inbiome.

molecular culture.

Instructions for Use Molecular Culture ID

catalogue number: MolCul 15000, 24 tests

MolCul 15000, rev 08, 03/2024

CE 2797 IVD EN

Instructions for use rev 12 Oktober 2025

Revisions from the previous version are highlighted in grey.

Instructions for use	https://inbiome.com/ifu
Safety Data Sheet	https://inbiome.com/sds
Summary of Safety and Performance (Applicable for EU customers)	To be found on https://ec.europa.eu/tools/eudamed/#/screen/search-device with: UDI-DI: 08720892099204
Software	https://antoni-research.inbiome.com
Technical support	Phone: +31 (0) 20 238 0320 E-mail: Techsupport@inbiome.com Website: https://inbiome.com

Complaints and improvements	https://forms.monday.com/forms/fb08dbf03b2cb724a16b74282b16b276?r=use1

Content

1. Intended purpose

Molecular Culture ID is a semi-automated and semi-quantitative in vitro diagnostic intended for the detection and identification of bacterial DNA isolated from specimens obtained from the bodily site of a suspected infection in humans, where that site is normally sterile (see Specimen types), for the aid in the diagnosis of bacterial infection.

The following species of the phyla *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Bacteroidetes* and *Proteobacteria* are claimed (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*.

Specimen 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

Intended patient population

Patients of all ages with a suspected bacterial infection, which may be diagnosed in the sample types described in Specimen types.

Intended user

For professional use. The intended user will be a specialized molecular diagnostic laboratory. The test will be carried out by trained laboratory personnel. Molecular Culture ID is intended as aid to diagnosis. Therefore, a trained healthcare professional should carefully interpret the results from Molecular Culture ID in conjunction with a patient's signs and symptoms, results from other diagnostic tests, and other relevant information.

2. Product description

Technical background

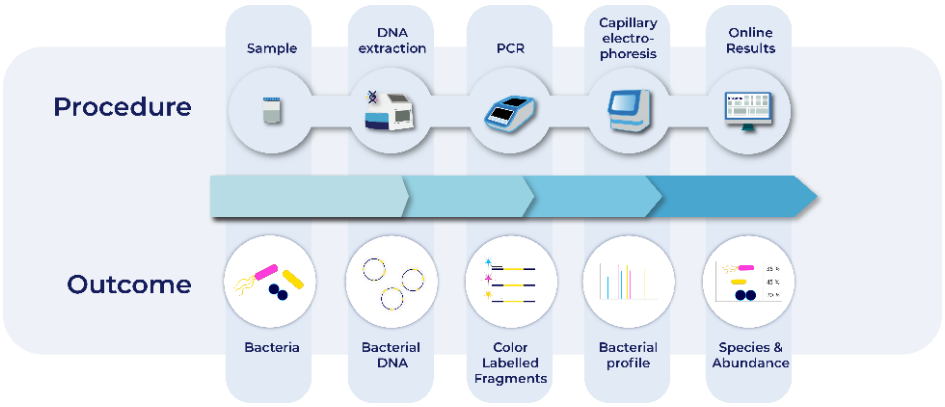
Molecular Culture ID is based on the InterSpace-profiling (IS-Pro™) technology. IS-pro™ is a bacterial profiling technique based on species-specific length polymorphisms of the IS (InterSpace) region and phylum-specific sequence polymorphisms of 16S rDNA. Molecular Culture ID consists of two multiplex 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 displayed on 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).

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™.

Validated sample types:

NucliSENS® easyMAG™ / eMAG™ (bioMerieux)

Effusions
Cerebrospinal fluid
Tissue biopsies
Drain fluids and pus

PCR
PCR machines validated are the Applied Biosystems™ Veriti from Thermo Fisher Scientific, the Applied Biosystems™ SimpliAmp™ Thermal Cycler from Thermo Fisher Scientific and the CFX Opus Real-Time PCR Systems from Bio-Rad
For machine validation, see instructions for use Molecular Culture Starter Kit for validation.

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.
For machine validation, see instructions for use Molecular Culture Starter Kit for validation.

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 labeled 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 Materials and machinery required for laboratory processes

- Sample collection devices
- DNA isolation equipment
- Input DNA
- PCR machines: Applied Biosystems™ Veriti from Thermo Fisher Scientific, the Applied Biosystems™ SimpliAmp™ Thermal Cycler from Thermo Fisher Scientific or the CFX Opus Real-Time PCR Systems from Bio-Rad
- Capillary electrophoresis machines: ABI 3500 XL machine, ABI 3500 machine, or SeqStudio Flex from Thermo Fisher Scientific
- Computer connected to the internet
- Bacterial Shock Buffers 1 and 2
- The required safety equipment to handle eMix as described on section 5.2

3.2.2 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 software requirements for the actual data analysis of the system required.

4. Storage

- Molecular Culture ID is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt or if the kit is damaged upon receipt, 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 in vitro diagnostic use.
- Molecular Culture ID is intended as an aid to diagnosis. Therefore, a trained healthcare professional should carefully interpret the results from Molecular Culture ID in conjunction with a patient's signs and symptoms, results from other diagnostic tests, and other relevant information
- 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, and protective lab coats.
- 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 patient samples as biohazardous material.
Handle samples as if capable of transmitting an infectious agent.

All clinical samples should be regarded as infectious. These samples should be handled at the Biosafety Level 2 as recommended for any potentially infectious specimen in the Centre for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories," 1984.

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.

Handle eMix in a well-ventilated area and wear safety goggles with side protection and protective gloves which meet the specification of standard norm EC directive 89/686/EEC and the resultant standard EN374. Disposal considerations: do not let eMix enter drains. Keep away from surface and groundwater. Disposal of contents/containers must be following local, regional, national, and international regulations.

NOTE: eMix (MolCul 15007) contains Formamide. See Safety Data Sheet and handle product accordingly!

Precautionary Statements
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
P281 - Use personal protective equipment as required
P314 - Get medical advice/attention if you feel unwell

NOTICE: any serious incident that has occurred in relation to the device must be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

6. Sample conditions and storage

In clinical routine, samples should be processed as soon as possible. If storage or transport are required, samples should be stored at -80°C. The maximum storage duration allowed is 7 years at -80°C. based on the clinical performance in section 12.

7. Sample collection and preparation

Collect samples in sterile 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, it is crucial to run a blank control (negative control) through the whole process starting from sample preparation to ensure that there is no bacterial contamination during the process.

A blank control is a sample that does not contain the target nucleic acid or genetic material being tested for but is subjected to the same procedures as the actual samples of interest. It is used to monitor and assess the presence of contamination, identify false positives, and establish a baseline for background noise in the molecular testing procedure.

Sample preparation validated for bioMérieux NucliSENS® easyMAG™:

Reagents

- Bacterial Shock Buffer 1 (IBB23000, inbiome)
- Bacterial Shock Buffer 2 (IBB24000, inbiome)
- bioMérieux NucliSENS® easyMAG™ reagents

Classification eMix according to regulation (EC) No 1272/2008 (CLP)					
Section	**Hazard Class**	**Category**	**Hazard class and category**	**Hazard statement Code**	**Hazard statement**
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

- Zirconia/Silica beads 0.1mm (11079101Z, BioSpec)
- Qiagen AL buffer (19075, Qiagen)
- UltraPure™ DNase/RNase-Free Distilled Water (10977035, Invitrogen)

Procedure

Tissue biopsies

- Add a pinhead of finely cut sample to a PCR grade vial containing 400 mg of Zirconia/Silica beads 0.1mm
  NOTE: the final input volume must be about a pinhead, HOWEVER make sure to cut several small pieces from different places of the biopsy.
- Add 100 µL of DNase free water to the sample
- Beadbeating: 1x 180 seconds for 4,5 m/s at room temperature.
- Spin down briefly.
- Add 250 µL of Bacterial Shock Buffer 1 to the vial
- Incubate for 10 minutes at 800 rpm and 95 °C / 203 °F in the thermomixer.
- Spin down briefly.
- Add 25 µL of Bacterial Shock Buffer 2 and vortex
- Spindown and collect the supernatant.
- Add 1 ml easyMAG™ lysis buffer + 1 ml AL buffer to an easyMAG™ vessel.
- Add complete volume of pretreated material to the easyMAG vessel

Synovial effusion and pus

- Add 50 µl sample and 250 µl Bacterial Shock Buffer 1 to the PCR grade vial.
- Vortex the samples.
- Incubate for 10 minutes at 800 rpm and 95 °C / 203 °F in the thermomixer.
- Spin down briefly.
- Add 25 µl Bacterial Shock Buffer 2 and vortex.
- Spin down briefly.
- Add 1 ml easyMAG™ lysis buffer + 1 ml AL buffer to the easyMAG™ vessels.
- Add complete volume of pretreated material to the easyMAG vessel

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

- Add to the PCR grade vial 250 µl of Bacterial Shock Buffer 1 and 200 µl sample.
- Vortex the samples.
- Incubate 10 minutes at 800 rpm and 95 °C / 203 °F in the thermomixer.
- Add 25 µl Bacterial Shock Buffer 2 and vortex.
- Spin down briefly.
- Add 1 ml easyMAG™ lysis buffer + 1 ml AL buffer to the easyMAG™ vessels.
- Add complete volume of pretreated material to the easyMAG vessel

Machine protocol

Please follow the manufacturer's instructions for a description of the usage of the easyMAG™ machine, system features, isolation protocols, and operational guidelines.

For each easyMAG™ vessel:

- Pipet 3 times up and down to mix the contents.
- Incubate for at least 10 minutes at room temperature.
- Remove visible particles.
- Add 70 µl of easyMAG™ magnetic silica.
- Mix the contents by pipetting.
- Start the isolation run as described in the easyMAG™ manual using the following run conditions:
  - Select the Specific A protocol with off-board lysis incubation.
  - Use 70 µl elution volume.

Store DNA at 2-8 °C.

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 15007). 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 Scan≤ 1

* The online software 'antoni' does not require a sizecalling protocol because it uses raw data from the .fsa. Since sizecalling is obliged 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

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 obliged 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	7500 sec	7.5 kVolts	180 sec	15 kVolt	45 sec	3 kVolts	200 sec

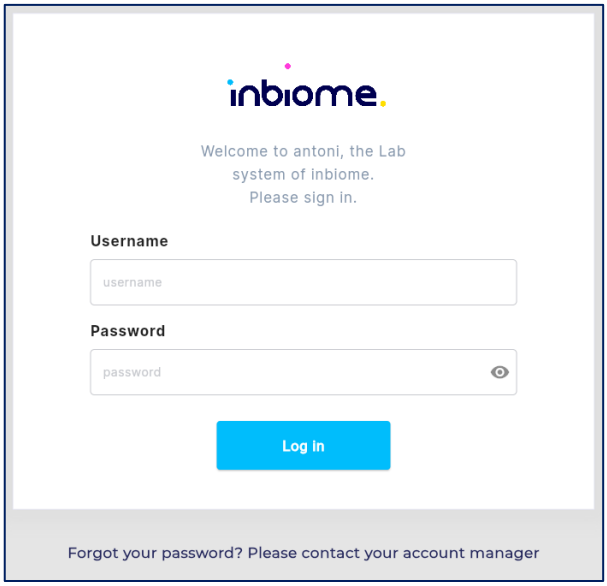
* The online software 'antoni' does not require a sizecalling protocol because it uses raw data from the .fsa. Since sizecalling is obliged in the instrument software, please select a LIZ sizemarker.

9.3 Data analysis

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

1. Login
2. Dashboard
3. Onboarding
4. Uploading
5. Groups / Results and 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.

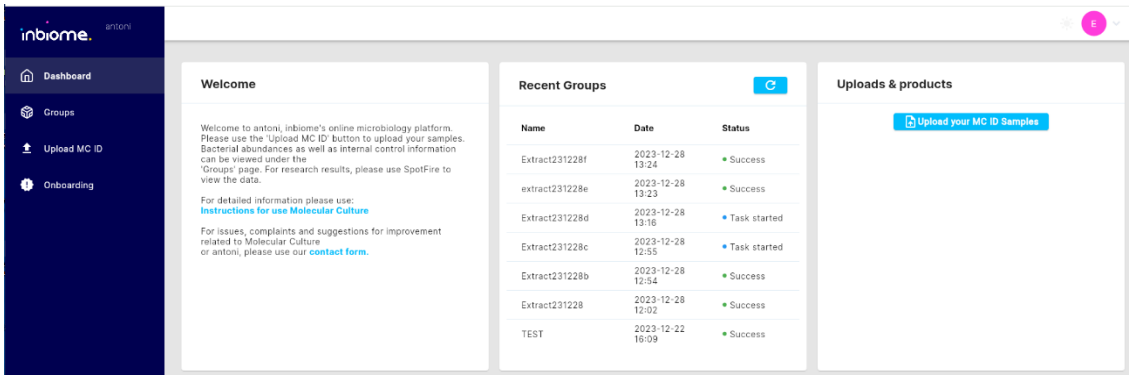
Note: If multiple antoni users need to share information across departments, studies or institutes, you have the option to request ‘Group studies’. The request for 'Group studies’ is possible via techsupport@inbiome.com. Please provide us with the department name or official acronym and the full name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

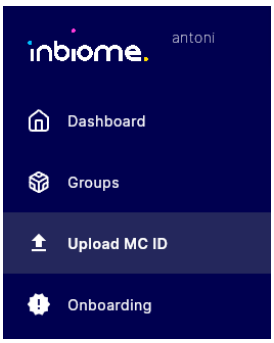
The top right corner contains two icons:

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.

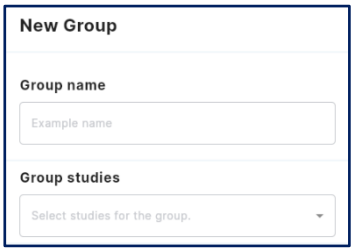
The main body of the dashboard contains three parts:

1. The left part 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 part 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.

Uploading

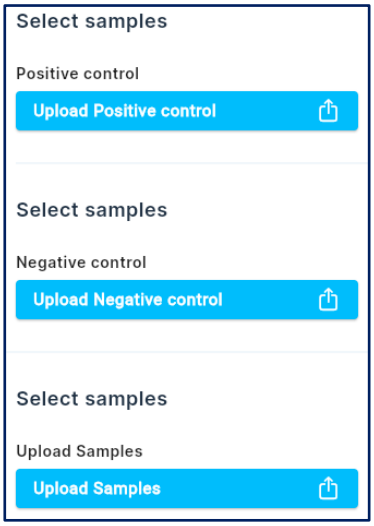


Uploading files for analysis is done through the “Upload MC ID” tab.
Once on this page, users can upload multiple fsa files from the capillary electrophoresis, corresponding to samples to be analyzed in one group. Such a group can later be viewed using the “Groups” page after analysis in antoni.



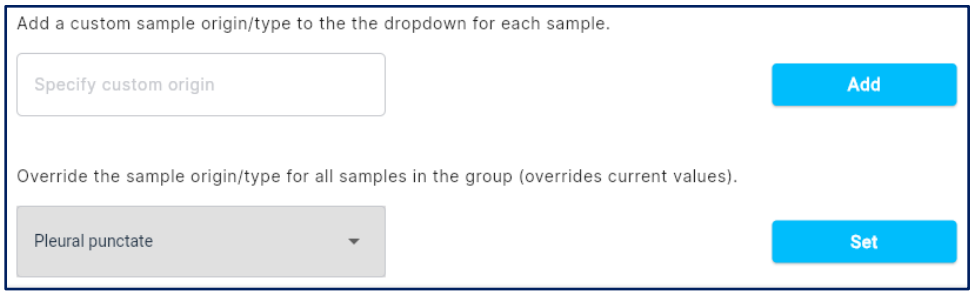
First, a **Group name** has to be provided. This can be any distinct name of your choice, facilitating easy identification of the specific batch of fsa files chosen for uploading.

