

**General Studies Contract ESTEC
Contract N° 14758/00/NL/PA**

**«Study of a Microbial Detection System for
Space Applications»**

Executive Summary

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July 2001

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1. AIMS OF THIS STUDY

The overall objectives of this study are to analyse the current state-of-the-art in relation to the microbiological contamination of closed space environments based on the Mir experience ; to assess the risk associated with such contamination and to recommend to ESA ways of controlling the associated risk(s). The study was divided into three Work Packages, the objectives of which are given below;

2. WORK PACKAGE 1 - OBJECTIVES

- To establish and classify factors which influence and/or reduce the risk of microbial contamination in microgravity
- To identify the limitations of current technology
- To identify synergies with terrestrial situation

3. WORK PACKAGE 2 - OBJECTIVES

- To establish a microbial risk assessment for the crew, based on current knowledge
- To comment and advise on microbial risk for hardware

4. WORK PACKAGE 3 - OBJECTIVES

- To outline the main limitations of the existing scientific knowledge
- To outline the main limitations of current hardware
- To propose a list of scientific and technical actions to solve these issues
- To propose a preliminary development plan

5. MICROBIAL CONTAMINATION IN CLOSED ENVIRONMENTS

The behaviour of micro-organisms in closed systems on Earth e.g. sub-marine environment, has been used as model for studying their behaviour in the closed environment of space flight. Such studies have provided valuable information which can be extrapolated to the closed micro-gravity environment. However, they do not reflect all of the conditions experienced in long-haul space flight.

5.1 The importance of microbiological monitoring of manned space environments

In closed systems, such as long-haul spaceflight, the microbiological risks associated with the crew are intimately involved with the technological risks for the hardware and equipment. Therefore, microbial monitoring of the closed environments of spacecraft and space stations is crucial to both maintaining crew health and system integrity. As the duration of space missions increases and re-supply intervals becomes longer, it is essential to be able to effectively monitor air, surface and water supplies for the build up of microbial contaminants. This is even more important due to the necessity to recycle air and water for many months at a time. In addition, several

studies have claimed that microbial growth is accelerated under microgravity conditions, that resistance to antibiotics increases and that genetic exchange augments.

5.2 The Russian Experience - Microbiological Monitoring of Salyut and Mir

Microbiological monitoring experiments began on Salyut in 1978 and were continued on Mir from the period 1987-1999. These experiments represent the most comprehensive studies carried out to date on microbiological contamination of the space environment. Sample types studied were mainly air and the surfaces of interiors and equipment. More than 1000 air samples from 12 locations were analysed for bacterial or fungal contamination and more than 1300 surface samples from 85 locations were investigated. Results for the microbiological status of crew members were not available at the time of this report.

5.2.1 Air monitoring on board Mir

During the lifetime of Mir a total of 986 air samples were taken, 468 for bacterial isolation and 518 for fungal isolation. The frequency of air sampling was either once a month or once bimonthly. A commercial aspiration-sedimentation system was used to collect samples. Specific culture media were then used depending on whether bacterial or fungal isolation was required. Culture plates were incubated on Salyut or Mir and the results given orally to Earth. On some occasions, plates were sent back to Earth for further analysis. The bacterial genera isolated from air samples included *Staphylococcus*, *Micrococcus* and *Coryneforme* bacteria. The main species included *S. aureus*, *S. capitis*, *S. haemolyticus*, *Flavobacterium meningisepticum*, *E. coli*, *Serratia marcescens*, *Streptococcus* spp, *B. cereus*.

The limits for bacterial air-borne contamination for manned space environments are set at 500cfu/m³. Ninety five per cent of samples were within these limits. The species found in samples exceeding these limits included : *S. aureus*, *S. capitis*, *S. simulans*, *Bacillus* spp, *B. cereus*, *Corynebacterium* spp and *Acinetobacter calcoaceticus*.

5.2.2 Surface monitoring on board Salyut and Mir

Sampling of Mir interior and equipment sites was carried out from 1987-1999. A total of 1300 surface samples were taken, 664 for bacterial isolation and 663 for fungal isolation.

