

# Current Progresses of MIDASS: The European Project for an Automated Microbial Identification Instrument

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Planetary Protection knowledge Gaps for Human Extraterrestrial  
Missions

# Needs for Space (Life Support & Planetary Protection)



- Assess microbial load (bacteria & fungi) on inner side of habitable module (i.e. Air, surfaces, water, Food (?)) and surface Landers,
- Insure the microbial innocuity for the crew safety (no pathogens in the habitable environment nor in spaceship)
- Identify microbial species & communities diversity (including VBNC microbes) and study their dynamics in relation with LSS, hardware, environment, crew activities, operators and cleaning procedure,
- Study colonization and establishment of opportunistic pathogens and technophiles & resistant/pathogenic profiles
- Study the emergence of strains with higher virulence, resistance to biocides & antibiotics (technophilic capabilities)

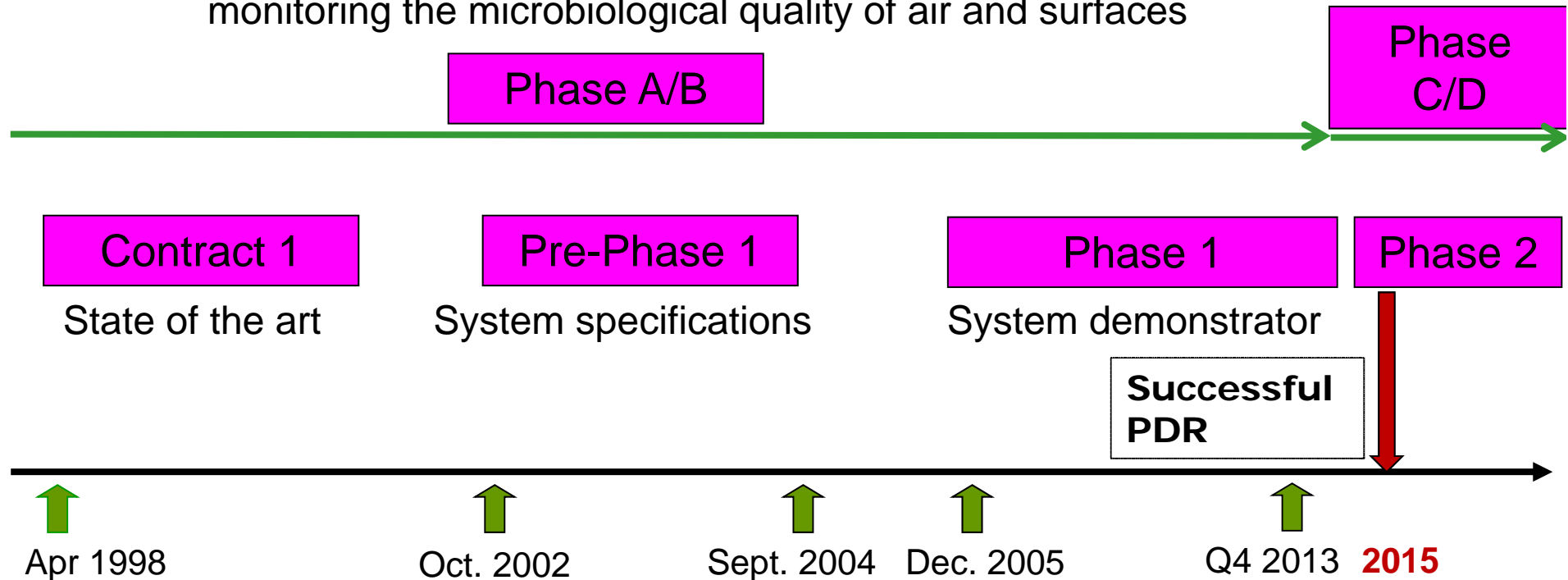
**→ Proper Microbial Risk assessment means Large Number of Samples (Statistical approach), not possible today onboard ISS.**

- Based on a 19th century technology: culture
  - Today needs sample return on Earth
  - Tomorrow for long haul flight, will lead to contamination and waste disposal issues
  - Results only valid for “culturable” micro-organisms
  
- Very long Time to Results: 48h to a week
- Requires too high crew time and expertise
- Requires up/download capabilities

