

Summary report – Training Course of Microbial Resources Information Management and Utilization for Developing Countries

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Personal introduction

Dr. Elizabeth Harummy Takagi is bachelor of Pharmacy-Biochemistry, emphasis in clinical analysis. Doctorate in Sciences, emphasis in clinical microbiology and molecular biology - Degree conferred 2011, August. Bachelor and doctorate at University of São Paulo, São Paulo, Brazil. Scientific Researcher at Instituto Adolfo Lutz (IAL), since 2014, February at “Núcleo de Coleção de Micro-organismos” (NCMO). Responsible for help to update catalogue of microorganism; continuous improvement of system of quality control; research at evaluation of a possible standard method for *Leptospira* long-term preservation and microbial identification. Experience in molecular typing (RAPD, PFGE and MLST), identification of bacteria, biofilm, cell adherence, quorum sensing, mechanism of resistance to carbapenems.

Núcleo de coleção de micro-organismos

ABSTRACT

Culture collections constitute centres of ex-situ genetic resources conservation, geared towards the acquisition, preservation, maintenance, and distribution characterization of authenticated microorganisms. The conservation of their biological material constitutes essential raw material that support the life sciences and biotechnology, both for research and for industry. The ideal preservation or long periods should avoid contamination, loss of viability, loss of physiological and genetic characteristics of the biological material. In this context, the “Núcleo de Coleção de Micro-organismos” in the Instituto Adolfo Lutz has the mission of be a service culture collection with the main goal to maintain and provide strains with quality and authenticity. In addition, to promoting partnerships within the Institution, assisting in research projects, educational activities and handling of strains and also contribute with other microbial culture collections around the world, by this way, allow communication between scientific groups involved in the characterization and protection of microbial diversity. “Núcleo de Coleção de Micro-organismos is a member of the World Federation Culture Collection, since 1973, under number 282 and it is considered a legal repositories by case no: 017/09-SECEX/CGEN/Ministry of environment, since 2009. Our participation in “Training Course of Microbial Resources Information Management and Utilization for Developing Countries” was very importante to improve our knowledge and disseminate, share the information related to our holdings, and also enjoy the networking microbial resource centers.

Key words: Culture collections, Instituto Adolfo Lutz, Brazil, “Núcleo de Coleção de Micro-organismos”

Contents

1. Brief introduction of your Culture Collection.

Due to the responsibilities of the Instituto Adolfo Lutz as Central Laboratory of public health – LACEN were progressively formed collections of microorganisms in different areas of the institution.

These collections are originated both the work in the lab, as the acquisition of strains in researchers trips abroad, as well as donations and exchanges with other collections. In order to organize and maintain these microorganisms was created in 1940 the collection of Cultures, established in 1970 (Decree of structuring the IAL).

In 2010, had its name changed to Núcleo de Coleção de Micro-organismos - NCMO, by Decree of administrative reorganization of the IAL.

Currently the NCMO has a collection of 3,700 lineages, consisting of protozoa, fungi and bacteria.

Technical Centers of the IAL use strains from NCMO for testing, quality control and scientific research. Strains are also available to the public, access industries, universities and laboratories.

The “Núcleo de Coleção de Micro-organismos” is a member of the World Federation for Culture Collections WFCC – since 1972, under number 282.

In Brazil, the Genetic Heritage Management Council (CGEN) created by MP 2186-16 (23rd August 2001) is the branch of the federal government responsible to address the activities that involves the use (access), shipment/transportation of genetic resources and the access to associated traditional knowledge. Thus, the access of components of genetic heritage for scientific research, bioprospecting and technological development needs authorization from CGEN in order to guarantee a fair benefit sharing from

the national biodiversity. It is required from the applicant institution to assign the national repository that will officially host the microbial cultures derived from their research activities.

Currently, there are a number of culture collections listed in the CGEN website (<http://www.mma.gov.br/patrimonio-genetico/conselho-de-gestao-do-patrimonio-genetico/instituicoes-fieis-depositarias>) considered legal repositories and the “Núcleo de Coleção de Micro-organismos” is one by case no: 017/09-SECEX/CGEN/Ministry of environment, since 2009.

Despite this history, between 1998 and 2008 the collection of Cultures, responsible for the collection of microorganisms, went through various problems that interfered directly in the incorporation of new strains and the preservation of the deposits. Strains preserved by cryopreservation method discontinued, remaining strains just by freeze-drying and subculturing methods.

Apart from the service collection maintained by NCMO, routine and research collections over the years in the laboratories of the institution by its researchers and currently about 200,000 microorganisms are stored in Bacteriology, Parasitology and Mycology and virology.

These collections are of the most importance for the institution because they are use as reference controls in diagnostic methods, research projects, such as the characterization of new clones related to microbial resistance and or epidemiological diversity, development for production and control of vaccine among other applications. The use of strains from routine and research collections are fundamental basis for the scientific and technical development of the IAL.

