The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan

PROJECT COMPLETION REPORT

February 2023

JAPAN INTERNATIONAL COOPERATION AGENCY (JICA) CHIBA UNIVERSITY

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23-011

Contents of the Project Completion Report

I. Basic Information of the Project

1. Country

Federative Republic of Brazil

2. Title of the Project

The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan

3. Duration of the Project (Planned and Actual)

September 15, 2017-September 14, 2022

4. Background (from Record of Discussions(R/D))

Fungal infections develop in immunocompromised patients with Acquired Immunological Deficiency Syndrome (hereinafter referred to as "AIDS") or others, and the incidence is increasing worldwide. The fungi's drug resistance has strengthened along with the increased frequency in use of antifungal drugs, and the mortality rate of the patients having contracted drug-resistant fungal infections is 80%, reportedly.

Brazil has twice the number of patients with pulmonary tuberculosis out of chronic respiratory diseases as Japan does. 10% of such patients developed chronic aspergillosis with poor prognosis among fungal infections, and the 5-year survival rate is estimated at 50% or less (Nam HS, et al. Int J Infect Dis 2010: 14: e479). The mechanism how aspergillus gains drug resistance has not been clarified. For example, it could be through the exposure to pesticides containing ingredients similar to medical antifungal drugs in the fields, or in the body of a patient with chronic pulmonary aspergillosis, etc. who has undergone a treatment using azole-based drugs for a long time. Moreover, there are few public data within Brazil that shows the frequency of identification regarding aspergillus strains that cause drug resistance.

It is thus necessary to clarify the mechanism of fungi's causing drug resistance first, then, develop a new diagnostic method, establish a medical treatment system at clinical sites, collect accurate epidemiological data, and build up a new therapy.

5. Project Purpose (from Record of Discussions(R/D))

Project Purpose: A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.

6. Implementing Agency

(Brazilian side)

University of Campinas, Instituto Adolfo Lutz (São Paulo Central Reference Laboratory), Hospital Mário Gatti, Centro Hospitalar Ouro Verde, Centro Infantil Boldrini Pediatric Oncology Hospital, Prefeitura Municipal de Campinas

(Japanese side)

Chiba University, University of Nagasaki, National Institute of Infectious Diseases, University of Tsukuba, Obihiro University of Agriculture and Veterinary Medicine

7.Methodology of the Joint Review

The Joint Review was carried out by the joint review team comprising of Brazilian and Japanese members in accordance with JICA Project Evaluation Guidelines, which basically follows DAC Principles for Evaluation of Development Assistance issued by Organization for Economic Co-operation and Development – Development Assistance Committee (OECD-DAC). The PDM Ver.9 with the statement of the project purpose, outputs and activities and the PO Ver.9 were used as the basic reference documents for the evaluation. Findings and information from discussions and reports were analyzed and assessed in line with the six evaluation criteria.

The evaluation criteria used for the review survey are the following: Relevance, Coherence, Effectiveness, Efficiency, Impact, and Sustainability (Table).

Table:	Evaluation	Perspectives	Using	the	Six	DAC	Criteria	for	Evaluating
Develo	pment Assis	stance							

	Validity with project implementation (development needs)
Polovanco	Focus on "Beneficiary."
Relevance	Consideration for inclusiveness and equity
	Appropriateness of the project plan and logic of approach
Cabaranaa	Consistency with development assistance policies of the
Conerence	Japanese Government and JICA

	Synergistic effect/mutual relations with JICA's other projects				
	(technical cooperation, loans, grant aid, etc.) Complementarity, harmonization, and coordination with othe				
assistance/projects in Japan, other devel					
	organizations, etc.				
Consistency with global framework (internation					
	initiatives, standards, etc.)				
	The degree of achievement of target level in target year of				
Effectiveness	expected project outcome (differential results across the				
	groups)				
	Positive and negative indirect and long-term effects (systems				
Impact	and norms, people's well-being, human rights, gender				
	equality, and the environment)				
Efficiency	Comparisons of planned and actual projects inputs, project				
Enciency	period, and project cost				
	Outlook on sustainability of effects that are realized by the				
Sustainability	project for aspects of policy/political,				
Sustainability	institutional/organizational, technical, financial, social &				
	environment, risk, and operation & maintenance				

II. Results of the Project

1. Results of the Project

1-1 Input by the Japanese side (Planned and Actual) (See ANNEX1.1: Results of the Project 1)

a) Dispatch of experts

Total number: 30 persons

Detection of fungal genes related to resistance, Development of detection methods for the resistant-related genes, epidemiology, Establishment of BioResource bank

JICA Project Coordinator Oct. 2017-Sep. 2022

b) Trainings and Lectures

Training courses in Brazil: 12 courses

Total number of Brazilian researchers received in Japan: 14 persons

Analyses of fungal genes, Epidemiological surveillance, Development of research network

c) Machinery and equipment

Equipment cost for a total of 3,792,506 Japanese yen and 3.934.827,68 Brazilian real. Most of the equipment was procured in Brazil except 'Storage System for fungal strains (L-dried)', 'Loopamp Realtime Turbidimeter (LAMP)' and 'Macbook Pro 13inches'. The breakdown and procurement cost of the equipment provided is shown in Annex 1.2: Results of the Project 2. The equipment will be handed over to the Brazilian side at the end of the Project.

1-2 Input by the Brazilian side (Planned and Actual)

- (a) Services of UNICAMP's counterpart personnel and administrative personnel
 Project Director, Project Manager, Project Co-Managers, and other researchers and students (see ANNEX1.3: Results of the Project 3)
- (b) Suitable office space with necessary equipment
- (c) Research space and facilities
- (d) Supply or replacement of machinery, equipment, instruments, vehicles, tools, spare parts and any other materials necessary for the implementation of the Project other than the equipment provided by JICA
- (e) Information as well as support in obtaining medical service
- (f) Credentials or identification cards
- (g) Available data (including maps and photographs) and information related to the Project
- (h) Running expenses necessary for the implementation of the Project
- (i) Expenses necessary for transportation within Brazil of the equipment as well as for the installation, operation and maintenance thereof
- (j) Necessary facilities to the JICA experts for the remittance as well as utilization of the funds introduced into Brazil from Japan in connection with the implementation of the Project

1-3 Local Activity Budget

The Brazilian side shared a total of R\$138.845,00, and the Japanese side shared a total of R\$1.726.751,00 as local cost incurred for the Project. For further details, see ANNEX 1.4: Local activity budget.

T-4 Activities (Flamed and Actual)				
1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance				
are epidemiologically elucidated.				
1-1. To perform classification and	Achieved			
identification of fungal strains by	Determination of species of fungal			
morphological and biochemical	clinical strains isolated in the			
properties with clinically-isolated	attending hospitals (UNICAMP, Mario			
fungal strains collected by UNICAMP-	Gatti Hospital, Boldrini Children			
related and other project cooperating	Hospital and Barretos Hospital) and			
medical facilities.	environmental strains collected by			
	Instituto Adolfo Luz and Federal			
	University of Mato Grosso do Sul are			
	ongoing (over 1,000 clinical strains			
	and 500 environmental strains).			
	Several environmental fungal strains			
	collected in Mato Grosso do Sul State			
	are confirmed to be antifungal			
	resistant.			
1-2. To perform more detailed	Almost achieved			
identification of fungal strains	Detailed species (cryptic species)			
including cryptic species using	identifications of fungal strains			
genetic techniques on the fungal	isolated from clinical and			
isolates identified on the above	environmental specimens using			
activity.	several methods such as DNA			
	sequencing, multiplex PCR and			
	LAMP are in progress and are			
	expected to be completed by the end			
	of the Project.			
1-3. To examine the susceptibility of	Achieved			
the fungal species (especially for	Antifungal susceptibility testing is			
Aspergillus, Candida, Cryptococcus,	performed on the strains isolated			
and Fusarium) including some cryptic	clinically in the attending hospitals.			
species in accordance with				
internationally-approved methods				
(e.g., the methods provided by the				

1-4 Activities (Planned and Actual)

Clinical and Laboratory Standards	
1.4. To analyze the relationship	Achieved
hetween fungel characteristics	The database program (PEDCap®)
(strains, species and drug	has been operated and over 350
(strains, species and drug	clinical cases of patients with various
characteristics (source of infection	fundal infection admitted to the
infected sites underlying and/or	attending health care institutions have
complicated diseases, clinical entity	altending ficaliti care institutions have
history of chemotherapy by	
antifungals etc.)	
1-5. To elucidate mechanism of drug	Achieved
resistance by analyzing gene regions	To elucidate the effect of fungicides
which are reported to be responsible	on mutagenicity of fungi, studies on
for drug resistances using the fungal	exposure of A. fumigatus strains to a
isolates with drug-resistant property.	fungicide in a simulated field are
confirmed by the susceptibility	ongoing.
testing.	The CRISPR/Cas9 system are used
Ŭ	to elucidate the effect of some
	mutated genes on antifungal
	resistance.
1-6. To analyze geographic	Achieved
characteristics of drug resistance	Compared with fungal strains isolated
genes by comparing identified drug-	in Japan, a different polymorphism in
resistant mechanisms against	cyp51A gene was revealed in
antifungals.	Brazilian isolates.
1-7. To elucidate mechanisms of drug	
resistance using advanced genetic	
analysis technologies such as next-	
generation sequencing techniques for	
the fungal isolates, which are	
suspected to have unidentified drug	
resistance mechanisms.	
1-7-1. To provide Brazilian	Achieved
researchers with the Training in	See ANNEX1.1.
Japan in the theme of genetic	

analysis techniques using the next-	
generation sequencer for elucidating	
drug-resistant mechanisms at the	
MMRC.	
1-7-2. To identify gene regions, which	Almost achieved
are suspected to be responsible for	Using a next generation sequencer,
drug resistance, by whole-genomic	gene expression analysis
comparison of drug-susceptive and	(transcriptome) of antifungal-
drug resistant fungal strains isolated	susceptible/resistant strains is
in Brazil, followed by the functional	ongoing and will continue after the
analyses of the region in the	Project.
UNICAMP.	
1-8. To compile research findings and	Achieved
outputs into scientific articles,	Based on the results of this project,
together with that gained in the	the existing clinical manual for
Output 2 and in Japan, to be used as	aspergillosis has been revised.
evidence for revising the existing	
guidelines and for newly-developing it	
for the management of mycoses,	
followed by the trial-based application	
to the hospitals in Campinas	
metropolitan areas and/or other areas	
in Brazil.	
2. Gene amplification technologies-bas	ed drug-resistance genes detection
methods with sufficient sensitivity, spec	ificity and operability are established
for the diagnoses of drug-resistant myc	coses.
2-1. To develop gene amplification	
(LAMP, PCR, etc.)-based gene	
detection methods for the diagnoses	
of drug-resistant mycoses.	
2-1-1. To develop basic testing	Achieved
condition of detection methods for	Detection procedures for mutations in
drug-resistant fungi particularly for	cyp51A gene using PCR or LAMP
Aspergillus including its cryptic	method is developed in Brazil and
strains, Candida and other fungi,	Japan.
which are suitable for applied gene	Development of identification

amplification techniques.	procedures of cryptic species using
	PCR or LAMP method are in
	progress.
2-1-2. To provide Brazilian	Achieved
researchers with the Training in	See ANNEX1.1.
Japan in the theme of gene	
amplification-based gene detection	
methods for the diagnoses of drug-	
resistant mycoses.	
2-1-3. To introduce the detection	Achieved
methods for drug-resistant fungi,	Introduction of new detection method
developed in the Activity 2-1-2, to the	for antifungal resistant fungi using
UNICAMP by the joint efforts of	Surveyor Nuclease has been started.
Japanese researchers (JICA experts)	
and the Brazilian researchers who	
participated the Training in Japan.	
2-2. To evaluate the clinical	Almost achieved
performance of the detection	The new detection method for
methods for drug-resistant fungi	antifungal resistant fungi using
statistically by analyzing its sensitivity	Surveyor Nuclease is regularly
and specificity using fungal strains	performed in Brazil.
clinically-isolated from patients with	
mycoses in Brazil.	
2-3. To evaluate the operability of the	Almost achieved
drug-resistant gene detection	Detection procedure for point
method(s), which are developed on	mutations in cyp51A gene using PCR
the basis of LAMP techniques, by	or LAMP method are developed in
introducing them into UNICAMP-	Brazil and Japan, and their operability
related hospitals and other selected	are evaluated now.
external cooperative health or testing	
facilities on a trial basis.	
2-4. To initiate discussions with	Achieved
Brazilian authorities concerned on the	Counterparts of this project are the
practical application of the drug-	official members of the Committee on
resistant gene detection methods,	Microbial Resistance of Sao Paulo
developed by the Project, to clinical	State Department of Development

laboratory testing	and Innovation.
(registration/accreditation of the	
methods as the "Standard Methods"	
in Brazil).	
3. A UNICAMP-centered research colla	boration system among research,
medical and administrative organization	ns is established.
3-1. To construct a banking system of	
fungal strains including drug-resistant	
ones in the UNICAMP, which is	
interoperable by the external	
cooperative agencies for mycoses	
research.	
3-1-1. To develop bylaws for the	Achieved
operation of the sample banking	Material Transfer Agreement (MTA)
system, which stipulates the	was contracted between UNICAMP
supply/transportation of samples to	and MMRC, and some notable fungal
the UNICAMP, preservation and	strains has already shared each
shared use of the fungal strains	other.
including drug-resistant ones, by	
holding meeting with external	
cooperative agencies of the Project	
such as universities, hospitals and	
testing facilities.	
3-1-2. To provide Brazilian	Achieved
researchers with the Training in	See ANNEX1.1.
Japan in the theme of the operational	
management of fungal strains	
preservation facility at the MMRC.	
3-1-3. To develop a facility for the	Achieved
preservation of fungal strains in the	Preservation of fungal strains in
UNICAMP.	UNCAMP are ongoing.
3-1-4. To construct an information	Achieved
database of clinically-isolated fungal	The database program (REDCap®)
strains, compromised of fungal and	has been operated and over 350
patient' characteristics in the	clinical cases of patients with various
UNICAMP.	fungal infection admitted to the

	attending health care institutions have
	already enrolled.
3-2. To hold regular conferences	Achieved
and/or seminars on drug-resistant	The enrolled cases were selected in
fungi including the reporting of project	clinical case conferences attended by
achievements, geared to project	various medical professions including
external collaborating agencies,	physicians, nurses and
health and medical facilities in the	microbiologists of UNICAMP hospital
project areas and the administration	and other health care institutions
organizations in charge of the control	under a video-conferencing system.
of fungal infections including drug-	
resistant ones.	
3-3. To hold regular meetings with the	Achieved
administration organizations in	Medical professions of other
charge of the control of fungal	institutions have attended to the
infection including drug-resistant ones	regular conference and received a
for sharing of the epidemiological	training to input clinical information.
information such as incidence and	
clinical entity, gained through the	
research activities of the Project.	
3-4. To set up a network of clinical	Almost achieved
laboratory testing and diagnosis of	As the research network activity
mycoses including drug-resistant	participated by several medical
ones among the UNICAMP (as the	institutions and research laboratories
reference laboratory) and health and	in Sao Paulo State and Mato Grosso
testing facilities in the State of São	do Sul State, collecting clinical and
Paulo with the support of the Institute	environmental fungal strains, the
of Adolfo Lutz (IAL).	regular conference, and scientific
	meetings are run.
3-5. To set up a network of clinical	Achieved
laboratory testing and diagnosis of	The regular conference held on every
mycoses including drug-resistant	week is profitable for development of
ones among research institutes,	young medical experts at the
health facilities and the MMRC as the	participating medical institutions.
reference laboratory also in Japan, by	
referencing the achievements of	

project collaborative research and	
technical cooperation in Brazil.	
3-6. To hold national and international	Achieved
conferences on the research of drug-	International training course or
resistant mycoses geared to	international conference were already
research, health and administrative	held during this project.
agencies in other provinces and	Based on research and multicultural
nations, in light of future	experiences of the Project, UNICAMP
establishment of a network of	has attended to an international
collaborative research on mycoses	research project of CDC, and the
including drug-resistant ones as well	molecular epidemiology and
as wide-sharing of research findings	infectious disease laboratory in
and outcomes of the Project.	UNICAMP was designated as a
	"reference laboratory in Latin
	America."

