Molecular Culture ID kit

CE

IVD

Complies with the Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices

For In Vitro Diagnostic Use Only

Test Instructions

Amplification DNA assay for the semiquantitative in vitro detection of bacterial species from the phyla *Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia, Bacteroidetes,* and *Proteobacteria* in pus, punctates, drain fluids, ascites fluids, joint aspirates, and tissues from humans.

Catalogue Number: MolCul 15000, 24 tests

Store at -20°C upon receipt

Content

1. Intended use

Molecular Culture ID is intended to be used for the detection and identification of bacteria isolated from pus, punctuates, drain fluids, ascites fluids, joint aspirates, and tissue samples, for use in clinical diagnostics in humans. Bacteria can be relatively quantified, based on their proportion of the total bacterial population within a specimen. The total bacterial signal in a sample is correlated to absolute quantification, which makes the results (i.e. bacterial species present in the sample) semi-quantitative.
The intended user will be a specialized molecular diagnostic laboratory. The test will be carried out by trained laboratory personnel. The test is semi-automated, as it requires some minor manual work on sample preparation pre- and inbetween automated processes. No special training will be required for professional routine diagnostic laboratories performing semi-quantitative PCR.

2. Product Description

Molecular Culture ID is based on the IS-pro™ technology. IS-pro™ is a bacterial profiling technique based on species-specific length polymorphisms of the IS 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 is used to control for PCR inhibition and efficiency.
A marker is added to the eMix as a length reference to determine the lengths of the PCR amplicons.

The IS region is amplified with fluorescently labeled phylum-specific primers targeting the 16S rDNA and unlabeled primers targeting the 23S rDNA. The Molecular Culture ID reaction yields peak profiles that provide two levels of information: the color of fragments sorts species into phyla and fragment lengths can be used to further identify bacteria to the genus, species, or subspecies level.

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™
Ascites fluid
Drain fluid
Joint aspirate
Punctate
Pus
Tissue

PCR amplification can be carried out with any validated/verified PCR machine. See instructions for use Molecular Culture Starter Kit for validation. For separation and detection of the PCR amplicons, an ABI Genetic Analyzer 3500 and 3500XL are validated.

A uni-directional workflow in the laboratory is strongly recommended:
Sample Preparation area: Dedicated area to prepare the samples. All materials (equipment, supplies, protection, gloves, etc.) have to 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.) have to be dedicated to this area.
Amplification area: Dedicated area for amplification. All materials (equipment, supplies, protection, gloves, etc.) have to 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 Specimen Preparation Area.

3. Molecular Culture ID kit components

3.1 Included in Molecular Culture ID kit

Mastermix FIRBAC
2 vials, labeled “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
2 vials labeled “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
2 vials labeled “Positive Control Firbac” containing 30 µl positive control. The positive control contains the bacterial DNA of *Firmicutes/Bacteroidetes*.

Positive Control PROTEO
2 vials labeled “Positive Control Proteo” containing 30 µl positive control. The positive control contains the bacterial DNA of *Proteobacteria*.

eMix
1 vial labeled “eMix” containing 480 µl eMix.

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 machine
- 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

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 https://antoni-research.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 tubes have been compromised during shipment, please contact inbiome.
- All components should be stored at -20°C upon arrival.
- Repeated thawing and freezing of Mastermixes (more than nine times), positive control (more than nine times), and eMix (more than nine 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

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.

A uni-directional workflow must be adhered to in the laboratory with different areas for sample preparation, pre-amplification, and post-amplification.

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

Use sterile, DNase-RNase-free, aerosol resistant pipette tips and wear protective gloves. Protect kit contents or generated PCR product from direct sunlight.

For handling eMix work in a well-ventilated area, 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 product 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 MSDS 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 collection and preparation

General collection devices can be used for standard DNA isolation procedures according to the manufacturer's protocols. This procedures must be performed in the Sample Preparation Area in a class 2 Biological Safety Cabinet (protection to user and material). Include a blank solution for every isolation run to control for contamination.

