



# **KOACH Information**

### A report presented to the 15th Annual Meeting of the Japanese Society of Extremophiles

# "A super clean environment of ISO Class 1 cleanliness will become common practice in the study of life sciences."

In the study of life sciences where breakthroughs have been achieved in such areas of regenerative medicine and DNA testing, researchers are competing intensively to seek for a research output and are aiming at achieving greater accuracy in their studies. Mr. Yuki Morono, who is engaged in research of microorganisms at Kochi Institute for Core Sample Research of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC), believes that contamination-free experiment environment is indispensable for microbiological study, and from that point of view, KOACH, which can form a local super-clean environment, has great potentialities. He reported the progress of verifying the effectiveness of the KOACH at the annual meeting of the Japanese Society of Extremophiles.



The Japanese Society of Extremophiles was established in October, 1999. It studies microorganisms living in extreme environments and its membership includes researchers from a wide range of study fields. Discussions are made from interdisciplinary multiple approaches.

The study of extremophiles can extend the usefulness of primeval life forms and microorganisms.

Microorganisms are the life forms which are too small to be seen by the naked eye, such as bacteria and viruses. They are thought of as the first living organisms born on Earth.

Microorganisms are living in all kinds of environments on Earth and their ecological mechanisms are used for the benefit of our daily lives in many situations.

A familiar example of this is lactic acid bacterium which is included in bakery yeast, yogurt and many other fermented foods. In the medical field, the study of microorganisms has led to the development of antibiotics and in the area of regenerative medicine, when producing iPS cells, mechanisms in retroviruses are used.

Microorganisms are thriving in extreme conditions on land, underground or below seabed. It is said that the number of microorganisms living on Earth are about  $10 \times 10^{29}$ . Microorganisms that are thriving in extreme conditions that are detrimental to most life on Earth such as deserts, volcanos, hot spring sources, highly-concentrated salt water lakes or deep oceans, have a unique mechanism that is well adapted to such severe environments. They are recognized as "precious biological resources," and are studied all over the world.

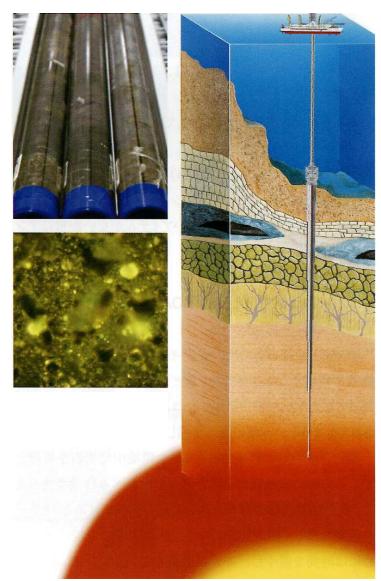
The Japanese Society of Extremophiles studies living organisms that are thriving in extreme conditions. It gives a platform for researchers who are engaged in production of drugs and useful products and application of its study to biotechnology.



Mr. Yuki Morono Sub leader Geomicrobiology Group Kochi Institute for Core Sample Research of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

### • What is "Core"?

The deep seabed layer is called "Core." The study of it helps understand the mechanism of earthquakes and the origin of life.



This photo shows observation through a microscope of DNA in cells stained by fluorescent reagent. You can tell microorganisms from others in different colors.

Kochi Institute for Core Sample Research is Japan's only laboratory that stores and studies sediment samples ("Cores") taken from deep earth by D/V CHIKYU, a Deep sea drilling vessel, or other ships. It also serves as one of the major core repositories in the world as part of the International Ocean Discovery Program (IODP) and its research achievements receive world attention.

KOKEN: In Geomicrobiology Group at which you are a sub leader, we understand that contamination prevention measures are given the highest priority to increase the research accuracy.

I am studying extremely rare microorganisms that thrive in "Cores," an extreme condition. What is challenging in this study is to prevent contamination by microorganisms that exist in the laboratory environment.

Various invisible microorganisms are floating in a laboratory. If they are mixed with the microorganisms under study, it becomes difficult to distinguish between them and you cannot achieve accuracy in the experimental data.

Microorganisms floating in a laboratory are regarded as "natural predators" for our study, and have been annoying the researchers for a long time.

KOKEN: I heard that you had used a clean bench and a biosafety cabinet to prevent contamination by indoor microorganisms.

At Kochi Institute, a clean bench was placed in a clean room with ISO Class 6 cleanliness to achieve ISO Class 3 clean local environment. It was very expensive though.

Some researchers involved in microorganism studies do not trust the clean equipment that is designed to circulate air in the equipment as a valid anti-contamination measure.

In my group, I thought it necessary to provide a stricter anti-contamination measure to achieve better experimental data. So, I have been on the watch for a better clean equipment.