2. Achievements of the Project

2-1 Outputs and indicators

(Target values and actual values achieved at completion)

1. Prevalence of drug-resistant fungi and its mechanisms of drug-			
resistance are epidemiologically elucidated.			
Indicator	Achievement		
(1) By December, 2019, the	Achieved		
proportion of drug-resistant fungal	The prevalence of antifungal-resistant		
strains in the fungal isolates (the	fungal strains among clinical isolates		
prevalence) in the target areas is	has been estimated, and scientific		
estimated.	articles have been published.		
(2) By March, 2020, principal	Achieved		
mechanisms of drug resistance	Main Mechanism of antifungal		
against antifungal agents are	resistance was identified and		
identified using clinical isolates	scientific articles have been		
gained in the Campinas metropolitan	published.		
area.			
(3) By March, 2022, the revising work	Achieved		
of the existing guidelines, and drafting	Based on the results of this project,		

work of the guidelines/booklets for the	the existing clinical manual for
management of mycoses are	aspergillosis has been revised.
commenced.	

2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.

Indicator	Achievement
(1) By December, 2020, basic testing	Achieved
condition and procedures of gene	The new detection method for
amplification (LAMP, PCR, etc.)-	antifungal resistant fungi using
based gene detection methods for the	Surveyor Nuclease was developed
diagnoses of drug-resistant	and reported as scientific articles.
Aspergillus, Candida and other fungi	
are determined.	
(2) By December, 2021, evaluation	Achieved
works of clinical performance of the	This detection method has already
above-mentioned methods are	been regularly performed in
completed.	UNICAMP.
3) By the time of the termination of	Achieved
the Project, concrete discussions with	The Brazilian specialists were
the Brazilian authorities concerned	discussing this issue through the
regarding practical procedures for the	research network system developed
application of the above-mentioned	in this project.
methods for clinical laboratory testing	
(registration/accreditation of the	
methods as the "Standard Methods"	
in Brazil) are commenced.	

3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.

Indicator	Achievement
(1) By the time of the termination of	Achieved
the Project, practical operation of the	Determination of species and
banking system of fungal strains	antifungal susceptibilities of fungal
including drug-resistant ones in the	clinical strains isolated in the

UNICAMP, which is interoperable by	attending hospitals (UNICAMP, Mario	
the external cooperative agencies for	Gatti Hospital, Boldrini Children	
mycoses research, is commenced.	Hospital and Barretos Hospital) and	
	environmental strains collected by	
	UNICAMP and Instituto Adolfo Luz	
	are ongoing (over 1,000 clinical	
	strains and 500 environmental	
	strains). After these evaluation,	
	preservation of the fungal strains	
	using the L-drying system (a	
	lyophilization method) in UNCAMP	
	are ongoing.	
(2) By December 2020,	Achieved	
epidemiological information on	The database program (REDCap®)	
mycoses including drug-resistant	has been operated and over 350	
ones, gained through the research	clinical cases of patients with various	
activities of the Project, is shared with	fungal infection admitted to the	
the Brazilian authorities concerned at	attending several health care	
a one-year interval.	institutions have already enrolled.	
(3) By the time of the termination of	Achieved	
the Project, practical operation of the	The enrolled cases were selected in	
network of clinical laboratory testing	regular clinical case conferences	
and diagnosis of mycoses including	attended by various medical	
drug-resistant ones, of which	professions including physicians,	
reference laboratory is the UNICAMP,	, nurses and microbiologists of	
is commenced.	UNICAMP hospital and other health	
	care institutions. Medical professions	
	of other institutions have also	
	attended to the regular conference	
	and received a training to input	
	clinical information.	

2-2 Project Purpose and indicators

(Target values and actual values achieved at completion)

A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.

Indicator	Achievement
(1) By the time of the termination of	Achieved
the Project, guidelines/booklets for	Based on the results of this project,
the management of mycoses are	the existing clinical manual for
subjected to trial-based application in	aspergillosis has been revising.
hospitals in the Campinas	
metropolitan area.	
(2) By the time of the termination of	Achieved
the Project, concrete discussions are	Counterparts of this project are the
commenced with Brazilian	official members of the Committee on
administrative organizations for the	Microbial Resistance of Sao Paulo
application of research findings and	State Department of Development
outcomes gained by the Project to	and Innovation.
policymaking and/or	
countermeasures for the control of	
fungal infections including drug-	
resistant ones.	
(3) By the time of the termination of	Achieved
the Project, at least 5 research	Total of 32 articles were published in
articles in the themes of project	this project (see ANNEX 2).
research topics such as	
epidemiological analyses of drug-	
resistant fungal infections,	
mechanisms of resistances of fungi to	
antifungals, and drug resistance-	
related genes detection methods are	
published.	

3. History of PDM Modification

Although the PDM has not been modified, part of the equipment procurement plan has been revised.

4. Others

As emergency support for the COVID-19 worldwide pandemic, we conducted the following activities, which were not planned in the PDM.

4-1. [Ad-hoc Panel of MIRE on COVID-19; AMC-19]

The Chief Advisor and counterparts started regular joint clinical conferences regarding COVID-19 and its-related issues to combat the COVID-19 pandemic as a special task force of the project since May 2020. As of August 2022, the meetings were held 23 times. Discussed issues are listed in ANNEX 6: List of the discussed issues in AMC-19.

4-2. [The Partnership for Accelerating COVID-19 Testing in Brazil (PACT Brazil)] Reliability verification test of the Loopamp 2019-nCoV detection kit was started in Brazil after exchanging the memorandum among JICA, Chiba University, UNICAMP and Eiken Co. Ltd. The objective of the PACT Brazil is to support the efforts of Brazil in increasing testing of SARS-Cov-2 through the quadripartite cooperation. The Participants worked together to conduct testing of the sample of test kits, and once the certification of clinical use is obtained, the Participants will consider the possibility of further cooperation to expand its clinical use.

III. Results of Joint Review			
1. Results of Revie	1. Results of Review based on DAC Evaluation Criteria		
Relevance	The relevance of the Project is high, since the Brazilian and Japanese development		
	policies/strategies on the health sectors in Brazil has		
	not been changed and the sector has been highly		
	prioritized since the commencement of the Project.		
	Antifungal resistance of fungal isolates has		
	strengthened worldwide, and the mortality rate of the		
	patients with fungal infections caused by resistant		
	fungus is 80%, reportedly. Fungal resistance is a big		
	public health problem; however, there were few public		
	data within Brazil. It was thus necessary to collect		
	accurate epidemiological data, to clarify the		
	mechanism of fungi's causing drug resistance, to		
	develop a new diagnostic method and to establish the		
	appropriate management at clinical sites.		
Coherence	The coherence of this project is high.		
	The objects of this project are consistent with the		
	Priority Policy "Building a sustainable and resilient		
	international community through efforts to address		
	global challenges." Moreover, this project is in		

	concord with Joint Statement of the Official Working			
	Visit of Prime-Minister Shinzo Abe to Brazil and on			
	the Establishment of the Strategic and Global			
	Partnership between Japan and Brazil (2014).			
	In 2015, WHO formulated a Global Action Plan			
	regarding antimicrobial resistance (AMR), and			
	thereafter, National Action Plans were formulated in			
	each country, including Japan, in 2016. In 2019, the			
	US CDC reported Antibiotic Resistant Threats, and			
	listed drug-resistant fungi in the document.			
	Some medical staff of MMRC regularly examine the			
	patients with fungal infection at Chiba University			
	Hospital using knowledge gained from the Project.			
	Furthermore, periodical clinical conference regarding			
	COVID-19 attending several medical institutions in			
	Brazil and Japan started.			
Effectiveness	The effectiveness of this project is high. As described			
	above, the perspective of achievement of the project			
	purpose is judged to be high since the objectively			
	verifiable indicators have been fulfilled appropriately.			
	Logical sequence of the causal relationships between			
	Outputs and the Project Purpose is enough strong			
	since (i) epidemiological elucidation of antifungal			
	resistant fungi and (ii) practicalities of rapid diagnostic			
	methods for the resistant fungi is indispensable to			
	enhanced capacities for diagnosis and treatment of			
	fungal infections between Chiba University and State			
	University of Campinas. The Brazilian side secured			
	additional budget for renovation of the molecular			
	epidemiology and infectious disease laboratory in			
	UNICAMP. The devoted leadership of Project			
	Manager and Chief Advisor have contributed to the			
	anticipated outcomes and enhanced research			
	capacities of CPs at Faculty of Medical Sciences,			
	UNICAMP.			
Impact	The impact of this project is high. Next generation			

	sequencing and genetic transformation technologies		
	have been developed by the Project and are		
	expected to be applied to other research areas		
	including virus and bacteria. Based on research and		
	multicultural experiences of the Project, UNICAMP		
	has attended to an international research project of		
	CDC, and the molecular epidemiology and infectious		
	disease laboratory in UNICAMP was designated as a		
	"reference laboratory in Latin America."		
Efficiency	The efficiency of this project is high. With regard to		
	quality and quantity input from the Brazilian and		
	Japanese sides such as allocation of CPs Japanese		
	experts provided equipment training in Japan at		
	Chiba University office and facilities are appropriate		
	Delegation of IICA experts in managerial aspects		
	medical aspects and research technical aspects is		
	appropriate since the Chief Advisor and Project		
	Appropriate since the Unier Advisor and Project		
	medical researchers took roles in sharing a lot of		
	research technologies		
Suctoinability	The prospect of sustainability is high from		
Sustainability	institutional technical and human resources		
	viewneint. The administrative and coordomic status of		
	Viewpoint. The administrative and academic status of		
	UNICAMP is sufficiently prestigious not only in Brazil		
	but also in Latin America, that it is expected to		
	disseminate adopted skills and technologies to other		
	institutions and to continue to be influential to society.		
	In fact, some experimental technologies had been		
	already introduced to other medical institution (Centro		
	Infantil Boldrini Pediatric Oncology Hospital). The		
	prestigious academic authority of the university that		
	may influence standardization of relevant regulations		
	Thay influence standardization of relevant regulations		
	and procedures is expected to obtain successive		
	and procedures is expected to obtain successive political support.		
	and procedures is expected to obtain successive political support. While the political and economic situation in Brazil		

agreed to take necessary measures to maintain the
machinery and equipment provided. Also, adopted
technologies are expected to be transferred to other
institutes in Brazil as well as other countries through
international training courses or international
conferences in the future. Such courses and
conferences were already held during this project.

2. Key Factors Affecting Implementation and Outcomes

(COVID-19 pandemic)

Part of the planned dispatches of JICA experts and training for Brazilian researchers in Japan could not performed because of the COVID-19 pandemic.

3. Evaluation on the results of the Project Risk Management

(COVID-19 pandemic)

Scientific meetings between the Chief Advisor, JICA experts and Brazilian researchers were performed periodically using a video-conferencing system and e-mail. Moreover, to keep up the efficient activities, Japanese team frequently conducted on-line training to Brazilian researchers as far as possible. Instead of the research activities in Brazil, a part of the relevant research works was performed in Japan. Furthermore, despite the pandemic, Brazilian researchers continued the research activities (ANNEX1.5: Other Activities in Brazil).

Through such united effort of both sides, the project was proceeded as planned.

(AMC-19)

The Chief Advisor and counterparts started regular joint clinical conferences regarding COVID-19 and its-related issues to combat the COVID-19 pandemic as a special task force of the project (Ad-hoc Panel of MIRE on COVID-19; AMC-19) since May 2020. As of August 2022, the meetings were held 23 times. Discussed issues are listed in ANNEX 6.

(PACT Brazil: The Partnership for Accelerating COVID-19 Testing in Brazil) Reliability verification test of the Loopamp 2019-nCoV detection kit was started in Brazil after exchanging the memorandum among JICA, Chiba University, UNICAMP and Eiken Co. Ltd. The objective of PACT Brazil is to support the efforts of Brazil in increasing testing of SARS-Cov-2, through the quadripartite cooperation. Participants worked together to conduct testing of the sample of test kits, and once the certification of clinical use is obtained, the Participants will consider the possibility of further cooperation to expand its clinical use.

4. Lessons Learnt

The Project indicates that it is indispensable for a scientific research-focused project to establish its research platform at the initial stage of project implementation including "hard" factors such as machinery, facility, office environment, and "soft" (human resource) factors such as communication, devotedness to project activities, harmonized teamwork among CPs and experts. In the Project, Brazilian and Japanese sides made possible efforts to establish and strengthen trust under the COVID-19 pandemic. It is indispensable to establish trust between Japanese and counterpart sides especially in an international co-research project with intercultural communication and cooperation.

During the Project implementation, early interventions and measures, such as holding frequent meeting to discuss the progress of the research and affirm the direction of the Project, were taken to prevent potential miscommunications caused by a multicultural communication gap between both sides. The long-term expert and the Project Manager have had a significant role in preventing potential miscommunication and alleviating the gap among the project members.

The Project successfully procured appropriate machinery that matches the needs of both the Japanese and Brazilian sides. As of this moment, a total of new 32 academic papers (See ANNEX 2: List of Products) on mycosis research have been published in peer-reviewed journals.

ANNEX 1.1: Results of the Project 1

(List of Dispatched Experts, List of Trainings)
ANNEX 1.2: Results of the Project 2
(List of Machinery and Equipment)
ANNEX 1.3: Results of the Project 3
(List of counterparts)
ANNEX 1.4: Local Activity Budget
ANNEX 1.5: Other Activities in Brazil
ANNEX 2: List of Products (Research articles) Produced by the Project
ANNEX 3: PDM (All versions of PDM)

ANNEX 4: R/D, M/M, Minutes of JCC (copy) (*) ANNEX 5: Monitoring Sheet (copy) (*) (Remarks: ANNEX 4 and 5 are for internal reference only.)

ANNEX.6: List of discussed issues in AMC-19

Annex 1.1. Results of the Project

JICA expert	Affiliation	Period of visiting
Akira Watanabe	MMRC, Chiba University	Sep. 13-26, 2017
(Chief Advisor)		Mar. 3-16, 2018
		Sep. 22-Oct. 5, 2018
		Feb.5-25, 2019
		May 12-Jun. 2, 2019
		Jan. 24-Feb. 16, 2020
Tetsuhiro Matsuzawa	University of Nagasaki	Sep. 13-26, 2017
		Mar. 3-25, 2018
		Sep. 15-Oct. 5, 2018
		Feb. 9- Mar. 2, 2019
		Sep. 14-Oct. 7, 2019
		Jul. 7-25, 2022
Teppei Arai	MMRC, Chiba University	Sep. 13-26, 2017
		Feb. 25-Mar. 25, 2018
		Sep. 15-Oct. 13, 2018
		Feb. 5-Mar. 18, 2019
		Sep. 8-Oct.7, 2019
		Jan. 24-Feb. 16, 2020
		Jul. 7-25, 2022
Yasunori Muraosa	MMRC, Chiba University	Mar. 3-16, 2018
		Sep. 15-Oct. 7, 2019
Hiroki Takahashi	MMRC, Chiba University	Nov. 3-16, 2019
Sayaka Ban	MMRC, Chiba University	Feb. 5-25, 2019
Yuzuru Mikami	MMRC, Chiba University	Mar. 3-16, 2018
		Jun. 8-30, 2018
		Jan. 24-Feb. 16, 2020
Tohru Gonoi	MMRC, Chiba University	Feb. 25-Mar.16, 2018
		Jun. 8-30, 2018
		Feb.6-16, 2020
Yutaro Hino	MMRC, Chiba University,	May 12-Jun. 2, 2019
	Chiba University Hospital	
Yukiko Tsuchiya	MMRC, Chiba University	May 12-Jun. 2, 2019

List of Dispatched Experts

JICA Project Coordinator

Oct. 2017-Sep. 2022

List of Brazilian Counterparts

See ANNEX1.3.

List of Trainings

<in Brazil>

- Antifungal Susceptibility Tests Workshop: Mar.14-15, 2018, Dr Mikami
- Training Course of LAMP: Mar. 19-21, 2018, Dr. Matsuzawa, Dr. Arai
- Lecture "Introductory medical mycology": Jun. 22, 2018, Dr. Mikami
- Lecture "Genomic and transcriptomic analyses of human pathogenic fungi, Aspergillus fumigatus": Jun. 22, 2018, Dr. Gonoi
- Lecture and Training "Phylogenetic analysis": Feb. 8, 2019, Dr Ban
- Training on L-drying system for fungal preservation: Feb. 12-15, 2019, Dr. Ban
- Training on Antifungal Susceptibility Testings and MIC Readings: May 20-24 and 27-30, 2019, Ms. Tsuchiya and Dr. Watanabe
- Lecture "Heterogeneity of stress response in pathogenic fungus": Nov. 7, 2019, Dr. Takahashi
- Workshop on the CRESPR/cas9: Feb. 10, 2020, Dr. Arai
- International Training Course in Fungal Infection: May 4-8, 2020, Dr.
 Watanabe, Dr. Ban
- 1st International Symposium FUNGI: FROM THE ENVIRONMENT TO THE HOST: Nov. 23-27, Dr. watanabe, Dr. Ban
- Training on CRISPR/Cas9: Jul. 12-14, 18, 2022, Dr. Arai

<in Japan>

- Analyses of fungal genes: Aug. 5-Sep. 10, 2018, C Tararam
- Epidemiology, Development of research network: Aug. 19-Sep. 10, 2018, MR Resende
- Analyses of fungal strains, Preservation of fungal isolates, Aug. 19-Sep. 10, 2018, A Schreiber
- Methods of next generation sequencing, Development of research network: Nov. 11-Dec. 14, 2018, P Trabasso
- Analyses of fungal genes: Nov. 11-Dec. 14, 2018, L Levy

- Development of research network: Nov. 25-Dec.14, 2018, ML Moretti
- Methods of next generation sequencing: Jul. 11-Sep. 5, 2019, C Tararam
- Development of research network, Analyses of fungal genes: Oct. 6-26, 2019, ML Moretti
- Analyses of fungal strains, Development of research network: Oct. 6-Nov. 3, 2019, P Trabasso
- Epidemiological surveillance, Development of research network: Nov. 18-Dec. 5, 2019, MR Resende, T Lima, EM Psaltikidis, IS Sauer, AC martins

Training courses provided by other institutions in Brazil and fulfilled by UNICAMP staff

See ANNEX1.5.