The **Group studies** is an optional tag that allows the user to easily find multiple groups uploaded with that same tag. A specific ‘Group studies’ 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.

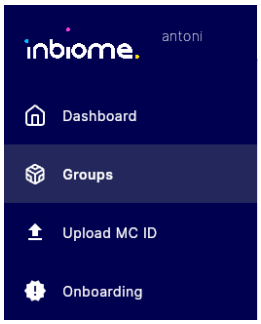
If multiple samples are selected with different sample types, an optional “Sample Origins File” can be used to automatically set the sample origin for these samples. The format of this file is described when pressing the info “**i**” button.

The group can be uploaded once the group has a name, and the samples are selected including positive and negative controls.

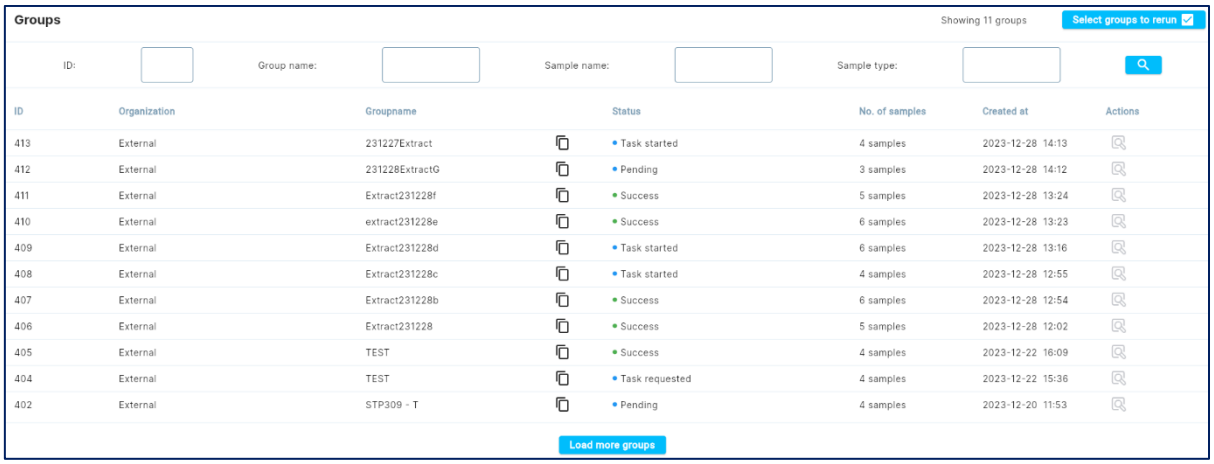
If any requirements are not met, the user will not be able to upload, and the unmet requirements are shown next to the upload button.

Groups

The Groups page can be accessed from the sidebar by pressing the “Groups” button.



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:

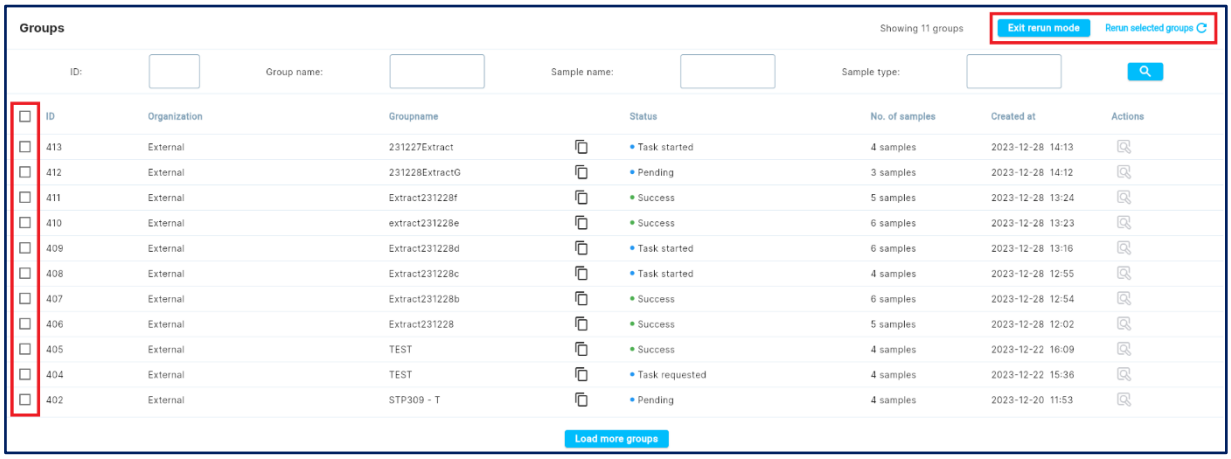
- **ID**: the internal group ID, a successive number created and used by the system.
- **Organization**: the name of the organization which uploaded the group
- **Groupname**: name of the group given by the user This column also includes an icon that allows the user to copy the groupname
- **Status**: 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.

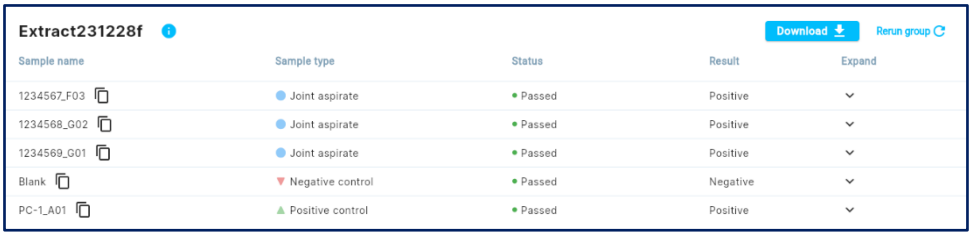
The top right corner of the page shows the number of groups currently displayed on the page. More groups can be loaded by pressing the "Load more groups" button on the bottom of the page.

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.

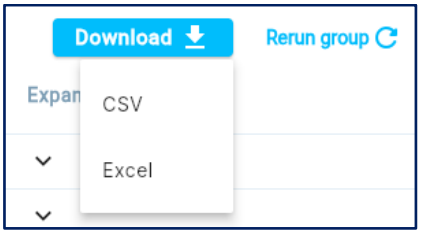


The top of the group page contains the name of the group, as well as an “i” icon which, when clicked, reveals additional metadata about the group.

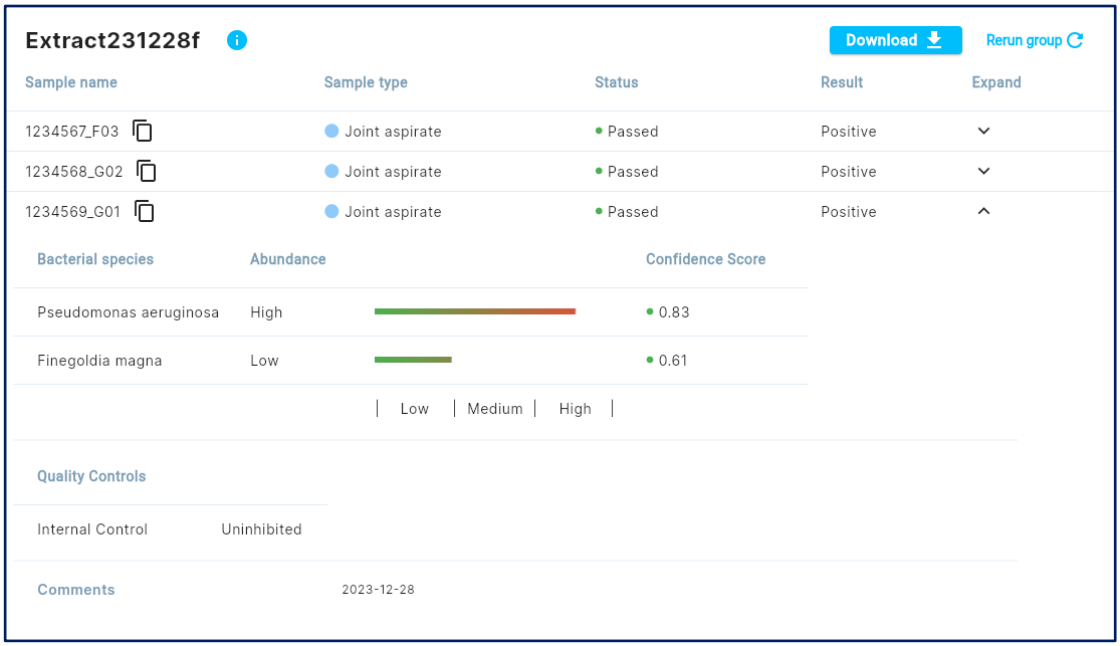
The group page for a specific group contains a list of all samples in that group. Each row represents a sample, and gives the following information:

- **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. Therefore, we strongly advice to always perform supplemental testing when an unexpected or rare species is encountered. 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** for the species identification is based on the match of the Molecular Culture profile with the reference data for a particular bacterium and on whether other bacterial species have similar reference data. The confidence score has a range between 0 and 1. A higher score means higher confidence, but the scale is non-linear. We have set the confidence score cut-off at 0.55. All species scoring above this threshold are considered

trustworthy outcomes, while species scoring below this threshold should be interpreted with caution, as their profile could potentially match other species within the same phylum.

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.
This 'Comments' section also contains a message when a bacterial peak was determined up to the phylum level and not yet to the species level. Then the message will inform you where to optionally ship the PCR product in order to have it sequenced and added to the bacterial reference data 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 analysed 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. Haemophilus influenzae / 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 clinical, epidemiological, or laboratory information for a comprehensive understanding.

3. Bacteria from other phyla than *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia, Bacteroidetes* and *Proteobacteria* are not detected For example, *Chlamydia spp.*, *Mycoplasma spp.*, and *Treponema spp.* fall outside the scope of detection and may require additional testing if clinically relevant.

4. Bacteria from other species than described in Annex I are not identified on species level but only on phylum level.

5. Good laboratory practices and strict adherence to these Test Instructions are indispensable to avoid contamination of reagents and/or specimens.

6. Results from Molecular Culture ID must be correlated with clinical history, epidemiological and other available data to the clinician evaluating the patient.

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

8. The likelihood of inaccurate results arising from interference by substances or competing microorganisms has only been assessed for those specified in section 10.6, cross-reactivity with non-bacterial organisms and section 10.7, interfering substances. Erroneous results may occur if there is interference or inhibition from substances or concentrations not covered in the mentioned sections.

9. 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

10. 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. Therefore, we strongly advice to always perform supplemental testing when an unexpected or rare species is encountered. The reference database is updated regularly to minimize this risk. For questions or assistance, you can contact our technical support at techsupport@inbiome.com.

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

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 table below shows the determination of the LoD with at least 95% confidence.

Limit of Detection per phylum

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 an 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 quantification classifications with 90% confidence. Low corresponds to <32 genomic copies, Medium corresponds to 16-512genomic 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 analysed and only cross-reactivity for the following species or equivalence* sets were observed when the bacterial load was very high. *Bifidobacterium breve, Clostridium tetani, Dialister pneumosintes, Staphylococcus aureus, Streptococcus constellatus, Streptococcus pneumoniae/mitis group and Odoribacter splanchnicus.* Only cross-reactivity between *Firmicutes/Bacteroidetes* and *Proteo primers* could be observed. The *Proteo peaks* are only observed when the Firmicutes/Bacteroidetes peak is also present. No bacterial signal was detected in other channels than expected for *Proteobacteria.* when the DNA concentration was very high (cultured bacteria)

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 presence or genetic similarities.

11.5 Cross-reactivity with human DNA

Cross-reactivity with human DNA can occur when 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

11.8 Inhibition

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

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. While intensities do not fluctuate significantly (3SD of peak deviation should be <0.5 (normalized intensity)).
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** is determined with a bacterium in the presence of another bacterium. 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 – Repeatablility and Reproducibility	Change in results from high to low or vice versa statistically occurs in less than 1 in a million detections. While intensities do not fluctuate significantly (3SD of peak deviation should be <0.5 (normalized intensity)). 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; 76% for species level determination.
Sensitivity - Functional (peak position (nc))	±1nc
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
Correlation study - Reference method	Culture
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
Assay format	**PCR machines:** Veriti from Thermo Fisher Scientific, SimpliAmp from Thermo Fisher Scientific, CFX Opus Real-Time PCR Systems from Bio-Rad Thermocycler, **Capillary Electrophoresis machines:** ABI3500, ABI3500 (XL), SeqStudio Flex
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 Clinical performance

The clinical performance of Molecular Culture ID was established during a prospective study conducted with two project partners in the Netherlands from 2013 to 2023.

12.1 Patient population

The patient population are patients of all ages with a suspected bacterial infection, which may be diagnosed in sample types described in specimen types. The age- and gender-distribution of the patients included in the clinical performance assesment is shown in Figure 1.

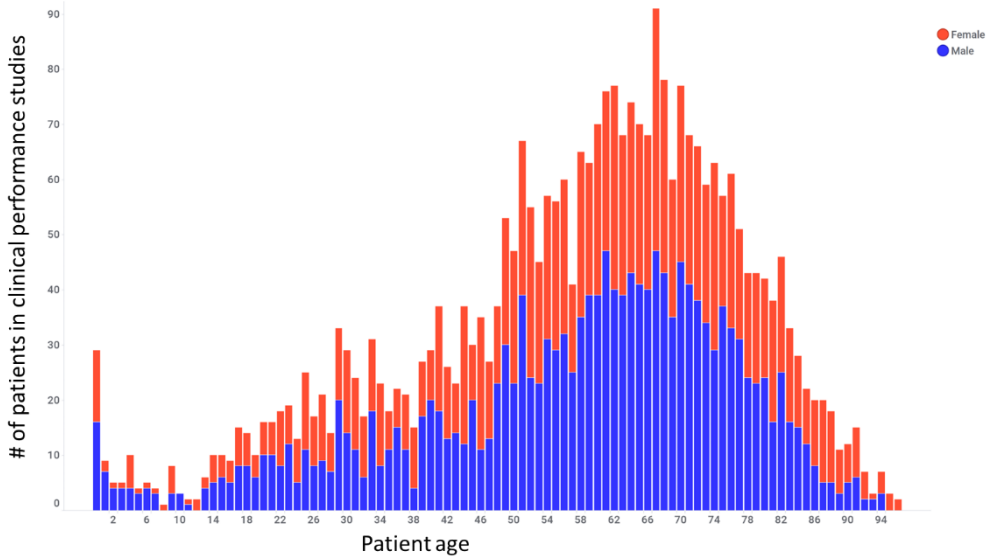


FIGURE 1 Distribution of gender and age for all patients contributing samples used in the clinical performance assessment of Molecular Culture ID.

Sample numbers

Sample type	Number of samples used for evaluation	Collection period	Project partner
Synovial effusion	591	2013-2016	A
Peritoneal effusion	247	2013-2016	A
Pleural effusion	440	2013-2016	A
Pericardial effusion	38	2013-2016	A
Cerebrospinal fluid	273	2023	B
Bone biopsies	53	2019-2022	A
Soft tissue biopsies	197	2013-2016	A
Drain fluid and pus	573	2013-2016	A
Total	2412		

Number of samples per sample type, collection period and study partners used to assess the performance of Molecular Culture ID

A: Department of Medical Microbiology of Amsterdam UMC, location VUmc at Amsterdam, The Netherlands.

B: Elisabeth-TweeSteden Ziekenhuis, Tilburg, The Netherlands.

12.2 Sample collection and processing

Samples used are consecutively collected specimens from clinical practice. This approach was chosen to eliminate selection bias and to estimate performance of Molecular Culture ID in a real-world clinical setting. Residual material of consecutive specimens that were sent for standard of care (SOC) microbiological diagnostics to the

Medical Microbiology laboratories of the study partners was collected and stored at -80°°C. This material was anonymized and moved in bulk to the laboratory of inbiome where DNA isolation followed by Molecular Culture ID testing was performed.

