg/mL)	No	Yes
Magnetic Silica	No	Yes
DNase/RNase free water	No	No

Possible Interfering compound	Dilution factor without interference FIRBAC PCR	Dilution factor without interference PROTEO PCR
0.5 M EDTA	10x	10x
bioMérieux Lysis buffer	100x	*All tested dilutions interfered with Proteo PCR*
96% EtOH	10x	100x
5M NaCl	10x	100x
Human haemoglobin 100mg/mL	100x	10x

11.8 Inhibition

Samples without interference or uninhibited samples are defined as samples with 2 internal control fragments present. Inhibition is defined as the absence of one or both internal control fragments.

11.9 Trueness

The systematic error (trueness of results) is defined as the performance of the Molecular Culture ID kit relative to two interlaboratory comparison programs available in the Netherlands; SKML and UK NEQAS, aimed at routine bacterial culture. The samples tested contained 63 simulated freeze-dried clinical samples. From these 63 samples, 44 contained unique species described in Annex I. In all 63 samples, Molecular Culture ID successfully detected bacterial signals in the correct phylum group (100%). The correct species level identification could be made in 53 samples of the 63 samples (84%). In 7 samples, species were determined as unknown species from the correct phylum group. In 3 samples, species were determined as another species from the same phylum group.

11.10 Precision

Repeatability (intra run variation) and **reproducibility** (inter run variation) was tested with a sample containing 94 bacterial peaks, tested 48 times.
Change in results from high to low or vice versa statistically occurs in less than 1 in a million detections. Peak intensities remain highly stable, with a standard deviation of less than 0.5 in normalized intensity (3SD). Furthermore, bacteria present in abundances close to the limit of detection (LoD) were detected in 95% of tests.

11.11 Dynamic range

The **dynamic range** was assessed by measuring the signal of one bacterium in the presence of another. The dynamic range of the FAFV group was 1:100, the dynamic range of the *Bacteroidetes* group was 1:1000 and the dynamic range of the *Proteobacteria* group was 1:1000. For the overall dynamic range, the lowest measured dynamic range was taken, being 1:100.

11.12 Assay list

Assay Performance	
Dynamic Range	1:100
Precision – Repeatability and Reproducibility	Change in results from high to low or vice versa statistically occurs in less than 1 in a million detections. Peak intensities remain highly stable, with a standard deviation of less than 0.5 in normalized intensity (3SD). Furthermore, bacteria present in abundances close to the limit of detection (LoD) were detected in 95% of tests.
Cross Reactivity - Bacterial species of other Phyla	No cross reactivity except for selected species
Cross Reactivity - Human DNA	Cross reactivity in high human DNA load samples have been identified and antoni automatically detects and deletes such fragments
Interference - Amplification inhibitors	Monitored by internal amplification control
Sensitivity - Analytical	100% for phylum level determination; 84% for species level determination.
Sensitivity - Functional (peak position (nc))	±1 nc
Sensitivity - Limit of Blank	4000 RFU
Quantification	Low corresponds to <32 genomic copies, Medium corresponds to 16-512 genomic copies, High corresponds to >512 genomic copies. (90% confidence)
Sensitivity - Limit of Detection	5 CFU per reaction
Assay Protocol	
Sample pretreatment	Lysis + DNA isolation, (Lysis + DNA isolation reagents are not included in the kit)
Type of sample	• Effusions: a) Synovial, b) Peritoneal, c) Pleural d) Pericardial • Cerebrospinal fluid • Tissue biopsies: a) Bone biopsies b) Soft tissue biopsies • Drain fluids and pus • EDTA anticoagulated whole blood