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.

6.1 Sample preparation validated for BioMérieux NucliSENS® easyMAG™:

Reagents
- Bacterial Shock Buffer 1 (IBB23000, inbiome)
- Bacterial Shock Buffer 2 (IBB24000, inbiome)
- BioMérieux NucliSENS® easyMAG™ reagents
- Zirconia/Silica beads 0.1mm (11079101Z, BioSpec)
- Qiagen AL buffer (19075, Qiagen)
- UltraPure™ DNase/RNase-Free Distilled Water (10977035, Invitrogen)

Sample preparation

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 at least for 10 minutes at room temperature.
- Remove visible particles with a sterile toothpick or by pipet.
- Add 70 ul 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 eluate volume.

Store DNA at 2-8 °C.

7. Molecular Culture ID Kit Test Procedure

This procedure must be performed in the Pre-Amplification Preparation Area. Use 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.

7.1 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. One Firbac and one Proteo reaction. NOTE: The first run of each kit should contain a Positive Control Firbac and Positive Control Proteo. 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 and 15 µl of Mastermix Proteo to the second reaction tube.

3. Vortex and spin down all DNA extracts. Add 10 µl DNA (Chapter 6) 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, spin down for 30 seconds at approximately 1000 rpm.

5. Place the reaction tubes/plate into the for Molecular Culture varified thermocycler and program the system as listed below:

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

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

7.2 Procedure Capillary Electrophoresis

1. Thaw and vortex eMix.
2. Per sample, two PCR reactions were performed previously (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. NB: 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. Put 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 can occur.
5. Replace the plate-cover with ABI septa
6. Store plate at 2-8°C until capillary electrophoresis. The plate can be stored for five days at 2-8°C in the dark. Capillary electrophoresis is started using the settings described in the tables below:
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 Treshold 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

* Molecular Lab Cloud /antoni (inbiome's free software service) does not require a sizecalling protocol because it uses raw data from the .fsa . but 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
FragmentAnalysis50_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 Treshold 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

* Molecular Lab Cloud /antoni (inbiome's free software service) does not require a sizecalling protocol because it uses raw data from the .fsa . but 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	4500 sec	11.0 kVolts	180 sec	15 kVolt	45 sec	3 kVolts	200 sec

Settings SeqStudio

Instrument protocol SeqStudio									
Application type:	Dye Set								
Fragment analysis	G5								
Instrument Protocol Properties									
Run Module	Capillary Temperature	Run Time	Run voltage	Run Ramp Duration	PreRun Time	PreRun Voltage	Injection Time	Injection Voltage	Data Delay
adjusted from LongFragAnalysis	60°C	5960 sec	4.25 kVolts	300 sec	180 sec	13 kVolt	18 sec	1.2 kVolts	350 sec

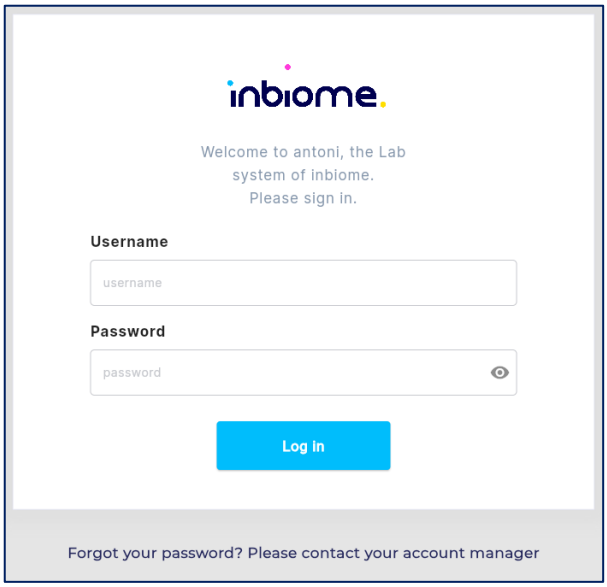
7.3 Analysing data

With Molecular Lab Cloud/ antoni >250 bacterial species from the phyla *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Bacteroidetes* and *Proteobacteria* can be identified. Species that are not in the current database are identified to this phylum level. The database is regularly updated. The most recent list of identifiable species is to be requested via techsupport@inbiome.com

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.