The Medical Ethical Review Board of the VU Medical Centre Amsterdam ruled that this study was not subject to the Dutch Medical Research involving human subjects act (WMO), since subjects were not subjected to investigational therapeutic or diagnostic interventions.

12.3 Clinical data collection

Clinical information was retrieved from the laboratory information systems from the project partners. This information included SOC results (species identification and load as obtained by culture followed by MALDI-TOF detection), leucocyte levels (from microscopic analysis after Gram-stain) and patient identifiers.

12.4 Performance analysis

Molecular Culture ID and SOC results are compared at the sample level, i.e. in terms of positive/negative and mono/polymicrobial outcomes. Samples that contain only low-load (0.1 or 1.0) common skin contaminants will not be counted as positive. The species considered contaminants are: Coagulase-Negative Staphylococci, *Micrococcus* species, and *Cutibacterium acnes*. The second comparison is at species detection level, i.e. each species detection in either assay will be classified as concordant/discordant.

These comparisons will yield a positive percent agreement (PPA) and negative percent agreement (NPA) with confidence intervals estimate of Molecular Culture ID performance. As SOC is an imperfect reference standard, PPA/NPA are reported with confidence intervals rather than sensitivity/specificity, following recommendations by the USA Food and Drug administration (1). Only bacterial species indicated in Annex I of the Molecular Culture ID IFU were taken into account for the comparison of SOC outcome to Molecular Culture ID outcome.

12.5 Discrepancy analysis

Discordant outcomes between Molecular Culture ID and SOC were analyzed based on the characteristics of the discordant bacterial species. E.g. whether it is known as a common contaminant, or as difficult to culture. When available, other samples of the same patient were examined for the presence of the discrepant species. Leucocyte levels were considered to support the absence or presence of an infection. Where possible, the presence of a discordant species in the Molecular Culture ID outcome was confirmed by an alternative identification method, such as species-specific qPCR or Next-generation sequencing.

12.6 Methods

Standard of care (SOC) diagnostics.

Partner A: Material was used for Gram stain, inoculation of bacteria on Columbia agar + 5% sheep blood (COS) and chocolate agar (PVX) incubated at 35–37 °C under aerobic conditions with carbon-dioxide (CO2). On all samples, anaerobic cultures were performed, i.e., inoculation of bacteria on COS incubated anaerobically at 35–37 °C for 4 days. To increase sensitivity, samples were placed in brain–heart infusion broth (BHI), inoculated for 7 days, and examined daily to evaluate growth. In case of bacterial growth in BHI, bacterial subcultures were inoculated on PVX and COS, incubated at 35–37 °C with CO2, and under anaerobic conditions. All morphologically distinct bacterial colonies were identified to species level with matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF, Bruker Microflex LT, Bruker). Culture loads were described in five categories: negative, 0.1 (positive only on secondary plates), 1 (limited growth, only in first streaking segment), 2 (intermediate growth into second streaking segment, 10-100 colonies), 3 (abundant growth, present in all three streaking segments, >100 colonies). Leucocyte counts were reported as none, few (1 or 2 per viewing field), medium (2-10 per viewing field), many (>10 per viewing field).

Partner B: Material was used for Gram stain, leucocyte count and culture. Culture was done on chocolate- and blood-agar plates in aerobic and anaerobic conditions, and in Brain Heart Infusion broth with vitox. In all cases secondary plates were inoculated from the broth culture on chocolate- and blood-agar plates in aerobic and anaerobic conditions. Colonies growing on plates were identified by a MALDI-TOF. Samples were sent for 16S amplicon sequencing for further diagnostics.

Sequencing**.** Whenever appropriate**,** Molecular Culture ID PCR products were sequenced on a MinION device (Oxford Nanopore Technologies, Oxford, UK). To this end, the PCR products obtained from the Molecular Culture ID reaction were diluted 1000x and subjected to the same PCR with non-fluorescently labelled primers targeting only the bacterial DNA (leaving out internal control and human DNA amplification). The resulting PCR products were barcoded using SQK-LSK109 and EXP-NBD196 kits (Oxford Nanopore Technologies, Oxford, UK) and sequenced on R9 flow cells. A custom script detected Molecular Culture ID primer sequences in the reads and

built consensus sequences from sequences containing both forward and reverse primer sequences. These sequences were classified with BLAST searches at NCBI on the nr/nt and whole genome shotgun databases. Only hits with >95% query coverage and identity were included in the Molecular Culture ID database.

12.7 Clinical performance analysis overview

Sample type	Positive Percent Agreement with SOC		Positivity rate*
	for presence of bacterial DNA	for bacterial species identification	Molecular Culture ID over SOC
Synovial effusion	96	71	1.36
Pleural effusion	94	67	3.04
Pericardial effusion	100	100	3.0
Peritoneal effusion	94	59	1.74
Pus and drain fluids	93	69	1.20
Soft tissue biopsies	86	52	1.12
Bone biopsies	83	50	1.17
Cerebrospinal fluid	95	57	1.54

*Positivity rate: number of positive samples found by Molecular Culture ID divided by number of SOC-positive samples

12.8 Clinical performance in synovial effusion

12.8.1 Detection of bacteria by SOC and Molecular Culture ID

A total of 591 samples derived from 411 unique patients was analyzed. Sample characteristics are shown in Table 1. Overall, 191 out of 591 (32.3%) samples were found positive by SOC, whereas 239 (40.4%) were positive by Molecular Culture ID. A total of 168 samples (28.4%) were found positive with both methods. PPA between Molecular Culture ID and SOC was 168/191 = 88.0% (95% CI 84.1 to 93.0%) and NPA was 330/400 = 82.5% (95% CI 79.6-86.4%) at sample level. Positivity rate for Molecular Culture ID over SOC was 239/191= 1.25x.

TABLE 1: Detection of bacterial presence by SOC and Molecular Culture ID

Sample score	SOC	Molecular Culture ID	Concordant
positive	191	239	168
polymicrobial	22	49	
negative	400	352	330
total	591	591	524

Table 1: Samples were scored as positive if one or more bacterial species were found. Samples were scored as polymicrobial when two or more different bacterial species were reported.

12.8.2 Discrepancy at sample level.

23 samples were SOC positive and Molecular Culture ID negative. Of the 23 discordant samples, 14 contained only low-load levels of skin-derived species, such as *Cutibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus warneri*, and *Staphylococcus hominis*. One discordant sample was SOC-positive for a low load *Bacillus* sp. Following removal of these contaminants, sample level PPA was 95.5% (168/176) and positivity rate was 1.36x. In contrast, the samples that were SOC-negative and Molecular ID positive, were often found to contain

12.8.3 Identification of bacteria by SOC and Molecular Culture ID.

All bacterial species identified by either method are shown in Table 2. A total of 158 out of the 221 SOC identifications were concordant with Molecular Culture ID identifications, yielding a PPA of 71.4% (95% CI 65.0 to 75.9%).

Excluding unknown phylum level detections, Molecular Culture ID yielded 68 additional bacterial detections compared to SOC. Aside *Cutibacterium acnes*, the most common additional detected bacteria were the pathogenic micro-organisms *S. aureus, E. coli*, and *S. dysgalactiae*. The remaining additional detected bacteria consisted of various gram-positive and gram-negative bacteria (Table 2).

TABLE 2: Identified bacterial species by SOC and Molecular Culture ID (MC-ID)

Species	SOC	MC-ID	Concordant	Additional detections by MC-ID	Additional detections by SOC
Staphylococcus aureus	59	61	53	8	6
Staphylococcus epidermidis	37	28	25	3	12
Cutibacterium acnes	18	27	6	21	12
Streptococcus dysgalactiae	14	19	13	6	1
Escherichia coli/Shigella spp.	11	14	10	4	1
Enterococcus faecalis	10	8	8	0	2
Pseudomonas aeruginosa	6	1	1	0	5
Streptococcus agalactiae	6	6	6	0	0
Streptococcus pneumoniae/mitis group	6	8	6	2	0
Klebsiella pneumoniae complex / Enterobacter cloacae complex	5	6	5	1	0
Morganella morganii	4	3	3	0	1
Bacteroides fragilis group	3	3	3	0	0
Corynebacterium striatum	3	2	1	1	2
Proteus mirabilis	3	2	2	0	1
Staphylococcus capitis	3	0	0	0	3
Streptococcus pyogenes/Corynebacterium sp.	3	4	3	1	0
Aerococcus urinae	2	0	0	0	2
Aerococcus viridans	2	2	2	0	0
Citrobacter sedlakii	2	0	0	0	2
Fusobacterium necrophorum	2	1	1	0	1
Klebsiella aerogenes/oxytoca	2	3	2	1	0
Staphylococcus caprae	2	2	1	1	1
Staphylococcus hominis	2	0	0	0	2
Staphylococcus lugdunensis	2	1	1	0	1
Staphylococcus pasteuri/warneri	2	2	1	1	1
Bacillus sp.	1	0	0	0	1

Citrobacter koseri/farmeri	1	2	1	1	0
Clostridium perfringens	1	1	1	0	0
Corynebacterium sp.	1	0	0	0	1
Finegoldia magna	1	1	1	0	0
Gemella morbillorum/haemolysans	1	0	0	0	1
Micrococcus luteus	1	0	0	0	1
Salmonella enterica	1	1	1	0	0
Staphylococcus Coagulase Negatieve	1	0	0	0	1
Staphylococcus sciuri	1	1	1*	0	0
Streptococcus anginosus/mutans	1	0	0	0	1
Veillonella sp.	1	0	0	0	1
Bacillus cereus	0	3	0	3	0
Burkholderia cepacia	0	1	0	1	0
Burkholderia sp.	0	1	0	1	0
Enterococcus cecorum	0	1	0	1	0
Lactococcus lactis	0	2	0	2	0
Lysinibacillus sp.	0	3	0	3	0
Prevotella denticola	0	1	0	1	0
Prevotella nigrescens/intermedia	0	1	0	1	0
Sneathia vaginalis	0	2	0	2	0
Streptococcus bovis group/*Streptococcus intermedius*	0	1	0	1	0
Turicibacter sanguinis	0	1	0	1	0
Unknown Bacteroidetes	0	2	0	2	0
Unknown FAFV	0	57	0	57	0
Unknown Proteobacteria	0	20	0	20	0
Total	221	305	158	147	63

Table 2: All bacterial identifications by SOC and Molecular Culture ID (MC-ID). Identifications were scored as concordant, when both methods identified the same bacterial species in a sample.

Data are the number of detections per assay and per species. *The SOC detection in this sample was Staphylococcus spp., which was considered concordant

12.8.4 Evaluation of discrepant detections

We looked for supporting clinical evidence for the additional Molecular Culture ID identifications that were not found by SOC, such as whether the species was present in SOC in other samples from the same patient or by examining qPCR and sequencing results (Table 3). 54.5% of extra detections had corroborative evidence.

TABLE 3 Extra Molecular Culture detections with confirmatory outcomes from corroborative patient evidence, sequencing or qPCR.

Bacterium	Count	Supporting Evidence	No Evidence
Cutibacterium acnes	21	0	21
Staphylococcus aureus	8	8	0
Streptococcus dysgalactiae	6	3	3
Escherichia coli/Shigella spp.	4	3	1
Staphylococcus epidermidis	3	2	1
Bacillus cereus	3	3	0
Lysinibacillus sp.	3	3	0
Streptococcus pneumoniae/mitis group	2	1	1
Lactococcus lactis	2	2	0
Sneathia vaginalis	2	2	0
Klebsiella pneumoniae complex / Enterobacter cloacae complex	1	1	0
Corynebacterium striatum	1	1	0
Streptococcus pyogenes/Corynebacterium sp.	1	1	0
Klebsiella aerogenes/oxytoca	1	1	0
Staphylococcus caprae	1	1	0
Staphylococcus pasteuri/warneri	1	0	1
Citrobacter koseri/farmeri	1	1	0
Burkholderia cepacia	1	0	1
Burkholderia sp.	1	0	1
Enterococcus cecorum	1	0	1
Prevotella denticola	1	1	0
Prevotella nigrescens/intermedia	1	1	0
Streptococcus bovis group/*Streptococcus intermedius*	1	1	0
Turicibacter sanguinis	1	1	0
Total	68	37	31

Table 3: Supporting evidence indicates the result was concordant with sequencing or qPCR or was present in a different sample from the same patient. No evidence indicates the sample was not found in a different patient sample and sequencing or qPCR was not performed.

12.8.5 DISCUSSION

In the present study, we evaluate the performance of Molecular Culture ID to aid in diagnosis of BJI in a large set of 591 synovial effusion samples. Molecular Culture ID showed a PPA of 88.0% (95% CI 84.1 to 93.0%) and NPA of 82.5% (95% CI 79.6-86.4%) to SOC at sample level. The PPA at species-level 71.4% (95% CI 65.0 to 75.9%). In cases where Molecular Culture ID missed the cultured species, SOC often showed a low load of cutaneous species. Interestingly, Molecular Culture ID detected a number of pathogens with high signals (e.g. S. aureus, E. coli, and S. dysgalactiae) that were missed by SOC.

Molecular Culture ID yielded 68 detections that were not reported by SOC. We found a number of uncommon species such as *Sneathia* and *Lysinibacillus* species. These were found in multiple samples from the same subject, arguing against them being contaminants. Both species have been recognized as human pathogens (17, 18). Overall, Molecular Culture ID outperformed SOC in terms of breadth of species detection. We also detected species, often in high loads, that should readily grow in culture. These might be bacteria affected by antibiotic

treatment or could represent DNA originating from biofilms. Regardless the origin, the identification of bacterial DNA in an infected joint may guide antibiotic treatment.

The most discordant outcomes between SOC and Molecular Culture ID were the *C. acnes* identifications. *C. acnes* occurs both as an infectious agent of BJI and as a common contaminant in BJI cultures, complicating the clinical interpretation of *C. acnes* detections. Furthermore, *C. acnes* is an often-encountered contaminant in molecular studies (19). Accordingly, we found that *C. acnes* signals required a higher background cut-off setting, (see Limit-of-blank report) than other species. The difficulty of interpreting a *C. acnes* finding in a suspected BJI is not new. It has been a longstanding issue in the diagnosis of BJI (20) for which guidelines for interpretation are in place (21). These guidelines require (among others) two or more positive samples, an elevated leucocyte count plus a load threshold to establish a confirmed infection. Of note, in our study four of the concordant *C. acnes* samples showed high loads, both in SOC and in Molecular Culture ID, and came from the same patient. Thus, load may be a discriminative factor in assessing the clinical significance of *C. acnes* detections and may further assist physicians in interpreting results.

12.9 Clinical performance of Molecular Culture ID in soft tissue biopsies

12.9.1 Detection of bacteria by SOC and Molecular Culture ID

The majority of the biopsies in the collection (173 out of 197) were not individually collected. Multiple samples were collected during one procedure, as is routine practice during periprosthetic joint infection surgery. Therefore, our collection contained multiple samples from the same patient taken at the same moment. Biopsies may not be homogeneously infected, resulting in potential differences in SOC versus Molecular Culture ID results which may not be fair to compare. We therefore analyzed the samples as episodes, i.e. all samples from the same patient taken at the same timepoint are combined as one episode. All bacterial species found in one episode are used for comparison. This resulted in a total of 74 episodes containing 1 up to 6 samples per episode.

Six episodes included only SOC-negative samples, except for one sample testing positive for a single low-load contaminant, such as *Cutibacterium acnes* or *Staphylococcus epidermidis*, indicating contamination. These six episodes were excluded as SOC-positive. Table 1 shows the positive/negative rate of the episodes. The PPA at episode level was 36/42, or 85.7% (95% CI 75.0 to 96.4%) and the NPA was 21/32 or 65.6% (95% CI 49.2 to 82.0%). Positivity rate was 1.12x.