It is important to note that the maintenance of routine and research collections is not suitable, because although freeze-drying or freezing preserve most strains, there is a periodic viability and control employees intended only to crop maintenance, in addition to inadequate

infrastructure, especially with respect to biosafety activities.

Because of the problems with the collection of NCMO service and the lack of structure of the collections for research and routine, since 2008 various actions are been developed in order to implement the service collection and organize the collections for research and routine, such as:

- the hiring of researchers in the areas of collections,
- purchasing equipment,
- aid for specific event participation (Collections Symposium),
- project approval infrastructure adequacy and
- authentication of NCMO's strains

In 2010:

- Policy Guidelines for the Biological Collections of IAL-order DG/IAL-17, of 12/22/2010 (DOE-SP Section I, p. 102, of 12/23/2010) were established
- Rules of procedure of the Committee of biological collections of the Instituto Adolfo Lutz-CCBIAL- 19, DG of Ordinance 12/28/2010 (DOE-SP Section I, p. 44, of 12/30/2010);
- Appointment of members of the Committee of biological collections of the Instituto Adolfo Lutz-Curatorships-DG-20 Ordinance, of 12/28/2010 (DOE-SP Section I, p. 43, of 12/30/2010); with republication for the biennium 2013-2015, Ordinance of 11 8/2/2013 IAL DG.

In 2011, the collection of Viruses of the institution created – Ordinance DG/IAL-3 of 4/14/2011 (DOE-SP Section I, p. 135, of 4/15/2011).

Other action developed was the inclusion of the "collection of cultures of microorganisms of public health laboratory reference" in the program of professional improvement of the SES/SP.

CCBIAL has worked in the Organization of collections of collections,

research and routine service, gradually incorporating the isolates characterized of interest to health and Science Collections established by Decree in order to receive an acronym, standardized methodologies and catalogued together with the associated information.

Despite the strains of interest already have been characterized in the centers of origin, when they are deposited in the collection, procedures are performed for confirmation of identification; maintenance such as freeze-drying and subculturing. After this manipulation is necessary to authenticate, to guarantee the purity of cultures and that its main features have been preserved. Currently, this authentication is not carrying out fully, especially in cases that depend on molecular biology techniques, once the NCMO has not yet deployed. The lack of equipment, supplies and human resources, has hindered not only the authentications, but also the expansion of the deposits. In addition, it is necessary to organize the collection of viruses, which along with the collection of microorganisms will enable the provision of collections for the whole community.

The methods of molecular biology are indispensable to any collection of microorganisms, as well as provide the identification based on genetics. In general, the cultures maintained for a long time can present such a problem, losing its original features, which leads to the fact become cultures that can no be used for quality testing or research with specific purposes. The deployment and implementation of maintenance and authentication methods in the “Núcleo de Coleção de Micro-organismos” in the IAL will provide the use of the strains in several areas of science in addition to ensure their quality and magnification.

The actions developed and intended this proposal come from meeting with the global movement of Biological resource centers training-BRC, coordinated by the German Government in Europe (Global Biological

Resource Centre Network – GBRCN), and in Brazil by the Ministry of science and technology-MCTI, with the participation of the INPI, INMETRO of Embrapa and the Oswaldo Cruz Foundation. The BRC defined in NIT-DICLA-061 – requirements on accreditation of testing laboratories and producers of reference materials of biological resource centers, such as:

"Essential part of the infrastructure that support biotechnology. Consist of service providers and repositories of living cells, organisms and genomes of heredity related information and functions of biological systems.

The BRC contain collections of cultivable organisms (e.g., microorganisms, cells, plants, animals and human); replicable parts of these (for example, genomes, plasmids, viruses, cDNAs), cells and tissues of viable organisms, but not yet cultivated, as well as databases containing molecular, physiological and structural information relevant to these collections and associated bioinformatics. "

In addition, the BRC should ensure the maintenance and supply of quality authenticated biological materials. In this order, the Organization for Economic Co-operation and development – OECD has been working together with the partner collections since 1998, editing multiple documents, being the most important: OECD best practice guidelines for biological resource centers, and best practice guidelines on Biosecurity for BRCS published in 2007. These guides are being used for the structuring of the BRC in the world and also in Brazil, once served as the basis for the drawing up, by INMETRO, of NIT-DICLA-061 – requirements on accreditation of testing laboratories and producers of reference materials of biological resource centers. This standard guides the accreditation of BRC for the production of certified reference Material – CRM. CRM strains used

in proficiency testing and validation of methods. Currently, few international collections license their lineages for the production of CRM for a few specialized industries. The implementation of the standard in the BRC of Brazil will enable the production of the CRM, to assist in the solution of this problem in our country. Although some institutions are BRC consolidated, there is still the possibility of inclusion of new collections, to be set by the BRC-coordination team Brazil. In this way, the IAL with their collections organized, can in the future to integrate the network of BRC in Brazil, in the area of health.