Assay format	**PCR machines:** Veriti from Thermo Fisher Scientific, SimpliAmp from Thermo Fisher Scientific, CFX Opus Real-Time PCR Systems from Bio-Rad, MiniAmp™ Thermal Cycler from Thermo Fisher Scientific, VeritiPro™ Thermal Cycler from Thermo Fisher Scientific Thermocycler, **Capillary Electrophoresis machines:** ABI3500, ABI3500 (XL), SeqStudio Flex (G5 (DS-33) dyeset, 50cm capillary and POP7)
Total assay time	<5 hr
Reagents, Calibrators and Controls	
Tests per kit	24
Positive control	
A. Matrix	Bacterial DNA
B. Physical form	Liquid frozen
C. Stability (shelf life) *	12 months
D. Number of freeze/thaw cycles	2
E. Stability after defrosting (2-8°C)	30 minutes between every freeze/thaw step
Mastermix	
A. Matrix	Contains sensitive DNA Polymerase
B. Physical form	Liquid frozen
C. Stability (shelf life)	12 months
D. Number of freeze/thaw cycles	2
E. Stability after defrosting (2-8°C)	30 minutes between every freeze/thaw step
eMix	
A. Matrix	Contains formamide and a fluorescent marker
B. Physical form	Liquid frozen
C. Stability (shelf life)	12 months
D. Number of freeze/thaw cycles	2
E. Stability after defrosting (2-8°C)	30 minutes between every freeze/thaw step
Shipping conditions	On dry ice
Storage conditions	between -25°C and -15°C

12. References

- Budding et al., (2010). IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota, FASEB J. 2010 Nov;24(11):4556-64. DOI: 10.1096/fj.10-156190
- Budding et al., (2016). Automated broad range molecular detection of bacteria in clinical samples, J Clin Microbiol. 2016 Apr;54(4):934-43. DOI: 10.1128/JCM.02886-15

List of symbols as used in labelling

	Manufacturer
	Catalogue number
	Batch code (Lot)
	Expiration date
	Temperature limitation
	Contains sufficient for <n> tests
	Consult Instructions for Use
	Contents
	Contains hazardous substance (Read SDS eMix)
	Danger. May damage fertility or the unborn child. Use personal protective equipment as required. If exposed or concerned: Get medical attention/advice. (Read SDS eMix)

List of Abbreviations

CDC	**Centers for Disease Control and Prevention (United States)**
CE-machine	**Capillary Electrophoresis Machine**
DNA	**Deoxyribonucleic acid**
DNase	**Deoxyribonuclease**
DQL	**Detection Quantitation Limit**
EDTA	**Ethylenediaminetetraacetic acid**
FAFV	**Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia**
IC	**Internal Control**
IS	**Interspace**
IS-pro™	**Interspace Profiling**
LoB	**Limit of Blank**
LoD	**Limit of Detection**
OSHA	**Occupational Safety and Health Administration (United States)**
SDS	**Safety Data Sheet**
NaCl	**Sodium Chloride**
Nc	**Nucleotide**
PC	**Positive Control**
PCR	**Polymerase Chain Reaction**
rDNA	**ribosomal DNA**
RNase	**Ribonuclease**

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REVISION HISTORY

Version	Revision date	Description
Rev 01	October 2026	Initial release

ANNEX I Bacteria list Molecular Culture ID

Bacteria	***Equivalence set*** *(Bacteria assigned to an equivalence set are those bacterial species whose IS-profiles are either consistently or conditionally indistinguishable, owing to various factors like low load presence or strain similarities)*
Abiotrophia defectiva	
Achromobacter deleyi	*Acinetobacter baumannii complex / Achromobacter xylosoxidans/denitrificans*
Achromobacter denitrificans	
Achromobacter ruhlandii	
Achromobacter xylosoxidans	
Acinetobacter baumannii	
Acinetobacter calcoaceticus	
Acinetobacter dijkshoorniae	
Acinetobacter nosocomialis	
Acinetobacter pittii	
Acinetobacter seifertii	
Acinetobacter johnsonii	
Acinetobacter lwoffii	
Actinobacillus rossii	
Actinomyces graevenitzii	
Actinomyces naeslundii	
Actinotignum schaalii	
Aerococcus urinae	
Aerococcus viridans	
Aeromonas caviae	
Aeromonas hydrophila	
Aeromonas salmonicida	
Aeromonas sobria	
Aeromonas veronii	
Aggregatibacter actinomycetemcomitans	
Aggregatibacter aphrophilus	
Aggregatibacter segnis	
Alistipes communis	*Alistipes group*
Alistipes finegoldii	
Alistipes onderdonkii	
Alistipes putredinis	
Alloiococcus otitis	
Alloprevotella tannerae	
Anaerococcus vaginalis	
Arcanobacterium haemolyticum	
Atopobium vaginae	
Bacillus cereus	
Bacillus coagulans	
Bacillus smithii	
Bacillus haynesii	*Bacillus spp./Parvimonas micra*
Bacillus licheniformis	
Bacillus paralicheniformis	