Table 1: Detection of bacteria by SOC and Molecular Culture ID

	SOC	Molecular Culture ID	Concordant
positive	42	47	36
negative	32	27	21
total	74	74	57

Table 1: Episodes were scored as positive if one or more bacterial species were found.

Six episodes were SOC-positive and MC-ID negative, involving low load (categories 0.1 or 1) detections of *Staphylococcus aureus* (3x), *Cutibacterium acnes* (3x), *Staphylococcus epidermidis* (1x), *Staphylococcus capitis* (1x), *Corynebacterium amycolatum* (1x), and *Lactococcus sakei* (1x)).

12.9.2 Identification of bacteria by SOC and Molecular Culture ID

All detections by SOC and Molecular Culture ID per episode were scored for concordance (Table 2). This analysis yielded a PPA at species level of 32/73 or 43.8% (95% CI 32.6 % - 55.1%). From the 41 detections missed by Molecular Culture ID, 20 were reported as low load (categories 0.1 or 1) in SOC. These missed detections may be caused by the different input levels in SOC versus Molecular Culture ID: for SOC the complete biopsy is used to inoculate agar plates, whereas only a small part of the biopsy was set aside for Molecular Culture ID. Also, only 1/7 of the total DNA yield of the biopsy is used as template in each Molecular Culture ID PCR.

Low load (0.1 or 1.0) contaminants, such as *Cutibacterium acnes* and *Staphylococcus epidermidis* were removed from the set (Table 3). Following removal of contaminants, PPA at the species level was 51.9% (95% CI 41.6 to 65.7%).

TABLE 2 Identification of bacterial species by SOC and Molecular Culture ID (MC-ID)

Species	SOC	MC-ID	Concordant	Missed	Extra
Staphylococcus aureus	14	8	7	1	7
Cutibacterium acnes	8	6	3	3	5
Staphylococcus epidermidis	7	4	2	2	5
Klebsiella aerogenes/oxytoca	4	2	2	0	2
Staphylococcus capitis	4	0	0	0	4
Enterococcus faecalis	3	3	3	0	0
Escherichia coli/Shigella spp.	3	2	2	0	1
Citrobacter freundii complex	2	2	2	0	0
Cutibacterium avidum / Mycobacterium sp.	2	0	0	0	2
Staphylococcus pasteuri/warneri	1	1	0	1	1
Streptococcus agalactiae	2	2	2	0	0
Streptococcus constellatus	2	2	2	0	0
Streptococcus pneumoniae/mitis group	2	5	2	3	0
Bacillus spp./Parvimonas micra	1	0	0	0	1
Citrobacter koseri/farmeri	1	1	1	0	0
Corynebacterium amycolatum/xerosis	1	0	0	0	1
Corynebacterium sp.	1	0	0	0	1
Dermabacter hominis	1	0	0	0	1
Finegoldia magna	1	4	1	3	0
Lactococcus sakei	1	0	0	0	1
Micrococcus luteus	1	0	0	0	1
Morganella morganii	1	1	1	0	0
Peptostreptococcus anaerobius	1	0	0	0	1
Porphyromonas asaccharolytica	1	0	0	0	1
Prevotella buccae	1	0	0	0	1
Pseudomonas aeruginosa	1	0	0	0	1
Staphylococcus Coagulase Negatieve	1	0	0	0	1
Staphylococcus caprae	1	1	1	0	0
Staphylococcus lugdunensis	1	0	0	0	1
Staphylococcus pseudintermedius	1	0	0	0	1
Staphylococcus saccharolyticus	1	1	1	0	0
Streptococcus pyogenes/Corynebacterium sp.	1	0	0	0	1
Abiotrophia defectiva	0	1	0	1	0
Bacteroides fragilis group	0	1	0	1	0
Eikenella corrodens	0	1	0	1	0
Gemella morbillorum/haemolysans	0	1	0	1	0
Haemophilus parainfluenzae	0	1	0	1	0
Prevotella disiens	0	2	0	2	0
Prevotella intermedia/pallens	0	1	0	1	0
Prevotella melaninogenica	0	1	0	1	0
Streptococcus bovis group/*Streptococcus intermedius*	0	1	0	1	0
Streptococcus equi subsp. zooepidemicus	0	1	0	1	0
Unknown Bacteroidetes	0	5	0	5	0
Unknown FAFV	0	27	0	27	0
Unknown Proteobacteria	0	10	0	10	0
Total	73	98	32	66	41

Table 2: Concordant detections represent those found in SOC and Molecular Culture ID (MC-ID) in the same episode. Detections not found in MC-ID are categorized as missed. Extra detections refer to the number of instances where the bacterium is only found by Molecular Culture ID.

TABLE 3 Detected species per patient by SOC and Molecular Culture ID (MC-ID) after removal of known low-load contaminants

Species	SOC	MC-ID	Concordant	Extra	Missed
Staphylococcus aureus	14	8	7	1	7
Klebsiella aerogenes/oxytoca	4	2	2	0	2
Escherichia coli/Shigella spp.	3	2	2	0	1
Enterococcus faecalis	3	3	3	0	0
Cutibacterium avidum	2	0	0	0	2
Cutibacterium acnes	2	5	1	4	1
Citrobacter freundii complex	2	2	2	0	0
Streptococcus agalactiae	2	2	2	0	0
Streptococcus constellatus	2	2	2	0	0
Streptococcus pneumoniae/mitis group	2	5	2	3	0
Bacillus spp./Parvimonas micra	1	0	0	0	1
Corynebacterium amycolatum/xerosis	1	0	0	0	1
Corynebacterium sp.	1	0	0	0	1
Dermabacter hominis	1	0	0	0	1
Lactococcus sakei	1	0	0	0	1
Micrococcus luteus	1	0	0	0	1
Peptostreptococcus anaerobius	1	0	0	0	1
Porphyromonas asaccharolytica	1	0	0	0	1
Prevotella buccae	1	0	0	0	1
Pseudomonas aeruginosa	1	0	0	0	1
Staphylococcus lugdunensis	1	0	0	0	1
Staphylococcus pseudintermedius	1	0	0	0	1
Streptococcus pyogenes/Corynebacterium sp.	1	0	0	0	1
Citrobacter koseri/farmeri	1	1	1	0	0
Finegoldia magna	1	4	1	3	0
Morganella morganii	1	1	1	0	0
Staphylococcus epidermidis	1	4	1	3	0
Staphylococcus saccharolyticus	1	1	1	0	0
Abiotrophia defectiva	0	1	0	1	0
Bacteroides fragilis group	0	1	0	1	0
Eikenella corrodens	0	1	0	1	0
Gemella morbillorum/haemolysans	0	1	0	1	0
Haemophilus parainfluenzae	0	1	0	1	0
Lactococcus lactis	0	1	0	1	0
Limosilactobacillus vaginalis	0	1	0	1	0
Prevotella disiens	0	2	0	2	0
Prevotella intermedia/pallens	0	1	0	1	0
Prevotella melaninogenica	0	1	0	1	0
Staphylococcus caprae	0	1	0	1	0
Staphylococcus pasteuri/warneri	0	1	0	1	0
Streptococcus bovis group/*Streptococcus intermedius*	0	2	0	2	0
Streptococcus equi subsp. zooepidemicus	0	1	0	1	0
Unknown Bacteroidetes	0	5	0	5	0
Unknown FAFV	0	26	0	26	0

Unknown Proteobacteria	0	10	0	10	0
TOTAL	54	99	28	71	26

Table 3: Concordant detections represent those found in SOC and Molecular Culture ID (MC-ID) in the same episode. Detections not found in MC-ID are categorized as missed. Extra detections refer to the number of instances where the bacterium is only found by Molecular Culture ID.

12.9.3 Discrepancy analysis

The extra detections reported by Molecular Culture ID were confirmed by sequencing (Table 4). Of the 24 extra species level identifications made by Molecular Culture ID, 18 were confirmed by sequencing (75%)

TABLE 4 Discrepancy analysis of extra detections by Molecular Culture ID

Species	Extra	Confirmed	Not confirmed	Not sequenced
Cutibacterium acnes	3	1	0	2
Streptococcus pneumoniae/mitis group	3	2	0	1
Finegoldia magna	3	3	0	0
Staphylococcus epidermidis	2	1	0	1
Prevotella disiens	2	2	0	0
Staphylococcus aureus	1	0	0	1
Staphylococcus pasteuri/warneri	1	1	0	0
Abiotrophia defectiva	1	1	0	0
Bacteroides fragilis group	1	1	0	0
Eikenella corrodens	1	1	0	0
Gemella morbillorum/haemolysans	1	1	0	0
Haemophilus parainfluenzae	1	1	0	0
Prevotella intermedia/pallens	1	1	0	0
Prevotella melaninogenica	1	1	0	0
Streptococcus bovis group/*Streptococcus intermedius*	1	1	0	0
Streptococcus equi subsp. zooepidemicus	1	0	1	0
Total	24	18	1	5

Table 4: Confirmed detections represent the extra Molecular Culture ID detections that were confirmed by sequencing. Not confirmed detections represent those where sequencing did not confirm the Molecular Culture ID result.

12.9.4 DISCUSSION

We found a PPA of 85.7% (95% CI 75.0 to 96.4%) and an NPA of 65.6% (95% CI 49.2 to 82.0%) for detection of bacterial DNA between Molecular Culture ID and SOC. At species identification level, the PPA was 43.8% (95% CI 32.6 % - 55.1%). Following removal of contaminants, PPA at the species level was 51.9% (95% CI 41.6 to 65.7%).

In this clinical performance validation on the detection of bacteria in tissue biopsies, we found that Molecular Culture ID detected more bacterial species than SOC. 75% of the additional Molecular Culture ID detections were confirmed by sequencing. Most of the discrepancies where SOC was positive and Molecular Culture ID was negative, involved low-load samples. Due to the set-up of this study, the input in Molecular Culture ID is inherently lower than the input in SOC, explaining why low-load infected samples may be missed. We conclude that Molecular Culture ID shows good performance to aid in diagnosis of infections that are analysed by tissue biopsies.

12.10 Clinical performance of Molecular Culture ID in bone biopsies

12.10.1 Detection of bacteria by SOC and Molecular Culture ID

The presence or absence of bacteria was scored as positive/negative for each sample. Fifty-three samples derived from 53 unique patients were analyzed. Sample characteristics are shown in Table 1. Overall, 28 out of 53 (52.8%) samples were found positive by SOC, whereas 27 (50.9%) were positive by Molecular Culture ID. Nineteen samples (10.5%) were found positive with both methods. PPA between Molecular Culture ID and SOC was 19 out of 28 samples, or 67.9% (95% CI 49.3%-81.1%) and NPA was 17 out of 25, or 68% (95% CI 51.3 to 87.3%) at the sample level.

TABLE 1 Sample Characteristics

	SOC	Molecular Culture ID	Concordant
Positive	28	27	19
Polymicrobial	12	16	
Negative	25	26	17
Total	53	53	36

Nine samples were SOC positive and Molecular Culture ID negative. All these discrepant samples were reported as low-load (loads 0.1 or 1) by SOC. Five samples were SOC-positive for low load (0.1) coagulase-negative Staphylococci – following removal of these detections, sample level PPA was 19 / 23 or 83% and positivity rate was 1.17x.

12.10.2 Identification of bacteria by SOC and Molecular Culture ID

All species identified by both methods are shown in Table 2. Low load skin contaminant detections were removed prior to species detection evaluation. Eighteen identifications were concordant between SOC and Molecular Culture ID yielding a PPA of 50% (95% CI 33.6 to 66.4%). Molecular Culture ID yielded 18 additional bacterial identifications compared to SOC (Table 2).

TABLE 2 All detections per species in SOC and Molecular Culture ID (MC ID)

Species	SOC	MC ID	Concordant	Missed	Extra
Staphylococcus aureus	10	7	5	5	2
Staphylococcus epidermidis/pettenkoferi	1	3	1	0	1
Streptococcus agalactiae	4	2	2	2	0
Streptococcus pneumoniae/mitis group	3	3	2	1	0
Corynebacterium striatum	2	1	1	1	0
Staphylococcus lugdunensis	2	1	1	1	0
Streptococcus dysgalactiae	2	1	1	1	0
Actinotignum schaalii	1	0	0	1	0
Citrobacter koseri	1	0	0	1	0
Enterococcus faecalis	1	2	0	1	2
Escherichia coli	1	3	1	0	2
Gram-positive rods*	1	1*	1	0	0
Haemophilus parainfluenzae	1	3	1	0	2
Morganella morganii	1	0	0	1	0
Peptostreptococcus anaerobius	1	1	0	1	1
Proteus mirabilis	1	0	0	1	0
Staphylococcus capitis	0	0	0	0	0
Staphylococcus caprae	0	1	0	0	1
Staphylococcus simulans	1	0	0	1	0
Streptococcus intermedius/bovis	1	1	1	0	0
Streptococcus species	1	1	1	0	0

Finegoldia magna	0	1	0	0	1
Lactobacillus fermentum	0	1	0	0	1
Prevotella melaninogenica	0	1	0	0	1
Streptococcus anginosus/mutans	0	1	0	0	1
Parabacteroides distasonis	0	1	0	0	1
Pediococcus acidilactici	0	1	0	0	1
Serratia marcescens	0	1	0	0	1
Unknown FAFV	0	18	0	0	18
Unknown Bacteroidetes	0	5	0	0	5
Unknown Proteobacteria	0	2	0	0	2
Total	36	63	18	18	43

Concordant: species was detected by both methods in the same sample.
Missed: species was detected in a sample by SOC but not by Molecular Culture ID
Extra: species was detected in a sample by Molecular Culture ID but not by SOC
* Molecular Culture ID detected various gram-positive species in this sample, thus it was deemed concordant

12.10.3 Discrepancy analysis

The bone biopsy samples analyzed in this clinical performance validation were collected simultaneously with an ulcer bed tissue biopsy, as part of the BeBoP study (11, 12). We analyzed whether any of the extra species-level identifications by Molecular Culture ID were present in the SOC results of the associated ulcer bed biopsy, as an indication of the true positivity of the Molecular Culture ID result. Furthermore, for a number of bone biopsy samples, the Molecular Culture ID PCR amplicons were sequenced to confirm the species identification. The results of this discrepancy analysis are shown in Table 3.

TABLE 3 Discrepancy analysis for the extra detections by Molecular Culture ID

Species	Extra	Confirmed by sequencing	Not found by sequencing	Not sequenced	Present in tissue
Staphylococcus aureus	2	1	0	0	1
Staphylococcus epidermidis/pettenkoferi	1	0	0	1	0
Enterococcus faecalis	2	1	0	0	1
Escherichia coli	2	1	0	0	1
Haemophilus parainfluenzae	2	1	0	1	0
Peptostreptococcus anaerobius	1	1	0	0	0
Staphylococcus caprae	1	1	0	0	0
Finegoldia magna	1	1	0	0	0
Lactobacillus fermentum	1	0	0	1	0
Prevotella melaninogenica	1	0	0	1	0
Streptococcus anginosus/mutans	1	0	0	1	0
Parabacteroides distasonis	1	0	1	0	0
Pediococcus acidilactici	1	0	0	1	0
Serratia marcescens	1	0	0	0	1
Total	8	7	1	6	4

Overall, supportive evidence for true positivity of the extra Molecular Culture ID detections was found for 11 out the 18 total extra detections (61%).

12.10.4 DISCUSSION

In the present study, we evaluate the performance of Molecular Culture ID as an aid in diagnosis of diabetic foot osteomyelitis in a set of 53 samples. Molecular Culture ID showed a detection PPA of 67.9% (95% CI 49.3%-81.1%) and NPA of 68% (95% CI 51.3 to 87.3%) at the sample level. Following removal of low load skin contaminants, sample level PPA was 19 / 23 or 83% and positivity rate was 1.17x. Molecular Culture ID yielded a species-level PPA of 50% (95% CI 33.6 to 66.4%).