**→Need for a rapid non-culture-based risk assessment system**

## MiDASS: Microbial Detection in Air System for Space

- Aim: To develop a miniaturized, automated system for sampling & monitoring the microbiological quality of air and surfaces



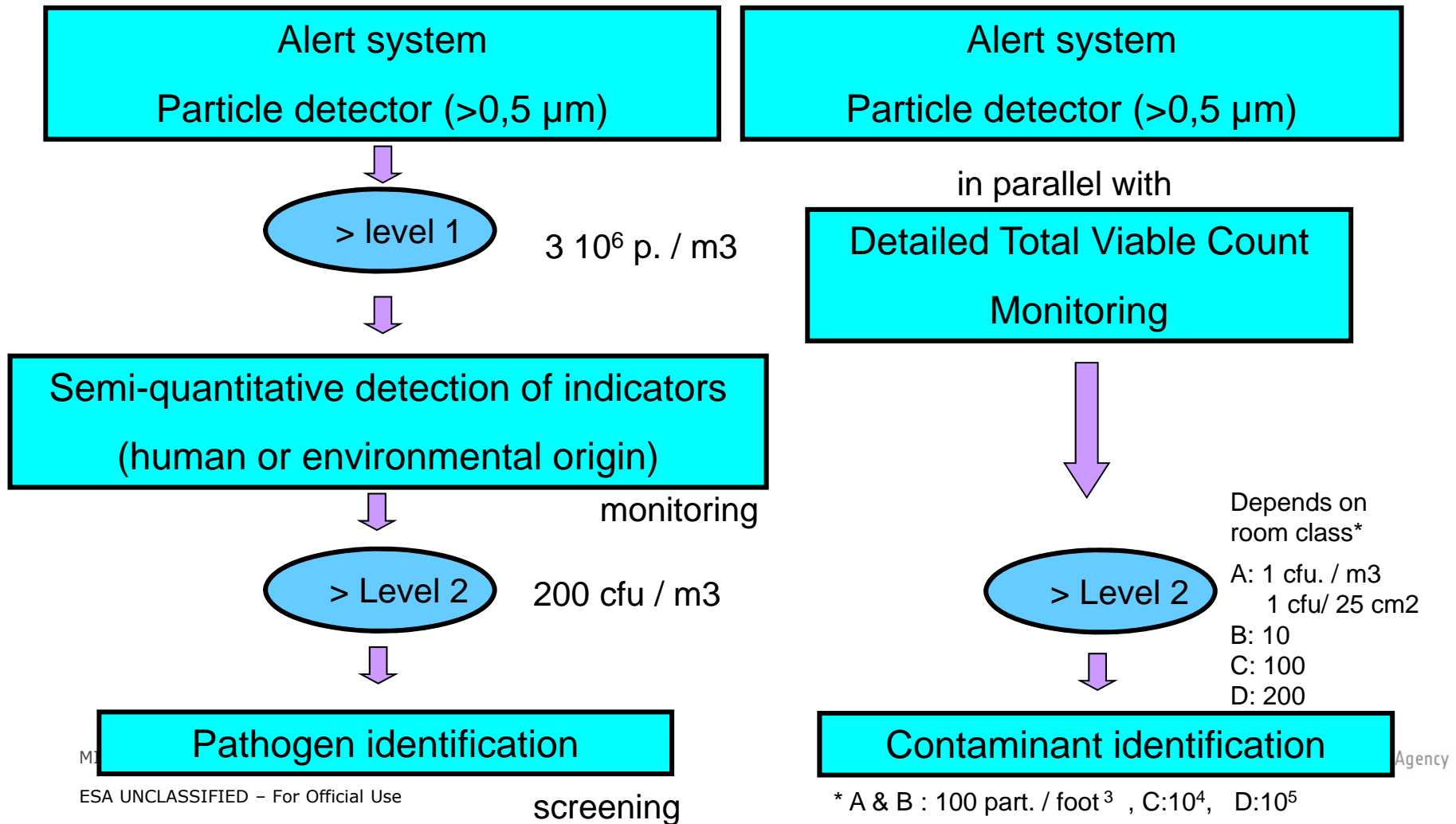
- In 1998, ESA initiated MIDASS project,
- Requirements elaboration was performed with our Russian colleagues from IBMP.
- ESA teams-up with the world leader for infectious *in vitro* diagnostics (i.e. bacteria, fungi, viruses) at 50/ 50 (~ 15 Meu) investment level: bioMerieux.