Annex 1.2 : List of Machinery and Equipment

No.	Name of Equipment	Quantity	Amount
1	CO2 Incubator	1	R\$ 29,900.00
2	Chemiluminescence imager	1	R\$ 189,999.23
3	Storage System for fungal strains (L-dried)	1	¥1,654,538
4	Thermal Cycler	2	R\$ 71,596.94
5	Capillary sequencer	1	R\$ 336,693.59
6	Biosafty Cabinet -1	2	R\$ 41,800.00
7	Biosafty Cabinet -2	1	R\$ 15,200.00
8	Biosafty Cabinet -3	1	R\$ 15,700.00
9	Clean Bench	1	R\$ 11,980.00
10	Biosafty Cabinet -4	2	R\$ 100,000.00
11	Refrigerated Centrifuge	1	R\$ 126,172.42
12	Ultrapure Water System	1	R\$ 69,723.49
13	Micro Refrigerated Centrifuge	1	R\$ 38,735.51
14	Macbook Pro 13inches	1	¥247,968
15	Loopamp Realtime Turbidimeter (LAMP)	1	¥1,890,000
16	Bead Beater	1	R\$ 45,125.21
17	Deep Freezer	1	R\$ 152,175.00
18	Refrigerator (glass-fronted)	2	R\$ 30,300.00
19	Optical Microscope	1	R\$ 34,229.76
20	Dry Heat Sterilizer	1	R\$ 6,352.41
21	Microplate Washer	1	R\$ 68,137.00
22	Block Incubator	2	R\$ 34,356.21
23	Incubator Shaker	1	R\$ 17,200.00
24	Real-time PCR	1	R\$ 236,619.99
25	Incubator	1	R\$ 3,910.00
26	Vertical Autoclave	2	R\$ 24,980.00
27	Eppendorf Repeater Electronic Pipette	1	R\$ 12,807.65
28	Electrophoresis System	2	R\$ 22,022.00
29	Fluorometer	1	R\$ 15,333.67
30	Freezer	1	R\$ 2,120.31
31	Refrigerator	1	R\$ 2,549.89
32	Rotary Homogenizer	1	R\$ 4,880.00
33	Schletter	1	R\$ 3,890.00
34	Ph Meter	1	R\$ 1,850.00
35	Small Burner	2	R\$ 12,800.00
36	UPS	1	R\$ 2,280.00
37	No Break	1	R\$ 3,015.00

1. List of Machinery and Equipment Provided on JICA Scheme

38	Computer	3	R\$ 18,960.00
39	Camera GPRO	1	R\$ 2,408.01
40	Mini-spinar	1	R\$ 12,202.29
41	Protective Gown	3000	R\$ 14,340.00
42	Protective Apron	3000	R\$ 25,260.00
43	Surgical Mask	3000	R\$ 2,820.10
44	Protective Gloves	3000	R\$ 17,076.00
45	PFF2 Surgical Mask	3000	R\$ 16,830.00
46	Cardioverter	10	R\$ 288,809.50
47	Electrocardiograph	10	R\$ 117,686.50
48	Multi-parameter Monitor System for ICU	20	R\$ 1,190,000.00
49	Portable Ultrasound Equipment	3	R\$ 444,000.00

Note: No.41 - 49 are ICU unit equipment has been donated for emergency assistance to combat the COVID-19.

ANNEX 1.3: Results of the Project 3 (List of counterparts) **Project MIRE** List of Brazilian Counterparts

					Updated August 1, 2022
	Institution	Name	Occupation	Position in the Project	Assigned to
1		Ivan Felizardo Contrera Toro	MD, PhD, Assistant Professor	Project Director (Dean of the FCM)	Coordination
2		Luís Carlos Zeferino	MD, PhD, Professor	Project Director (Dean of the FCM)	Coordination
3		Cláudio Saddy Rodrigues Coy	MD, PhD, Professor	Project Director (Dean of the FCM)	Coordination
4		Maria Luiza Moretti	MD, PhD, Professor	Project Manager (Vice-Rector of UNICAMP)	Coordination
5		Plinio Trabasso	MD, PhD, Associate Professor	Project Co-Manager	Molecular Biology studies
6		Angélica Zaninele Schreiber	PharmD, PhD, Assistant Professor	Project Co-Manager	Microbiology studies
7		Mariângela Ribeiro Resende	MD, PhD, Associate Professor	Project Co-Manager	Epidemiology studies
8	Unicamp	Lucieni de Oliveria Conterno	MD, PhD, Assistant Professor	Researcher	Epidemiology studies
9	onicamp	Elisa Donalísio Teixeira Mendes	MD, PhD, Infection Disease Practitioner	Researcher	Epidemiology studies
10		Renata Fagnani	RN, PhD, Infection Control Nurse	Researcher	Epidemiology studies
11		Eliane Molina Psaltikidis	RN, PhD, Infection Control Nurse	Researcher	Epidemiology studies
12		Tiago Lima	RN, PhD, Infection Control Nurse	Researcher	Epidemiology studies
13		Cibele Aparecida Tararam	BS, PhD, Biologist at LEMDI	Researcher	Molecular Biology studies
14		Larissa Ortolam Levy	BS, Biologist at LEMDI	Researcher	Molecular Biology studies
15		Erivan Olinda de Oliveira	Technician at LEMDI	Laboratory technician	Molecular Biology studies
16		Luzia Lyra	Technician at LIF	Laboratory technician	Microbiology studies
17	Campinas Municipal	Cármino Antônio de Souza	MD, PhD, Professor	Secretary of Health, Campinas Municipality	Administrative
18	Health	Andrea von Zubem	DMV	Health Surveillance Team	Epidemiology studies
19	Surveillance	Marcela Torres de Lolo	MD	Health Surveillance Team	Epidemiology studies
20	Surveinance	Roberta Yabu-uti do Valle	RN	Health Surveillance Team	Epidemiology studies
21		Marcos Eurípides Pimenta	MD	Director of HMMG	Administrative
22		Sergio Bisogni	MD	Director of HMMG	Administrative
23	Hospital Mário Gatti	Christiane Ambrósio	RN, Infection Control Nurse	Researcher	Epidemiology studies
24	(Municipal General	Inês Helena Leal Saraiva	MD, PhD, Infection Control Physician	Researcher	Molecular Biology studies
25	Hospital)	Christian Cruz Hofling	MD, PhD, Infection Control Physician	Researcher	Epidemiology studies
26		Daisy de Souza Araújo	MD, Clinical Pathology Physician	Researcher	Microbiology studies
27		Rogério Hakio Kuboyama	BS, PhD, Biologist at HMMG Laboratory	Researcher	Molecular Biology studies
28	Centro Hospitalar Ouro Verde	Roseli Aparecida de Lima	RN, Infection Control Nurse	Researcher	Epidemiology studies
29	(Municipal General Hospital)	Antônio Camargo Martins	MD, Infection Control Physician	Researcher	Molecular Biology studies
30	Centro Infantil Boldrini	Sílvia Regina Brandalise	MD, PhD, Professor	Researcher	Molecular Biology studies
31	Pediatric Oncology	Hellen Caroline Alves Caldeira	RN, Infection Control Nurse	Researcher	Epidemiology studies
32	Hospital	Flávio Henrique Gilli	MD, Infection Control Physician	Researcher	Epidemiology studies
33		Rosângela Aparecida Mendes	BS, Biologist at CIB Laboratory	Researcher	Microbiology studies
34		Márcia M.C. Melhem	PharmD, PhD	Researcher	Environmental studies
35	Instituto Adolfo Lutz	Claudete Rodrigues Paula	PharmD, PhD	Researcher (USP)	Environmental studies
36	(São Paulo Central	Rogério Antônio Oliveira	Laboratory Technician	Researcher	Environmental studies
37	Reference Laboratory)	Lucas Xavier Bonfietti	BS, PhD	Researcher	Environmental studies
38	ļ!	Juliana P. Fernandes Takanashi	BS, PhD	Researcher	Environmental studies
39		Paulo de Tarso Oliveira e Castro	MD, PhD	Researcher	Epidemiology studies
40	Barretos' Cancer Hospital	Jose Carlos Ignacio Júnior	MD, PD	Researcher	Epidemiology studies
41	Fordered University (Valeria Cristina Faustinoni	BS BS	Researcher	Microbiology studies
42	Federal University of	James venturini	Bivieasc, PhD	Researcher	Microbiology studies
43	Mato Grosss do Sul	Marilene Rodrigues Chang	Pharmu, Phu	Researcher	Microbiology studies

The following post-graduation students are also included in the Project

	Institution	Student's Name	Mentor's Name	Post-Graduation Level	Assigned to
44		Elisa Teixeira Mendes		Post-Doc	Environmental studies
45		Pamella Stivanelli*		Master's	Molecular Biology studies
46		Laís Sturaro*		Master's	Molecular Biology studies
47		Dayane Ruiz Costa		Trainee	Molecular Biology studies
48		Carolini Marcelli Oliveira	Maria Luiza Moretti	Trainee	Molecular Biology studies
49		Ana Paula Nascimento*		Trainee	Epidemiology studies
50		Anna Kim		Master's	Molecular Biology studies
51		Felipe W. Freire		Master's	Molecular Biology studies
52		Carlos Alberto Campos Passinho		PhD	Microbiology studies
53		Sarah Martins Craveiro		Master's	Molecular Biology studies
54	Unicamp	Fernanda Christina Reis		PhD	Molecular Biology studies
55		Ellen Recco		Master's	Environmental studies
56		Douglas Zamarin		Master's	Epidemiology studies
57		Dayana Fontoura	Plínio Trabasso	Master's	Epidemiology studies
58		Mariana Balduzzi		Undergraduate Research	Epidemiology studies
59		Mayara Lara Brabo		Undergraduate Research	Molecular Biology studies
60		Sarah Jarschel de Camargo		Master's	Environmental studies
61		Franqueline Reichert Lima*		PhD	Microbiology studies
62		Laís Pontes	Angélica Zaninele Schreiber	MsC	Microbiology studies
63		Caio Augusto Beraquet		Master's	Microbiology studies
64		Antônio Camargo Martins	Mariângela Ribeiro Resende	Doctorate	Molecular Biology studies
65		Lidiane de Oliveira		Master's	Environmental studies
66	Terretitudes Andriffe I uter	Carlos Alberto Campos Passinho*		Master's	Environmental studies
67	67 Instituto Adolfo Lutz (São Paulo Central 68 Reference Laboratory)	Joana de Souza Pereira Barrel	Márcia M.C. Melhem	Master's	Environmental studies
68		Alan Antônio Kubo	Marcia M.O. Meinenn	PhD	Environmental studies
69		Milena Bronze Macioni		PhD	Environmental studies
70		Dálity Keffelen de Barros Rodrigues		PhD	Environmental studies
71	Federal University of Mato	Suellen Raquel da Silva Ferreira	Marilene Rodrigues Chang	Master's	Environmental studies
72	Grosso do Sul (UFMS)	Francine de Sales Dorneles	Márcia M.C. Melhem	Doctorate	Environmental studies

 $\ensuremath{\mathsf{Obs.:}}$ *Students that finished the post-graduation program during the Project.

ANNEX1.4. Local Activity Budget

Brazilian Side: (Currency: R								(Currency: Real)
	Evenediture		FY2018	FY2019	FY2020	FY2021	FY2022	Total
Expenditure		(Set-Dec)	(Jan-Dec)	(Jan-Dec)	(Jan-Dec)	(Jan-Dec)	(Jan-Set)	TOTAL
UNICAMP	Project Implementation Budget ¹⁾	2.150,00	8.374,00	17.930,00	7.673,00	3.215,00	1.717,00	41.059,00
	Laboratory Renovation Budget ²⁾	1.335,00	27.420,00	56.280,00	5.290,00	4.485,00	2.976,00	97.786,00
								138.845,00

Note 1) Includes desks, chairs, meeting table, shelves, racks, and other minor furniture items, projection screen, data show equipment, microcomputers (laptop and desktops), and office supplies. Dedication of human

2) Includes structural design project, architectural and technical projects, basic materials (e.g.: sand and cement, bricks, pipes, electricity wires, LAN wires, electrical and LAN network sockets, light bulbs, gas pipes and terminals), benches, cabinets, shelves, air conditioning equipment, cooling for the cold chamber, and miscellaneous.

Japanese Side:								(Currency: Real)	
Expenditure		FY2017	FY2018	FY2019	FY2020	FY2021	FY2022	Total	
		(Set-Mar)	(Apr-Mar)	(Apr-Mar)	(Apr-Mar)	(Apr-Mar)	(Apr-Set)	TOLAT	
	JICA	General Expenses ³⁾	95.569,00	360.885,00	501.225,00	137.085,00	409.570,00	222.237,00	1.726.571,00

Note 3) General expenses incudes reagents, antifungal drug, consumable items for fugal preservation, travel expenses, and miscellaneous.

ANNEX1.5. Other activities in Brazil

Training courses provided by other institutions in Brazil and fulfilled by LEMDI staff

Title	Institution	Year	Name
Methods for Preservation of Micro-Organisms: Which is Most Efficient?	Brazilian Society of	2017	Larissa Ortolan Levy
	Microbiology		
Logical Method for Scientific Writing	Unicamp	2018	Larissa Ortolan Levy
VI International Course on Molecular methodologies for epidemiology and	"Oswaldo Cruz" Foundation	2018	Cibele Aparecida Tararam
diagnosis of invasive fungal infections	(Fiocruz – Rio de Janeiro)		Larissa Ortolan Levy
Fragment Sequencing and Analysis on the SeqStudio Genetic Analyzer	Thermo Fisher Scientific	2018	Cibele Aparecida Tararam
platform			Larissa Ortolan Levy
VIII International Course on Molecular Methodologies for Epidemiology	"Oswaldo Cruz" Foundation	2020	Larissa Ortolan Levy
and Diagnosis of Invasive Fungal Infections	(Fiocruz – Rio de Janeiro)		
Lecture series on the cellular and molecular basis of the host-fungus	Unicamp	2020	Cibele Aparecida Tararam
interaction			
Environmental education and sustainability	Federal University of Santa	2020	Cibele Aparecida Tararam
	Catarina		
Training in Environmental Waste Management	Unicamp	2021	Cibele Aparecida Tararam
			Larissa Ortolan Levy
Laboratory Diagnosis in Mycology	Microlab advisoring in	2021	Larissa Ortolan Levy
	Microbiology		
Molecular and MALDI Identification of Molds	Association of Public Health	2021	Larissa Ortolan Levy
	Laboratories		
Good Laboratory Practices	Unicamp	2021	Cibele Aparecida Tararam
			Larissa Ortolan Levy
Introductory Real-Time PCR (qPCR) online training for the QuantStudio5	Thermo Fisher Scientific	2021	Cibele Aparecida Tararam
platform			Larissa Ortolan Levy

Scientific research methodology	"Oswaldo Cruz" Foundation	2022	Larissa Ortolan Levy
	(Fiocruz – Rio de Janeiro)		
Biosafety in focus	"Oswaldo Cruz" Foundation	2022	Larissa Ortolan Levy
	(Fiocruz – Pernambuco)		
Identification of Candida auris	Ministry of Health, Brazil	2022	Larissa Ortolan Levy
Update in Medical Mycology: Mycological diagnosis of opportunistic	Universidade Paulista Júlio	2022	Larissa Ortolan Levy
fungal infections prevalent in tertiary hospitals	de Mesquita		
Invasive Fungal Infections	Rio de Janeiro Society of	2022	Cibele Aparecida Tararam
	Infectious Diseases		Larissa Ortolan Levy
XX Bioinformatics Course "Identification, annotation and analysis of	Unicamp	2022	Cibele Aparecida Tararam
transcript expression using RNA-Seq"			
II International Workshop "Curation in Microbial Collections"	Unicamp	2022	Cibele Aparecida Tararam
			Larissa Ortolan Levy

Participation in Congresses with presentation of poster by Brazilian staff

Cibele Aparecida Tararam

• The gene expression profiles of susceptible and resistant strains of *Aspergillus fumigatus* after exposure to azoles appraised by RNAseq. 31st Brazilian Congress of Microbiology, ISBN: 2176-414X, October, 2021.