A significant proportion of the missed detections by Molecular Culture ID involved low-load SOC detections, which could be a result of the lower input available for Molecular Culture ID compared to SOC. Molecular Culture ID yielded 18 additional species-level identifications over SOC, with 11 of these confirmed by sequencing or by SOC results from simultaneously taken tissue samples.

In conclusion, although only a limited number of samples was available for the clinical performance assessment, we conclude that Molecular Culture ID is a promising alternative for culture for use in diagnosis of bone infections.

Part of this clinical performance analysis was published in BMC Infectious Diseases.

Gramberg MCTT, Knippers C, Lagrand RS, van Hattem JM, de Goffau MC, Budding AE, Davids M, Matamoros S, Nieuwdorp M, de Groot V, Heijer MD, Sabelis LWE, Peters EJG. 2023. Concordance between culture, Molecular Culture and Illumina 16S rRNA gene amplicon sequencing of bone and ulcer bed biopsies in people with diabetic foot osteomyelitis. BMC Infect Dis 23:505.

12.11 Clinical performance of Molecular Culture ID in cerebrospinal fluid

12.11.1 Detection of bacteria by SOC and Molecular Culture ID

The presence or absence of bacteria was scored as positive/negative for each sample. Samples were scored as polymicrobial when two or more different bacterial species were reported. Sample characteristics are shown in Table 1. Overall, 53 out of 273 (19.4%) samples were found positive by SOC, whereas 60 (22.0%) were positive by Molecular Culture ID. 39 samples were found positive by both methods (14.2%). PPA between Molecular Culture ID and culture was 73.5% (95% CI 64.6 to 84.6%) and NPA was 90.5% (95% CI 87.3 to 94.4%) at the sample level. Positivity rate for Molecular Culture ID over SOC was 60/53 = 1.13x.

TABLE 1 Sample Characteristics

	SOC	Molecular Culture ID	Concordant
Positive	53	60	39
Monomicrobial	48	50	
Polymicrobial	5	10	
Negative	220	213	199
Total	273	273	238

12.11.2 Bacterial detection discrepancy.

Fourteen samples were SOC positive and Molecular Culture ID negative. The bacteria present in these samples, as identified by SOC, were *Staphylococcus epidermidis* (x6), *Staphylococcus haemolyticus* (x4), *Cutibacterium acnes* (x1), *Micrococcus luteus* (x1), *Staphylococcus aureus* (x1), *Staphylococcus cohnii* (x1), *Staphylococcus hominis* (x1), and Enterococcus faecalis (x1). Following removal of low load skin contaminants, PPA at the sample level was 95% (39/41) and positivity rate was 1.54x.

12.11.3 Identification of bacteria by SOC and Molecular Culture ID

All species detected by both methods are shown in Table 2. Of the 58 SOC identifications, 33 were concordant with Molecular Culture ID identifications, yielding a PPA of 56.9% (95% CI 45.0 to 68.8%). Molecular Culture ID yielded 37 additional bacterial detections compared to SOC (Table 2).

TABLE 2 Detected species per patient by SOC and Molecular Culture ID (MC-ID)

Species	SOC	MC-ID	Concordant	Missed	Extra
Staphylococcus epidermidis	31	21	19	2	12
Staphylococcus haemolyticus	8	3	2	1	6
Enterococcus faecalis	6	5	5	0	1
Enterococcus faecium	2	3	2	1	0
Klebsiella pneumoniae complex / Enterobacter cloacae complex	2	3	2	1	0
Escherichia coli/Shigella spp.	2	1	1	0	1
Staphylococcus aureus	2	1	1	0	1
Staphylococcus hominis	1	2	0	2	1
Acinetobacter baumannii complex / Achromobacter xylosoxidans/denitrificans	1	1	1	0	0
Cutibacterium acnes	1	1	0	1	1
Micrococcus luteus	1	0	0	0	1
Staphylococcus cohnii	1	0	0	0	1
Streptococcus bovis group/*Streptococcus intermedius*	0	2	0	2	0
Streptococcus pneumoniae/mitis group	0	1	0	1	0
Unknown FAFV	0	16	0	16	0
Unknown Proteobacteria	0	9	0	9	0
Unknown Bacteroidetes	0	1	0	1	0
TOTAL	58	70	33	37	25

12.11.4 DISCUSSION

In the present study, we evaluate the performance of Molecular Culture ID for diagnosis of bacterial meningitis in a set of 273 samples. Molecular Culture ID showed a detection PPA of 73.5% (95% CI 64.6 to 84.6%) and NPA was 90.5% (95% CI 87.3 to 94.4%) at the sample level as compared to SOC. The PPA at species-level identification was 56.9% (95% CI 45.0 to 68.8%).
Molecular Culture ID yielded 37 additional bacterial detections that were not reported by SOC.

Due to the invasive nature of the cerebrospinal fluid collection procedure, obtaining many samples is difficult. Furthermore, CSF is normally a sterile fluid, meaning it should not contain any bacteria under healthy conditions. The central nervous system, including the brain and spinal cord, is well-protected by the blood-brain barrier, which limits the passage of microorganisms from the bloodstream into the cerebrospinal fluid. Therefore, the interpretation of results containing bacterial species known to form biofilms and present in normal skin flora, such as Staphylococci limits the clinical understanding of these results (6-7). Limited information from SOC such as antibiotic use and culture load further complicate the interpretation of these results.
Regardless, Molecular Culture ID identified an additional 21 culture-negative samples as positive, including instances of *Klebsiella pneumoniae* and *Staphylococcus haemolyticus*, improving the overall diagnostic sensitivity.

12.12 Clinical performance of Molecular Culture ID in pericardial effusions

12.12.1 Detection of bacteria by SOC and Molecular Culture ID

The presence or absence of bacteria was scored as positive/negative for each sample. Thirty-eight samples derived from 36 unique patients were analyzed. Sample characteristics are shown in Table 1. Three samples that were reported as containing only low-load *Cutibacterium acnes* in SOC, were scored as negative, as this result is not considered clinically relevant for this sample type. Overall, 4 out of 38 (10.5%) samples were found positive by SOC, whereas 12 (31.6%) were positive by Molecular Culture ID. 4 samples (10.5%) were found positive with both methods. PPA between Molecular Culture ID and SOC was 4 out of 4 samples, or 100% (95% CI 51 to 100%) and NPA was 26 out of 31, or 76.5% (95% CI 60.0 to 87.6%) at the sample level. Positivity rate or Molecular Culture ID over OSC was 12/4= 3.0.

TABLE 1 Sample Characteristics

	SOC	Molecular Culture ID	Concordant
Positive	4	12	4
Monomicrobial	3	10	
Polymicrobial	1	2	
Negative	34	26	26
Total	38	38	30

12.12.2 Identification of bacteria by SOC and Molecular Culture ID

All species detected by both methods are shown in Table 2. 5 of the 5 SOC identifications were concordant with Molecular Culture ID identifications, yielding a species-level PPA of 100.0% (95% CI 57 to 100%). Molecular Culture ID yielded 11 additional bacterial detections compared to SOC (Table 2). When possible, additional bacterial identifications by Molecular Culture ID were subjected to sequencing to confirm the bacterial identification. The results of this investigation are available in Table 3. Many of the additional detections by Molecular Culture ID were not able to be sequenced.

TABLE 2 Detected species per patient by SOC and Molecular Culture ID (MC-ID)

Species	SOC	MC-ID	Concordant	Missed	Extra
Cutibacterium acnes	0	1	0	0	1
Escherichia coli/Shigella spp.	2	3	2	0	1
Staphylococcus aureus	1	1	1	0	0
Streptococcus bovis group/*Streptococcus intermedius*	1	2	1	0	1
Streptococcus pneumoniae/mitis group	1	4	1	0	3
Enterococcus cecorum	0	1	0	0	1
Streptococcus pyogenes/Corynebacterium sp.	0	1	0	0	1
Unknown FAFV	0	2	0	0	2
Unknown Proteobacteria	0	1	0	0	1
Total	5	16	5	0	11

TABLE 3 Summary of extra bacterial detections in Molecular Culture ID. The confirmation was performed by sequencing of the IS-Pro fragments. Inconclusive results refer to unavailable sequencing results.

Species	No. of results	MC-ID confirmed	Not confirmed	Inconclusive
Cutibacterium acnes	1	1	0	0
Escherichia coli/Shigella spp.	1	1	0	0
Streptococcus bovis group/*Streptococcus intermedius*	1	1	0	0
Streptococcus pneumoniae/mitis group	3	0	0	3
Enterococcus cecorum	1	1	0	0
Streptococcus pyogenes/Corynebacterium sp.	1	1	0	0
Unknown FAFV	2	0	0	2
Unknown Proteobacteria	1	0	0	1
Total	11	5	0	6
% of total Extra Results		45.4%	0%	54.5%

12.12.3 DISCUSSION

In the present study, we evaluate the performance of Molecular Culture ID for diagnosis of pericardial effusion infection in a set of 38 samples. Molecular Culture ID showed a detection PPA of 100% (95% CI 51 to 100%) and negative percent agreement (NPA) was 76.5% (95% CI 61.9 to 91.1%) to SOC at the sample level. The PPA at species-level identification was 100.0% (95% CI 57 to 100%).

Molecular Culture ID yielded 11 additional bacterial detections that were not reported by SOC. Overall, Molecular Culture ID outperformed SOC in terms of breadth of species detection, detecting 7 unique species as compared to the 5 bacterial species reported in SOC.

Due to the invasive nature of the pericardial effusion fluid procedure and the potential for complications, obtaining numerous samples is very difficult. In this study, samples were collected from a medium-sized hospital over the span of 7 years, resulting in the 38 samples for analysis. While ideal to have increased sample numbers, in this situation it's highly unlikely. Nonetheless, Molecular Culture ID identified three times as many positive samples as SOC.

12.13 Clinical performance of Molecular Culture ID in peritoneal effusions

12.13.1 Detection of bacteria by SOC and Molecular Culture ID

The presence or absence of bacteria was scored as positive/negative for each sample. Samples were scored as poymicrobial when two or more different bacterial species were reported. Out of 247 samples, 72 were positive in SOC (29.1%) whereas 118 samples (47.7%) were positive for Molecular Culture ID, with 64 samples concordant (Table 1). PPA between Molecular Culture ID and SOC at the sample level was 88.9% (IC 95%, 79.1% to 94.6%), and NPA was 69.1% (IC 95%, 64.5% to 75.1%). Positivity rate of Molecular Culture ID over SOC was 118/72 = 1.64x.

TABLE 1 Sample level results from SOC and Molecular Culture ID.

	SOC	Molecular Culture ID	Concordant
Positive	72	118	64
Monomicrobial	38	49	
Polymicrobial	34	69	
Negative	175	129	121
Total	**247**	**247**	**185**

12.13.2 Bacterial detection discrepancy

Eight samples were SOC positive and Molecular Culture ID negative. Four of the discrepant samples were reported as low-load SOC outcomes (0.1-1) containing potential skin contaminants like *Staphylococcus haemolyticus, Staphylococcus warneri, Staphylococcus pasteuri* and *Cutibacterium acnes*. Three samples containing *Enterococcus faecium*, *Escherichia coli*, or *Staphylococcus aureus* were reported as low load SOC outcomes (0.1). One sample contained a high SOC load (3) of *Mycoplasma hominis,* a bacterium undetectable, and therefore not claimed, by Molecular culture ID (see Bacteria list Molecular Culture ID (pdf: Part A - Device Description and Specifications including variants and accessories)). Following removal of these contaminants, PPA at the sample level was 94.1% (64/68) and positivity rate was 1.74x. Of the additional 54 Molecular Culture ID-positive samples, 30 had high to medium levels of leukocytes, indicative of a true infection.

12.13.3 Identification of bacteria by SOC and Molecular Culture ID

Detections involving potential skin contaminants *(Staphylococcus epidermidis, S. capitis, S. hominis, S. warneri, S. pasteuri and Cutibacterium acnes*) at low loads (0.1-1) were removed from this analysis as these were most likely not clinically relevant.

SOC detected 35 unique species, which were compared with Molecular Culture ID in **Table 2.** Of the 121 SOC identifications, 71 were concordant with Molecular Culture ID identifications, yielding a PPA of 58.7% (95% CI 51.4 to 65.9%). One of the missed detections was *Actinomyces neuii,* a species not claimed by Molecular Culture ID, i.e. not present in Bacteria list Molecular Culture ID (pdf: P*art A - Device Description and Specifications including variants and accessories*).

TABLE 2 Number of detections per species. Only species found in SOC are taken into account. Concordant detections represent those found in SOC and Molecular Culture ID (MC-ID) in the same sample. Detections not found in MC-ID are called missed. Extra detections represent when the bacterium is only found by Molecular Culture ID.

Species	SOC	MC-ID	Concordant	Missed	Extra
Enterococcus faecium	20	21	15	6	5
Enterococcus faecalis	19	18	12	6	7
Escherichia coli/Shigella spp.	18	31	16	15	2
Klebsiella pneumoniae complex / Enterobacter cloacae complex	10	12	8	4	2
Staphylococcus aureus	6	3	2	1	4
Staphylococcus epidermidis	5	5	3	2	2
Stenotrophomonas maltophilia	4	2	2	0	2
Klebsiella aerogenes/oxytoca	3	2	2	0	1
Proteus vulgaris/penneri	3	1	1	0	2
Bacteroides fragilis group	2	7	0	7	2
Citrobacter freundii complex	2	4	1	3	1
Morganella morganii	2	0	0	0	2
Staphylococcus haemolyticus	2	1	1	0	1
Streptococcus anginosus/intermedius	2	2	2	0	0
Streptococcus mutans	2	1	1	0	1
Streptococcus pneumoniae/mitis group	2	8	0	8	2
Acinetobacter baumannii complex / Achromobacter xylosoxidans/denitrificans	1	0	0	0	1
Acinetobacter sp.	1	0	0	0	1
Actinomyces neuii	1 [a]	0	0	0	1
Bacillus sp.	1	0	0	0	1
Citrobacter koseri/farmeri	1	0	0	0	1
Clostridium perfringens	1	4	1	3	0
Corynebacterium tuberculostearicum	1	0	0	0	1
Cutibacterium acnes	1	16	1	15	0
Enterococcus gallinarum	1	0	0	0	1
Lactobacillus sp.	1	0	0	0	1
Neisseria subflava group	1	0	0	0	1
Pseudomonas aeruginosa	1	0	0	0	1
Sphingomonas paucimobilis	1	0	0	0	1
Staphylococcus capitis	1	1	1	0	0
Staphylococcus Coagulase Negatieve	1	0	0	0	1
Staphylococcus pasteuri/warneri	1	1	0	1	1
Streptococcus agalactiae	1	2	1	1	0
Streptococcus bovis group/*Streptococcus intermedius*	1	8	0	8	1

Streptococcus pyogenes/Corynebacterium sp.	1	1	1	0	0
Total	121	151	71	80	90

12.13.4 Evaluation of extra bacterial detections

In total, Molecular Culture ID yielded 165 extra species level bacterial detections described in Table 3. In addition, Molecular Culture ID yielded 119 phylum level detections. Molecular Culture ID identified 52 unique species, 17 more than culture. As much as possible, samples with additional identifications by Molecular Culture ID were sequenced to confirm the presence of these species. Additional sequencing of Molecular Culture ID amplicons confirmed 105 (63.6%) additional detections. For 22 detections, the sequencing results were not available or inconclusive due to lack of sequencing coverage.

TABLE 3 Extra bacterial detections in Molecular Culture ID. The confirmation was performed by sequencing of the IS-Pro fragments. Inconclusive results refer to unavailable sequencing results.