Bacillus pumilus	
Bacillus subtilis	
Parvimonas micra	
Bacteroides caccae	
Bacteroides eggerthii	
Bacteroides caecimuris	*Bacteroides fragilis group*
Bacteroides fragilis	
Bacteroides uniformis	
Bacteroides ovatus	
Bacteroides spp.	
Bacteroides stercoris	
Bacteroides thetaiotaomicron	
Bacteroides ureolyticus	
Bacteroides vulgatus	
Bacteroides zoogleoformans	*Bacteroides zoogleoformans / Barnesiella merdipullorum*
Barnesiella merdipullorum	
Barnesiella merdipullorum	
Bifidobacterium animalis	*Bifidobacterium bifidum/animalis*
Bifidobacterium bifidum	
Bifidobacterium breve	
Bifidobacterium longum	
Bordetella bronchiseptica	*Bordetella bronchiseptica/parapertussis*
Bordetella parapertussis	
Bordetella holmesii	*Bordetella pertussis/holmesii*
Bordetella pertussis	
Bulleidia extructa	
Bulleidia sp.	*Bulleidia sp.*
Bulleidia extructa	
Burkholderia cenocepacia	
Burkholderia cepacia	
Campylobacter coli	
Campylobacter jejuni	
Capnocytophaga canimorsus	
Capnocytophaga sputigena	
Cardiobacterium hominis	
Citrobacter amalonaticus	
Citrobacter braakii	*Citrobacter freundii complex*
Citrobacter freundii	
Citrobacter gillenii	
Citrobacter murliniae	
Citrobacter portucalensis	
Citrobacter werkmanii	
Citrobacter youngae	
Citrobacter braakii	*Citrobacter freundii complex / Raoultella ornithinolytica/planticola*
Citrobacter freundii	

Citrobacter gillenii	
Citrobacter murliniae	
Citrobacter portucalensis	
Citrobacter werkmanii	
Citrobacter youngae	
Raoultella ornithinolytica	
Raoultella planticola	
Citrobacter farmeri	*Citrobacter koseri/farmeri*
Citrobacter koseri	
Citrobacter sedlakii	
Clostridium butyricum	
Clostridium difficile	
Clostridium innocuum	
Clostridium perfringens	
Clostridium septicum	
Clostridium sporogenes	
Clostridium tertium	
Clostridium tetani	
Corynebacterium amycolatum	*Corynebacterium amycolatum/xerosis*
Corynebacterium xerosis	
Corynebacterium coyleae	
Corynebacterium diphtheriae	
Corynebacterium kefirresidentii	
Corynebacterium macginleyi	
Corynebacterium accolens	*Corynebacterium segmentosum/accolens*
Corynebacterium segmentosum	
Corynebacterium striatum	
Corynebacterium tuberculostearicum	
Corynebacterium ulcerans	
Corynebacterium urealyticum	
Cutibacterium acnes	
Cutibacterium avidum	*Cutibacterium avidum / Mycobacterium sp.*
Mycobacterium kansasii	
Mycobacterium malmoense	
Mycobacterium marinum	
Mycobacterium tuberculosis	
Cutibacterium granulosum	
Delftia acidovorans	
Dermabacter hominis	
Dialister micraerophilus	
Dialister pneumosintes	
Dolosigranulum pigrum	
Eikenella corrodens	
Enterococcus avium	
Enterococcus cecorum	

