Science

In London, we visited the world's oldest biobank: it stores and despatches thousands of viruses and bacteria all over the planet, from plague to Ebola. All in aid of research.

The most dangerous bank on Earth

Photos by Christian Sinibaldi - Translation by Simon Knight

HANDLE WITH CARE. A biologist at the Culture Collections in London, where thousands of viruses, bacteria, cells and fungi are conserved.

Their website is simplicity itself: you just type in the name of the product you are looking for and the details are displayed, along with the price. But the catalogue is terrifying: for € 390 you can order the SARS coronavirus. More affordable is *Yersinia pestis*, the bacillus responsible for bubonic plague: € 348. But the real bargain is the Ebola virus (*Zaire Ebolavirus*): it comes free; you only have to pay the cost of carriage.

You might think this was a website run by bio-terrorists, but the masthead features the reassuring logo of the British NHS. The UK's public health agency provides an extraordinary service: conserving and sending by post, in unbreakable packaging, thousands of micro-organisms –including some of the most deadly – to scientists worldwide.

Culture Collections (www.phe-culturecollections.org.uk) is in fact one of many bio-banks established in different parts of the world (*see box on following page*). Rather than conserving agricultural seeds, their freezers contain dangerous infectious agents: 400 viruses and 5,000 bacterial cultures, as well as 40,000 cell lines and 4,000 strains of fungi. One of the most important international collections of its kind, and by far the oldest, it has been going for almost a century. How does it operate? What purpose does it serve? And, above all, is it safe? *Focus* magazine went to find out.

DEEP-FROZEN. Access is permitted only after a lengthy bureaucratic process. The most deadly viruses, those with a bio-security rating of 4 (fatal and infectious even when airborne), are kept at Porton Down, to the west of London, in airtight rooms, which can be entered only after strict security checks via armoured doors with combination locks. The bacteria, meanwhile, are stored by the Health Protection Agency in Colindale, north London. At these facilities, the cells, handled with long rubber gloves protruding into sealed biosafety cabinets, are carefully monitored and conserved in vials: first they are frozen in liquid nitrogen at -196°C, then kept in special freezers at -80°C.

«To keep the cultures alive and free from contamination, we need to carry out lots of tests» explains Julie Russell, the bank's director. «First we have to get them to ▸

PREPARATION. Vials of bacteria in a dryer: without water they can be stored for many years.

There is a bacterium that Fleming extracted from his nose

proliferate and, in some cases, as with the Koch bacillus, which causes tuberculosis, this may take up to six weeks. Then we perform a whole battery of bio-chemical, genomic and morphological tests. It takes three months to carry out all the checks on just one strain of micro-organism: all it takes to contaminate them is one micro-plasma (a very small bacterium - *ed.*), which is invisible using an optical microscope. We then freeze and store them, checking each year to make sure they are still alive».

BUDGET. So what is the purpose of this complex organization? Conserving this delicate biological material is only part of the work. The crucial phase is the shipping. «Our collections», explains Russell, «are used by scientists who study these pathogens to perform diagnostic tests or develop new treatments, whether antibiotics or vaccines».

One of the items most in demand this year is the Zika virus, which has raised enormous concern in Brazil because it causes malformations of the foetus. «We have three strains in our collection», continues Russell. «One was extracted in 1962 from a mosquito captured in the Zika Forest in Uganda. A second strain was isolated in the USA from a man from Puerto Rico in 2015, and a third was taken from the sperm of a patient from Guadeloupe, hospitalized in the United Kingdom: it will be available shortly, for € 298.50».

But why make people pay? Maintaining a biobank is expensive. For the first fifty years of its existence, the British institution was financed by the Ministry of Health. «But since 1970 we have been self-supporting», says Russell. «Our annual budget is £ 5 million (€ 5.83 million): 80% is derived from sales of biological material, the remainder from funding by other organizations».