# MiDASS Risk Assessment Approach



## ECLSS

## Planetary Protection/Pre-flight Ground Integration Process



M1

# MiDASS parameters detection requirements



## Life Support

	Microorganism	Gram	Origin
Indicators	<i>Propionibacterium acnes</i>	+	Human commensal Environment
	<i>Bacillus</i> spp.	+	Environment
	<i>Legionella</i> spp.	-	Water (air condition.)
	<i>Candida</i> spp.	yeasts	Human
	<i>Aspergillus</i> spp.	moulds	Environment
Pathogens	<i>L. pneumophila</i>	-	Water (air condition.)
	<i>S. aureus</i>	+	Environment
	<i>S. pyogenes</i>	+	Human commensal
	<i>S. pneumoniae</i>	+	Environment
	<i>C. albicans</i>	yeast	Human commensal
	<i>A. fumigatus</i>	mould	Environment

## Planetary Protection

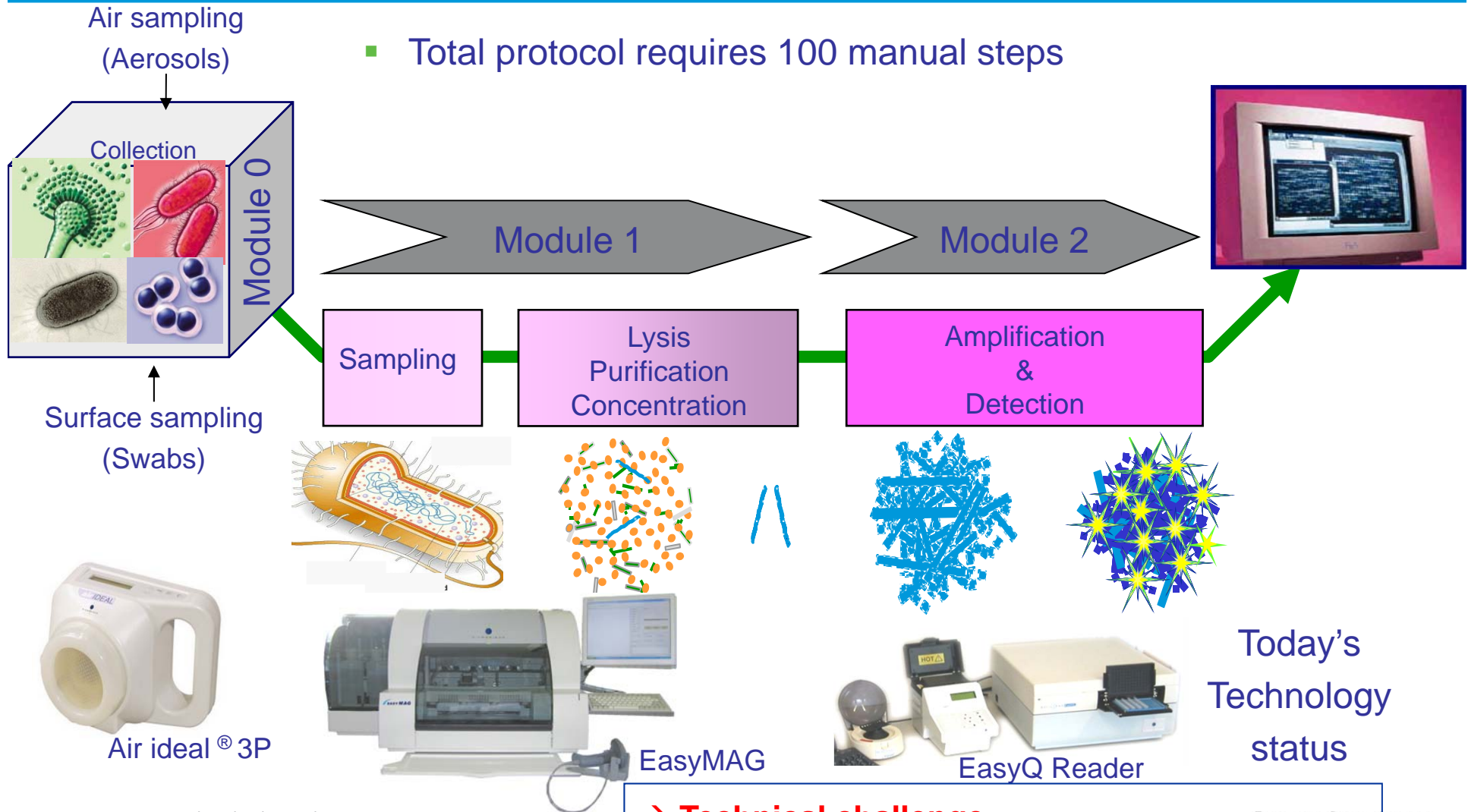
	Microorganism
TVC*	Pan bacteria
	Pan fungi
Major Microbial Groups	Gram+, spore forming ( <i>Bacillus</i> , ...)
	Gram+, <u>non</u> - spore forming ( <i>Staph</i> -like...)
	Gram-, non-fermenters ( <i>Ps. aeruginosa</i> ...)
	<i>Enterobacteriaceae</i>
	Yeasts
	Moulds