Larissa Ortolan Levy

 Prevalence of fungal isolates in respiratory material and blood cultures from hospitalized patients with and without COVID-19 diagnosis. Levy, L. O.; Pontes, L.; Luz, E. A. de Sá, C. F.; Watanabe, A.; Trabasso, P.; Moretti, M. L.; Schreiber A. Z. 31st Brazilian Congress of Microbiology, ISBN: 2176-414X, October, 2021.

Cibele Aparecida Tararam and Larissa Ortolan Levy

- Cases of fungemia caused by *Candida haemulonii var. vulnera* resistant to Amphotericin B among children at philanthropic hospital Boldrini Child Center. Freire, F.W.; Tararam, C.A; Levy, L.O.; Silva, R.A.M.; Caldeira, H.C.A; Gilli, F.H.; Brandalise, S.R.; Watanabe, A.; Trabasso, P.; Moretti, M.L. 31st Brazilian Congress of Microbiology, ISBN: 2176-414X, October, 2021.
- Visible DNA microarray system as an adjunctive molecular test to differentiate Candida dubliniensis from Candida albicans. Levy, L. O., Tararam, C. A., Sturaro, L. L., Busso-Lopes, A. F., Lyra, L., Schreiber, A. Z., Trabasso, P., Moretti, M. L. 28th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Madrid, Spain, 04/21 to 04/24/2018.

ANNEX 2 List of products

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Project Monitoring Sheet I (Revision of Project Design Matrix)

Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resist: Version 1

Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)

Dated 07 March, 2018

Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

Period of Project: From September 2017 to September 2022

Project Site:	Model Site:				
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
	(1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to	 Reports developed by the Project Meeting minutes with relevant organizations Research articles published in international 			
A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.	 trial-based application in hospitals in the Campinas metripolitan area. (2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. (3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug-resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance- 	journals		Achievement of these indicators will be verified at the end of this Project.	
Outputs	related genes detection methods are				
1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	(1) By Descember, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	 Reports developed by the Project A draft of revised guidelines 	1. The Project gains the cooperation of the Brazilian health administration organizations such as the MOLL the QEE David Of the	Among the strains	
	(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.	 Reports developed by the Project Minutes of meetings with the Brazilian authorities concerned 	Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug- resistant ones for	hospital, an antifungal- resistant <i>Aspergillus</i> <i>fumigatus</i> strain has been found. Species identification of fungal	
	(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.	 Reports developed by the Project Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned 	 implementing meetings with the Project regarding policy advocacy etc. 2. Political superiority of the control of fungal infections including drug-resistant ones is not diminished so as to impair the achievement of the Project 	strains isolated from clinical asn environmental specimens using a microarray method is in progress.	
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2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.	 By Descember, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug- resistant <i>Aspergillus, Candida</i> and other fungi are determined. By Descember, 2021, evaluation works of clinical performance of the above-mentioned methods are completed. By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced. 		Purpose.	Detection experiments for mutations in antifungal-resistant genes were started using gene amplification (LAMP and PCR) methods.	

Activities	In	puts	Pre-Conditions	1
	The Japanese Side	The Brazilian Side		
	Experts	Counterparts		
1. Prevalence of drug-resistant fungi	(1) Chief Advisor (Short-term Expert/double as	(1) Project Director	1 Approvals are obtained by	
and its mechanisms of drug-resistance	an expert for Diagnostics-related studies)	(2) Project Manager	the ethical committees of each	
are epidemiologically elucidated.	(2) Project Coordinator/Specialist for	(3) Project Co-managers (3 persons)	project implementing agoney	
	Research Institutes Cooperation (Long-term	(4) Researchers (Infectious Diseases Division of	for the medical and/or	
1-1. To perform classification and	Expert)	the Internal Medicine Department and the	epidemiological research	
identification of fungal strains by	(3) Diagnostics-related studies (Short-term	Clinical Pathology Department, the Faculty of	which are supposed to be	
morphological and biochemical properties	Experts)	Medical Sciences, the UNICAMP)	performed in the Project (if	
with clinically-isolated fungal strains	(4) Molecular epidemiological studies (Short-		perossary)	
collected by UNICAMP-related and other	term Experts)	Facilities, equipment and materials	necessary).	
project cooperating medical facilities.	(5) Gene amplification-related studies (Short-	(1) Office space or facilities for JICA experts in		
1.0. To perform more detailed identification	term Experts)	the UNICAMP		
1-2. To perform more detailed identification	(6) Bioinformatics (Short-term Experts)	(2) Research space and facilities in the		
or fungal strains including cryptic species	(7) Epidemiological investigation and optimal	UNICAMP		
isolaton identified in the Activity 1.1	administration-related studies (Short-term	(3) Existing research instruments and		
Isolales identified in the Activity 1-1.	Experts)	equipment for research activities, etc.		
1-3. To examine the susceptibility of the	(8) Management of fungal strains-related			
fungal species (especially for Aspergillus,	study (Short-term Experts)	Local Costs		
Candida, Cryptococcus, and Fusarium)	(9) Other persons with necessary expertise for	- Running expenses for research activities, etc.;		
including some cryptic species in	the project activities (Short-term Experts)	- Assignment of counterpart personnel;		
accordance with internationally-approved	Tasisian in Janan	- Utility costs (electricity, water, etc.);		
methods (e.g. the methods provided by the	I raining in Japan	- Personnel costs (to the work of counterpart);		
Clinical and Laboratory Standards Institute	(1) Training for operating Next Concretion	Operational costs		
(CLSI)).		- Operational costs.		
	(3) Training for genome comparison and gene		lssues and countermesures>	
	disruption			
1-4. To analyze the relationship between	(4) Training for management of collected fungi			
fungal characteristics (strains, species and	(5) Other necessary training		A capillary sequencer and	The equipment is re-
drug susceptibility) and patients'	(c) e li ci necescary li ali li g		other equipment for research	planned to be procured
characteristics (source of infection,	Equipment and materials		which were planned to be	in 2018FY and both
infected sites, underlying and/or	Necessary equipment and materials for		donated to UNICAMP, could	sides try to do their best
complicated diseases, clinical entity,	research activities in the Project		not be procured in 2017FY	to minimize the impact
history of chemotherapy by antifungals,			because of budgetary limitation	of delays on activities
etc.).	Local cost		of Japanese side.	concerned.
4.5. To alwaidate markenians of down				
1-5. To elucidate mechanism of drug				
resistance by analyzing gene regions,				
drug registeness using the fungel isoletes				
uity resistances using the fungal isolates				
the Activity 1-3				
1.6 To analyzo goographycal				
characteristict of drug registance genes by				
comparing identified drug-resistant				
mechanisms against antifungals				
	1	l	I	I

1-7. In parallel with the Activity 1-5, to elucidate mechanisms of drug resistance using advanced genetic analysis technologies such as next-generation sequencing techniques for the fungal isolates, which are suspected to have unidentified drug resistance mechanisms.

1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the nextgeneration sequencer for elucidating drugresistant mechanisms at the MMRC.

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptive and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newlydeveloping it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan ares and/or other areas in Brazil.

2. Gene amplification technologiesbased drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drugresistant mycoses.

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drugresistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan. 2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drugresistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.
2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. Io develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.		It will take more amount of time than expected to bring out fungal strains from Brazil to Japan because of treaty ratification of Convention on Biological Diversity in May 2017.
3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.		
3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.		
3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP.		
3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant		
ones. 3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.		
3-4. To set up a network of clinical		

Project. 3-4. To set up a network of clinical

laboratory testing and diagnosis of	1	
mycococ including drug resistant ones		
among the UNICAMP (on the reference		
among the UNICAMP (as the relefence		
laboratory) and health and testing facilities		
In the State of Sao Paulo with the support		
of the Institute of Adolfo Lutz (IAL).		
3-5. To set up a network of clinical		
laboratory testing and diagnosis of		
mycoses including drug-resistant ones		
among research institutes, health facilities		
and the MMRC as the reference laboratory		
also in Japan, by referencing the		
achievements of project collaborative		
research and technical cooperation in		
Brazil.		
3-6. To hold national and international		
conferences on the research of drug-		
resistant mycoses geared to research,		
health and administrative agencies in other		
provinces and nations, in light of future		
establishment of a network of collaborative		
research on mycoses including drug-		
resistant ones as well as wide-sharing of		
research findings and outcomes of the		
Project.		
-1		

Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant On Version 2

Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)

Dated 26 September, 2018

Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

Period of Project: From September 2017 to September 2022

Project Site: Model Site:

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Durain at Durana a					
Floject Fulpose	(1) By the time of the termination of the Project.	- Paparta davalapad by the			
	guidelines/booklets for the management of	Project			
	mycoses are subjected to trial-based	- Meeting minutes with relevant			
	application in hospitals in the Campinas				
	metripolitan area.	- Possarch articles published in international			
	(2) By the time of the termination of the Project	inumpele			
	concrete discussions are commenced with	journais			
	Brazilian administrative organizations for the				
A research and reference collaborative	application of research findings and outcomes				
system for the diagnoses of fungal	gained by the Project to policymaking and/or			Achievement of these indicators will be verified at the	
infections including drug-resistant ones is	countermeasures for the control of fungal			end of this Project.	
established both in Brazil and Japan.	infections including drug-resistant ones.			· · · · · · · · · · · · · · · · · · ·	
	(3) By the time of the termination of the Project,				
	at least 5 research articles in the themes of				
	project research topics such as				
	epidemiological analyses of drug-resistant				
	fungal infections, mechanisms of resistances				
	of fungi to antifungals, and drug resistance-				
	related genes detection methods are				
	published.				
Outputs					
1. Prevalence of drug-resistant fungi and its	(1) By December, 2019, the proportion of drug-	Reports developed by the Project	1. The Project gains the		
mechanisms of drug-resistance are	resistant fungal strains in the fungal isolates	A draft of revised guidelines	cooperation of the Brazilian health		
epidemiologically elucidated.	(the prevalence) in the target areas is		administration organizations such	Among the strains isolated in UNICAMP, antifungal-	
	estimated.		as the MOH, the Sao Paulo State	resistant Aspergillus fumigatus strains have been	
	(2) By March, 2020, principal mechanisms of	 Reports developed by the Project 	Health Office and the Campinas	found both clinically and environmentally. These	
	drug resistance against antifungal agents are	 Minutes of meetings with the Brazilian 	City Health Office in charge of the	strains are now analyzed genetically to find the	
	identified using clinical isolates gained in the	authorities concerned	control of fungal infections including	mechanism for antifungal-resistance. Training for	
	Campinas metropolitan area.		drug-resistant ones for	whole-genome analyses using a next generation	
			Project regarding policy educes	sequencer was performed at MMRC in August to	
	(3) By March, 2022, the revising work of the	 Reports developed by the Project 		September, 2018. Species identifications of fungal	
	existing guidelines, and drafting work of the	 Documents of information sharing regarding 	610.	strains isolated from clinical and environmental	
	guidelines/booklets for the management of	epidemiological data with the Brazilian authorities	2 Political superiority of the control	specimens using several methods such as multiplex	
	mycoses are commenced.	concerned	of fundal infections including the	PCH, microarray and LAMP are in progress.	
			ones caused by drug-resistant	Relationship between environmental strain isolated in	
			etraine is not diminished so as to	nospital and clinical strain isolated from patients have	
			impair the achievement of the	been analyzed using DINA sequencing, susceptibility	
			Project Purpose	testing and DNA fingerprinting.	
			4		

2. Gene amplification technologies-based drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.	 By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant Aspergillus, Candida and other fungi are determined. By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed. By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above- mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) 		Evaluation of automatic identification methods for differentiation between <i>Candida albicans</i> and <i>C.dubliniensis</i> and antifungal susceptibility testing of these strains are in progress. Training for gene analyses was performed at MMRC in August to September, 2018. Detection procedures for mutations in antifungal-resistant genes are developing using some genetic analytical methods such as mismatch- specific nucleases (Surveyor Nuclease) and LAMP.	
3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.	 By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced. By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval. By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced 	A L-drying system was selected as the fungal preservation method. For the epidemiological study the REDCap software was chosen and the database system based on it has been developed. REDCap (Research Electronic Data Capture) is a secure and open web application for building and managing online surveys and databases.	Mycological laboratory in UNICAMP (LEMDI) has been expanded the space and upgraded. Two workshops for microbiology and a training course for LAMP were held in UNICAMP. Reactivation of fungal strains stored in UNICAMP is ongoing. Training for fungal preservation procedure using a L- drying system was performed at MMRC in August to September, 2018. Brazilian researchers attended training courses on REDCap in São Paulo in April and June, 2018. The database program has been developed and is now tested in UNICAMP by entering the information of real clínical cases of patients hospitalized in the Hospital de Clinicas of Unicamp with different fungal infection. The cases were selected by an assistance physician and three hospital epidemiology nurses are responsible to input the clinical data. Brazilian researchers were given lectures on NESID (the National Epidemiological Surveillance of Infectious Diseases) and JANIS (Japan Nosocomial Infections Surveillance) in Japan in August to September, 2018. Activity of building research network including workshops, meetings and visit to other institutes were in progress.	

Activities	In	puts	Pre-Conditions
	The Japanese Side	The Brazilian Side	
	Exporte	Countorparte	
1. Brovalance of drug registent fungi and		Counterparts	
1. Frevalence of drug-resistant lungrand	(1) Chief Advisor (Short-term Expert/double as	(1) Project Director	
its mechanisms of drug-resistance are	an expert for Diagnostics-related studies)	(2) Project Manager	1. Approvals are obtained by the
epidemiologically elucidated.	(2) Project Coordinator/Specialist for Research	(3) Project Co-managers (3 persons)	ethical committees of each project
	Institutes Cooperation (Long term Export)	(4) Pessarehore (Infectious Diseases Division of	implementing agency for the
1-1 To perform classification and		(4) Researchers (Intectious Diseases Division of	mplementing agency for the
identification of function later in a later	(3) Diagnostics-related studies (Short-term	the Internal Medicine Department and the	medical and/or epidemiological
identification of fungal strains by	Experts)	Clinical Pathology Department, the Faculty of	research, which are supposed to
morphological and biochemical properties	(4) Molecular epidemiological studies (Short-	Medical Sciences, the UNICAMP)	be performed in the Project (if
with clinically-isolated fungal strains	torm Exporte)		necessary)
collected by UNICAMP-related and other			noocooury).
project aconcrating medical facilities	(5) Gene amplification-related studies (Short-	Facilities, equipment and materials	
project cooperating medical facilities.	term Experts)	 Office space or facilities for JICA experts in 	
	(6) Bioinformatics (Short-term	the UNICAMP	
1-2. To perform more detailed identification	Experts)	(2) Besearch space and facilities in the	
of fungal strains including cryptic species	Lyperts)		
using genetic techniques on the fungal	(7)	UNICAMP	
isolatos identified in the Activity 1.1	Epidemiological investigation and optimal	(3) Existing research instruments and equipment	
Isolates identified in the Activity 1-1.	administration-related studies (Short-term	for research activities, etc.	
1.2. To overning the susceptibility of the	Experts)		
1-3. To examine the susceptibility of the	(0) Management of function in a late of a task	Level Orete	
fungal species (especially for Aspergillus,	(8) Management of fungal strains-related study	Local Costs	
Candida, Cryptococcus, and Fusarium)	(Short-term Experts)	- Running expenses for research activities, etc.;	
including some cryptic species in	(9) Other persons with necessary expertise for	- Assignment of counterpart personnel	
	(c) called percenter mail necescal (conperate for	I tility costs (clostricity water sta):	
accordance with internationally-approved	the project activities (Short-term Experts)	- Otinity costs (electricity, water, etc.),	
methods (e.g. the methods provided by the		- Personnel costs (to the work of counterpart);	
Clinical and Laboratory Standards Institute	Training in Japan	and	
(CLSI))	(1) Training for gene analyses	- Operational costs.	
(0201)).	(2) Training for operating Next Generation		decuse and countermosures
			<issues and="" countermesures=""></issues>
	Sequencer		
1.4. To analyze the relationship between	(3) Training for genome comparison and gene		
1-4. To analyze the relationship between	disruption		
fungal characteristics (strains, species and	(4) Training for management of collected fungi		
drug susceptibility) and patients'	(F) Other and a second training for management of conceted range		
characteristics (source of infection infected	(5) Other necessary training		
sites, underlying and/or complicated			
sites, underlying and/or complicated	Equipment and materials		
diseases, clinical entity, history of	Necessary equipment and materials for		
chemotherapy by antifungals, etc.).	Necessary equipment and materials for		
	research activities in the Project		
	Local cost		
1. E. To obvidete mechanism of drug			
1-5. To elucidate mechanism of drug			
resistance by analyzing gene regions,			
which are reported to be responsible for			
drug resistances using the fungal isolates			
uiug resistances using the lungarisolates			
with drug-resistant property, confirmed in			
the Activity 1-3.			
1-6 To analyse geographycal			
characteristict of drug registance genes by			
characteristict of drug resistance genes by			
comparing identified drug-resistant			
mechanisms against antifungals.			
1.7 In percellal with the Activity 1.5 to			
1-7. In parallel with the Activity 1-5, to			
elucidate mechanisms of drug resistance			
using advanced genetic analysis			
technologies such as next-generation			
teeningies such as here the former			
sequencing techniques for the fungal			
isolates, which are suspected to have			
unidentified drug resistance mechanisms.			