For bacteria, the limits set for surfaces were 1000 cfu/ 100cm². Thirty percent of samples analysed exceeded these limits. A wide diversity of bacterial species were found compared to air-borne isolates. The predominant bacterial species isolated were *Corynebacteria* spp and *S. epidermidis*. In addition, opportunistic pathogens were isolated including: *Enterobacter*, *Escherichia*, *Proteus*, *Serratia*. For fungi, the limits set for surfaces were 100 cfu/100 cm². Sixty per cent of samples analysed exceeded these limits.

5.3 Water monitoring

Water monitoring on board Salyut and Mir

The criteria used for safety were total viable counts, with absence of pathogens. During its lifetime little quality control testing of Mir water was carried out on board. The regenerated water from atmospheric condensate was tested on the Mir mock-up. It was found to have a total viable count of 10⁴- 10⁶cfu/ml. After membrane filtration, the counts were reduced to 10²-10³ cfu/ml and subsequent treatment with silver ion reduced the counts even

further to 10^1 - 10^2 cfu/ml. The microflora of the condensate regenerated water was composed mainly of saprophytes and opportunistic organisms. These included members of the families *Pseudomonadaceae*, *Enterobacteriaceae*, *Micrococaceae* and *Neisseriaceae*. It should be noted that water samples were not tested for the presence of *Legionella pneumophila*, a pathogenic organism which is associated with both hospital- and community-acquired legionellosis. This organism proliferates in aquatic environments where it develops intracellularly in amoeba cells in biofilms. Infection of humans is due to the inhalation of aerosols contaminated with *L. pneumophila*.

6. SOURCE OF MICROBIAL CONTAMINATION ON MIR

A number of potential sources of microbial contamination existed on Mir. These include: the initial contamination of materials during manufacturing and assembly ; the delivery of supplies to Mir ; the supplies themselves ; secondary contamination during the lifetime of the orbital station ; the crew and any other biological material on board e.g. animals, plants, microbes used in scientific experiments. The environmental factors on board Mir would have selected the particular types of microbes colonising Mir. The nature of the bacterial species isolated on Salyut and Mir indicate that the primary source of bacterial contamination was the crew. For fungal isolates, the main source of contamination was probably the microflora of structural materials although biotechnology experiments involving plants on board may have contributed to the fungal contamination. In addition, monitoring experiments themselves may have amplified the contamination problem as they involved the culture of microbes on board.

Two other fundamental aspects need to be taken into account about when considering the risk of microbial infection of the crew on board long-haul space flight: immune status and microbial virulence.

7. MICROBIAL INDUCED CORROSION OF MATERIAL AND HARDWARE ON BOARD MIR

The particular environmental conditions on board Mir encouraged the growth and colonisation of the orbital station by microbes. More than 234 microbial species were isolated, including 108 bacterial and 126 fungal species. Amongst these, potential biodegrading organisms were identified. These organisms are sometimes referred to as technophiles as they have the potential to cause damage to various materials on board leading to the malfunctioning of equipment and hardware. Microbial growth on Mir was demonstrated to be responsible for changes in the colour and structure of materials, leakage and short circuits, and deterioration of optical characteristics. One of the best-documented cases describes the progressive destruction of a window on Mir's descent vehicle. This was largely due to the growth of a biofilm composed of *B. polymyxa*, *P. chrysogenum* and *Aspergillus* spp. Other materials affected included elements of the air conditioning system, the cable network, and the oxygen electrolysis block.

In addition, biofilms were reported to block water distribution channels of the water regeneration system. These biofilms were shown to be composed of a complex mix of bacterial and fungal associations.

8. FACTORS WHICH INFLUENCE AND/OR REDUCE MICROBIAL CONTAMINATION IN MICROGRAVITY

A number of factors have been shown to influence microbial contamination in microgravity conditions. The closed environment of long-haul space flight influences both the types of microbes which colonise crew members and their virulence capabilities. This environment also facilitates the spread of microbes from person-to-person, from person-to-environment and from environment to person.

In addition, environmental conditions such as air speed (0.2 m/sec) lead to the rapid dissemination of microbes from one location to another. The humidity of Mir (20-80%) favoured the growth of microbes, especially behind panels where the build up of condensation and hence availability of water allowed microbes, particularly fungi, to proliferate. Finally, the temperature of Mir was within the range (20-35°C) required for microbial growth. Taken together, these environmental conditions favoured the growth of micro-organisms on board Mir.