- Near continuous monitoring, larger air volumes
- Automated as much as possible
- Challenge: large spectrum test requires ultra-clean reagents

# MiDASS Process Description



- Total protocol requires 100 manual steps



→ **Technical challenge:**  
**Miniaturization/Integration/Automation**



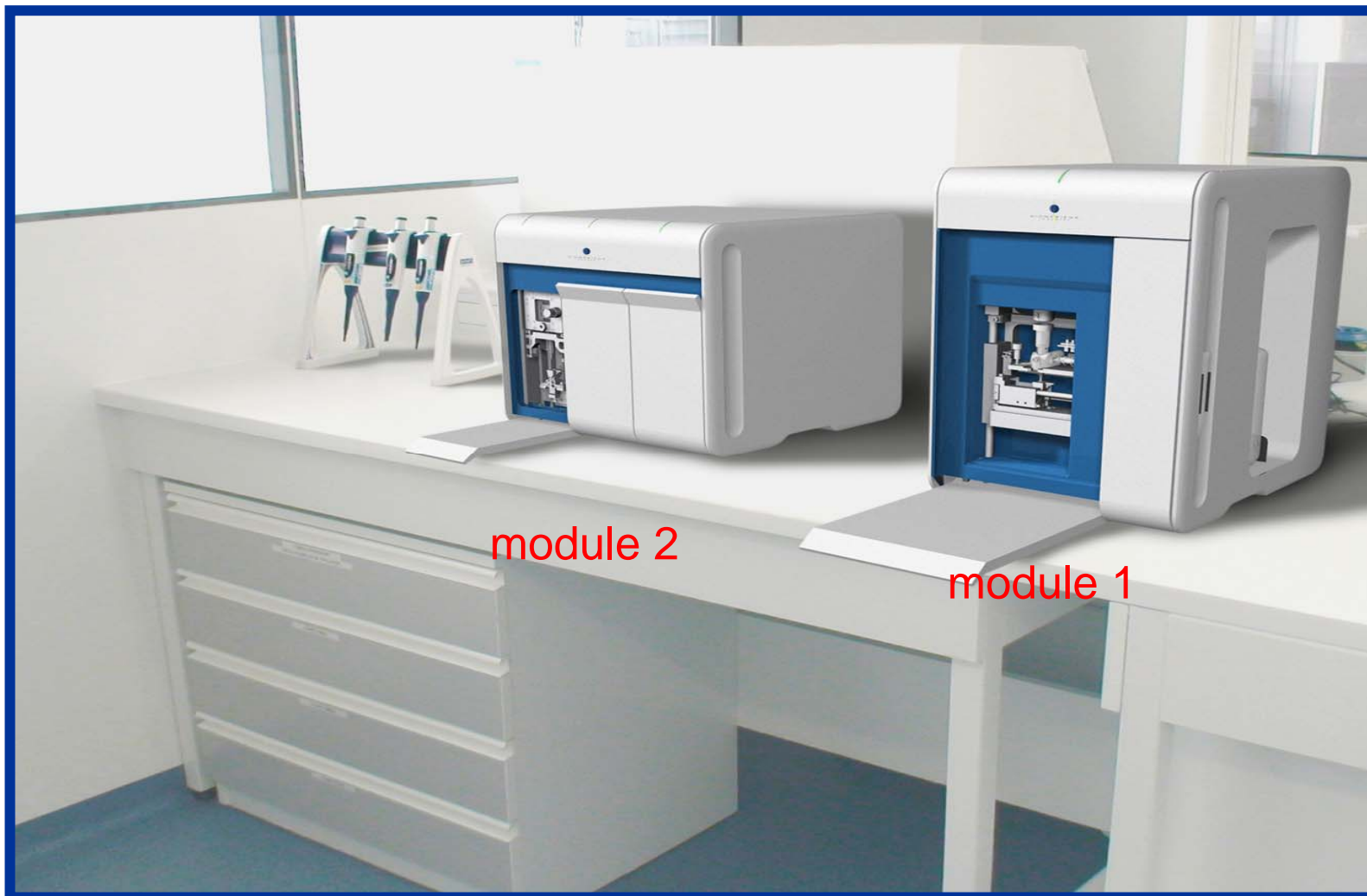
# Specifications of the MiDASS Ground prototype