Species	MC-ID	Confirmed	Not-confirmed	Inconclusive
Abiotrophia defectiva	1	0	1	0
Alistipes group	14	14	0	0
Alloiococcus otitis	1	1	0	0
Bacteroides caccae	1	1	0	0
Bacteroides eggerthii	1	0	1	0
Bacteroides fragilis group	7	7	0	0
Bacteroides vulgatus	9	7	2	0
Bacteroides zoogleoformans / Barnesiella merdipullorum	1	1	0	0
Barnesiella merdipullorum	5	4	0	1
Bulleidia extructa	1	0	0	1
Citrobacter freundii complex	3	3	0	0
Citrobacter sedlakii	1	0	1	0
Clostridium innocuum	1	0	1	0
Clostridium perfringens	3	3	0	0
Cutibacterium acnes	15	6	1	8
Enterococcus avium	3	1	2	0
Enterococcus cecorum	1	1	0	0
Enterococcus faecalis	6	5	1	0
Enterococcus faecium	6	5	1	0
Escherichia coli/Shigella spp.	15	8	3	4
Finegoldia magna	1	0	1	0
Fusobacterium necrophorum	2	0	2	0
Gemella morbillorum/haemolysans	2	2	0	0
Granulicatella adiacens	1	1	0	0
Klebsiella pneumoniae complex / Enterobacter cloacae complex	4	4	0	0
Lactobacillus (para)gasseri	1	0	0	1
Lactobacillus casei/paracasei	1	0	1	0
Lactobacillus fermentum	2	0	2	0
Lactobacillus pentosus	1	0	1	0
Leptotrichia amnionii	1	0	1	0
Moraxella catarrhalis	1	0	1	0
Neisseria mucosa/sicca	1	0	0	1
Odoribacter splanchnicus	6	5	0	1
Parabacteroides distasonis	1	1	0	0
Peptostreptococcus anaerobius	2	2	0	0

Prevotella buccae	1	1	0	0
Prevotella denticola	1	0	0	1
Prevotella histicola	10	0	9	1
Prevotella intermedia	1	1	0	0
Prevotella intermedia/pallens	2	2	0	0
Prevotella melaninogenica/jejuni	1	1	0	0
Prevotella nigrescens/intermedia	1	1	0	0
Pseudomonas putida	1	0	1	0
Serratia marcescens	1	0	0	1
Staphylococcus aureus	1	1	0	0
Staphylococcus caprae	1	0	1	0
Staphylococcus epidermidis	2	1	0	1
Staphylococcus pasteuri/warneri	1	0	1	0
Streptococcus agalactiae	1	1	0	0
Streptococcus bovis group/*Streptococcus intermedius*	8	7	0	1
Streptococcus constellatus	2	1	1	0
Streptococcus pneumoniae/mitis group	8	6	2	0
Unknown Bacteroidetes	34	-	-	-
Unknown FAFV	55	-	-	-
Unknown Proteobacteria	30	-	-	-
total	**165**	**105**	**38**	**22**

12.13.5 DISCUSSION

In the present study, we evaluate the performance of Molecular Culture ID for the diagnosis of spontaneous bacterial peritonitis in a set of 247 samples. Molecular Culture ID showed a detection PPA of 88.9% (IC 95%, 79.1% to 94.6%) and NPA of 69.1% (IC 95%, 64.5% to 75.1%) at the sample level.
Upon exclusion of low-load contaminant species, we obtained a PPA of 58.7% (95% CI 51.4 to 65.9%) at the species level. Molecular Culture ID successfully identified the most prevalent species causing SBP (*Escherichia coli/Shigella* spp., *Enterococcus faecalis, Enterococcus faecium and Klebsiella pneumoniae* complex / *Enterobacter cloacae* complex) (18).

Molecular Culture ID yielded 165 additional bacterial detections that were not reported by SOC. After confirmation via sequencing of Molecular Culture ID amplicons, 105 of these extra detections were confirmed.
Therefore, Molecular Culture ID overall outperforms SOC in terms of species identification, especially for polymicrobial detections, demonstrating its potential to accelerate SBP diagnosis.

12.14 Clinical performance of Molecular Culture ID in pleural effusions

12.14.1 Detection of bacteria by SOC and Molecular Culture ID

The presence or absence of bacteria was scored as positive/negative for each sample. Samples were scored as polymicrobial when two or more different bacterial species were reported. 440 samples were analyzed. Sample characteristics are shown in Table 1. Overall, 54 out of 440 (12.3%) samples were found positive by SOC, whereas 137 (31.1%) were positive by Molecular Culture ID. 45 samples were found positive by both methods (10.2%). PPA between Molecular Culture ID and culture was 83.3% (95% CI 71.7 to 91.1%) and NPA was 79.3% (95% CI 72.3 to 81.2%) at the sample level. Positivity rate for Molecular Culture ID over SOC was 137/55= 2.49x.

TABLE 1 Sample Characteristics

	SOC	Molecular Culture ID	Concordant
Positive	54	137	45
Monomicrobial	40	100	
Polymicrobial	14	37	
Negative	386	306	297
Total	440	440	382

12.14.2 Bacterial detection discrepancy

Nine samples were SOC positive and Molecular Culture ID negative (Figure 1). All discrepant samples were reported as low-load SOC outcome, with six of them positive for common contaminant skin-derived bacteria, such as *S. epidermidis*, *S. hominis*, *C. acnes*, and *S. capitis*. Three samples were reported as low load *E. coli*, *S. aureus* or *S. sanguinis* SOC outcomes. Following removal of these contaminants, PPA at the sample level was 93.8% (45/48) and positivity rate was 3.04x. Of the additional 88 Molecular Culture ID-positive samples, 52 had high levels of leukocytes, indicative of a true infection.

FIGURE 1 Discrepant Molecular Culture ID-negative samples.

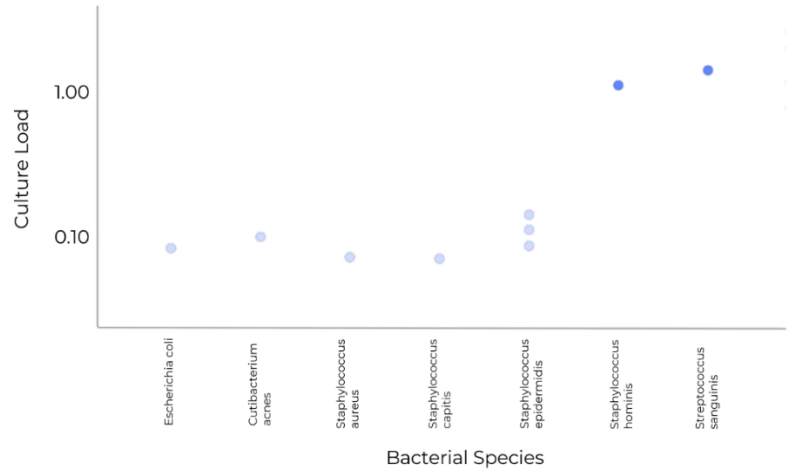


Figure 1. The species and load as reported by SOC are indicated by the X- and Y-axis, respectively.

12.14.3 Identification of bacteria by SOC and Molecular Culture ID

All species detected by both methods are shown in Table 2. Forty-three of the 73 SOC identifications were concordant with Molecular Culture ID identifications, yielding a PPA of 58.9% (95% CI 47.7 to 70.1%). Molecular Culture ID yielded 99 additional bacterial detections compared to SOC (Table 2).

Discrepancies were more prevalent in the polymicrobial samples: PPA at the species level between SOC and Molecular Culture ID was 24/40 (60.0%, 95% CI 45.1 to 74.9%) for SOC monomicrobial samples, whereas this was 14/33 (42.4%, 95% CI 25.8 to 59.0 %) for SOC polymicrobial samples.

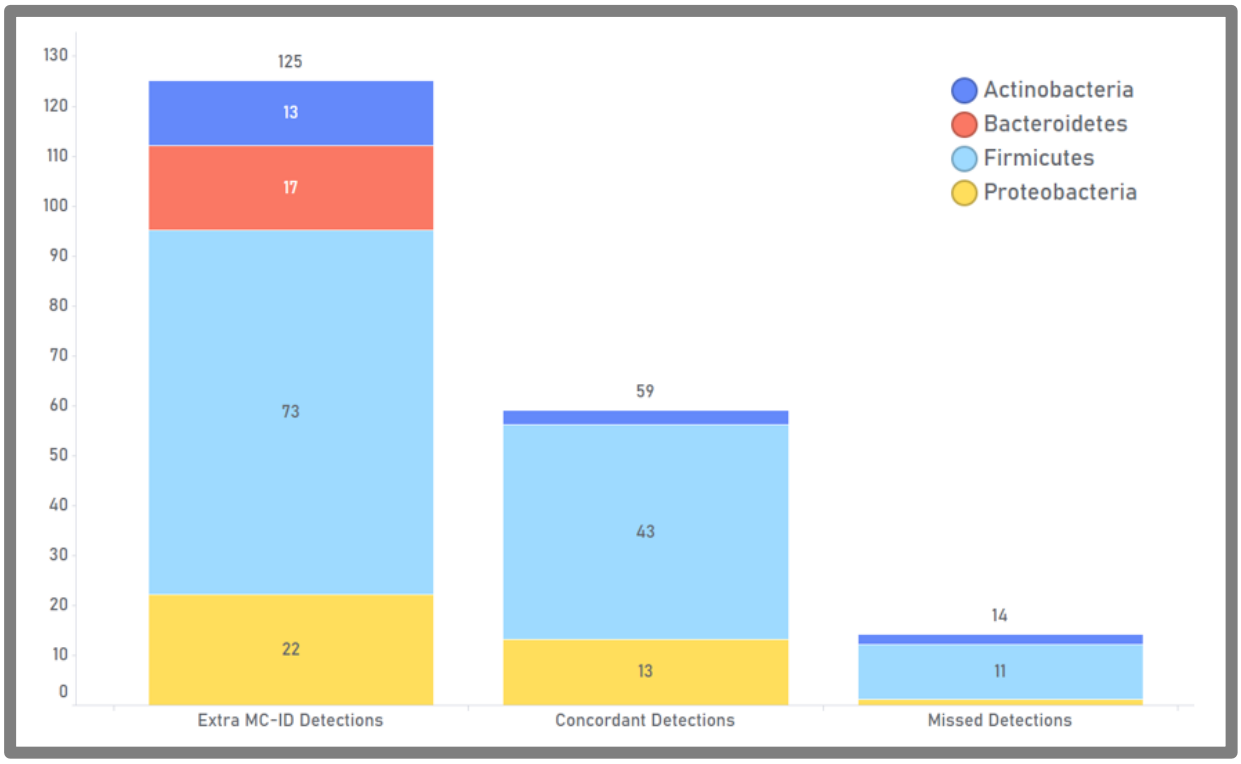
TABLE 2 Detected species per patient by SOC and Molecular Culture ID (MC-ID)

Species	SOC	MC-ID	Concordant	Missed	Extra
Staphylococcus aureus	8	11	5	6	3
Streptococcus bovis group/*Streptococcus intermedius*	8	22	7	15	1
Escherichia coli/Shigella spp.	6	14	5	9	1
Staphylococcus epidermidis	11	7	5	2	6
Citrobacter koseri/farmeri	4	5	4	1	0
Cutibacterium acnes	4	14	2	12	2
Streptococcus pneumoniae/mitis group	4	23	3	20	1
Enterococcus faecalis	3	0	0	0	3
Streptococcus pyogenes/Corynebacterium sp.	3	9	3	6	0
Bacillus cereus	2	0	0	0	2
Enterococcus faecium	2	1	1	0	1
Klebsiella pneumoniae complex / Enterobacter cloacae complex	2	3	2	1	0
Lactococcus sp.	2	0	0	0	2
Staphylococcus capitis	2	0	0	0	2
Staphylococcus lugdunensis	2	0	0	0	2
Streptococcus anginosus/intermedius	2	5	2	3	0
Corynebacterium tuberculostearicum	1	0	0	0	1
Eikenella corrodens	1	3	1	2	0
Enterococcus durans	1	0	0	0	1
Klebsiella aerogenes/oxytoca	1	1	1	0	0
Lactobacillus sp.	1	0	0	0	1
Staphylococcus hominis	1	1	0	1	1
Streptococcus agalactiae	1	3	1	2	0
Streptococcus constellatus	1	2	1	1	0
Alistipes group	0	1	0	1	0
Alloprevotella tannerae	0	2	0	2	0
Bacteroides zoogleoformans / Barnesiella merdipullorum	0	2	0	2	0
Bulleidia sp.	0	2	0	2	0
Dialister pneumosintes	0	1	0	1	0
Granulicatella adiacens	0	3	0	3	0
Haemophilus influenzae	0	1	0	1	0
Haemophilus parainfluenzae	0	1	0	1	0
Lactobacillus (para)gasseri	0	3	0	3	0
Lactobacillus fermentum	0	3	0	3	0
Lautropia mirabilis	0	1	0	1	0

Limosilactobacillus vaginalis	0	1	0	1	0
Moraxella catarrhalis	0	1	0	1	0
Neisseria subflava group	0	1	0	1	0
Prevotella intermedia/pallens	0	1	0	1	0
Prevotella melaninogenica/jejuni	0	1	0	1	0
Prevotella nigrescens/intermedia	0	4	0	4	0
Prevotella oralis	0	1	0	1	0
Prevotella oris	0	1	0	1	0
Prevotella sp.	0	4	0	4	0
Proteus vulgaris/penneri	0	1	0	1	0
Pseudomonas putida	0	1	0	1	0
Stenotrophomonas maltophilia	0	2	0	2	0
Streptococcus anginosus	0	2	0	2	0
Streptococcus ilei group	0	1	0	1	0
Streptomyces microflavus	0	1	0	1	0
Turicibacter sanguinis	0	1	0	1	0
Unknown Bacteroidetes	0	8	0	8	0
Unknown FAFV	0	28	0	0	0
Unknown Proteobacteria	0	11	0	0	0
TOTAL	**73**	**215**	**43**	**172**	**30**

At the phylum-level, PPA between SOC and Molecular Culture ID was 59 out of 73 detections or 80.8% (95% CI 71.9 to 89.7%). Missed detections were entirely from the phylum Firmicutes, aside from one Proteobacteria (Figure 2). Excluding Unknown detections, 17 additional detections by Molecular Culture ID were from the phylum Bacteroidetes, which are known to be difficult to culture. Molecular Culture ID detected 22 additional Proteobacteria as compared to SOC.

FIGURE 2 Phylum concordance of bacterial detections by Molecular Culture ID. Bars depict additional MC-ID detections, concordant detections, and detections missed by MC-ID



12.14.4 Evaluation of discrepant identifications.

Samples with additional identifications by Molecular Culture ID were sequenced to confirm the presence of species. 16 of the detections by SOC were reported as very low load (0.1) and 19 detections were reported as low load (1.0), including 6 detections of *Staphylococcus epidermidis*, a known skin contaminant. A corrected version of Table 2, with the exclusion of low load (0.1-1.0), known contaminants, including *Cutibacterium acnes*, *Staphylococcus* species (excluding *Staphylococcus aureus*), and *Micrococcus* species can be found in Table 3. Samples with non-reported SOC load were included in this table.