1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the nextgeneration sequencer for elucidating drugresistant mechanisms at the MMRC.

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptive and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newlydeveloping it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan ares and/or other areas in Brazil.

2. Gene amplification technologiesbased drug-resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drugresistant mycoses.

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drug-resistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of

the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinicallyisolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drugresistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or

It will take more amount of time than expected to bring out fungal strains from Brazil to Japan because of treaty ratification of Convention on Biological Diversity in May 2017.

seminars on drug-resistant fungi including
the reporting of project achievements,
geared to project external collaborating
agencies, health and medical facilities in the
project areas and the administration
organizations in charge of the control of
fungal infections including drug-resistant
ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drugresistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).

3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.

3-6. To hold national and international conferences on the research of drugresistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drugresistant ones as well as wide-sharing of research findings and outcomes of the Project.

Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan Version 3

Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)

Dated 20 February, 2019

Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

Period of Project: From September 2017 to September 2022

Project Site:	Model Site:				
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal Project Purpose		Paporte developed by the Project			
A research and reference collaborative system for the diagnoses of fungal infections including drug- resistant ones is established both in Brazil and Japan.	 By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metripolitan area. By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug- resistant fungal infections, mechanisms of resistance-related genes detection methods are published. 	Meeting minutes with relevant organizations Research articles published in international journals		Achievement of these indicators will be verified at the end of this Project.	
Outputs 1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	 Reports (including some articles or abstracts of academic meeting) developed by the Project A draft of revised guidelines 	 The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug- resistant ones for implementing meetings with the Project regarding policy advocacy etc. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose. 	Classification and identification of fungal strains collected in UNICAMP are ongoing (over 500 strains). Lecuture course on molecular epidemiology in National Institute of Infectious Diseases, Japan (NIID) were held on December. 2018.	

	(2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area.	Reports developed by the Project Minutes of meetings with the Brazilian authorities concerned	
	(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.	Reports developed by the Project Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned	
2. Gene amplification technologies-based drug- resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.	(1) By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant Aspergillus, Candida and other fungi are determined. (2) By December, 2021, evaluation works of clinical performance of the above- mentioned methods are completed. (3) By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced.		

Detailed species identifications of fungal phylogenetic analysis were held in UNICAMP in February 2019. Detailed species identifications of fungal strains isolated from clinical and environmental specimens using several methods such as multiplex PCR, microarray and LAMP are in progress. Among the strains isolated in UNICAMP, antifungal-resistant <i>Aspergillus fumigatus</i> strains have been found both clinically and environmentally. This environmental resistant strain possesses a unique mutation in <i>cyp51A</i> gene. To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of environmental fungal strains to some fungicides are on going in Instituto Adolfo Lutz. Introduction of whole-genome analyses using a next generation sequencer was performed at MMRC in November to December, 2018.	
Training for gene analyses was performed at MMRC in November to December, 2018. Lecture on the advanced LAMP method was held in UNICAMP in February 2019. Detection method for mutations in antifungal- resistant genes cyp51A using mismatch- specific nucleases (Surveyor Nuclease) is validated. Development of a detection procedure for point mutatitions in cyp51A gene using ARMS-LAMP method is in progress.	

system among research, medical and adm organizations is established.	 Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced. (2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval. (3) By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones, of which reference laboratory is the UNICAMP, is commenced. 	Workshops for phylogenetic an LAMP were held in UNICAMP. L-drying system for fungal press installed in UNICAMP. The train fungal preservation using this s held in UNICAMP inFebruary 20 The database program (RedCa operated and over 30 clinical c with various fungal infection ad Hospital de Clinicas of Unicamp enrolled. These cases were sel case conferences attended by professions including physiciam microbiologists. This conferenc week in the hospital, and is proi development of young medical Activity of building research net workshops, meetings and visit t institutes were in progress.	alysis and for arvation was ing course for system were D19. b) has been uses of patients mitted to the have already ected in clinical various medical s, nurses and b held every triable for experts. work including o other
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A stiuities		Inputo	Dre Conditions
Activities	The Jananasa Cida	Inputs The Dranilian Cide	Pre-Conditions
	The Japanese Side	The Brazilian Side	
4. Describer as of development for all reading	Experts	Counterparts	
1. Prevalence of drug-resistant fungi and its	 Chief Advisor (Short-term 	(1) Project Director	
mechanisms of drug-resistance are	Expert/double as an expert for	(2) Project Manager	
epidemiologically elucidated.	Diagnostics-related studies)	(3) Project Co-managers (3 persons)	
	(2) Project Coordinator/Specialist for	(4) Researchers (Infectious Diseases Division of	 Approvals are obtained by the ethical committees of each
	Because Institutes Conservation (Long	(+) Researchers (intesticus Diseases Division of	project implementing agency for the medical and/or
 1-1. To perform classification and identification of 	Research institutes Cooperation (Long-	the internal Medicine Department and the Clinical	epidemiological research, which are supposed to be performed
fungal strains by morphological and biochemical	term Expert)	Pathology Department, the Faculty of Medical	in the Project (if necessary).
properties with clinically-isolated fungal strains	(3) Diagnostics-related studies (Short-	Sciences, the UNICAMP)	
collected by UNICAMP-related and other project	term Experts)		
cooperating modical facilities	(4) Molecular epidemiological studies	Facilities, equipment and materials	
cooperating medical facilities.	(Short-term Experts)	(1) Office space or facilities for JICA experts in the	
	(5) Gene amplification-related studies	UNICAMP	
1-2. To perform more detailed identification of fungal	(Short form Exports)	(2) Research space and facilities in the LINICAMP	
strains including cryptic species using genetic	(C) Disinformation (Chart term Eurorte)	(2) Research space and racinges in the ONICAMF	
tochniques on the fungal isolates identified in the	(6) Bioinformatics (Short-term Experts)	(3) Existing research instruments and equipment	
A strate 4 4	(7) Epidemiological investigation and	for research activities, etc.	
ACTIVITY 1-1.	optimal administration-related studies		
	(Short-term Experts)	Local Costs	
1-3 To examine the susceptibility of the fungal	(8) Management of fungal strains-related	- Running expenses for research activities, etc.:	
species (especially for Asperaillys, Candida	study (Short-term Experts)	- Assignment of counterpart personnel:	
Species (especially for Aspergillus, Carloida,		I tility agets (cleatricity water, etc.)	
Cryptococcus, and Fusarium) including some	(9) Other persons with necessary	- Otility costs (electricity, water, etc.);	
cryptic species in accordance with internationally-	expertise for the project activities (Short-	 Personnel costs (to the work of counterpart); 	
approved methods (e.g. the methods provided by	term Experts)	and	
the Clinical and Laboratory Standards Institute		 Operational costs. 	
(CLSI)).	Training in Japan		
())	(1) Training for gene analyses		
	(2) Training for operating Next		lssues and countermesures>
	Concretion Sequencer		
4.4. To each month a collection while the two each formula	(3) I raining for genome comparison and		
1-4. To analyze the relationship between fungal	gene disruption		
characteristics (strains, species and drug	(4) Training for management of collected		
susceptibility) and patients' characteristics (source	fungi		
of infection, infected sites, underlying and/or	(5) Other necessary training		
complicated diseases, clinical entity, history of	(5) Other necessary training		
chemotherapy by antifungals, etc.)	Equipment and materials		
chemotherapy by antifungais, etc.).	Equipment and materials		
	Necessary equipment and materials for		
	research activities in the Project		
	Local cost		
1-5. To elucidate mechanism of drug resistance by			
analyzing gene regions, which are reported to be			
responsible for drug resistances using the fungal			
isolates with drug-resistant property confirmed in			
the Activity 1.2			
the Activity 1-5.			
1-6. To analyse geographycal characteristict of drug			
resistance genes by comparing identified drug-			
resistant mechanisms against antifungals.			
1-7. In parallel with the Activity 1-5, to elucidate			
mechanisms of drug resistance using advanced			
genetic analysis technologies such as next-			
generation acquencing techniques for the fungel			
generation sequencing techniques for the rungar			
isolates, which are suspected to have unidentified			
drug resistance mechanisms.			
1-7-1. To provide Brazilian researchers with the			
Training in Japan in the theme of genetic analysis			
techniques using the next-generation sequencer for			
elucidating drug-resistant mechanisms at the			
MMDC			
WIWING.			
I1-7-2. To identify gene regions, which are suspected	1	ļ	

to be responsible for drug resistance, by wholegenomic comparison of drug-susceptive and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newlydeveloping it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan ares and/or other areas in Brazil.

 Gene amplification technologies-based drugresistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drugresistant mycoses.

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplificationbased gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drugresistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established. 3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research. 3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities. 3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC. 3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP. 3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP. 3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones. 3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project. 3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).

3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.		
3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.		

Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Japan Version 4

Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)

Dated 20 September, 2019

Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people).

Period of Project: From September 2017 to September 2022

Project Site:	Model Site:				
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Narrative Summary Overall Goal Project Purpose A research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones is established both in Brazil and Japan.	Objectively Verifiable Indicators (1) By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metripolitan area. (2) By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant	Means of Verification • Reports developed by the Project • Meeting minutes with relevant organizations • Research articles published in international journals	Important Assumption	Achievement Achievement of these indicators will be verified at the end of this Project.	Remarks
	ones. (3) By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug- resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published.				
1. Prevalence of drug-resistant fungi and its	(1) By December, 2019, the proportion of	Reports (including some articles or abstracts of	1. The Project gains the cooperation of the Brazilian health		
mechanisms of drug-resistance are epidemiologically elucidated.	Irrug-resistant tungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	academic meeting) developed by the Project • A draft of revised guidelines	administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug- resistant ones for implementing meetings with the Project regarding policy advocacy etc. 2. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose.	Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals and environmental strains collected by instituto Adolfo Luz are ongoing (over 1,000 clinica strains and 500 environmental strains). Lecture and training course on antifuncal	

	 (2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area. (3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced. 	Reports developed by the Project Minutes of meetings with the Brazilian authorities concerned Reports developed by the Project Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned	susceptibility testing according to CLSI methods to researchers of UNICAMP and other atending institutions were held on May, 2019 in UNICAMP. Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress. Among the strains isolated in UNICAMP, another antifungal-resistant <i>Aspergillus</i> <i>fumigatus</i> clinical strains have been found. Compared with fungal strains isolated in Japan, a different polymorphism in cyp51A gene was revealed in Brazilian isolates. To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of A. fumigatus strains to some fungicides are on going in Instituto Adolfo Lutz. Using a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains was started from July 2019.
 Gene amplification technologies-based drug- resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses. 	 By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant Aspergillus, Candida and other fungi are determined. By December, 2021, evaluation works of clinical performance of the above- mentioned methods are completed. By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced. 		Validation of detection method for mutations in antifungal-resistant genes <i>cyp51A</i> by mismatch-specific nucleases (Surveyor Nuclease) using antifungal- susceptible/resistant strains were finisihed. Development of a detection procedure for point mutations in <i>cyp51A</i> gene using ARMS- LAMP method is in progress. Microbiome analysis using clinical samples (bronchoalveolar lavage fluid) was started from May 2019. The CRISPR/Cas9 system, a new method for transgenesis, was started to perform to clarify the function of mutated gene.

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methods to researchers of UNICAMP and	
other atending institutions were held on May,	
2019 in UNICAMP.	
Detailed species (cryptic species)	
identifications of fungal strains isolated from	
clinical and environmental specimens using	
several methods such as DINA sequencing,	
Among the strains isolated in LINICAMP	
another antifungal-resistant Aspergillus	
<i>fumigatus</i> clinical strains have been found.	
Compared with fungal strains isolated in	
Japan, a different polymorphism in cyp51A	
gene was revealed in Brazilian isolates.	
To elucidate the effect of fungicides on	
mutagenicity of fungi, studies on exposure of	_
A. rumigatus strains to some rungicides are o going in Instituto Adolfo Lutz	"
Using a next generation sequencer, gene	
expression analysis (transcriptome) of	
antifungal-susceptible/resistant strains was	
started from July 2019.	
Validation of detection method for mutations i	n
antifungal-resistant genes cyp51A by	
mismatch-specific nucleases (Surveyor	
suscentible/resistant strains were finisibed	
Development of a detection procedure for	
point mutations in cyp51A gene using ARMS	-
LAMP method is in progress.	
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Microbiome analysis using clinical samples	
Microbiome analysis using clinical samples (bronchoalveolar lavage fluid) was started	
Microbiome analysis using clinical samples (bronchoalveolar lavage fluid) was started from May 2019.	
Microbiome analysis using clinical samples (bronchoalveolar lavage fluid) was started from May 2019. The CRISPR/Cas9 system, a new method for transgenesis, was started to perform to clarify	r /
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5. A OnleAdmr-Centered research realablication system among research, medical and administrative organizations is established.	 (1) by the line of the fermination of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced. (2) By December 2020, epidemiological information on mycoses including drug-resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval. (3) By the time of the termination of the Project, practical operation of the Project, practical operation of the Project, sistant ones, of which reference laboratory is the UNICAMP, is commenced. 			The database program (RedCap) has been operated and over 110 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled. These cases were selected in clinical case conferences attended by various medical professions including physicians, nurses and microbiologists of UNICAMP hospital and other health care institutions under a video-conference system. This conference held every week, and is profitable for development of young medical experts. As the research network activity participated by several health care and research institutes in Sao Paulo State, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run.	
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A stiuities		Inputo	Dre Conditions
Activities	The Jananasa Cida	Inputs The Dranilian Cide	Pre-Conditions
	The Japanese Side	The Brazilian Side	
4. Describer as of development for all reading	Experts	Counterparts	
1. Prevalence of drug-resistant fungi and its	Chief Advisor (Short-term	(1) Project Director	
mechanisms of drug-resistance are	Expert/double as an expert for	(2) Project Manager	
epidemiologically elucidated.	Diagnostics-related studies)	(3) Project Co-managers (3 persons)	
	(2) Project Coordinator/Specialist for	(4) Researchers (Infectious Diseases Division of	 Approvals are obtained by the ethical committees of each
	Because Institutes Cooperation (Long	(+) Researchers (intesticus Diseases Division of	project implementing agency for the medical and/or
 1-1. To perform classification and identification of 	Research institutes Cooperation (Long-	the internal Medicine Department and the Clinical	epidemiological research, which are supposed to be performed
fungal strains by morphological and biochemical	term Expert)	Pathology Department, the Faculty of Medical	in the Project (if necessary).
properties with clinically-isolated fungal strains	(3) Diagnostics-related studies (Short-	Sciences, the UNICAMP)	
collected by UNICAMP-related and other project	term Experts)		
cooperating modical facilities	(4) Molecular epidemiological studies	Facilities, equipment and materials	
cooperating medical facilities.	(Short-term Experts)	(1) Office space or facilities for JICA experts in the	
	(5) Gene amplification-related studies	UNICAMP	
1-2. To perform more detailed identification of fungal	(Short form Exports)	(2) Research space and facilities in the LINICAMP	
strains including cryptic species using genetic	(C) Disinformation (Chart term Eurorte)	(2) Research space and racinges in the ONICAMF	
tochniques on the fungal isolates identified in the	(6) Bioinformatics (Short-term Experts)	(3) Existing research instruments and equipment	
A strate 4 4	(7) Epidemiological investigation and	for research activities, etc.	
ACTIVITY 1-1.	optimal administration-related studies		
	(Short-term Experts)	Local Costs	
1-3 To examine the susceptibility of the fungal	(8) Management of fungal strains-related	- Running expenses for research activities, etc.:	
species (especially for Asperaillys, Candida	study (Short-term Experts)	- Assignment of counterpart personnel:	
Species (especially for Aspergillus, Carloida,		I tility agets (cleatricity water, etc.)	
Cryptococcus, and Fusarium) including some	(9) Other persons with necessary	- Otility costs (electricity, water, etc.);	
cryptic species in accordance with internationally-	expertise for the project activities (Short-	 Personnel costs (to the work of counterpart); 	
approved methods (e.g. the methods provided by	term Experts)	and	
the Clinical and Laboratory Standards Institute		 Operational costs. 	
(CLSI)).	Training in Japan		
())	(1) Training for gene analyses		
	(2) Training for operating Next		lssues and countermesures>
	Concretion Sequencer		
4.4. To each month a collection while the two each formula	(3) I raining for genome comparison and		
1-4. To analyze the relationship between fungal	gene disruption		
characteristics (strains, species and drug	(4) Training for management of collected		
susceptibility) and patients' characteristics (source	fungi		
of infection, infected sites, underlying and/or	(5) Other necessary training		
complicated diseases, clinical entity, history of	(5) Other necessary training		
chemotherapy by antifungals, etc.)	Equipment and materials		
chemotherapy by antifungais, etc.).	Equipment and materials		
	Necessary equipment and materials for		
	research activities in the Project		
	Local cost		
1-5. To elucidate mechanism of drug resistance by			
analyzing gene regions, which are reported to be			
responsible for drug resistances using the fungal			
isolates with drug-resistant property confirmed in			
the Activity 1.2			
the Activity 1-5.			
1-6. To analyse geographycal characteristict of drug			
resistance genes by comparing identified drug-			
resistant mechanisms against antifungals.			
1-7. In parallel with the Activity 1-5, to elucidate			
mechanisms of drug resistance using advanced			
genetic analysis technologies such as next-			
generation acquencing techniques for the fungel			
generation sequencing techniques for the rungar			
isolates, which are suspected to have unidentified			
drug resistance mechanisms.			
1-7-1. To provide Brazilian researchers with the			
Training in Japan in the theme of genetic analysis			
techniques using the next-generation sequencer for			
elucidating drug-resistant mechanisms at the			
MMDC			
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I1-7-2. To identify gene regions, which are suspected	1	ļ	