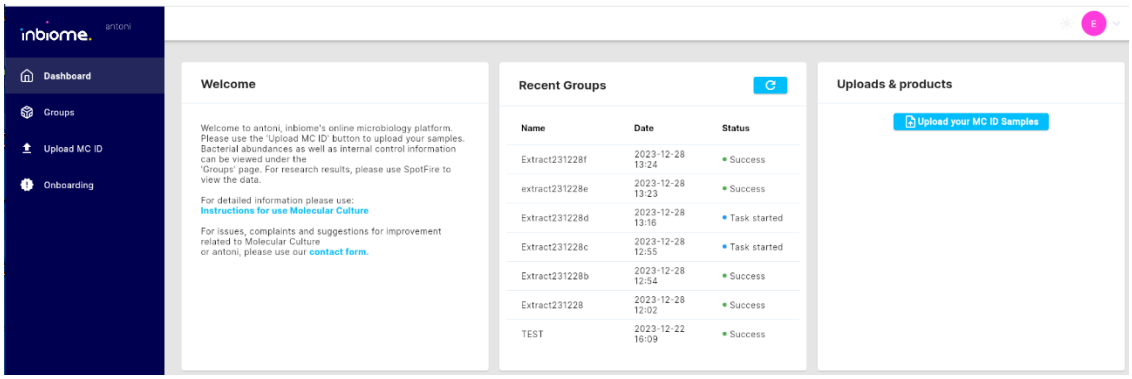
Note: If multiple antoni users need to share information across departments, studies or institutes, you have the option to request ‘Group studies’. The request for ‘Group studies’ is possible via techsupport@inbiome.com. Please provide us with the department name or official acronym and the full name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

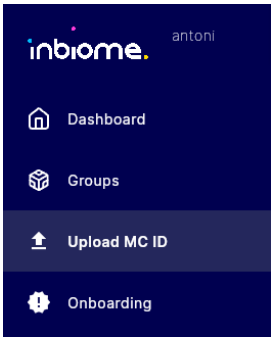
The top right corner contains two icons:

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.

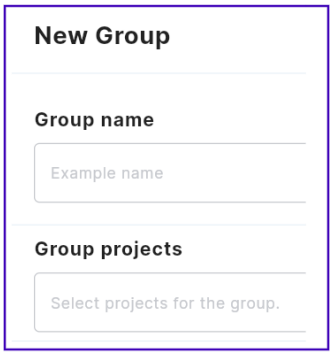
The main body of the dashboard contains three parts:

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

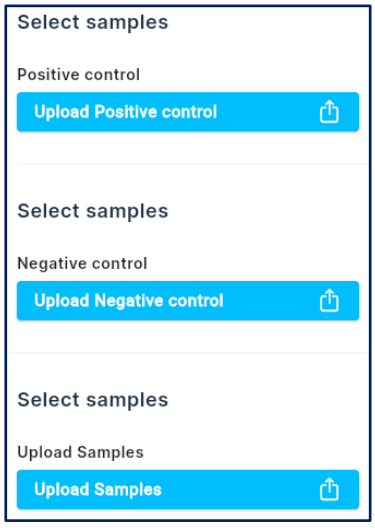


Uploading files for analysis is done through the “Upload MC ID” tab.
Once on this page, users can upload multiple fsa files from the capillary electrophoresis, corresponding to samples to be analyzed in one group. Such a group can later be viewed using the “Groups” page after analysis in antoni.