TABLE 3 Detected species per patient by SOC and Molecular Culture ID (MC-ID) after removal of known low-load contaminants

Species	SOC	MC-ID	Concordant
Staphylococcus aureus	8	11	5
Streptococcus bovis group/*Streptococcus intermedius*	8	22	7
Escherichia coli/Shigella spp.	6	14	5
Staphylococcus epidermidis	5	7	4
Citrobacter koseri/farmeri	4	5	4
Streptococcus pneumoniae/mitis group	4	23	3
Enterococcus faecalis	3	0	0
Streptococcus pyogenes/Corynebacterium sp.	3	9	3
Bacillus cereus	2	0	0
Enterococcus faecium	2	1	1
Klebsiella pneumoniae complex / Enterobacter cloacae complex	2	3	2
Lactococcus sp.	2	0	0
Staphylococcus lugdunensis	2	0	0

Streptococcus anginosus/intermedius	2	5	2
Corynebacterium tuberculostearicum	1	0	0
Cutibacterium acnes	1	14	1
Eikenella corrodens	1	3	1
Enterococcus durans	1	0	0
Klebsiella aerogenes/oxytoca	1	1	1
Lactobacillus sp.	1	0	0
Streptococcus agalactiae	1	3	1
Streptococcus constellatus	1	2	1
Alistipes group	0	1	0
Alloprevotella tannerae	0	2	0
Bacteroides zoogleoformans / Barnesiella merdipullorum	0	2	0
Bulleidia sp.	0	2	0
Dialister pneumosintes	0	1	0
Granulicatella adiacens	0	3	0
Haemophilus influenzae	0	1	0
Haemophilus parainfluenzae	0	1	0
Lactobacillus (para)gasseri	0	3	0
Lactobacillus fermentum	0	3	0
Lautropia mirabilis	0	1	0
Limosilactobacillus vaginalis	0	1	0
Moraxella catarrhalis	0	1	0
Neisseria subflava group	0	1	0
Prevotella intermedia/pallens	0	1	0
Prevotella melaninogenica/jejuni	0	1	0
Prevotella nigrescens/intermedia	0	4	0
Prevotella oralis	0	1	0
Prevotella oris	0	1	0
Prevotella sp.	0	4	0
Proteus vulgaris/penneri	0	1	0
Pseudomonas putida	0	1	0
Staphylococcus hominis	0	1	0
Stenotrophomonas maltophilia	0	2	0
Streptococcus anginosus	0	2	0
Streptococcus ilei group	0	1	0
Streptomyces microflavus	0	1	0
Turicibacter sanguinis	0	1	0
Unknown Bacteroidetes	0	8	0
Unknown FAFV	0	28	0
Unknown Proteobacteria	0	11	0
TOTAL	61	215	41

Following correction for these species, 20 bacterial identifications were found for SOC which were not identified by Molecular Culture ID. Following correction for contaminants, PPA between Molecular Culture ID and SOC was 67.2% (95% CI 55.4 to 79.0%). Samples with extra detections in Molecular Culture were sequenced, when possible, to confirm the presence of bacterial species (Table 4). 76% of additional detections by MC-ID were corroborated with qPCR or sequencing.

TABLE 4 Extra Molecular Culture detections with outcomes from sequencing

		No. of investigations		
Species	**No. of result**	**SOC result confirmed**	**MC result confirmed**	**Inconclusive**
Streptococcus pneumoniae/mitis group	20	20	0	0
Streptococcus bovis group/Streptococcus intermedius	15	11	4	0
Cutibacterium acnes	12	4	8	0
Escherichia coli/Shigella spp.	9	6	3	0
Streptococcus pyogenes/Corynebacterium sp.	6	4	2	0
Staphylococcus aureus	6	0	6	0
Prevotella nigrescens/intermedia	4	4	0	0
Prevotella sp.	4	4	0	0
Granulicatella adiacens	3	3	0	0
Lactobacillus (para)gasseri	3	3	0	0
Lactobacillus fermentum	3	3	0	0
Streptococcus anginosus/intermedius	3	2	1	0
Alloprevotella tannerae	2	2	0	0
Bacteroides zoogleoformans / Barnesiella merdipullorum	2	2	0	0
Bulleidia sp.	2	2	0	0
Stenotrophomonas maltophilia	2	0	2	0
Streptococcus anginosus	2	2	0	0
Eikenella corrodens	2	2	0	0
Streptococcus agalactiae	2	2	0	0
Staphylococcus epidermidis	2	2	0	0
Alistipes group	1	1	0	0
Prevotella intermedia/pallens	1	1	0	0
Prevotella melaninogenica/jejuni	1	1	0	0
Prevotella oralis	1	0	0	1
Prevotella oris	1	1	0	0
Dialister pneumosintes	1	1	0	0
Limosilactobacillus vaginalis	1	1	0	0
Haemophilus influenzae	1	1	0	0
Haemophilus parainfluenzae	1	1	0	0
Lautropia mirabilis	1	1	0	0
Moraxella catarrhalis	1	1	0	0
Neisseria subflava group	1	1	0	0
Proteus vulgaris/penneri	1	0	1	0
Pseudomonas putida	1	0	0	1
Streptococcus ilei group	1	1	0	0
Streptomyces microflavus	1	1	0	0
Turicibacter sanguinis	1	1	0	0
Streptococcus constellatus	1	1	0	0
Klebsiella pneumoniae complex / Enterobacter cloacae complex	1	1	0	0
Citrobacter koseri/farmeri	1	1	0	0
Staphylococcus hominis	1	1	0	0
Total	125	95	27	3
% of total results		76%	21.6%	2.4%

Table 4: Sequencing results of the extra Molecular Culture ID detections. Inconclusive: the sequencing result was insufficiently clear to draw any conclusion.

12.14.5 DISCUSSION

In the present study, we evaluate the performance of Molecular Culture ID for diagnosis of pleural infection in a set of 503 samples. Molecular Culture ID showed a detection PPA of83.3% (95% CI 71.7 to 91.1%) and negative percent agreement (NPA) was 77.1% (95% CI 72.7 to 81.3%) to SOC at the sample level. The PPA at species-level identification was better for monomicrobial infections (60.0%) than for polymicrobial samples (42.4%), with overall PPA of 58.9% (95% CI 47.7 to 70.1%). Upon exclusion of low-load contaminants, the species identification PPA was 67.2% (95% CI 55.4 to 79.0%).

Molecular Culture ID yielded 125 additional bacterial detections that were not reported by SOC. 76% of these detections were confirmed by amplicon sequencing or qPCR. Interestingly, Molecular Culture ID detected several pathogens, such as *Haemophilus influenzae,* that were missed by SOC (Table 3).

The most discordant outcomes between SOC and Molecular Culture ID were the *Staphylococcus epidermidis* identifications. *S. epidermidis* occurs as both an important infectious agent and a common contaminant in bacterial cultures, complicating the clinical interpretation of detections. Additionally, the input for Molecular Culture ID (50-200 µl) was much lower than the SOC input (900 µl) explaining why low-load SOC results may be missed by Molecular Culture ID.

12.15 Clinical performance of Molecular Culture ID in drain fluid and pus

12.15.1 Detection of bacteria by SOC and Molecular Culture ID

The presence or absence of bacteria was scored as positive/negative for each sample. Samples were scored as polymicrobial when two or more different bacterial species were reported. 574 samples derived from 452 unique patients were analyzed. Sample characteristics are shown in Table 1. Overall, 335 out of 574 (58.4%) samples were found positive by SOC, whereas 391 (68.1%) were positive by Molecular Culture ID. 304 samples were found positive by both methods (53.0%). PPA between Molecular Culture ID and SOC was 90.7% (95% CI 87.6 to 93.9%) and NPA was 63.6% (95% CI 57.5 to 69.7%) at the sample level. Positivity rate for Molecular Culture ID over SOC was (391/335) 1.17x higher.

TABLE 1 Sample Characteristics

	SOC	Molecular Culture ID	Concordant
Positive	335	391	304
Monomicrobial	182	129	
Polymicrobial	153	262	
Negative	239	183	152
Total	574	574	456

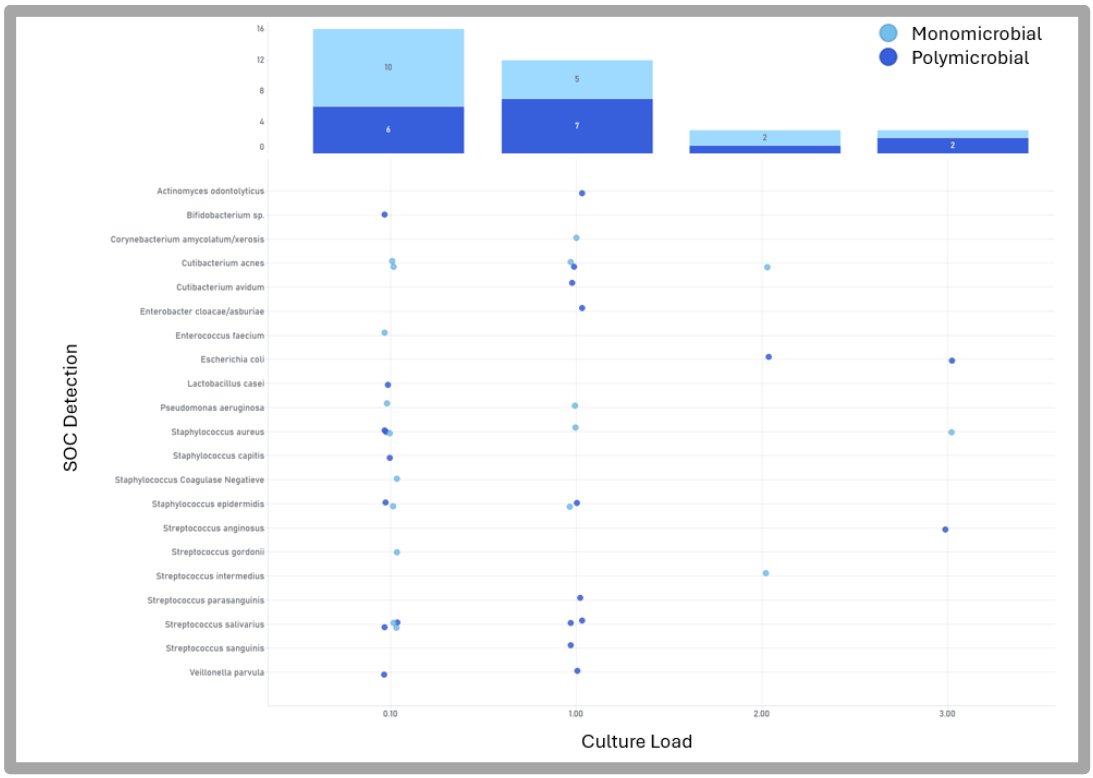
12.15.2 Bacterial detection discrepancy

Thirty-one samples were SOC positive and Molecular Culture ID negative. Twenty-four of these samples contained only very low or low loads. Eight samples were monomicrobial and low-load positive for common skin bacteria, such as *Cutibacterium acnes* and *Staphylococcus epidermidis*, which could indicate contamination (Figure 1). Following removal of these contaminants, sample level PPA was 93.0% (304/327) and positivity rate was 1.2x.

Molecular Culture ID detected 87 additional positive samples compared to SOC. Fourty-seven (54%) of these samples had high or medium levels of leukocytes, indicative of a true infection.

FIGURE 1 Missed bacterial detections by Molecular Culture ID.

The scatterplot represents the missed detections per species by SOC load. The total count of missed detections per SOC load is represented in the bar plot. The colours indicate whether the sample was polymicrobial or monomicrobial in SOC.



12.15.3 Identification of bacteria by SOC and Molecular Culture ID

All species detected by both methods are shown in Table 2. 314 of the 553 SOC identifications were concordant with Molecular Culture ID identifications, yielding a PPA of 56.8% (95% CI 52.6 to 60.9%). Excluding Unknowns, Molecular Culture ID yielded 469 additional bacterial detections compared to SOC (Table 2).

TABLE 2 Identified species count per patient by SOC and Molecular Culture ID (MC-ID)

Species	SOC count	MC-ID count	Concordant	Missed	Extra
Escherichia coli/Shigella spp.	83	85	66	19	17
Enterococcus faecalis	76	63	49	14	27
Enterococcus faecium	56	49	37	12	19
Klebsiella pneumoniae complex / Enterobacter cloacae complex	48	50	37	13	11
Staphylococcus aureus	38	37	26	11	12
Pseudomonas aeruginosa	17	3	3	0	14
Staphylococcus epidermidis/Streptococcus sanguinis	21	15	6	9	15
Streptococcus bovis group/*Streptococcus intermedius*	16	39	6	33	10
Streptococcus anginosus/mutans	15	0	0	0	15
Klebsiella aerogenes/oxytoca	13	15	9	6	4
Streptococcus pneumoniae/mitis group	11	30	6	24	5
Lactobacillus sp.	9	0	0	0	9
Citrobacter freundii complex / Raoultella ornithinolytica/planticola	8	2	2	0	6
Cutibacterium acnes	8	32	2	30	6
Serratia marcescens	8	4	4	0	4
Citrobacter koseri/farmeri	7	6	6	0	1
Streptococcus constellatus	7	10	5	5	2
Citrobacter freundii complex	6	13	6	7	0
Staphylococcus haemolyticus	6	2	1	1	5
Stenotrophomonas maltophilia	6	4	4	0	2
Lactobacillus rhamnosus/zeae/acidophilus	5	8	5	3	0
Proteus vulgaris/penneri	4	1	1	0	3
Staphylococcus lugdunensis	4	2	2	0	2
Streptococcus agalactiae	4	6	3	3	1
Aerococcus urinae	3	2	2	0	1
Bacteroides fragilis group	3	15	1	14	2
Enterococcus avium	3	2	0	2	3
Finegoldia magna	3	15	2	13	1
Haemophilus influenzae	3	2	2	0	1
Haemophilus parainfluenzae	3	16	0	16	3
Proteus mirabilis	3	3	3	0	0
Streptococcus anginosus/intermedius	3	4	3	1	0
Streptococcus dysgalactiae	3	3	2	1	1
Streptococcus pyogenes/Corynebacterium sp.	3	4	3	1	0
Veillonella parvula	3	0	0	0	3
Actinomyces odontolyticus	2	0	0	0	2
Bacillus spp./Parvimonas micra	2	1	1	0	1
Clostridium perfringens	2	17	1	16	1
Corynebacterium striatum	2	2	1	1	1
Fusobacterium necrophorum	2	4	1	3	1
Lactobacillus casei/paracasei	2	3	1	2	1
Morganella morganii	2	0	0	0	2

Prevotella buccae	2	3	0	3	2
Abiotrophia defectiva	1	1	0	1	1
Acinetobacter baumannii complex / Achromobacter xylosoxidans/denitrificans	1	0	0	0	1
Acinetobacter lwoffii	1	0	0	0	1
Actinomyces radingae	1	0	0	0	1
Actinomyces sp.	1	0	0	0	1
Aeromonas sobria	1	0	0	0	1
Bacillus cereus	1	0	0	0	1
Bacteroides ovatus	1	0	0	0	1
Bacteroides vulgatus	1	14	1	13	0
Bifidobacterium sp.	1	0	0	0	1
Citrobacter amalonaticus	1	0	0	0	1
Citrobacter sp.	1	0	0	0	1
Clostridium difficile	1	0	0	0	1
Clostridium tertium	1	1	0	1	1
Corynebacterium amycolatum/xerosis	1	3	0	3	1
Cutibacterium avidum / Mycobacterium sp.	1	1	0	1	1
Enterococcus sp.	1	0	0	0	1
Fusobacterium nucleatum	1	0	0	0	1
Hafnia alvei	1	0	0	0	1
Lactobacillus fermentum	1	13	1	12	0
Pediococcus pentosaceus	1	2	0	2	1
Prevotella denticola	1	7	1	6	0
Salmonella enterica	1	6	1	5	0
Staphylococcus capitis	1	0	0	0	1
Staphylococcus caprae	1	2	1	1	0
Staphylococcus Coagulase Negatieve	1	0	0	0	1
Streptococcus sp.	1	0	0	0	1
Veillonella sp.	1	0	0	0	1
Aerococcus viridans	0	1	0	1	0
Aggregatibacter aphrophilus	0	1	0	1	0
Alistipes group	0	19	0	19	0
Alloprevotella tannerae	0	1	0	1	0
Anaerococcus vaginalis	0	4	0	4	0
Bacillus coagulans	0	2	0	2	0
Bacillus smithii	0	3	0	3	0
Bacteroides caccae	0	2	0	2	0
Bacteroides eggerthii	0	1	0	1	0
Bacteroides thetaiotaomicron	0	2	0	2	0
Bacteroides zoogleoformans / Barnesiella merdipullorum	0	2	0	2	0
Barnesiella merdipullorum	0	3	0	3	0
Bordetella pertussis/holmesii	0	1	0	1	0
Bulleidia extructa	0	2	0	2	0
Bulleidia sp.	0	4	0	4	0
Capnocytophaga sputigena	0	1	0	1	0
Clostridium innocuum	0	6	0	6	0
Corynebacterium coyleae	0	1	0	1	0
Cutibacterium granulosum	0	1	0	1	0
Dialister micraerophilus	0	1	0	1	0
Dialister pneumosintes	0	2	0	2	0
Enterococcus cecorum	0	5	0	5	0