KOKEN: Could you tell us the reason why you have chosen the KOACH as an anti-contamination measure?

I had thought it was natural to use clean equipment such as a biosafety cabinet before knowing the KOACH. However, it was difficult to remove contaminants generated inside the equipment due to its wall structure. That was a headache.

In our study, in particular, there was a process of determining if there existed microorganisms in a rock that was broken in a clean environment. If a rock was contaminated with microorganisms on the surface, breaking the rock would significantly contaminate the surrounding clean environment. It took a lot of time and effort to restore the damaged environment.

In 2012 we saw for the first time the KOACH, the concept of which is based on a completely novel idea compared with conventional equipment. It pushes out contaminants with a highly purified airflow and forms a super clean environment quickly. I thought this would be a fundamental solution for removing contaminants generated during work.

It was decided that the KOACH should be the best selection for our study where very rare microorganisms are subjects under study.

# KOKEN: We heard that successful implementation of anti-contaminant measures during experiment would directly lead to satisfactory research outcomes.

I have to admit that it is rare to conduct work involving breaking a rock like our study. But, it may be quite common that contaminants are generated by workers during work. We cannot get away from the problem of contamination in any research.

Because KOACH can provide a high level of clean environment easily, I can think of many situations where it can be used. We are conducting various performance experiments to make the best use of its use value.

In an experiment to verify if floating microorganisms indoors will not cause contamination in a local clean zone formed by the KOACH, we obtained a successful result that showed that contamination from unnecessary microorganisms that are not subjects under study was effectively prevented. We reported this result at this year's meeting.

Utilization of KOACH that can exhaust contaminants without enclosing walls must be a valid anti-contamination measure and a formula for a successful study not only for study in microorganisms but also for all areas of research.

## ■ ISO 14664-1 Cleanroom Standards

Air cleanliness		Maximum particles/m³					
ISO14664-1	FED STD	Particle size					
	209E	$0.1\mu$ m	$0.2\mu$ m	$0.3\mu$ m	$0.5\mu$ m	$1.0\mu\ \mathrm{m}$	$5.0\mu$ m
	Equivalent						
Class 1		10	2				
Class 2		100	24	10	4		
Class 3	1	1,000	237	102	35	8	
Class 4	10	10,000	2,370	1,020	352	83	
Class 5	100	100,000	23,700	10,200	3,520	832	29
Class 6	1,000	1,000,000	237,000	102,000	35,200	8,320	293
Class 7	10,000				352,000	83,200	2,930
Class 8	100,000				3,520,000	832,000	29,300
Class 9					35,200,000	8,320,000	293,000

# Presentation at the 15th Annual Meeting of the Japanese Society of Extremophiles

Mr. Morono thinks that it is useful to his study to use the KOACH that can form a local super clean environment all over the place. To verify the usefulness of the KOACH, he conducted an experiment to confirm that there would be no contamination from DNAs existing outside the clean zone formed by the KOACH.

### Presentation Title:

Evaluation of DNA contamination level in a clean experimental environment

The following are excerpts edited by KOKEN with the approval of Mr. Morono.

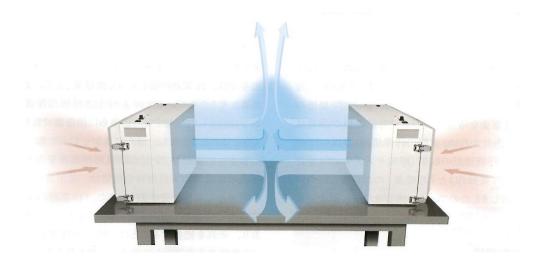
To evaluate the levels of cleanliness in a clean experiment space and its surrounding environment, DNA aerosols will be generated artificially to contaminate the space. They will pass over a plate placed upstream of the KOACH and then over a plate placed downstream. Exposure to each place will take 30 minutes and after that, contaminated DNA will be quantified.

As a result, it was found that the Table KOACH can capture aerosol DNA molecules and the usefulness of the KOACH was verified for a high-precision detection of very small amount of DNA including sample analysis of extremophiles.



To verify the purification ability of KOACH and the levels of contamination, aerosols containing a large amount of DNAs are generated in front of the air intake surface of the KOACH.

### Mechanism of KOACH that can form a local clean zone



KOACH will remove contaminants such as microorganisms floating in the environment with a uniquely developed high efficiency FERENA \*filter.

KOACH consists of two push hoods set opposite each other. By making purified coherent airflows generated by the two opposing push hoods collide at the middle of the push hoods, thus creating powerful "exhaust ability," KOACH can maintain a super clean environment without surrounding walls.

\*FERENA shows the same amount of pressure drop as HEPA filters while having the same level of filtering efficiency as ULPA filters.