Enterococcus durans	
Enterococcus casseliflavus	*Enterococcus faecalis*
Enterococcus faecalis	
Enterococcus faecium	
Enterococcus gallinarum	
Escherichia coli	*Escherichia coli/Shigella spp.*
Shigella spp.	
Shigella boydii	
Shigella dysenteriae	
Shigella flexneri	
Shigella sonnei	
Eubacterium limosum	
Finegoldia magna	
Fusobacterium necrophorum	
Fusobacterium nucleatum	
Gardnerella vaginalis	
Gemella haemolysans	*Gemella morbillorum/haemolysans*
Gemella morbillorum	
Gemella sanguinis	
Granulicatella adiacens	
Haemophilus haemolyticus	
Haemophilus influenzae	
Haemophilus influenzae	*Haemophilus influenzae / Pseudomonas aeruginosa*
Pseudomonas aeruginosa	
Haemophilus parahaemolyticus	
Haemophilus parainfluenzae	
Hafnia alvei	
Herbasprillium huttiense	
Kingella kingae	
Klebsiella aerogenes	*Klebsiella aerogenes/oxytoca*
Klebsiella grimontii	
Klebsiella michiganensis	
Klebsiella oxytoca	
Klebsiella pasteurii	
Enterobacter amnigenus	*Klebsiella pneumoniae complex / Enterobacter cloacae complex*
Enterobacter asburiae	
Enterobacter cloacae	
Enterobacter hormaechei	
Enterobacter kobei	
Enterobacter ludwigii	
Enterobacter nimipressuralis	
Klebsiella pneumoniae	
Klebsiella quasipneumoniae	
Klebsiella quasivariicola	
Klebsiella variicola	

Lacticaseibacillus rhamnosus	
Lactobacillus gasseri	*Lactobacillus (para)gasseri*
Lactobacillus paragasseri	
Lactobacillus casei	*Lactobacillus casei/paracasei*
Lactobacillus paracasei	
Lactobacillus crispatus	
Lactobacillus fermentum	
Lactobacillus helveticus	
Lactobacillus iners	
Lactobacillus jensenii	
Lactobacillus pentosus	
Lactobacillus acidophilus	*Lactobacillus rhamnosus/zeae/acidophilus*
Lactobacillus rhamnosus	
Lactobacillus zeae	
Lactococcus lactis	
Lautropia mirabilis	
Legionella pneumophila	
Leptotrichia amnionii	
Leptotrichia sp.	
Limosilactobacillus vaginalis	
Listeria marthii	*Listeria monocytogenes/marthii*
Listeria monocytogenes	
Lysinibacillus sp.	
Megasphaera micronuciformis	
Microbacterium lacticum	
Micrococcus luteus	
Moraxella catarrhalis	
Moraxella lacunata	
Moraxella nonliquefaciens	
Moraxella osloensis	
Morganella morganii	
Mycobacterium abscessus	
Neisseria cinerea	*Neisseria gonorrhoeae/cinerea*
Neisseria gonorrhoeae	
Neisseria meningitidis	
Neisseria mucosa	*Neisseria mucosa/sicca*
Neisseria sicca	
Neisseria flavescens	*Neisseria subflava group*
Neisseria mucosa	
Neisseria perflava	
Neisseria sicca	
Neisseria subflava	
Nocardia brasiliensis	
Nocardia farcinica	
Odoribacter laneus	