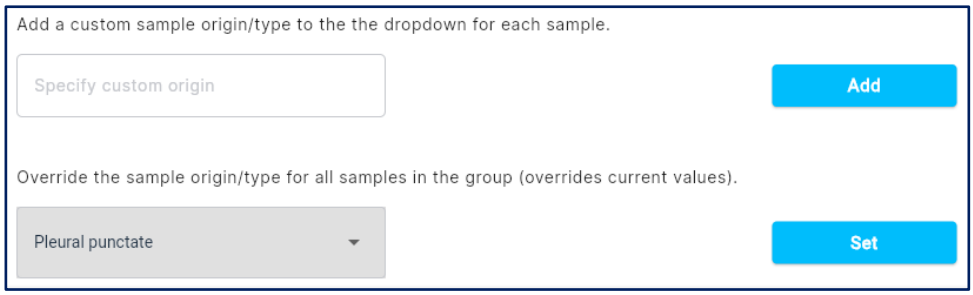
First, a **Group name** has to be provided. This can be any distinct name of your choice, facilitating easy identification of the specific batch of fsa files chosen for uploading.

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

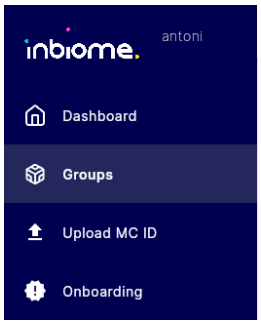
If multiple samples are selected with different sample types, an optional "Sample Origins File" can be used to automatically set the sample origin for these samples. The format of this file is described when pressing the info "**i**" button.

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

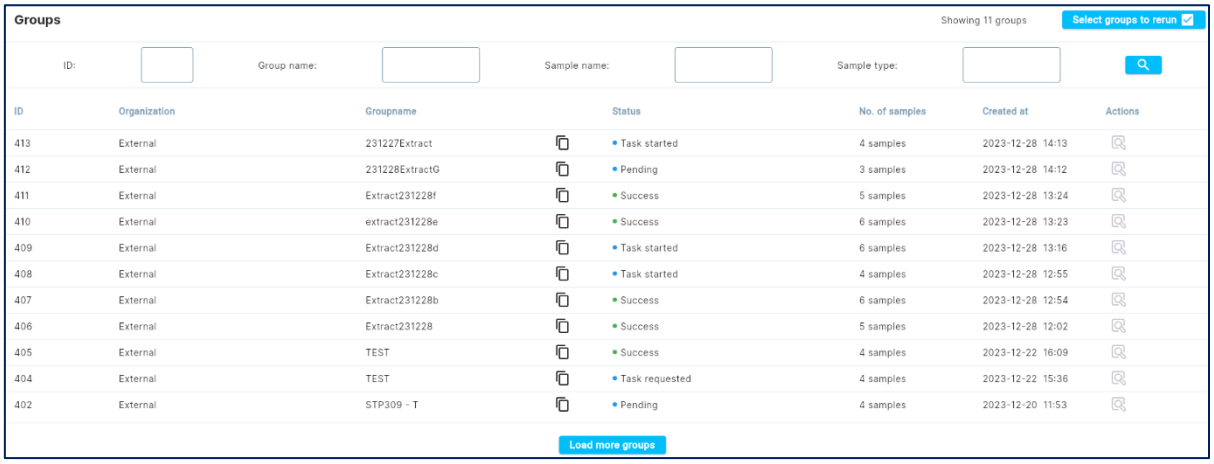
If any requirements are not met, the user will not be able to upload, and the unmet requirements are shown next to the upload button.

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:

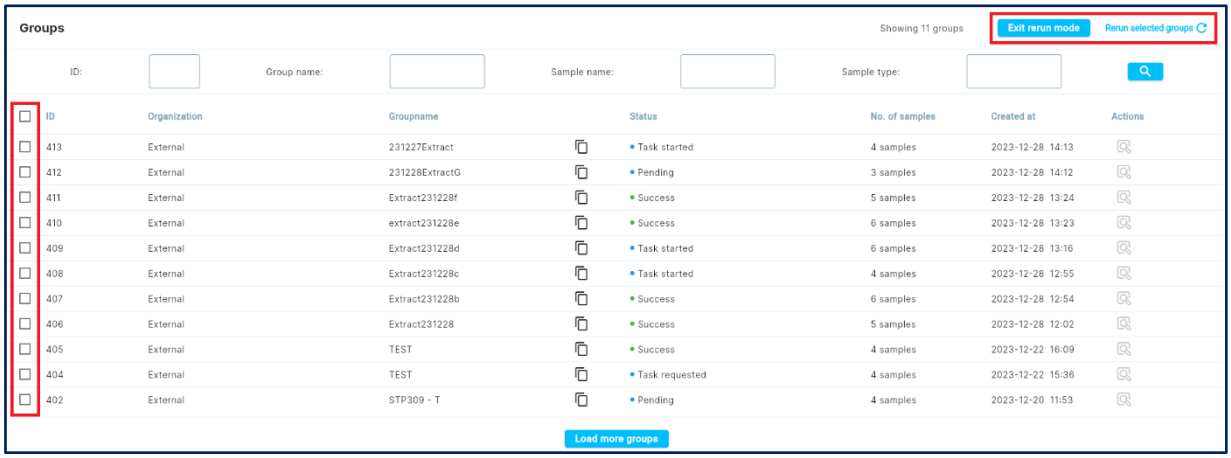
- **ID**: the internal group ID, a successive number created and used by the system.
- **Organization**: the name of the organization which uploaded the group
- **Groupname**: name of the group given by the user This column also includes an icon that allows the user to copy the groupname:
- **Status**: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

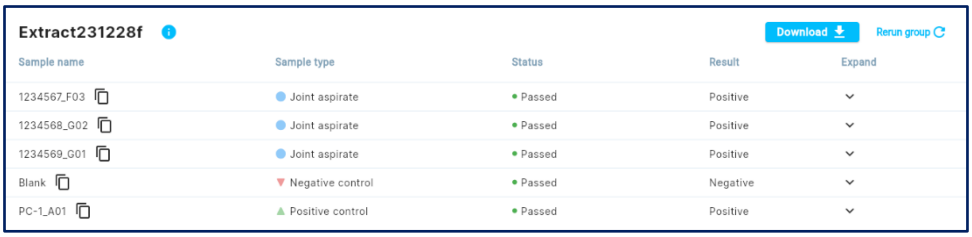
The top right corner of the page shows the number of groups currently displayed on the page. More groups can be loaded by pressing the "Load more groups" button on the bottom of the page.

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.

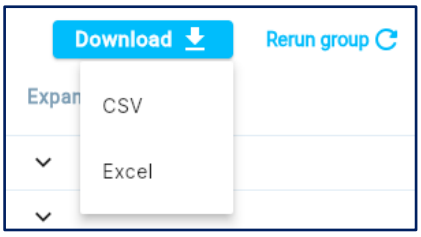


The top of the group page contains the name of the group, as well as an “i” icon which, when clicked, reveals additional metadata about the group.

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 the most recent species list.
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.

7.4 Procedural notes

1. Be extremely careful when handling materials to prevent contamination. Always mix and spin down reagents and samples before opening. In case of any suspicion of contamination, discard the materials.

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

3. Samples with equivocal results must be verified by repeat assays or isolation.

4. Do not pool reagents from different lots.

5. If the kit is damaged upon receipt, please contact your local distributor and/or inbiome.

8. 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.

4. Bacteria from other species than described in the most recent version of the species list 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. Bacteria from other species than described in Annex I are not identified on species level but only on phylum level.

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

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

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

10. 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.

11. 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

12. 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.

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

9. Performance characteristics

All results of the following performance characteristics have been obtained with Molecular Lab Cloud / antoni:

9.1 Analytical specificity

Cross-reactivity with non-bacterial organisms

The analytical specificity of the Molecular Culture ID kit 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 *Cytomegalovirus.* All tested organisms gave negative results, indicating no cross-reactivity with DNA from the tested cultured organism.