Enterococcus gallinarum	0	1	0	1	0
Eubacterium limosum	0	2	0	2	0
Gemella morbillorum/haemolysans	0	4	0	4	0
Granulicatella adiacens	0	2	0	2	0
Haemophilus influenzae / Pseudomonas aeruginosa	0	1	0	1	0
Haemophilus parahaemolyticus	0	1	0	1	0
Lactobacillus (para)gasseri	0	11	0	11	0
Lactobacillus crispatus	0	2	0	2	0
Lactobacillus iners	0	3	0	3	0
Lactobacillus jensenii	0	1	0	1	0
Lactococcus lactis	0	1	0	1	0
Lautropia mirabilis	0	2	0	2	0
Limosilactobacillus vaginalis	0	3	0	3	0
Moraxella catarrhalis	0	1	0	1	0
Moraxella lacunata	0	1	0	1	0
Odoribacter splanchnicus	0	4	0	4	0
Peptostreptococcus anaerobius	0	3	0	3	0
Prevotella bivia	0	2	0	2	0
Prevotella disiens	0	3	0	3	0
Prevotella histicola	0	6	0	6	0
Prevotella intermedia	0	3	0	3	0
Prevotella intermedia/pallens	0	2	0	2	0
Prevotella melaninogenica	0	9	0	9	0
Prevotella melaninogenica/jejuni	0	1	0	1	0
Prevotella nigrescens/intermedia	0	8	0	8	0
Prevotella oris	0	1	0	1	0
Prevotella sp.	0	3	0	3	0
Prevotella veroralis	0	2	0	2	0
Pseudomonas oryzihabitans	0	2	0	2	0
Pseudomonas putida	0	3	0	3	0
Rothia mucilaginosa/aeria	0	3	0	3	0
Sarcina ventriculi	0	1	0	1	0
Staphylococcus schleiferi	0	1	0	1	0
Staphylococcus sciuri	0	1	0	1	0
Streptococcus ilei group	0	1	0	1	0
Streptococcus mutans	0	1	0	1	0
Unknown Bacteroidetes	0	90	0	90	0
Unknown FAFV	0	186	0	186	0
Unknown Proteobacteria	0	103	0	103	0
Total	553	1162	314	848	239

12.15.4 DISCUSSION

In the present study, we evaluate the performance of Molecular Culture ID for diagnosis of pus, bile, drain fluids, and punctates in a set of 574 samples across 452 unique patients. Molecular Culture ID showed a detection PPA 90.7% (95% CI 87.6 to 93.9%) NPA of 63.6% (95% CI 57.5 to 69.7%) to SOC at the sample level. Molecular Culture ID identified 87 additional positive samples compared to SOC.

Molecular Culture ID yielded 469 additional bacterial detections that were not reported by culture. Overall, Molecular Culture ID outperformed SOC in terms of breadth of species detection, detecting 107 unique species as compared to the 72 bacterial species reported in SOC. At the species-level, PPA was 56.8% (95% CI 52.6 to 60.9%).

In conclusion, the study demonstrates that Molecular Culture ID surpasses SOC in the diagnosis of pus, bile, drain fluids, and punctates, exhibiting a higher detection at the sample level, identifying additional positive samples and bacterial species, thus enhancing the breadth of diagnostic outcomes.

13 References

Budding et al., (2010). IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota, FASEB J. 2010 Nov;24(11):4556-64.
Budding et al., (2016). Automated broad range molecular detection of bacteria in clinical samples, JClin Microbiol. 2016 Apr;54(4):934-43.
Bos et al., (2023). Rapid Diagnostics of Joint Infections Using IS-Pro, JClin Microbiol.2023 61(6): e0015423.

List of symbols as used in labeling


Manufacturer

Catalogue number

Batch code (Lot)


Expiration date


Temperature limitation

Contains sufficient for <n> tests


Consult Instructions for Use

Contents


Contains hazardous substance (Read MSDS eMix)


Danger. May damage fertility or the unborn child. Use personal protective equipment as required. If exposed or concerned: Get medical attention/advice. (Read MSDS eMix)

List of Abbreviations

ABI	**ABI Genetic Analyser**
CE-machine	**Capillary Electrophoresis Machine**
DNA	**Deoxyribonucleic acid**
DNAse	**Deoxyribonuclease**
EDTA	**Ethylenediaminetetraacetic acid**
FAFV	**Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia**
IC	**Internal Control**
IS	**Interspace**
IS-pro™	**Interspace Profiling**
MSDS	**Material Safety Data Sheet**
NaCl	**Sodium Chloride**
Nc	**Nucleotide**
NPA	**Negative percent agreement**
PC	**Positive Control**
PCR	**Polymerase Chain Reaction**
PPA	**positive percent agreement**
rDNA	**ribosomal DNA**
RNAse	**Ribonuclease**
SOC	**Standard of care**

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REVISION HISTORY

Version	Revision date	Description
Rev 01	Oktober 2015	initial release
Rev 02	May 2016	Section 3 and 6.2 rephrased for more clarity
		Section 4: precautions to use sterile Dnase/Rnase free aerosol resistent pipette tips was added
		Minor formatting changes and typograpical errors corrected
		Company name corrected to current status. IS Diagnostics to IS Diagnostics Ltd.
Rev 03	June 2016	Section 4.2 and 9.6: validated PCR machines added
		Minor formatting changes
Rev 04	June 2017	Section 2: Summary and explanation of the test was changed to Product description
		Section 2: Validated sample types added per extraction machine
		Reorganising sections of 'Warning and Precausions', 'Storage'
		Section 4: Shipping conditions added
		Section 4: Freeze/Thaw cycles added
		Section 4: Protect all components to light was added
		Section 5: Warning and precausions; classification of emix is updated to regulation (EC) No 1272/2008 (CLP)
		Section 6.1: Machine protocol; incubation for 1h extra for drain fluid
		Section 7: Instructions about thawing components adjusted to keep on ice when in use
		Section 7.2: Centifuging time and speed added
		Section 7.2: Use an appropiate cover for the ABI plate and replacing the septa
		Section 7.3: Advice about filenameconvention of .fsa files adjusted
		Section 8: Quality control: Information added that quality criteria results are obtained with IS-Diagnostics software service
		Section 8: Negative control criteria For Firmicutes and Bacteroidetes >145 updated from 100IFU to 500 IFU
		Section 10: Information added that performance characteristics are obtained with IS-Diagnostics software service
		Section 10.6: Validated PCR machine added: Tadvanced Thermal Cycler (Biometra)
		Shipping conditions changed from -78°C to dry ice.
		List of symbols as used in labelling added, manufacturer, catalogue number, batch code /lot), Use by date, temperature limitation, contains sufficinet for <n> tests, consult instructions for use, danger
		Legal manufacturer; internal address added
		Minor formatting changes and typograpical errors corrected
Rev 05	May 2019	Company name changed to inbiome
		Section 5: Warnings and precaucians: NOTICE: any serious incident that has occurred in relation to the device must be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established. Was added
		Section 7: Remark to keep reaction tubes cooled/on ice during procedure
		Section 7.2: The model of the ABI machine was specified to 3500
		Section 8: Clarification that 2 controls are provided per kit.
		Section 8: Criteria for internal control adjusted
		Section 8: a Note was added to verify low E. coli signals with external assay
		Section 9: a limitation was added that Molecular Cultrue has a reduced amplification efficiency for Actiomycetes
Rev 06	May 2022	New version of the product developed

		Update in intended use: to use in clinical diagnostics in humans. Clarification about the test being semi-automated is added. Cerebrospinanal fluids were removed as sample due to limited samples available for validation.
		Update in product description: Machines to use in combination with the product updated
		Kit components updated
		Section 3.2 added: materials not included in Molecular Culture ID kit
		Section 4: In use conditions updated
		Section 4: Clarification about the storage locations of kit components in different areas
		Sections 5: Precautionary statements of eMix added
		Sections 6.1: Reagents for sample preparation updated
		Section 6.1: Sample preparation updated
		Section 6.1: Machine protocol updated
		Section 7.1: Procedure PCR updated
		Section 7.2: Procedure (ABI) CE machine updated
		Section 7.3: Analysing data; Data analysis platform changed to Molecular Lab Cloud
		Section 7.3: Change from genera which can be identified to phyla that can be identified.
		Section 7.3: Clarification about species not being in the database will be identified on phylum level.
		Section 8: Change of fluorescent dyes in use
		Section 8: Quality control: Inhibition criteria updated
		Section 9: Limitations of the procedure: a limitation was removed that Molecular Cultrue has a reduced amplification efficiency for Actinomycetes
		Section 10: Update of performance characteristics due to new validations performed for the new product
		List of symbols as used in labelling: Directive 98/79/ EC remove because not in force anymore
		Branding
Rev 07	April 2023	Small structural changes in content
		amount of emix tubes in kit corrected to one
		Bacterial Shock Buffer 2 name corrected
		Section 6: Extraction volume corrected for Synovial Fluid/ Joint Aspirate
		Section 7: Information added about varivied PCR machines
		Section 7: Informatin added to run CE-machine according the settings described
		Section 7: Sizecalling information added
		Section 7: Seqstudio settings clarified
		Renaming Molecular Lab Cloud to Molecular Lab Cloud/antoni
Rev 08	March 2024	Mayor revision for IVDR (send in to the notified body)
Rev 08.01	Sept 2024	Updates due to IVDR round one
		Link added for the Instructions for Use,
		Link added for the Safety Data Sheets
		Location for link added for the Summary of Safety and Performance
		Software location added
		Customer Technical Support email added
		Link added for complaints and improvement
		Freeze thaw cycles of all components of the kit changed to two freeze/thaw cycles
		Storage temperature of all kit components changed from -20°C to 'between -25°C and -15°'

		Section 3.2: materials not included in Molecular Culture ID kit updated
		Section 9.3: a note added for potential misidentification of rare bacteria due to limitations in the reference library.
		Section 10.10: a limitation added added for potential misidentification of rare bacteria due to limitations in the reference library.
		Section 11.4: Anaerococcus prevotii added for potential cross-reactivity
		Section 12.1: Intented patient population changed to patient population
Rev 08.02	Okt 2024	Updates due to IVDR round two
		Searchoption added for Summary of Safety and Performance on the Eudamed website.
		Section 10:11 a limitation added for bacteria lower than Limit of Detection having a high risk of not being detected
		Section 6: Information added for maximum storage time of frozen samples.
Rev 08.03	Dec 2024	Section 8 Sample preparation: Procedure biopsies adjusted
Rev 08.04	Dec 2024	List of symbols as used in labeling updated with new symbol to align with eMix tubes
Rev 08.05	Dec 2024	List of symbols as used in labeling updated with new symbol to align with eMix tubes. Reviewed and also old symbol included for danger.
Rev 08.06	Jan 2025	Cross reactivity with *Odoribacter splanchnicus* added.
Rev 08.07	Jan 2025	Cross reactivity with *Cereibacter sphaeroides, Faecalibacterium prausnitzii added.*
Rev 09	Aug 2025	URL to online software changed
Rev10	Aug 2025	Updated ANNEX I Bacteria list Molecular Culture ID to reflect current software version. This version of the bacteria list has been utilized for updating the clinical performance reports . Removed the following species from previous published version: • *Acinetobacter haemolyticus* • *Acinetobacter ursingii* • *Aeromonas hydrophila subsp. hydrophila* • *Aeromonas hydrophila subsp. ranae* • *Aeromonas spp* • *Alcaligenes faecalis* • *Bacillus megaterium* • *Bacteroides pyogenes* • *Bordetella trematum* • *Brevundimonas diminuta* • *Brevundimonas vesicularis* • *Brevundimonas spp* • *Burkholderia gladioli* • *Burkholderia multivorans* • *Burkholderia sp.* • *Buttiauxella agrestis* • *Campylobacter fetus* • *Campylobacter lari* • *Campylobacter rectus* • *Cedecea lapagei* • *Clostridium cadaveris* • *Clostridium clostridioforme* • *Clostridium ramosum* • *Clostridium sordelii* • *Comamonas testosteroni* • *Corynebacterium glucuronolyticum* • *Corynebacterium pseudodiphtheriticum* • *Corynebacterium pseudotuberculosis* • *Corynebacterium spp* • *Cronobacter sakazakii* • *Eggerthella lenta*

		• *Fusobacterium mortiferum* • *Fusobacterium spp* • *Fusobacterium varium* • *Haemophilus spp* • *Halomonas sp.* • *Kluyvera intermedia* • *Leclercia adecarboxylata* • *Leuconostoc spp* • *Listeria rocourtiae* • *Mycobacterium haemophilum* • *Mycobacterium kansassii* • *Myroides spp* • *Neisseria lactamica* • *Nocardia abscessus* • *Nocardia cyriacigeorgica* • *Nocardia spp.* • *Ochrobactrum anthropi* • *Pantoea spp* • *Porphyromonas gingivalis* • *Prevotella baroniae* • *Prevotella loescheii* • *Pseudomonas fluorescens* • *Pseudomonas lactis* • *Pseudomonas spp* • *Rahnella aquatilis* • *Rhizobium radiobacter* • *Sphingomonas sp.* • *Staphylococcus carnosus* • *Staphylococcus chromogenes* • *Staphylococcus cohnii* • *Staphylococcus equorum* • *Staphylococcus hyicus* • *Staphylococcus intermedius* • *Staphylococcus lentus* • *Staphylococcus xylosus* • *Streptococcus equinis* • *Streptococcus gwangiuense* • *Tannerella forsythensis* • *Vibrio vulnificus*
Rev 11	Sept 2025	Safety precautions; added information about guanidine salts
		Limitations of the procedure added: clarified species outside scope
		Updated sizecalling information for seqstudio Flex.
		Cross reactivity updated based on current bacteria list Annex I
Rev 12	Okt 2025	Typographical correction

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 animalis subsp. lactis	
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	*Salmonella enterica*
Salmonella enterica subsp. choleraesuis	
Salmonella enterica subsp. enteritidis	
Salmonella enterica subsp. paratyphi	
Salmonella enterica subsp. typhi	
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 subsp. gallolyticus	
Streptococcus gallolyticus subsp. macedonicus	
Streptococcus gallolyticus subsp. pasteurianus	
Streptococcus infantarius subsp. coli	
Streptococcus infantarius subsp. infantarius	
Streptococcus intermedius	
Streptococcus pasteurianus	
Streptococcus salivarius	
Streptococcus thermophilus	
Streptococcus vestibularis	
Streptococcus constellatus	
Streptococcus criceti	*Streptococcus cristatus/criceti*
Streptococcus cristatus	
Streptococcus dysgalactiae	
Streptococcus equi subsp. zooepidemicus	
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	