Odoribacter splanchnicus	
Pantoea agglomerans	
Parabacteroides distasonis	
Paraburkholderia phytofirmans	
Parvimonas micra	
Pasteurella canis	
Pasteurella multocida	
Pediococcus acidilactici	
Pediococcus pentosaceus	
Peptostreptococcus anaerobius	
Plesiomonas shigelloides	
Prevotella bivia	
Prevotella buccae	
Prevotella denticola	
Prevotella disiens	
Prevotella histicola	
Prevotella intermedia	
Prevotella intermedia	*Prevotella intermedia/pallens*
Prevotella pallens	
Prevotella melaninogenica	
Prevotella jejuni	*Prevotella melaninogenica/jejuni*
Prevotella melaninogenica	
Prevotella intermedia	*Prevotella nigrescens/intermedia*
Prevotella nigrescens	
Prevotella oralis	
Prevotella oris	
Prevotella sp.	
Prevotella veroralis	
Proteus mirabilis	
Proteus penneri	
Proteus vulgaris	
Proteus penneri	*Proteus vulgaris/penneri*
Proteus vulgaris	
Providencia rettgeri	
Providencia stuartii	
Pseudomonas aeruginosa	
Pseudomonas alcaligenes	
Pseudomonas oryzihabitans	
Pseudomonas putida	
Pseudomonas sp.	
Pseudomonas stutzeri	
Pseudomonas viridiflava	
Ralstonia insidiosa	
Ralstonia mannitolilytica	
Rothia dentocariosa	

Rothia aeria	*Rothia mucilaginosa/aeria*
Rothia mucilaginosa	
Salmonella enterica	
Sarcina maxima	
Sarcina ventriculi	
Serratia fonticola	
Serratia marcescens	
Serratia proteamaculans	
Shewanella putrefaciens	
Sneathia vaginalis	
Sphingomonas paucimobilis	
Staphylococcus arlettae	
Staphylococcus aureus	
Staphylococcus auricularis	
Staphylococcus capitis	
Staphylococcus caprae	
Staphylococcus epidermidis	
Staphylococcus epidermidis	*Staphylococcus epidermidis/Streptococcus sanguinis*
Streptococcus sanguinis	
Staphylococcus haemolyticus	
Staphylococcus hominis	
Staphylococcus lugdunensis	
Staphylococcus pasteuri	*Staphylococcus pasteuri/warneri*
Staphylococcus warneri	
Staphylococcus pseudintermedius	
Staphylococcus saccharolyticus	
Staphylococcus saprophyticus	
Staphylococcus schleiferi	
Staphylococcus sciuri	
Staphylococcus simulans	
Staphylococcus vitulus	
Stenotrophomonas maltophilia	
Streptococcus agalactiae	
Streptococcus mutans	*Streptococcus anginosus/mutans*
Streptococcus anginosus	
Streptococcus anginosus	*Streptococcus anginosus/intermedius*
Streptococcus intermedius	
Streptococcus alactolyticus	*Streptococcus bovis group/Streptococcus intermedius*
Streptococcus bovis	
Streptococcus equinus	
Streptococcus gallolyticus	
Streptococcus gallolyticus	
Streptococcus infantarius	
Streptococcus intermedius	
Streptococcus pasteurianus	

Streptococcus salivarius	
Streptococcus thermophilus	
Streptococcus vestibularis	
Streptococcus constellatus	
Streptococcus criceti	*Streptococcus cristatus/criceti*
Streptococcus cristatus	
Streptococcus dysgalactiae	
Streptococcus equi	
Streptococcus australis	*Streptococcus ilei group*
Streptococcus ilei	
Streptococcus koreensis	
Streptococcus rubneri	
Streptococcus gordonii	*Streptococcus pneumoniae/mitis group*
Streptococcus gwangjuense	
Streptococcus infantis	
Streptococcus lactarius	
Streptococcus mitis	
Streptococcus oralis	
Streptococcus parasanguinis	
Streptococcus pneumoniae	
Streptococcus pseudopneumoniae	
Corynebacterium jeikeium	*Streptococcus pyogenes/Corynebacterium sp.*
Corynebacterium riegelii	
Streptococcus pyogenes	
Streptomyces microflavus	
Turicibacter sanguinis	
Veillonella parvula	
Vibrio alginolyticus	
Vibrio parahaemolyticus	
Yersinia enterocolitica	
Yersinia pseudotuberculosis	