How do you send a test tube containing millions of Ebola viruses or anthrax bacilli weighing just 0.15 grams? «We run a very tightly controlled operation, largely automated, in which time is the crucial factor: we cannot allow the material to be destroyed or lost. Or end up in the wrong hands», replies Ana Deheer-Graham, scientific coordinator of the Bacteria Collection.

The first step is to check the scientific credentials of those requesting micro-organisms. Recipients must be scientists working for a public or private laboratory equipped with the necessary safety structures. They must also complete a number of forms explaining why the virus or bacterium is needed, and undertake not to pass it on to others. «We do not send anything until we have checked every detail», adds Russell. «All request must be authorized by the Foreign Office. Checks can take two or three months, and not even we know what factors they take into account. Authorization may also depend on political circumstances».

PERMITS. The most critical countries? «Arab States and Iran. And less developed countries, which often do not have adequate laboratories for storing these organisms», replies Deheer-Graham. «But even shipping material to the USA or Australia is beset with bureaucratic complications: they have very rigid legislation on imports of biological material. It takes three months to obtain all the necessary permits, then you have to add the delivery time».

The bacteria are transported in vials, dehydrated, with 97% of the water removed. We then extract the air and seal the vial under intense heat. Treated in this way, bacteria can survive for up to 50 years. Viruses, on the other hand, are freeze-dried in small plastic test tubes, or in a liquid suspension, and placed in a container with dry ice, which keeps them at a temperature of –80°C. Clients can request just the RNA extracted from the virus, which is not infectious and can be used for diagnosing infections. Both types of product are transported in unbreakable packaging. A team of 20 people work exclusively on the logistics: preparing the material, packaging it and handing it over it to one of the two specialized courier firms approved by the British government. And shipments are tracked up to the moment of delivery.

Micro-organisms also travel in the opposite direction: scientists who discover new sources of infection, or mutations of al- ▸

DROP BY DROP. A biologist prepares a culture: she must take care not to contaminate it and herself avoid getting infected.

LIVING ARCHIVE. A filing cabinet full of test tubes: each contains a strain of bacteria, which are stored in refrigerated rooms.

THE 9 MOST DANGEROUS MICRO-ORGANISMS IN THE COLLECTION

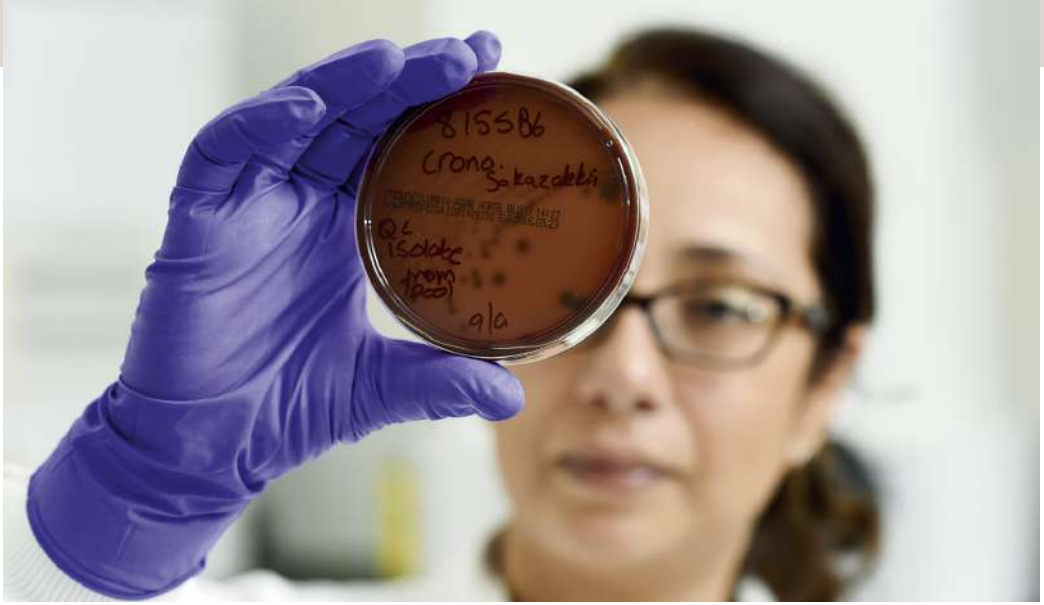
BEWARE. The British Culture Collections conserve many deadly organisms. The most high-risk bacteria are: ***Bacillus anthracis*** (which causes anthrax), ***Francisella tularensis*** (tularemia), ***Yersinia pestis*** (plague), ***Salmonella typhi*** (typhoid fever), ***Mycobacterium tuberculosis*** (tuberculosis), ***Brucella*** (brucellosis). The most dangerous viruses are: ***Zaire ebolavirus*** (Ebola), the yellow fever virus and the dengue virus.