Focus on performances for terrestrial applications : i.e. environmental control of Ground Integration Processes/Lander Assembly

- Non growth-based system
- TTR < 3 hours
- Throughput 20 samples/day (prototype)
- Fully integrated protocol (contamination-free)
- Samples: air & surface (and potentially water),
- Parameters: TVC (Pan-bacteria & Pan-fungi: screening & quantification)
- Sensitivity: 1 cell per m<sup>3</sup>
- Store RNA/DNA for subsequent ID and sequencing if required,
- Functional and operable by a non-biologist technician,

# Terrestrial Prototype (Today in bioMerieux)



# Space Application: Objectives and technical requirements



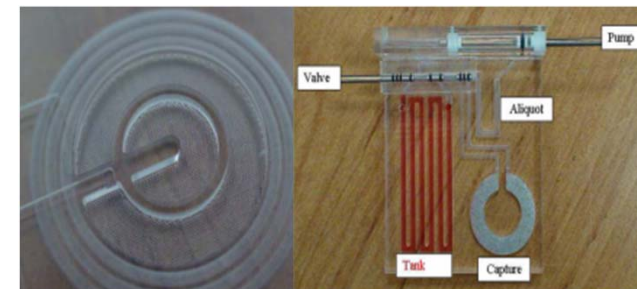
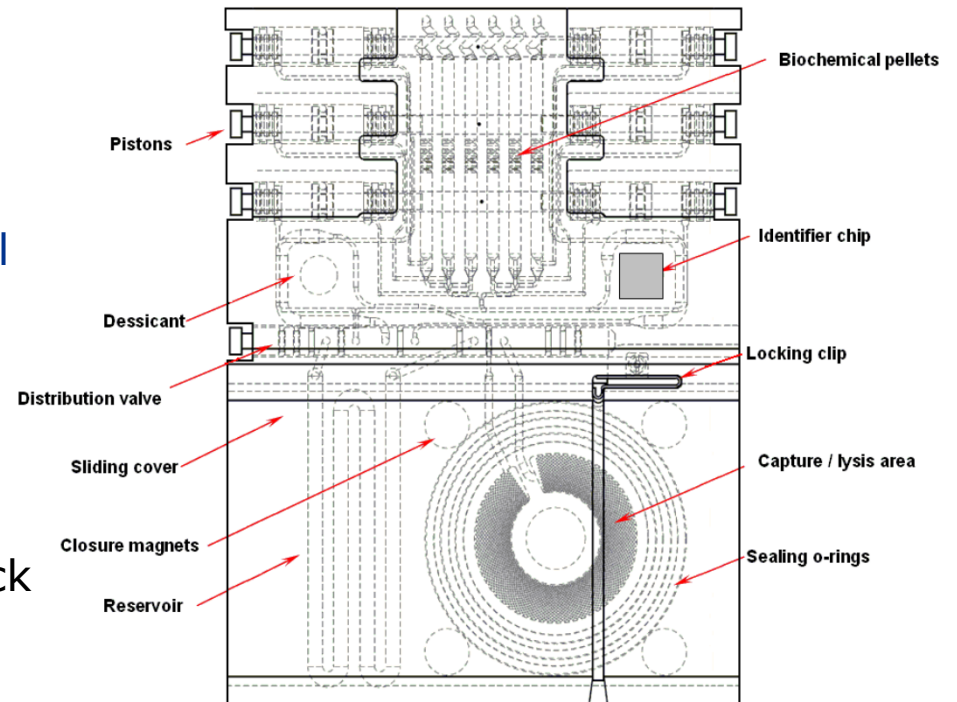
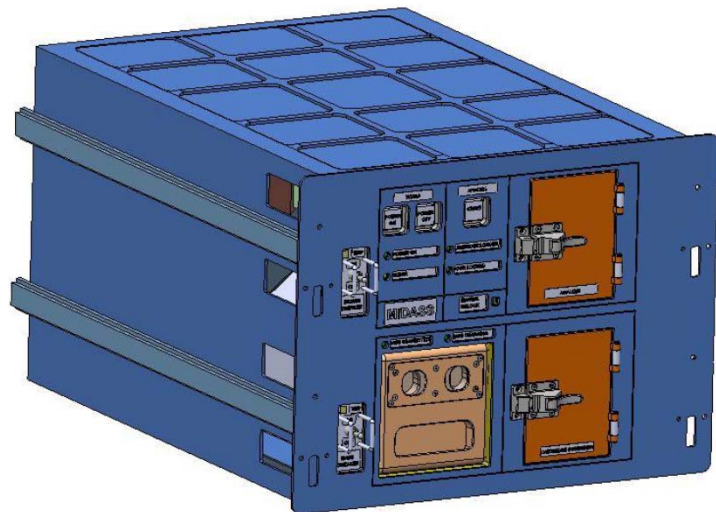
Key specifications (performance):