to be responsible for drug resistance, by wholegenomic comparison of drug-susceptive and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newlydeveloping it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan ares and/or other areas in Brazil.

 Gene amplification technologies-based drugresistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drugresistant mycoses.

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplificationbased gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drugresistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drug-resistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established. 3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research. 3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities. 3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC. 3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP. 3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP. 3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones. 3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project. 3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).

3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug-resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.		
3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.		

Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Jap Version 5

Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)

Dated 30 January, 2020

Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

Period of Project: From September 2017 to September 2022

Floject Site.	Model Site.				
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal	-				
Project Purpose A research and reference collaborative system for the diagnoses of fungal infections including drug- resistant ones is established both in Brazil and Japan.	 By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metripolitan area. By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug- resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods. 	Reports developed by the Project Meeting minutes with relevant organizations Research articles published in international journals		Achievement of these indicators will be verified at the end of this Project.	
1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	 By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated. By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area. 	 Reports (including some articles or abstracts of academic meeting) developed by the Project A draft of revised guidelines Reports developed by the Project Minutes of meetings with the Brazilian authorities concerned 	 The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose. 	The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated. Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Ouro Verde Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are ongoing (over 1,000 clinical strains and 500 environmental strains). Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress. A paper regarding antifungal-resistant A. <i>fumigatus</i> clinical strains has been accepted for publication.	

	(3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced.	Reports developed by the Project Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned	
 Gene amplification technologies-based drug- resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses. 	 By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant Aspergillus, Candida and other fungi are determined. By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed. By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazili) are commenced. 		
3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.	 By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced. By December 2020, epidemiological information on mycoses including drug- resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval. By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones, of which reference laboratory is the UNICAMP, is commenced. 		

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mutagenicity of fungi, studies on exposure	
of A <i>fumigatus</i> strains to a fungicide in a	
simulated field are on going	
maining of next-generation sequencer was	
performed from July to September, 2019 in	
MMRC.	
Training on antifungal susceptibility test	
according to CLSI methods was performed	
on November, 2019 in MMRC.	
Using a next generation sequencer, gene	
expression analysis (transcriptome) of	
antifungal-susceptible/resistant strains are	
ongoing	
Light a payt concretion acquirmer	
Using a next generation sequencer,	
metagenomic analysis of clinical specimens	
are ondoind.	
A paper regarding a simple method for	
detecting gone mutations of A furning for	
detecting gene mutations of A. tumigatus	
using Surveyor Nuclease assay has been	
accepted for publication.	
Introduction of new detection method for	
antifungal resistant fungi using Surveyor	
Nuclease has been started.	
Development of a detection procedure for	
point mutations in cvp51A gene using PCR	
or I AMP method is in progress	
Developments of identification procedures	
of cruptic opening DCB or LAMP	
or cryptic species using FCR or LAWF	
method are in progress.	
I raining on gene amplification methods	
including LAMP was performed from	
October to November, 2019 in MMRC.	
The CRISPR/Cas9 system are usd to	
elucidate the effect of some mutated genes	
on antifungal resistance.	
The database program (RedCap) has been	
operated and over 160 clinical cases of	
patients with various fungal infection	
admitted to the attending health care	
institutions have already aprolled	
As the research petwork activity perticipants	
As the research network activity participated	
by several medical institutions and a	
research laboratory in Sao Paulo State,	
collecting clinical and environmental fungal	
strains, the regular conference, and	
scientific meetings are run.	
Training on the preservation method of	
fungel strains was performed from Ostebor	
Turigal strains was performed from October	
to November, 2019 In MINIRC.	
Preparation of the freeze-dried ampouls for	
preservation of fungal strains in UNCAMP	
are on going.	
Training on epidemiological and multi-	
center collaborative studies to Brazillian	
researchers in LINICAMP and other medical	
institutions was performed from Nevember	
Insulutions was benottied from inovember	
to December 2010 by several leases	
to December, 2019 by several Japanese	
to December, 2019 by several Japanese researchers in Tokyo and Chiba.	

Activities		Inputs	Pre-Conditions
	The Japanese Side	The Brazilian Side	
	Experts	Counterparts	
1. Prevalence of drug-resistant fungi and its	(1) Chief Advisor (Short-term	(1) Project Director	
mechanisms of drug-resistance are	Expert/double as an expert for	(2) Project Manager	
epidemiologically elucidated.	Diagnostics-related studies)	(3) Project Co-managers (3 persons)	
	(2) Project Coordinator/Specialist for	(4) Researchers (Infectious Diseases Division of	 Approvals are obtained by the ethical committees of
	Research Institutes Cooperation (Long-	the Internal Medicine Department and the Clinical	each project implementing agency for the medical and/or
1-1. To perform classification and identification of	term Expert)	Pathology Department, the Eaculty of Medical	epidemiological research, which are supposed to be
fungal strains by morphological and biochemical	(3) Diagnostics-related studies (Short-	Sciences, the UNICAMP)	performed in the Project (if necessary).
properties with clinically-isolated fungal strains	(3) Diagnostics-related studies (Short-	Sciences, the UNICAMP)	
collected by UNICAMP-related and other project	(4) Melocular apidemiological studios	Excilition, aquipment and materials	
cooperating medical facilities.	(4) Molecular epidemiological studies	(4) Office encode or facilities for UCA synants in	
	(Shon-term Expens)	(1) Once space or facilities for JICA experts in	
1-2 To perform more detailed identification of	(5) Gene amplification-related studies		
fungal strains including cryptic species using	(Short-term Experts)	(2) Research space and facilities in the	
genetic techniques on the fungal isolates identified	(6) Bioinformatics (Short-term Experts)	UNICAMP	
in the Activity 1-1	(7) Epidemiological investigation and	(3) Existing research instruments and equipment	
	optimal administration-related studies	for research activities, etc.	
	(Short-term Experts)		
1-3. To examine the susceptibility of the fungal	(8) Management of fungal strains-related	Local Costs	
species (especially for Aspergillus, Candida,	study (Short-term Experts)	 Running expenses for research activities, etc.; 	
Cryptococcus, and Fusarium) including some	(9) Other persons with necessary	 Assignment of counterpart personnel; 	
cryptic species in accordance with internationally-	expertise for the project activities (Short-	 Utility costs (electricity, water, etc.); 	
approved methods (e.g. the methods provided by	term Experts)	- Personnel costs (to the work of counterpart) ;	
the Clinical and Laboratory Standards Institute		and	
(CLSI)).	Training in Japan	- Operational costs.	
	(1) Training for gene analyses		
	(2) Training for operating Next		<issues and="" countermesures=""></issues>
	Generation Sequencer		
	(3) Training for genome comparison and		
1-4. To analyze the relationship between fungal	gene disruption		
characteristics (strains, species and drug	(4) Training for management of collected		
susceptibility) and patients' characteristics (source	fungi		
of infection, infected sites, underlying and/or	(5) Other necessary training		
complicated diseases, clinical entity, history of	(b) other necessary training		
chemotherapy by antifungals, etc.).	Equipment and materials		
	Necessary equipment and materials for		
	recessary equipment and materials for		
	research activities in the Project		
	Local cost		
1.5 To elucidate mechanism of drug resistance by			
1-5. To elucidate mechanism of drug resistance by			
analyzing gene regions, which are reported to be			
responsible for drug resistances using the fungal			
isolates with drug-resistant property, confirmed in			
the Activity 1-3.			
1-6. To analyse geographycal characteristict of			
drug resistance genes by comparing identified			
drug-resistant mechanisms against antifungals.			
1-7 In parallel with the Activity 1-5 to elucidate			
mechanisms of drug resistance using advanced			
genetic analysis technologies such as next-			
deneration sequencing techniques for the fungel			
isolates which are suspected to have unidentified			
drug registeres mechanisms			
urug resistance mechanisms.			
1-7-1. To provide Brazilian researchers with the			
Training in Japan in the theme of genetic analysis			
techniques using the next-generation sequencer for			
elucidating drug-resistant mechanisms at the			
MMRC			
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1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptive and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newlydeveloping it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan ares and/or other areas in Brazil.

2. Gene amplification technologies-based drugresistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drugresistant mycoses.

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drugresistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drugresistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2.4. To initiate discussions with Provilian authorities

2-4. To initiate discussions with brazilian autonities concerned on the practical application of the drugresistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. Io set up a network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).		
3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.		
3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.		

Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Jap Version 6

Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)

Dated 30 September, 2020

Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

Period of Project: From September 2017 to September 2022

Project Site:

Model Site:

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal	-				
Project Purpose A research and reference collaborative system for the diagnoses of fungal infections including drug- resistant ones is established both in Brazil and Japan.	 by the time of the termination of the management of mycoses are subjected to trial-based application in hospitals in the Campinas metripolitan area. by the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug- resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes 	Reports developed by the Project Meeting minutes with relevant organizations Research articles published in international journals		Achievement of these indicators will be verified at the end of this Project.	
1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	Reports (including some articles or abstracts of academic meeting) developed by the Project A draft of revised guidelines	 The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose. 	The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated. Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Ouro Verde Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are	

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	 (2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area. (3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced. 	 Reports developed by the Project Minutes of meetings with the Brazilian authorities concerned Reports developed by the Project Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned
2. Gene amplification technologies-based drug- resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.	 By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant Aspergillus, Candida and other fungi are determined. By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed. By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced. 	

Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress. Discussion on clinical handbook/manual for aspergillosis was started on the Web. To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of <i>A. fumigatus</i> strains to a fungicide in a simulated field are on going. Using a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains are ongoing. Using a next generation sequencer, metagenomic analysis of clinical specimens are ongoing. A paper regarding genetic basis of antifungal resistance in clinical <i>Candida glabrata</i> was published.	
Introduction of new detection method for antifungal resistant fungi using Surveyor Nuclease has been started. Development of a detection procedure for point mutations in <i>cyp51A</i> gene using PCR or LAMP method is on going in Brazil and Japan. Developments of identification procedures of cryptic species using PCR or LAMP method are in progress. The CRISPR/Cas9 system are usd to elucidate the effect of some mutated genes on antifungal resistance.	

5. A ONICAMP Contract research medical and administrativ organizations is established.	 by the time of the termination of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced. By December 2020, epidemiological information on mycoses including drug- resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval. By the time of the termination of the network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones, of which reference laboratory is the UNICAMP, is commenced. 			The database program (RedCap) has been operated and over 200 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled. As the research network activity participated by several medical institutions and a research laboratory in Sao Paulo State, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run. One medical institution has newly joined to the research network. Preparation of the freeze-dried ampouls for preservation of fungal strains in UNCAMP are on going. A paper regarding a potential cause of invasive fusariosis in hospitals or residences was published.	
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Activities		Inputs	Pre-Conditions		
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	The Japanese Side	The Brazilian Side			
	Experts	Counterparts			
1. Prevalence of drug-resistant fungi and its	(1) Chief Advisor (Short-term	(1) Project Director			
mechanisms of drug-resistance are	Expert/double as an expert for	(2) Project Manager			
epidemiologically elucidated.	Diagnostics-related studies)	(3) Project Co-managers (3 persons)			
	(2) Project Coordinator/Specialist for	(4) Researchers (Infectious Diseases Division of	1. Approvals are obtained by the ethical committees of		
4.4. To manform allocation and identification of	Besearch Institutes Cooperation (Long-	the Internal Medicine Department and the Clinical	each project implementing agency for the medical and/or		
1-1. To perform classification and identification of	term Expert)	Pathology Department, the Eaculty of Medical	epidemiological research, which are supposed to be		
rungal strains by morphological and biochemical	(3) Diagnostics-related studies (Short-	Sciences the UNICAMP)	performed in the Project (if necessary).		
properties with clinically-isolated fungal strains	torm Exports)				
collected by UNICAMP-related and other project	(4) Molecular epidemiological studies	Excilition oquipment and materials			
cooperating medical facilities.	(Short torm Exports)	(1) Office space or facilities for IICA experts in			
	(5) Gono amplification related studios	the UNICAMP			
1-2. To perform more detailed identification of	(Short-term Experts)	(2) Besearch space and facilities in the			
fungal strains including cryptic species using	(6) Bioinformatics (Short torm				
genetic techniques on the fungal isolates identified	(0) Diolinomatics (Short-term	(2) Evicting research instruments and equipment			
in the Activity 1-1.	Experts)	(3) Existing research instruments and equipment			
	(7) Epidemiological investigation and	ior research activities, etc.			
1-3. To examine the susceptibility of the fungal	(7) Epidemiological investigation and	Lagel Casta			
species (especially for Asperaillus Candida	(Short torm Exports)	Durning our page for research activities at a			
Cryptococcus and Fusarium) including some	(8) Management of fungal strains related	- Running expenses for research activities, etc.;			
cryptic species in accordance with internationally-	(6) Management of fungal strains-related	- Assignment of counterpart personnel;			
approved methods (e.g. the methods provided by	(0) Other persons with personal	- Utility costs (electricity, water, etc.);			
the Clinical and Laboratory Standards Institute	(9) Other persons with necessary	- Personnel costs (to the work of counterpart) ;			
(CLSI))	expertise for the project activities (Short-	and			
(020.)).	term Expens)	- Operational costs.			
	Training in Japan		lssues and countermesures>		
	(1) Training for gono analysos				
	(1) Training for energing Next				
1-4. To analyze the relationship between fungal	(2) Training for operating Next		(Issues)		
characteristics (strains, species and drug	Generation Sequencer		Due to the pandemic of COVID-19 worldwide, Japanse		
susceptibility) and patients' characteristics (source	(3) Training for genome comparison and		researchers' visiting to Brazil and receiving Brazilian		
of infection, infected sites, underlying and/or	(4) Training for more provided to the start		trainees in Japan were deferred.		
complicated diseases, clinical entity, history of	(4) framing for management of collected		(Countermeasures)		
chemotherapy by antifungals, etc.).			Research meetings between the chief advisor/Japanese		
	(5) Other necessary training		experts and Brazilian researches were performed		
	Environment and materials		periodically using a video-conferencing system.		
	Equipment and materials				
	Necessary equipment and materials for				
1.5. To olucidate mechanism of drug resistance by	research activities in the Project				
analyzing gone regions, which are reported to be	L L				
responsible for drug resistances using the fungal	Local cost				
isolates with drug resistant preparty, confirmed in					
the Activity 1.2					
the Activity 1-3.					
1.C. To engly a record hypel characteristics of					
1-6. To analyse geographycal characteristict of					
drug resistance genes by comparing identified drug-					
resistant mechanisms against antirungais.					
1-7. In parallel with the Activity 1-5, to elucidate					
mechanisms of drug resistance using advanced					
genetic analysis technologies such as next-					
generation sequencing techniques for the fungal					
isolates, which are suspected to have unidentified					
drug resistance mechanisms.					
1.7.1. To provide Brazilian researchers with the					
Training in Japan in the theme of genetic enclusion					
techniques using the port apporation converses for					
lectiniques using the next-generation sequencer for					
MMPC					
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1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptive and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newlydeveloping it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan ares and/or other areas in Brazil.