BIOBANKS: 700 AROUND THE WORD (15 IN ITALY)

STRAINS. According to the World Federation for Culture Collections (WFCC), there are currently 710 biobanks in 72 different countries. Together they conserve more than 2.5 million bacteria, fungi, viruses and cell lines. The largest, in the USA, is the ATCC, which has 18,000 strains of bacteria and more than 2 million viruses. In Europe, there are biobanks in Germany, Sweden, Belgium, Spain, France and the Netherlands. And many are joining together to form pan-European institutions: France hosts the virus archive (Evag), the United Kingdom the stem-cell biobank (Ebisc).

So what about Italy? It has 15 biobanks, mostly concerned with food and agriculture. The Umcc at the University of Reggio Emilia, for example, conserves the yeasts and bacteria used in producing wines, vinegars and cheeses, especially Parmesan.

WAREHOUSE. Right, the Collection's logistics department: the cylindrical containers are liquid-nitrogen freezers, operating at temperatures as low as -196°C. Below, testing to ensure that the vials are hermetically sealed.



CATALOGUED AND CONTROLLED. A culture of *Cronobakter sakazakii*, a bacterium that infects new-born babies and may be fatal. Bacteria can be stored for up to 50 years.

ready known bacteria or viruses, can send them to the Collection, which will store them for future research. One such sender was Australian doctor Barry Marshall: in 1984 he hypothesized that ulcers and gastritis were caused by a bacterium, *Helicobacter pylori*. But the scientists of the day disagreed with him, convinced that it was killed off by the natural acidity of the stomach. To demonstrate the validity of his theory, Marshall drank a culture of the bacterium: two weeks later, he was afflicted with gastritis. Marshall won the Nobel Prize and sent his Heliobacters to London! Indeed, a number of organisms that have made history are conserved in the Collection: 16 cultures were deposited by Alexander Fleming, the discoverer of penicillin. He extracted *Haemophilus influenzae*, a bacterium that can cause meningitis, directly from his nostrils. The earliest samples in the Collection, founded in 1920 by the bacteriologist Frederick William Andrewes, are specimens of another bacterium, Shigella dysenteriae, taken from the body of Ernest Cable, the first British soldier to die of dysentery in the trenches of the First World War.