- R-5.2.1-01: MiDASS shall have a **collection efficiency higher than 80% for relevant microorganisms**
- R-5.2.3-02: Diagnostic **specificity of pathogens shall be better than 95%, with a false positive rate lower than 5%**
- R-5.2.5-01: MiDASS detection time shall be less than **150 minutes**
- R-5.2.6-01: MiDASS shall be able to monitor in continuous mode the concentration of particles suspended in the air whose size fall in the size range of common bacteria (from 0.5  $\mu\text{m}$  to 10  $\mu\text{m}$ )
- R-5.2.7-01: MiDASS shall provide storage for consumables and waste for operate for a 6 months period

# Space Application: Current preliminary design



- ❑ Integrated “lab-on-a-chip” cartridge with all the necessary elements to perform the following operations:
  - Capture of microorganisms from aerosol
  - Lysis of microorganisms to release their nucleic acids
  - Amplification/detection of the target nucleic acid *via* NASBA
- ❑ MIDASS Analyser integrated in EDR rack



European Space Agency

→ Successful PDR review in Q4 2013

- MIDASS quantifies rRNA copies of the bio-contaminants,
- The results are expressed in **Geq**: Genome equivalent

Based on literature review, today, MIDASS is calibrated for:

$$1 \text{ CFU} = 1 \text{ Geq}$$

- As today, CFU remains the Golden Standard Unit, numerous tests have been initiated to statistically confirm the proposed “units”