 Gene amplification technologies-based drugresistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drugresistant mycoses.

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplificationbased gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drugresistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

9.4. To initiato discussions with Provilian authoritian

2-4. To initiate discussions with brazinan automites concerned on the practical application of the drugresistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).		
3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.		
3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.		

Project Monitoring Sheet I (Revision of Project Design Matrix)

Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Jaj Version 7

Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)

Dated 31 March, 2021

Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

Period of Project: From September 2017 to September 2022

Project Site:	Model Site:				
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal	-				
Project Purpose A research and reference collaborative system for the diagnoses of fungal infections including drug- resistant ones is established both in Brazil and Japan.	 by the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metripolitan area. By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug- resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes 	Reports developed by the Project Meeting minutes with relevant organizations Research articles published in international journals		Achievement of these indicators will be verified at the end of this Project.	
1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	Reports (including some articles or abstracts of academic meeting) developed by the Project A draft of revised guidelines	 The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose. 	The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated. Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Barretos Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are onoging (over 1.000 clinical strains and 500	

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	 (2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area. (3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced. 	 Reports developed by the Project Minutes of meetings with the Brazilian authorities concerned Reports developed by the Project Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned
2. Gene amplification technologies-based drug- resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses.	 By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant <i>Aspergillus</i>, <i>Candida</i> and other fungi are determined. By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed. By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced. 	

Detailed spe identification colinical and several mett multiplex PC Discussion of aspergillosis conference To elucidate mutagencity of <i>A. fumiga</i> simulated fit Using a nex expression a antifungal-s ongoing. Using a nex metagenom are ongoing FKS genes was publish A paper reg FKS genes	cices (cryptic specie is so ffungal strains is environmental speci- nods such as DNA s 2R and LAMP are in on clinical handbook was continued usir system. the effect of fungici of fungi, studies or <i>tus</i> strains to a fung eld are on going. t generation sequen analysis (transcripto usceptible/resistant t generation sequen c analysis of clinica arding a rapid identi mutations in <i>Candid</i> ed.	s) solated from mens using equencing, progress. //manual for ng a video- des on exposure icide in a cer, gene me) of strains are cer, I specimens fication of a glabrata of enes was	
Introduction antifungal re Nuclease ha Developmer Japan. Developmer of cryptic sp method are The CRISPf elucidate the on antifunga	of new detection me esistant fungi using S as been started. It of a detection pro- ons in <i>cyp51A</i> gene thod is on going in I hts of identification p ecies using PCR or in progress. R/Cas9 system are u e effect of some mut I resistance.	ethod for Surveyor cedure for using PCR Brazil and roccedures LAMP usd to ated genes	

3. A UNICAMP-centered research collaboration system among research, medical and administrati organizations is established.	 By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced. By December 2020, epidemiological information on mycoses including drug- resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval. By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones, of which reference laboratory is the UNICAMP, is commenced. 		The database program (RedCap) has been operated and over 250 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled. Several medical and research institution not only in Sao Paulo State but also in Mato Grosso do Sul State are presently participating the research network. As the research network activity, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run. Preparation of the freeze-dried ampouls for preservation of fungal strains in UNCAMP are on going.	

Г	Activities		Inputs	Pre-Conditions
-	Activities	The Japanese Side	The Brazilian Side	T Te-Conditions
		Evporto	Counterparte	
1	Prevalence of drug-resistant fungi and its	(A) Object Ashiever (Object to real	(4) Device the Director	
	achanisms of drug-resistance are	(1) Chief Advisor (Short-term	(1) Project Director	
		Expert/double as an expert for	(2) Project Manager	
e	bidemiologically elucidated.	Diagnostics-related studies)	(3) Project Co-managers (3 persons)	1. Approvals are obtained by the ethical committees of
		(2) Project Coordinator/Specialist for	(4) Researchers (Infectious Diseases Division of	each project implementing agency for the medical and/or
1	1 To perform classification and identification of	Research Institutes Cooperation (Long-	the Internal Medicine Department and the Clinical	enidemiological research, which are supposed to be
fı	ingal strains by morphological and biochemical	term Expert)	Pathology Department, the Faculty of Medical	performed in the Project (if pecessary)
n	constries with clinically-isolated fundal strains	(3) Diagnostics-related studies (Short-	Sciences, the UNICAMP)	penormed in the Project (in necessary).
P	lested by UNICAMP related and other project	term Experts)	· · · · · · · · · · · · · · · · · · ·	
		(4) Molecular epidemiological studies	Facilities, equipment and materials	
C	operating medical facilities.	(Short-term Experts)	(1) Office space or facilities for JICA experts in	
		(5) Gene amplification-related studies	the UNICAMP	
1	To perform more detailed identification of	(Short-term Experts)	(2) Research space and facilities in the	
fu	ngal strains including cryptic species using	(6) Bioinformatics (Short-torm Exports)		
g	enetic techniques on the fungal isolates identified	(7) Epidemiological investigation and	(2) Evicting research instruments and equipment	
ir	the Activity 1-1.	(7) Epidemiological investigation and	(5) Existing research instruments and equipment	
	,	Object terms Free arts)	for research activities, etc.	
	2. To exercise the execeptibility of the funcel	(Short-term Experts)		
T.	3. To examine the susceptionity of the fungal	(8) Management of fungal strains-related	Local Costs	
S	becies (especially for Aspergillus, Candida,	study (Short-term Experts)	 Running expenses for research activities, etc.; 	
C	ryptococcus, and Fusarium) including some	(9) Other persons with necessary	 Assignment of counterpart personnel; 	
C	yptic species in accordance with internationally-	expertise for the project activities (Short-	 Utility costs (electricity, water, etc.); 	
а	oproved methods (e.g. the methods provided by	term Experts)	 Personnel costs (to the work of counterpart); 	
th	e Clinical and Laboratory Standards Institute		and	
(0	CLSI)).	Training in Japan	- Operational costs.	
		(1) Training for gene analyses		deques and countermosures
		(2) Training for operating Next		
		Generation Sequencer		
	4. To such as the selection which between forward	(3) Training for genome comparison and		
1	4. To analyze the relationship between fungal	gene disruption		(Issues)
C	haracteristics (strains, species and drug	(4) Training for management of collected		Due to the pandemic of COVID-19 worldwide, Japanse
S	isceptibility) and patients' characteristics (source	fungi		researchers' visiting to Brazil and receiving Brazilian
0	infection, infected sites, underlying and/or	(5) Other necessary training		trainees in Japan were deferred.
C	omplicated diseases, clinical entity, history of	(5) Other necessary training		(Countermeasures)
c	nemotherapy by antifungals, etc.).	Equipment and materials		Research meetings between the chief advisor/Japanese
		Equipment and materials		experts and Brazilian researches were performed
		Necessary equipment and materials for		periodically using a video-conferencing system.
		research activities in the Project		
		Local cost		
1	5. To olucidate mechanism of drug resistance by			
	-3. To elucidate mechanism of drug resistance by			
a	any zing gene regions, which are reported to be			
ie	elates with drug resistances using the rungal			
15	a Astivity 4.2			
u	e Activity 1-3.			
	• To another an encoderate the second state of			
1.	6. To analyse geographycal characteristict of			
a	ug resistance genes by comparing identified			
a	rug-resistant mechanisms against antifungals.			
1	7. In parallel with the Activity 1-5, to elucidate			
m	echanisms of drug resistance using advanced			
a	enetic analysis technologies such as next-			
a	eneration sequencing techniques for the fungal			
9 ic	olates, which are suspected to have unidentified			
d	rug resistance mechanisms			
u	ug resistance mechanisms.			
1	7-1. To provide Brazilian researchers with the			
Т	raining in Japan in the theme of genetic analysis			
te	chniques using the next-generation sequencer for			
е	ucidating drug-resistant mechanisms at the			
N	MRC.			

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptive and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newlydeveloping it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan ares and/or other areas in Brazil.

2. Gene amplification technologies-based drugresistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drugresistant mycoses.

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drugresistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drugresistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2.4. To initiate discussions with Provilian authorities

2-4. To initiate discussions with brazilian autonities concerned on the practical application of the drugresistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. I o set up a network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).		
3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.		
3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.		

Project Monitoring Sheet I (Revision of Project Design Matrix)

Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Jaj Version 8

Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)

Dated 30 September, 2021

Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

Period of Project: From September 2017 to September 2022

Project Site:	Model Site:				
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose A research and reference collaborative system for the diagnoses of fungal infections including drug- resistant ones is established both in Brazil and Japan.	 by the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metripolitan area. by the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug- resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes 	Reports developed by the Project Meeting minutes with relevant organizations Research articles published in international journals		Achievement of these indicators will be verified at the end of this Project.	
Outputs 1. Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated.	(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	Reports (including some articles or abstracts of academic meeting) developed by the Project A draft of revised guidelines	 The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose. 	The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated. Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Barretos Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are	

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	 (2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area. (3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced. 	 Reports developed by the Project Minutes of meetings with the Brazilian authorities concerned Reports developed by the Project Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned
 Gene amplification technologies-based drug- resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses. 	 By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant Aspergillus, Candida and other fungi are determined. By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed. By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods as the "Standard Methods" in Brazil) are commenced. 	

0 0 0 N D is c s n D a o T n o s J e a d J n a A b ()	ongoing (over 1,000 clinical strains and 500 environmental strains). Environmental fungal strains collected in Mato Grosso do Sul State are analyzing. Detailed species (cryptic species) dentifications of fungal strains isolated from dinical and environmental specimens using several methods such as DNA sequencing, nultiplex PCR and LAMP are in progress. Discussion on clinical handbook/manual for ispergillosis was continued using a video- conference system. To elucidate the effect of fungicides on nutagenicity of fungi, studies on exposure of <i>A. fumigatus</i> strains to a fungicide in a simulated field are on going. Jsing a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains are ongoing. Jsing a next generation sequencer, netagenomic analysis of clinical specimens are ongoing. A paper regarding novel mutations caused by the exposure to a fungicide tebuconazole) was published.	
III a N C C C C C C C C C C C C C C C C C C	ntroduction of new detection method for antifungal resistant fungi using Surveyor Nuclease has been started. Development of a detection procedure for point mutations in <i>cyp51A</i> gene using PCR or LAMP method is on going in Brazil and Iapan. A paper regarding a detection test for point nutation in cyp51A gene using LAMP nethod has been published. Developments of identification procedures of cryptic species using PCR or LAMP nethod are in progress. The CRISPR/Cas9 system are usd to plucidate the effect of some mutated genes on antifungal resistance.	

3. A UNICAMP-centered research collaboration	(1) By the time of the termination of the		1	1
system among research, medical and administrativ	e Project, practical operation of the			1
organizations is established.	banking system of fungal strains		The database program (RedCap) has been	1
	including drug-resistant ones in the		operated and over 300 clinical cases of	1
	UNICAMP, which is interoperable by the		patients with various fungal infection	1
	external cooperative agencies for		admitted to the attending health care	1
	mycoses research, is commenced.		institutions have already enrolled.	1
	(2) By December 2020, epidemiological		Several medical and research institution	1
	information on mycoses including drug-		not only in Sao Paulo State but also in Mato	1
	resistant ones, gained through the		Grosso do Sul State are presently	1
	research activities of the Project, is		participating the research network.	1
	shared with the Brazilian authorities		As the research network activity, collecting	1
	concerned at a one-year interval.		clinical and environmental fungal strains,	1
	(3) By the time of the termination of the		the regular conference, and scientific	1
	Project, practical operation of the		meetings are run.	1
	network of clinical laboratory testing and		Preparation of the freeze-dried ampouls for	1
	diagnosis of mycoses including drug-		preservation of fungal strains in UNCAMP	1
	resistant ones, of which reference		are on going.	1
	laboratory is the UNICAMP, is		A paper regarding a case series of the	1
	commenced.		COVID-19 associated fungal infections at	1
			UNICAMP was published.	1
				1
				1
				1

Activities		Inputs	Pre-Conditions
	The Japanese Side	The Brazilian Side	
	Experts	Counterparts	
1. Prevalence of drug-resistant fungi and its	(1) Chief Advisor (Short-term	(1) Project Director	
mechanisms of drug-resistance are	Expert/double as an expert for	(2) Project Manager	
epidemiologically elucidated.	Diagnostics-related studies)	(3) Project Co-managers (3 persons)	
	(2) Project Coordinator/Specialist for	(4) Researchers (Infectious Diseases Division of	1. Approvals are obtained by the ethical committees of
	Research Institutes Cooperation (Long-	the Internal Medicine Department and the Clinical	each project implementing agency for the medical and/or
1-1. To perform classification and identification of	term Expert)	Pathology Department, the Eaculty of Medical	epidemiological research, which are supposed to be
fungal strains by morphological and biochemical	(3) Diagnostics-related studios (Short-	Sciences, the UNICAMP)	performed in the Project (if necessary).
properties with clinically-isolated fungal strains	torm Exports)	Sciences, the UNICAMIT /	
collected by UNICAMP-related and other project	(4) Molecular epidemiological studies	Facilities, equipment and materials	
cooperating medical facilities.	(Short-torm Exports)	(1) Office space or facilities for IICA experts in	
	(Short-term Experts)	the UNICAMP	
1-2. To perform more detailed identification of	(5) Gene amplification-related studies	(2) Desearch appear and facilities in the	
fungal strains including cryptic species using	(Short-term Experts)	(2) Research space and facilities in the	
genetic techniques on the fungal isolates identified	(6) Bioinformatics (Short-term Experts)		
in the Activity 1-1.	(7) Epidemiological investigation and	(3) Existing research instruments and equipment	
	Optimal administration-related studies	for research activities, etc.	
1.2. To everying the everyntibility of the function	(Short-term Experts)		
1-3. To examine the susceptibility of the fungal	(8) Management of fungal strains-related	Local Costs	
species (especially for Aspergillus, Candida,	study (Short-term Experts)	 Running expenses for research activities, etc.; 	
Cryptococcus, and Fusarium) including some	(9) Other persons with necessary	 Assignment of counterpart personnel; 	
cryptic species in accordance with internationally-	expertise for the project activities (Short-	 Utility costs (electricity, water, etc.); 	
approved methods (e.g. the methods provided by	term Experts)	 Personnel costs (to the work of counterpart); 	
the Clinical and Laboratory Standards Institute		and	
(CLSI)).	Training in Japan	 Operational costs. 	
	Training for gene analyses		
	(2) Training for operating Next		
	Generation Sequencer		(1001100)
1.4. To apply the relationship between fungel	(3) Training for genome comparison and		(ISSUES)
1-4. To analyze the relationship between rungar	gene disruption		Due to the pandemic of COVID-19 wondwide, Japanse
characteristics (strains, species and drug	(4) Training for management of collected		researchers' visiting to Brazil and receiving Brazilian
susceptibility) and patients' characteristics (source	fungi		trainees in Japan were deferred.
of infection, infected sites, underlying and/or	(5) Other necessary training		(Countermeasures)
complicated diseases, clinical entity, history of	(-),,		Research meetings between the chief advisor/Japanese
chemotherapy by antifungals, etc.).	Equipment and materials		experts and Brazilian researches were performed
	Necessary equipment and materials for		periodically using a video-conferencing system.
	research activities in the Project		The experts perform the preliminary experiments in Japan
			as a replacement for inputs to Brazil.
	Local cost		
1-5. To elucidate mechanism of drug resistance by			
analyzing gene regions, which are reported to be			
responsible for drug resistances using the fungal			
isolates with drug-resistant property, confirmed in			
the Activity 1-3.			
1-6. To analyse geographycal characteristict of			
drug resistance genes by comparing identified			
drug-resistant mechanisms against antifungals			
a so sector and a sector a s			
1-7. In parallel with the Activity 1-5, to elucidate			
mechanisms of drug resistance using advanced			
genetic analysis technologies such as next-			
generation sequencing techniques for the fungal			
isolates, which are suspected to have unidentified			
drug resistance mechanisms.			
1-7-1. To provide Brazilian researchers with the			
Training in Japan in the thome of genetic applying			
tochniques using the port concretion acquerers for			
elucidating drug resistant mochanisms at the			
I	1		

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptive and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newlydeveloping it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan ares and/or other areas in Brazil.