In addition, the requirement for recycling of water and air no doubt contributed to the microbial load on Mir.

9. MICROBIAL RISK ASSESSMENT FOR THE CREW BASED ON CURRENT KNOWLEDGE

The particular environmental conditions on Mir no doubt contributed to the microbiological problems encountered during its lifetime, in particular the high humidity levels, air speed and closed environment. This was further complicated by the lack of a systematic use of an effective air filtration system. The Russian report on the consequences of the microbiological conditions on the health of cosmonauts is eagerly awaited. In the meantime, we conclude that, based on the Mir experience coupled to the specific conditions encountered in long-haul space flight (closed environment, depressed immune system, re-cycling of waste products...), the risk of microbial contamination of long-haul manned space missions poses a real risk to both the safety of the crew and the integrity of the hardware on board. The severity of these risks is difficult to assess due to the lack of epidemiological data. However, it can be concluded that, for the crew, these risks are real and may be divided between short-to-middle-term and long-term risks. Short term risks include the development of respiratory tract infections, including legionellosis, skin infections, gastro-enteritis or other alimentary tract infections and allergies. Most of these illnesses can be easily spread amongst other crew members, and the risk of spread is increased due to the closed environment. In addition, reports on augmented antibiotic resistance of bacteria isolated from microgravity complicate the treatment of these illnesses. Long-term risks associated with microgravity conditions include the development of certain cancers due to the activation of latent viruses in astronauts which has been reported and is probably due to the stress associated with space flight.

10. . MICROBIAL RISK FOR HARDWARE-COMMENT

In closed systems, such as long-haul space-flight, the microbiological risks associated with the crew are intimately involved with the technological risk for the hardware and equipment (see TN1). The Mir experience demonstrated the real risk of microbial contamination and growth to the functioning of the hardware and life support systems on board. As for the risk to crew health, the design of the ISS (and subsequent long-haul space systems) will decrease, but not eliminate this risk. In the context of the ISS, and above all for inter-planetary space missions, any system designed to reduce the microbiological risk to the crew will also contribute reducing the risk to hardware.

11. MAIN LIMITATIONS OF EXISTING SCIENTIFIC KNOWLEDGE

11.1 Areas for further study

Two main areas have been identified which require further experimentation in order to fulfil the current lack of scientific knowledge and which impact on the development of a microbial detection system. These are:

- Basic microbiology (microbial growth, resistance to antibiotics, origin of strains...)
- Microbial genetics (genetic analysis of "Mir" isolates, phenotypic expression, plasmid-mediated gene transfer, genetic stability...)

It should be noted the experimental techniques and expertise required to supplement the current lack of scientific knowledge for both areas could be fulfilled by one expert laboratory.

11.1.1 Basic microbiology

Areas that require further study include:

- Effects of microgravity on bacterial growth
- Effects of microgravity on bacterial resistance to antibiotics
- Effects of microgravity on bacterial virulence
- Effects of microgravity on the evolution of bacterial strains
- The origin of microbial strains under long-haul space conditions

11.1.2 Microbial genetics

Areas that require further study include:

- Phenotypic expression
- Plasmid-mediated gene transfer
- Genetic stability and stress-induced functions

12. MAIN LIMITATIONS OF CURRENT HARDWARE

Current microbial air contamination monitoring methods are based on the culture of microbes on specific media. This implies a long incubation time e.g. 2 days for bacteria, but this becomes longer for stressed bacteria or fungal strains. For microgravity applications, both the requirement for culture (and hence amplification of the microbes present) and the long time-to-result response are not suitable.

The capacity for recovery of microbes from air by any method should ideally be 100%. However, currently there is no known method capable of achieving this. For example, with solid impact devices, when the stream of air passes over the nutrient medium, some dehydration occurs resulting in shrinkage of the agar and reducing the efficiency of the sampler. Drying may also cause a film to form over the agar which can retard microbial growth and cells already deposited on the agar surface may desiccate and die. When the microbial level in the air of a controlled environment is not expected to be high, several cubic meters of air should be sampled in order to give

precise and accurate results. However, current air sampling devices are limited in the size of the air sample that can be handled.