# Principle:

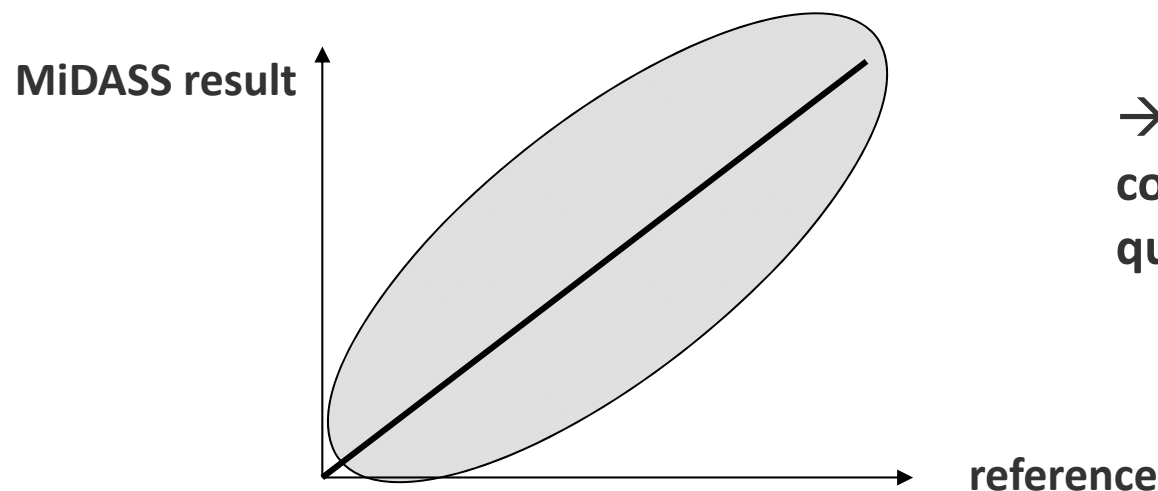
## 1/ Qualitative analysis

		MiDASS	
		positives	negatives
reference	positives	x %	?
	negatives	?	y %

### Assessment of :

- Sensi: Nb of Positive results with Midass among positives samples according to ref method
- Specif: Nb of Negative results with Midass among negative samples according to ref method

## 2/ Quantitative analysis



→ Assessment of the correlation between both quantifications

**Is the relation linear?**

# Real-context testing: Hematology ward for “Pan-fungi” surface testing



## ■ Method:

- Surfaces samples size: 25 cm<sup>2</sup>
- 3 zones targeted:
  - Hematology patient rooms: screening specification = 0 Mold/ 25 cm<sup>2</sup>
  - Hematology corridor: specifications = quantification of < 5 Molds/ 25 cm<sup>2</sup>
  - Elevator floor: positive control with high fungal load

## ■ Sampling plan:

- Hematology patient rooms: 8 samples x 12 days over 12 weeks: n= 96
- Hematology corridor: 3 samples x 12 days over 12 weeks: n= 36
- Elevator floor: 1 sample (or more) x 12 days over 12 weeks: n= 18

## ■ Midass results expressed as Geq/25 cm<sup>2</sup>

->Brenier-Pinchart M.P. et al, Medical Mycology 2014: Usefulness of pan-fungal NASBA test for surveillance of environmental fungal contamination in a protected hematology unit

# Results: Elevators floors as positive control



n = 1-2 samples x 12 different days (12 weeks) = 18

		Culture-based method	
		+	-
Midass	+	18 (100%)	0 (0%)
	-	0 (0%)	0 (0%)

- Same positive results for culture and Midass
- Negative process controls for Midass were negative
- Midass - 3 hours *versus* culture - 1 week



# Results: Hematology patient rooms



Specifications: 0 cfu molds/25 cm<sup>2</sup> sample by culture or < 1 Geq pan-fungi/25 cm<sup>2</sup> for Midass test  
n = 8 samples x 12 different days (12 weeks) = 96

		Culture-based method	
		+	-
Midass	+	0 (0%)	6 (6.25%) * [1.6 to 4.6 Geq]
	-	2 (2.8%) - [1 cfu]	88 (91%)

- **\* More positive results with Midass pan-fungi compared to culture-based test**
- **Midass - 3 hrs *versus* culture - 1 week**

# Results: Hematology corridors



Specifications:  $\leq 5$  CFU molds/25 cm<sup>2</sup> sample by culture or  $\leq 5$  Geq pan fungi /25 cm<sup>2</sup> for Midass test

n = 3 samples x 12 different days (12 weeks) = 36

		Culture-based method	
		+	-
Midass	+	0 (0%)	3 (8.33%) * [5.6 to 8 Geq]
	-	1 (2.77 %) [7 cfu]	32 (88.88%)

- More positive results with Midass pan-fungi compared to culture-based test
- Midass - 3 hrs *versus* culture - 1 week

- MIDASS is a complete & generic solution to meet ESA needs (LS & PP) for future exploration:
  - Air & surface sampling. Water is ongoing
  - Generic sample preparation (G- & G+ bacteria, Fungi and spores)
  - Panel of molecular detection for space LS ready(sensitivity and specificity)
  - Total automation (minimal hands-on time) & Traceability
  - Key requirements have been demonstrated and validated→ Successful PDR
  
- Solution that allows ESA to be in a position to provide life scientists a platform aboard the ISS supporting molecular diagnostics capabilities
  
- Phase C/D will start in 2015→ Technology demonstrator to verify the capability to perform microbial detection onboard ISS

# Thank You