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

Cross-reactivity between *Firmicutes* (*Clostridium tertium, Peptoniphilus assacharolyticus, and Staphylococcus haemolyticus)* and *Proteoprimers* could be observed when the DNA concentration was very high (cultured bacteria), which gives the primers a chance to bind aspecifically. The *Proteopeaks* are only created when the Firmicutes peak is also present. No bacterial signal was detected in other channels than expected for *Bacteroidetes* and *Proteobacteria.* In practice, the risk of cross-reactivity between the three different phyla is very low, but if it happens in clinical samples it may be used as additional information to identify a bacterial species.

Cross-reactivity with human DNA

Cross-reactivity with human DNA can occur when human DNA load is high and bacterial DNA absent or in very low concentrations relative to human DNA. Human cross-reactivity peaks may occur alone or in various combinations. The freely available Molecular Lab Cloud can automatically detect and delete such fragments.

9.2 Analytical sensitivity

The **limit of detection** (LoD) of the Molecular Culture ID kit was determined using quantified stock cultures (by counting CFU). The table below shows the determination of the LoD with at least 95% confidence.

Limit of Detection per phylum

Phylum	Bacteria	LoD	Confidence
Firmicutes	*Streptococcus cristatus* (DSM 8249T / ATCC 13637)	5 CFU	>95%
Firmicutes	*Streptococcus bovis* (IS-I, clinical sample, no strain known)	5 CFU	>95%
Bacteroidetes	*Bacteroides fragilis* (NCTC 9343 / DSM 2151)	1 CFU	>95%
Bacteroidetes	*Bacteroides thetaiotaomicron* (VPI 5483/DSM 2079)	5 CFU	>95%
Proteobacteria	*Stenotrophomas maltophilia* (DSM 50170 / ATCC 13637)	5 CFU	>95%
Proteobacteria	*Pseudomonas aeruginosa* (ATCC 27853)	5 CFU	>95%

To determine the **dynamic range** of a bacterium in the presence of another bacterium from the same phylum. The dynamic range of the FAFV group was 1:100, the dynamic range of the *Bacteroidetes* was 1:1000 and the dynamic range of the *Proteobacteria* was 1:1000. For the overall dynamic range the lowest measured dynamic range was taken, 1:100.

9.3 Precision

The **repeatability** (within run values) was tested with one bacterium per phylum in 15 fold. Results were within the range.

Repeatability of positive control per phylum log2 transformed

	-2StDev (log2intensity)	-1StDev (log2intensity)	Average (log2intensity)
Firmicutes	14,06	14,42	14,78
Bacteroidetes	13,36	13,94	14,53
Proteobacteria	13,94	14,34	14,74

Repeatability of 10 CFU bacterial analyte per phylum log2 transformed

	-2StDev (log2intensity)	-1StDev (log2intensity)	Average (log2intensity)
Firmicutes	13,64	14,10	14,56
Bacteroidetes	12,17	12,9	13,62
***Proteobacteria* + IC**	11,26	12,17	13,07

For the **reproducibility**, the effects of the variables were evaluated for two values (different run dates, different operators, different PCR machines, and different ABI machines). Different variables were tested simultaneously. Results were within the range of 2SD (0.93 log2RFU) which has been set as a reference for reproducibility as described in 'IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota.

9.4 Accuracy

The systematic error (trueness of results) is defined as the performance of the Molecular Culture ID kit relative to culture (golden standard).

In a recent study in which 591 joint aspirate samples were tested , a total of 177 samples were positive by culture, and 213 were positive with Molecular Culture ID. 8 samples were positive with culture and negative with Molecular Culture ID. These were all likely contaminated samples with low loads of skin commensals. 44 samples were positive with Molecular Culture ID and negative with traditional culture. In most cases, clinical contest supported these additional findings. In conclusion, Molecular Culture ID outperformed traditional culture for diagnostics of infections. Combined sensitivity for Molecular Culture ID was 96% (213/221), and 80% (177/221) for traditional culture.