For any controlled environment, identification of the flora obtained during sampling is important. Currently, in order to identify microbes isolated during air sampling procedures, additional culture steps are required, followed by biochemical or other methods of identification. This requires both additional experimentation, expertise on the part of the operator and a longer time-to-result. These requirements mean that such methods are not appropriate for microgravity applications.

13. PROPOSAL FOR SCIENTIFIC AND TECHNICAL ACTIONS

13.1 Suggestions for further experimental approach

Proposed experiments include:

- An evaluation of the best available technique for DNA extraction and amplification (PCR and possibly RT-PCR) from surface samples (metal, plastic polymers, etc..) and aerosols .
- An evaluation of the most appropriate techniques to analyse, at the DNA level, the Russian samples as they now exist. This should include an inventory of the storage methods that were used to store the Mir samples.
- A study of some key Russian samples taking into account the observed changes in the microbial composition of Mir samples.

13.2 Suggestions for bacterial genetics in space conditions

Suggested future experiments include those concerning :

- Phenotypic expression
- Definition of the bacterial material to be tested
- Phenotypic markers
- Gene transfer
- Mutagenesis
- Assay of the mutator phenotype of *R. metallidurans* under space conditions
- Environment-responding and stress-induced functions on DNA microarrays

14. PROPOSAL FOR A PRELIMINARY DEVELOPMENT PLAN

The preliminary development plan should be divided into two main areas; scientific requirements and technical requirements. Both areas should be carried out in parallel and completed within 24 months.

14.1 Scientific requirements

The areas to be covered here include a review and study of the specific micro-organisms and/or genetic elements which need to be considered when developing a microbial detection system for microgravity applications. In

addition, complementary experimentation necessary for the development of a microbial detection system appropriate to space applications should be identified and proposed (see 5). The expertise required to carry this out include; microbiology, microbiology of extreme environments, microbial genetics, molecular biology.

14.2 Technical requirements

A review and study of the technical requirements based on the scientific requirements mentioned above should be carried out. The expertise required to carry this out include; microbiology (both medical and industrial), microbial diagnostics, automated systems, sample preparation, data analysis etc.

Aspects such as the types of microbes/genetic elements to be detected, the required sensitivity and specificity of the detection system, the frequency of detection and the time to results should be included.

This should be followed by a proposal of the most appropriate technology which responds to these particular requirements. The involvement of the European Space Agency (ESA) as a consultant in this is important in order to ensure that the technology proposed will be appropriate to both the specific requirements and constraints of the microgravity environment. Parameters to be included cover; the detection technology, air sampling method, sample preparation and data analysis.

It is likely that the choice of technology will require further scientific investigations in order to ensure that any development will be both relevant to and workable in the microgravity environment. Therefore, such complementary investigations should be both identified and carried out. A preliminary design plan of the completed instrument should be proposed, including sufficient details to ensure its suitability for microgravity applications. Again, ESA should play a consultancy role here. The critical technologies required for the completed instrument should be identified and any complementary tests required should be proposed and carried out.

Based on the outcome of these tests, a detailed design of the completed microbial detection system for microgravity applications should be proposed. These details should be sufficient to allow the construction of a breadboard. Based on the above, an evaluation of the costs of the development of such a system should be carried out. In addition, as the detection system proposed will be developed specifically for space applications, it will be necessary to select the most appropriate European space industry partner to carry out microgravity specific areas of the development.

15. OVERALL CONCLUSIONS

- The most comprehensive information on microbial contamination in long-haul space flight to date comes from the «Russian Study». However, there is still a great deal of basic scientific knowledge that can be obtained using well designed experiments that have been validated for the space environment and with appropriate controls
- The results of the Russian study indicate that microbial contamination of long-haul manned space missions may pose a real threat to both the safety of the crew and the integrity of the hardware on board
- The specific conditions of long-haul space flight pose a risk to the health of the crew and to the integrity of the hardware on board

- These risks need to be quantified and systems of both control and monitoring put in place
- Current systems of microbial monitoring are not the most appropriate tools for long-haul manned space missions and any method based on culture may increase the risk of contamination.
- Monitoring requires the development of a system which responds to the specific needs of long-haul space missions