2. Gene amplification technologies-based drugresistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drugresistant mycoses.

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for *Aspergillus* including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drugresistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil.

2-3. To evaluate the operability of the drugresistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2.4. To initiate discussions with Provilian authorities

2-4. To initiate discussions with brazilian autonities concerned on the practical application of the drugresistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.

3-4. I o set up a network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).		
3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.		
3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.		

Project Monitoring Sheet I (Revision of Project Design Matrix)

Project Title: The Project for the Establishment of a Research and Reference Collaborative System for the Diagnoses of Fungal Infections including Drug-Resistant Ones both in Brazil and Jar Version 9

Implementing Agency: University of Campinas, Sao Paulo (UNICAMP)

Dated 31 March, 2022

Target Group: Residents of the area covered by Campinas University hospital and affiliated hospitals (Approximately 3 million people)

Model Site:

Period of Project: From September 2017 to September 2022

Project Site:

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumption	Achievement	Remarks
Overall Goal					
Project Purpose					
A research and reference collaborative system for the diagnoses of fungal infections including drug- resistant ones is established both in Brazil and Japan.	 By the time of the termination of the Project, guidelines/booklets for the management of mycoses are subjected to trial-based application in hospitals in the Campinas metripolitan area. By the time of the termination of the Project, concrete discussions are commenced with Brazilian administrative organizations for the application of research findings and outcomes gained by the Project to policymaking and/or countermeasures for the control of fungal infections including drug-resistant ones. By the time of the termination of the Project, at least 5 research articles in the themes of project research topics such as epidemiological analyses of drug- resistant fungal infections, mechanisms of resistances of fungi to antifungals, and drug resistance-related genes detection methods are published. 	 Reports developed by the Project Meeting minutes with relevant organizations Research articles published in international journals 		Achievement of these indicators will be verified at the end of this Project.	
Outputs					
 Prevalence of drug-resistant fungi and its mechanisms of drug-resistance are epidemiologically elucidated. 	(1) By December, 2019, the proportion of drug-resistant fungal strains in the fungal isolates (the prevalence) in the target areas is estimated.	Reports (including some articles or abstracts of academic meeting) developed by the Project A draft of revised guidelines	 The Project gains the cooperation of the Brazilian health administration organizations such as the MOH, the São Paulo State Health Office and the Campinas City Health Office in charge of the control of fungal infections including drug-resistant ones for implementing meetings with the Project regarding policy advocacy etc. Political superiority of the control of fungal infections including the ones caused by drug-resistant strains is not diminished so as to impair the achievement of the Project Purpose. 	The prevalence of antifungal-resistant fungal strains among clinical isolates has been estimated. Determination of species and antifungal susceptibilities of fungal clinical strains isolated in the attending hospitals (UNICAMP, Mario Gatti Hospital, Boldrini Children Hospital and Barretos Hospital) and environmental strains collected by UNICAMP and Instituto Adolfo Luz are ongoing (over 1,000 clinical strains and 500 environmental strains). Several environmental fungal strains	

	 (2) By March, 2020, principal mechanisms of drug resistance against antifungal agents are identified using clinical isolates gained in the Campinas metropolitan area. (3) By March, 2022, the revising work of the existing guidelines, and drafting work of the guidelines/booklets for the management of mycoses are commenced. 	 Reports developed by the Project Minutes of meetings with the Brazilian authorities concerned Reports developed by the Project Documents of information sharing regarding epidemiological data with the Brazilian authorities concerned 	collected in Mato Grosso do Sul State are confirmed to be antifungal resistant strains. Detailed species (cryptic species) identifications of fungal strains isolated from clinical and environmental specimens using several methods such as DNA sequencing, multiplex PCR and LAMP are in progress. Discussion on clinical handbook/manual for aspergillosis was continued using a video- conference system. To elucidate the effect of fungicides on mutagenicity of fungi, studies on exposure of <i>A. fumigatus</i> strains to a fungicide in a simulated field are on going. Using a next generation sequencer, gene expression analysis (transcriptome) of antifungal-susceptible/resistant strains are ongoing. Using a next generation sequencer, metagenomic analysis of clinical specimens are ongoing. A paper regarding the antifungal resistance of <i>Candida tropicalis</i> was published. A paper regarding denetic differences of <i>Aspergillus fumigatus</i> among several countries was published. A paper regarding detailed mechanism of Hmg1 mutations in <i>A. fumigatus</i> was published.	
 Gene amplification technologies-based drug- resistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drug-resistant mycoses. 	 By December, 2020, basic testing condition and procedures of gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant Aspergillus, Candida and other fungi are determined. By December, 2021, evaluation works of clinical performance of the above-mentioned methods are completed. By the time of the termination of the Project, concrete discussions with the Brazilian authorities concerned regarding practical procedures for the application of the above-mentioned methods for clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil) are commenced. 		ca The new detection method for antifungal resistant fungi using Surveyor Nuclease is regularly performed. Development of a detection procedure for point mutations in <i>cyp51A</i> gene using PCR or LAMP method is on going in Brazil and Japan. Developments of identification procedures of cryptic species using PCR or LAMP method are in progress. The CRISPR/Cas9 system are usd to elucidate the effect of some mutated genes on antifungal resistance.	

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3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.	 By the time of the termination of the Project, practical operation of the banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research, is commenced. By December 2020, epidemiological information on mycoses including drug- resistant ones, gained through the research activities of the Project, is shared with the Brazilian authorities concerned at a one-year interval. By the time of the termination of the Project, practical operation of the network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones, of which reference laboratory is the UNICAMP, is commenced. 		The database program (RedCap) has been operated and over 350 clinical cases of patients with various fungal infection admitted to the attending health care institutions have already enrolled. Several medical and research insritution not only in Sao Paulo State but also in Mato Grosso do Sul State are presently participating the research network. As the research network activity, collecting clinical and environmental fungal strains, the regular conference, and scientific meetings are run. Preparation of the freeze-dried ampouls for preservation of fungal strains in UNCAMP are on going.	

Activities	Inputs		Pre-Conditions	
	The Japanese Side	The Brazilian Side		
	Experts	Counterparts		
1. Prevalence of drug-resistant fungi and its	(1) Chief Advisor (Short-term	(1) Project Director		
mechanisms of drug-resistance are	Expert/double as an expert for	(2) Project Manager		
epidemiologically elucidated.	Diagnostics-related studies)	(3) Project Co-managers (3 persons)		
	(2) Project Coordinator/Specialist for	(4) Researchers (Infectious Diseases Division of	 Approvals are obtained by the ethical committees of 	
	Research Institutes Cooperation (Long-	the Internal Medicine Department and the Clinical	each project implementing agency for the medical and/or	
1-1. To perform classification and identification of	term Expert)	Pathology Department, the Faculty of Medical	epidemiological research, which are supposed to be	
fungal strains by morphological and biochemical	(3) Diagnostics-related studies (Short-	Sciences, the UNICAMP)	performed in the Project (if necessary).	
properties with clinically-isolated rungal strains	term Experts)			
collected by UNICAMP-related and other project	(4) Molecular epidemiological studies	Facilities, equipment and materials		
cooperating medical facilities.	(Short-term Experts)	(1) Office space or facilities for JICA experts in		
	(5) Gene amplification-related studies	the UNICAMP		
1-2. To perform more detailed identification of	(Short-term Experts)	(2) Research space and facilities in the UNICAMP		
fungal strains including cryptic species using	(6) Bioinformatics (Short-term Experts)	(3) Existing research instruments and equipment		
genetic techniques on the fungal isolates identified	(7) Epidemiological investigation and	for research activities, etc.		
in the Activity 1-1.	Optimal administration-related studies	Lagal Casta		
	(8) Management of fungel strains	- Punning expenses for research activities, etc.		
1.2 To exemine the suggestivity of the function	(o) management or rungal strains-felated	- Assignment of counterpart personnel		
1-3. To examine the susceptibility of the fungal	(Q) Other persons with pecessory	- Itility costs (electricity water etc.)		
species (especially for Aspergillus, Candida,	expertise for the project activities (Short-	- Personnel costs (to the work of counterpart)		
cryptococcus, and Fusarium) including some	term Experts)	and		
cryptic species in accordance with internationally-	com Expondy	- Operational costs		
the Clinical and Laboratory Standards Institute	Training in Japan	opolational coole.		
	(1) Training for gene analyses			
(0201)).	(2) Training for operating Next			
	Generation Sequencer		lssues and countermesures>	
	(3) Training for genome comparison and			
	gene disruption		(1991)	
1-4. To analyze the relationship between fungal	(4) Training for management of collected		Due to the pandemic of COVID-19 worldwide Japanse	
characteristics (strains, species and drug	fungi		researchers' visiting to Brazil and receiving Brazilian	
susceptibility) and patients' characteristics (source	(5) Other necessary training		trainees in Japan were deferred.	
of infection, infected sites, underlying and/or			(Countermeasures)	
complicated diseases, clinical entity, history of	Equipment and materials		Research meetings between the chief advisor/Japanese	
chemotherapy by antifungals, etc.).	recessary equipment and materials for		experts and Brazilian researches were performed	
	research activities in the Project		periodically using a video-conferencing system.	
	Local cost		The experts perform the preliminary experiments in Japan	
			as a replacement for inputs to Brazil.	
1.5. To churidate markenians of dura projetance but				
1-5. To elucidate mechanism of drug resistance by				
responsible for drug resistances using the fungel				
isolates with drug-resistant property, confirmed in				
the Activity 1-3.				
,				
1-6. To analyse geographycal characteristict of				
drug resistance genes by comparing identified				
drug-resistant mechanisms against antifungale				
and a solution moon and an against antitungals.				
1-7. In parallel with the Activity 1-5, to elucidate				
mechanisms of drug resistance using advanced				
genetic analysis technologies such as next-				
generation sequencing techniques for the fungal				
isolates, which are suspected to have unidentified				
drug resistance mechanisms.				
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1-7-1. To provide Brazilian researchers with the Training in Japan in the theme of genetic analysis techniques using the next-generation sequencer for elucidating drug-resistant mechanisms at the MMRC.

1-7-2. To identify gene regions, which are suspected to be responsible for drug resistance, by whole-genomic comparison of drug-susceptive and drug resistant fungal strains isolated in Brazil, followed by the functional analyses of the region in the UNICAMP.

1-8. To compile research findings and outputs into scientific articles, together with that gained in the Output 2 and in Japan, to be used as evidences for revising the existing guidelines and for newlydeveloping it for the management of mycoses, followed by the trial-based application to the hospitals in Campinas metropolitan ares and/or other areas in Brazil.

2. Gene amplification technologies-based drugresistance genes detection methods with sufficient sensitivity, specificity and operability are established for the diagnoses of drugresistant mycoses.

2-1. To develop gene amplification (LAMP, PCR, etc.)-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-1. To develop basic testing condition of detection methods for drug-resistant fungi particularly for Aspergillus including its cryptic strains, *Candida* and other fungi, which are suitable for applied gene amplification techniques.

2-1-2. To provide Brazilian researchers with the Training in Japan in the theme of gene amplification-based gene detection methods for the diagnoses of drug-resistant mycoses.

2-1-3. To introduce the detection methods for drugresistant fungi, developed in the Activity 2-1-2, to the UNICAMP by the joint efforts of Japanese researchers (JICA experts) and the Brazilian researchers who participated the Training in Japan.

2-2. To evaluate the clinical performance of the detection methods for drug-resistant fungi statistically by analyzing its sensitivity and specificity using fungal strains clinically-isolated from patients with mycoses in Brazil. 2-3. To evaluate the operability of the drug-resistant gene detection method(s), which are developed on the basis of LAMP techniques, by introducing them into UNICAMP-related hospitals and other selected external cooperative health or testing facilities on a trial basis.

2-4. To initiate discussions with Brazilian authorities concerned on the practical application of the drugresistant gene detection methods, developed by the Project, to clinical laboratory testing (registration/accreditation of the methods as the "Standard Methods" in Brazil).

3. A UNICAMP-centered research collaboration system among research, medical and administrative organizations is established.

3-1. To construct a banking system of fungal strains including drug-resistant ones in the UNICAMP, which is interoperable by the external cooperative agencies for mycoses research.

3-1-1. To develop bylaws for the operation of the sample banking system, which stipulates the supply/transportation of samples to the UNICAMP, preservation and shared use of the fungal strains including drug-resistant ones, by holding meeting with external cooperative agencies of the Project such as universities, hospitals and testing facilities.

3-1-2. To provide Brazilian researchers with the Training in Japan in the theme of the operational management of fungal strains preservation facility at the MMRC.

3-1-3. To develop a facility for the preservation of fungal strains in the UNICAMP.

3-1-4. To construct an information database of clinically-isolated fungal strains, compromised of fungal and patient' characteristics in the UNICAMP.

3-2. To hold regular conferences and/or seminars on drug-resistant fungi including the reporting of project achievements, geared to project external collaborating agencies, health and medical facilities in the project areas and the administration organizations in charge of the control of fungal infections including drug-resistant ones.

3-3. To hold regular meetings with the administration organizations in charge of the control of fungal infection including drug-resistant ones for sharing of the epidemiological information such as incidence and clinical entity, gained through the research activities of the Project.		
3-4. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones among the UNICAMP (as the reference laboratory) and health and testing facilities in the State of São Paulo with the support of the Institute of Adolfo Lutz (IAL).		
3-5. To set up a network of clinical laboratory testing and diagnosis of mycoses including drug- resistant ones among research institutes, health facilities and the MMRC as the reference laboratory also in Japan, by referencing the achievements of project collaborative research and technical cooperation in Brazil.		
3-6. To hold national and international conferences on the research of drug-resistant mycoses geared to research, health and administrative agencies in other provinces and nations, in light of future establishment of a network of collaborative research on mycoses including drug-resistant ones as well as wide-sharing of research findings and outcomes of the Project.		

ANNEX 6 List of the discussed issues in AMC-19

1st AMC-19 (June 3, 2020): Clinical cases of COVID-19 in Japan

2nd AMC-19 (June 17, 2020): Clinical cases of COVID-19 in Japan and Brazil

3rd AMC-19 (July 1, 2020): Comprehensive health care (incl. mental health care) for medical staff on COVID-19

4th AMC-19 (July 22, 2020): Clinical practice on COVID-19 at HC/UNICAMP – focusing on PBPM-Protocol-Based Pharmacotherapy Management –

5th AMC-19 (August 12, 2020): Clinical Practice on COVID-19 at Chiba University Hospital – focusing on Antiviral and anticoagulant therapy –

6th AMC-19 (September 2, 2020): Comprehensive health care (incl. mental health care) for medical staff on COVID-19 at UNICAMP

7th AMC-19 (September 23, 2020): Persistent symptoms after acute COVID-19 (Japan)

8th AMC-19 (October 14, 2020): Persistent symptoms after acute COVID-19 (Brazil)

9th AMC-19 (November 4, 2020): COVID-19 disease in Children – Japan

10th AMC-19 (November 25, 2020): COVID-19 disease in Children – Brazil

11th AMC-19 (December 16, 2020): Extrapulmonary manifestations and impact of COVID-19

12th AMC-19 (February 24, 2021): Management of non-ICU COVID-19 patients in Chiba University Hospital

13th AMC-19 (March 17, 2021): Current Status of a new SARS-CoV-2 lineage,

named P.1 (20J/501Y.V3) and Epidemiological Surveillance in Brazil

14th AMC-19 (April 28, 2021): COVID-19 - Epidemiological situation in Japan

15th AMC-19 (May 26, 2021): Clinical features of COVID-19 variants including P.1 lineage

16th AMC-19 (June 30, 2021): The organization policy of higher education facilities in the COVID-19 pandemic

17th AMC-19 (July 21, 2021): Campinas State University: pioneering the fight against Covid-19

18th AMC-19 (September 8, 2021): The infodemic about COVID-19 vaccines

19th AMC-19 (September 29, 2021): The infodemic; Why? How? And so what?

20th AMC-19 (October 12, 2021): Campinas and COVID-19: Current Overview of Cases, Vaccines and Variants

21st AMC-19 (November 9, 2021): Two cases of rare adverse event after COVID-19 vaccination

22nd AMC-19 (December 15, 2021): After-Effect of COVID-19; Psychiatric Disorders and Post Traumatic Growth

23rd AMC-19 (March 15, 2022): Epidemiological Aspects of VOC Omicron in São Paulo State and the City of Campinas

24th AMC-19 (May 10, 2022): Treatment strategies for mild to moderate COVID-19 in Japan, 2022 May