Finally, the structuring of the collections of IAL's scientific community will allow the so-called "quadrangle of health work in partnership, with access to qualified holdings. Microbiological diversity and volume of strains currently cataloged, combined with the possibility of incorporating samples of medical importance of partner institutions that do not have established collections, refer to the perspective of one of the largest collections of Latin America.

General objectives of “Núcleo de coleção de Micro-organismos” – IAL:

The structuring of biological collections of IAL, in both its physical and technical part, aims to enable the development of actions that will allow the certification of your holdings in accordance with national and international legislation in force for the formation of Biological resource centers. The adequacy of the physical space with reforms should ensure the conditions of biosecurity and bioprospection, as well as the appropriate storage of lineages, i.e. two different methodologies of conservation. In addition to creating specific spaces for procedures with molecular biology and new techniques that currently are indispensable in taxonomic studies.

These actions will also benefit the Bacteriology Center, Food Center, Parasitology and Mycology Center and Virology Center, which will transfer

its collections and research work to the same locations as the collection of service and research to the same location as the collection of service in order to maintain the same standard of operation and authentication.

Before the objectives, the organization will modernize and become official of all collections of IAL promote advances in the scientific development of the Institution and other entities that make use of the deposits strains.

Specific objectives:

- Reintroduce the methods of cryopreservation: freezing (-80°C and freezing in liquid nitrogen -196°C, targeting mainly microorganisms such as protozoa, bacteria of the genus *Leptospira* and viruses).
- Deploy the collection of viruses
- Introduce freeze-drying for viruses.
- Conduct genotype IDs when necessary, by rDNA sequencing, and the other specific genes microorganisms;
- Conduct control of mutations through genotyping, using techniques such as ERIC-PCR, RAPD, REP-PCR, RFLP, Pulsed-Field Gel Eletroforesis, and sequencing.
- Deploy barcode system for production, inventory control and supply of products.
- Computerize the deposits
- Expand public access providing the catalog on the web
- Deploy systems that ensure the safety of the deposits
- Deploy systems that guarantee the biosafety
- Deploy methodology to provide freeze-dried with at least 3 known concentrations of unit forming colony (UFC)
- Form DNA database with known concentrations for internal and external supply
- Form Bank of genetic identity through molecular typing

- Develop inputs as reference material certified with attention to pathogens that cause infectious emergencies (Bioterrorism)
- Deploy molecular identification service of bacteria not covered by other Technical Centers of IAL, e.g. *Staphylococcus aureus*, *Lactobacillus* spp., *Bacillus* spp., *Listeria* spp., among other public healthcare pathogens.
 - Deploy the ISO Guide 34 – production of certified reference material
 - Deploy the NIT-DICLA 061 – accreditation of laboratory assays and products producers of referente materials of Biological resource centers.
- Expand the collection from the collections of the IAL with focus on strains of importance regarding the characteristics of virulence and resistance aimed at research and technological development, as production and immunobiological products, vaccines and control processes.

2. Benefit from the training courses.

Meet other collections in various countries, different types of culture collections, different types of preservation used, what types of microorganisms preserved, the size of staff of the cultures collections and the type of services provided and also the researches developed in microbial collections.

It was fantastic to see the infrastructure of China General Microbiological Culture Collection (CGMCC).

Learn about the several tools provided by WDCM that help give visibility to collections and bioinformatic tools.

Practice data were helpful to see and resolve problems in class.

Oportunity to share opinios, future works, challenges by a networking culture collections centers.

Improve our knowledge and disseminate and share the information related to our holdings, we hope that some

3. Suggestion on WDCM work.

- Promoting a training course in Brazil, because we have many research collections throughout the country, besides enabling the awareness of the need to submit for strains collections that can administer that data. Currently, many data is lost due to the end of the survey or for lack of people to perform maintenance work.
- A link in website about opportunities to get funding to culture collections.
- Forum online to talk about culture collections.
- If possible, make video explanation about culture collections, Nagoya protocol, TRUST, GCM and other.
- Online meetings

4. Comments or suggestion on the training courses.

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Thank you for the opportunity to enjoy this relevant training course. I am so grateful for all.

Suggestions:

- promote integration between all participants of the course, know which site came and what type of collection (service and/or research), what type strains have and also the contact e-mail address.

- Perhaps this information could be included on the list of attendees delivered to one of us. If it were possible to have a small picture of each participant would be quite interesting to get to know each other.
- It would be interesting if we could get access to the list of participants before coming to China, in this way we could contact previously.
- The presentations of each participant could be divided into several days, with 5 or 6 presentations per day, probably at the end of the day, maybe this will be more favorable for there to be discussions about the different collections.

5. Suggestion on further cooperation between WDCM and your collections.

Continuous cooperation to help “Núcleo de Coleção de Micro-organismos” to manage, disseminate and share the information related to our holdings by this way we will can promote scientific and industrial usages of holdings. Increase our knowledge about bioinformatics and microbial information data management.