The entire dataset of this study can be requested via sales@inbiome.com

9.5 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	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
EDTA	10x	10x
Biomerieux Lysis buffer	100x	*All tested dilutions interfered with Proteo PCR*
96% EtOH	10x	100x
NaCl	10x	100x

9.6 Assay list

Assay Performance	
Dynamic Range	1:100
Precision - Repeatablility	Positive control, -2*stdev: *Firmicutes*: 14.06 (log2intensity), *Bacteroidetes*: 13.36 (log2intensity), *Proteobacteria*: 13.94 (log2intensity) Positive control, -1*stdev: *Firmicutes*: 14.42 (log2intensity), *Bacteroidetes*: 13.94 (log2intensity), *Proteobacteria*: 14.34 (log2intensity) 10 CFU, -2*stdev: *Firmicutes*: 13.64 (log2intensity), *Bacteroidetes*: 12.17 (log2intensity), *Proteobacteria*: 11.26 (log2intensity) 10 CFU, -1*stdev: *Firmicutes*: 14.1 (log2intensity), *Bacteroidetes*: 12.9 (log2intensity), *Proteobacteria*: 12.17 (log2intensity)
Precision - Reproducibility	2SD log2RFU(0.93) for 95% of the peaks
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.
Interference - Amplification inhibitors	Preferably monitored by internal amplification control
Sensitivity - Analytical	At least 95% sensitivity compared to culture for species included in assay
Sensitivity - Functional (peak position (nc))	±1nc
Sensitivity - Limit of Blank (RFU)	Intensity < 4000
Sensitivity - Limit of Detection (RFU)	1 to 5 CFU (>95% confidence).

Correlation study - Reference method	Culture
Correlation study - Reference standard	Clinical samples
Assay Protocol	
Sample pretreatment	Lysis + DNA isolation, (Lysis + DNA isolation reagents are not included in the kit)
Reagent pretreatment	NA
Type of sample	DNA derived from pus, punctates, drain fluids, ascites fluids, joint aspirates, tissues from humans.
Sample size limitations (w/o dead volume)	NA
Assay format	Thermocycler, ABI3500 (XL), ABI SeqStudio, ABI SeqStudio Flex
Automatic/Manual Dilution	NA
Total assay time	<5 hr
Reagents, Calibrators and Controls	
Tests per kit	24
Positive control	
A. Matrix	Liquid frozen inactivated DNA
B. Physical form	Liquid frozen
C. Stability (shelf life)*	12 months
D. Number of freeze/thaw cycles	9
E. Stability after defrosting (2-8°C)	30 minutes between every freeze/thaw step
Mastermix	
A. Matrix	Liquid frozen with Taq Polymerase and no DNA control
B. Physical form	Liquid frozen
C. Stability (shelf life)	12 months
D. Number of freeze/thaw cycles	9
E. Stability after defrosting (2-8°C)	30 minutes between every freeze/thaw step
Shipping conditions	On dry ice
Storage conditions	-20°C
Legal/Regulatory	
Intended use	Molecular Culture ID is intended to be used for the detection and identification of bacteria isolated from pus, punctuates, drain fluids, ascites fluids, joint aspirates, and tissue samples, for use in clinical diagnostics in humans. Bacteria can be relatively quantified, based on their proportion of the total bacterial population within a specimen. The total bacterial signal in a sample is correlated to absolute quantification, which makes the results (i.e. bacterial species present in the sample) semi-quantitative. The intended user will be a specialized molecular diagnostic laboratory. The test will be carried out by trained laboratory personnel. The test is semi-automated, as it requires some minor manual work on sample preparation pre- and inbetween automated processes. No special training will be required for professional routine diagnostic laboratories performing semi-quantitative PCR.

10. 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.

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

List of Abbreviations

ABI	**ABI Genetic Analyser**
DNA	**Deoxiribonucleic acid**
DNAse	**Desoxyribonuclease**
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**
PC	**Positive Control**
PCR	**Polymerase Chain Reaction**
rDNA	**ribosomal DNA**
RNAse	**Ribonuclease**

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TECHNICAL ASSISTANCE

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