PROFILES. «Those old strains are useful for studying the evolution of micro-organisms», comments Russell. «We supplied them for an international study of 330 strains of Shigella isolated during the last century. It was discovered that dysentery, now a scourge of Asia and Africa, in fact originated in Europe. Ninety-eight per cent of its genome has remained unchanged, but in the meantime it has become resistant to antibiotics».
Since 2014, the biobank has begun studying the genome (DNA and RNA) of every micro-organism. Using cutting-edge computerized sequencers, it tales just 24 hours to trace the genetic profile of each of the items in the catalogue. The project, known as NCTC3000, was funded by a grant of £1 million from the Wellcome Trust Sanger Institute, and the results are published free of charge on its website for the benefit of the international scientific community.
The Collection is perfectly placed to monitor new sources of infection, reported by hospitals in the United Kingdom and through international contacts. In 2011, for example, the Collection was involved when more than 3,000 people in Germany were affected by an epidemic of *Escherichia coli*, a food-borne bacterium that eventually claimed 53 victims. Microbiologists discovered that the source was a producer of salad vegetables who was using contaminated sainfoin seeds imported from Egypt. And this particular strain had developed a gastro-resistant capsule that made it all the more virulent.
The Collection also includes the fearsome new virus that causes Middle East respiratory syndrome coronavirus infection (MERS-COV), which has a death rate higher even than SARS. «It was first isolated in 2012, in the lungs of a patient from Arabia. It may be that this disease is disseminated via camels».
Unfortunately, it is becoming more difficult to send these micro-organisms abroad: anti-terror laws, and legislation governing biological materials and GMOs, are making the biobank's work more problematic. «And», adds Deheer-Graham, «there are no international regulations on shipping bio-material. In the past, it was easier to send material abroad; nowadays, the Nagoya Protocol (2010) stipulates that any economic benefits deriving from the use of an organism must be shared between the sending and the receiving countries. It is easier to do this within Europe than elsewhere».

TISSUES. Despite these difficulties, the Culture Collections have sent 25,000 items to all parts of the world (especially Europe) over the last year. Those most in demand, however, are not bacteria or viruses, but cell lines, i.e. samples of human tissue, both sick and healthy. At Porton Down, they have over 40,000 such cell lines, covering 50 different kinds of tissue. And this section includes the biobank's most expensive item: cancer of the cervix, costing € 574.
«Cell lines are essential for testing new drugs and checking the toxicity of substances without having to use animals», explains Russell. «Many discoveries concerning breast cancer, diabetes or the effects of cosmetics on the skin have been made using such samples. But they must of course be certified».
This is something that cannot be taken for granted. In 2012, the authoritative scientific review *Nature* invited 56 laboratories to replicate their cancer experiments in the presence of an independent expert. It emerged that only 11% had used appropriate cell models. «Some biologists think they are studying cells from breast cancer tumours, when instead they are experimenting on cervical cancer cultures», says Russell. «For this reason laboratories are increasingly turning to us for genuine samples».
What will happen to the Culture Collections after Brexit? «We don't know», replies Russell. «We are worried, like all British scientists: cooperation with Europe is fundamental and continues for the time being. We were established in 1920 to supply certified resources for the progress of science. And we still believe in this mission. This is why we send the Ebola virus free of charge: to support the search for a cure».

Vito Tartamella

Artificial viruses? Possible, but...

EXPENSIVE. These biobanks are not accessible to criminals. But could a terrorist create an artificial virus or bacterium? The question is certainly not fanciful: as long ago as 2002, the University of New York was successful in creating the poliomyelitis virus – whose RNA has only 7,500 bases – in the laboratory. But would it be possible to create and more complex and powerful virus? «Nowadays various companies are synthesizing the genome for payment», replies Massimo Pizzato, virologist at the Integrated Biology Centre of the University of Trento, Italy. «Assembling the genome of a virus might cost between 2 and 3 million euro. But in some cases, such as the smallpox virus, assembling the DNA is a long process: it has 200,000 bases, and the cost would be much greater». However, adds Pizzato, simply replicating a virus or bacterium's DNA or RNA is not enough: «Bacteria have membranes, walls and cytoplasm that cannot be reconstructed artificially. So it would be necessary to transfer the DNA to an existing bacterium. This was achieved by Craig Venter in 2010, but very few laboratories have the resources and skills to do so. Viruses, meanwhile, contain enzymes and have a proteinic and often also lipoid involucre, which is what makes them infectious. To replicate them, a criminal would need to have an in-depth knowledge of molecular and cellular biology. And it would take months. Not to mention the fact that he or she would need expensive equipment to manipulate these organisms safely».

Every year they send 25,000 biological samples all over the world. Under strict conditions.