NATIONAL ACADEMIES Sciences Engineering Medicine

FORUM ON MICROBIAL THREATS

The Role of Plant Agricultural Practices on Development of Antimicrobial Resistant Fungi Affecting Human Health

A Workshop

21., 22. and 27. June 2022

LatAsp CP/HM

Aspergillus fumigatus azole resistance survey by air sampling through a citizen science approach

Principle Investigators:

PI-1: **Prof. Wieland Meyer**, Fundação Oswaldo Cruz (FIOCRUZ), Brazil/Curtin University/University of Sydney, Australia

PI-2: **A/Prof. Plinio Trabasso**, University of Campinas (UNICAMPI), Campinas, Brazil

PI-3: **Prof. Marcia Melhem**, Universidade Federal de Mato Grosso do Sul (UFMS)/Instituto Adolfo Lutz (IAL), Brazil

PI-4: **Prof. Maria-Luisa Moretti**, UNICAMPI, Campinas, Brazil

PI-5: **Prof. Luciana Trilles**, FIOCRUZ, Rio de Janeiro, Brazil

PI-6: **Dr. Beatriz Bustamante**, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru

PI-7: **Prof. Claudia M. Parra-Giraldo**, Pontificia Universidad Javeriana (PUJ), Bogota, Colombia

PI-8: **Prof. Laura Castañón-Olivera**, Universidad Nacional Autónoma de Mexico (UNAM), Mexico City, Mexico

FIOCRUZ Fundação Oswaldo Cruz · Curtin University · The University of Sydney · UNICAMP · UFMS · Instituto Adolfo Lutz · Universidad Peruana Cayetano Heredia · Pontificia Universidad Javeriana · UNAM

LatAsp

Azole Resistance in A. *fumigatus* in Latin America

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

SUSCEPTIBILITY

March 2020 Volume 64 Issue 3 e02059-19 Antimicrobial Agents and Chemotherapy aac.asm.org 1

Aspergillus fumigatus Clinical Isolates Carrying CYP51A with TR34/L98H/S297T/F495I Substitutions Detected after Four-Year Retrospective Azole Resistance Screening in Brazil

Laís Pontes,[a] Caio Augusto Gualtieri Beraquet,[a] Teppei Arai,[b] Guilherme Leite Pigolli,[a] Luzia Lyra,[a] Akira Watanabe,[b] Maria Luiza Moretti,[a] Angélica Zaninelli Schreiber[a]

1% (2/199) in Brazil

ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2021, 0, 1–9
https://doi.org/10.1093/mmy/myab031
Advance Access Publication Date: 22 May 2021
Original Article

Original Article

Species distribution and antifungal susceptibility patterns of *Aspergillus* isolates from clinical specimens and soil samples in Mexico

Rogelio de J. Treviño-Rangel[1,†], Hiram Villanueva-Lozano[1,†], Alexandro Bonifaz[2], Laura R. Castañón-Olivares[3], Angel Andrade[1], Miguel A. Becerril-García[1], Michel F. Martínez-Reséndez[4], Jacobo Ayala-Gaytán[4], Alexandra M. Montoya[1] and Gloria M. González[1,*]

6.1-10.2% (3-5/49) in Mexico

ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2020, 58, 54–60
doi: 10.1093/mmy/myz032
Advance Access Publication Date: 23 April 2019
Original Article

Original Article

Azole resistance among clinical isolates of *Aspergillus fumigatus* in Lima-Peru

Beatriz Bustamante[1,*], Luis Ricardo Illescas[2], Andrés Posadas[3] and Pablo E. Campos[4]

2% (3/143) in Peru

Journal of *Fungi* MDPI

Article

Triazole-Resistance in Environmental *Aspergillus fumigatus* in Latin American and African Countries

Agustin Resendiz-Sharpe [1], Klaas Dewaele [2], Rita Merckx [1], Beatriz Bustamante [3], Maria Celeste Vega-Gomez [4], Miriam Rolon [4], Jan Jacobs [1,5], Paul E. Verweij [6,7], Johan Maertens [1,8] and Katrien Lagrou [1,2,*]

J. Fungi 2021, 7, 292. https://doi.org/10.3390/jof7040292

6.9% (7/102) in Mexico
8.3% (3/36) in Paraguay
9.8% (6/61) in Peru

LatAsp

CDC call: ***Topic 4:*** *International Transmission, Colonization, and Prevention of Antibiotic Resistant Pathogens*
4.3. Estimating the prevalence of antifungal-resistant *Aspergillus fumigatus* in low- and middle-income countries

Project Objectives

- To evaluate the presence of azole-resistant *A. fumigatus* isolates in air samples in Latin America.
- To assess the presence of mutations in the *Cyp51A* gene of azole-resistant *A. fumigatus* isolates.
- To compare the genetic relationship between resistant strains found in the air of different countries of Latin America.
- To evaluate the relationship between the use of azole agricultural fungicides and the presence of azole-resistant *A. fumigatus* isolates.
- Establish a Network in Latin America for resistance monitoring of *Aspergillus* and other studies that can be carried out afterwards.

Latin American Medical Mycology Network Laboratories (LAMMN)

Latin American
Aspergillus fumigatus
Azole Resistance Survey
in 12 countries, ~~20~~ 26 laboratories
+ 2

+

CF/WM

Brazil - Argentina – Venezuela - Antarctica

Brazil			
	University of Campinas (UNICAMP)	Campinas	Maria-Luiza Moretti
			Silviane Duarte Rodrigues
			Plinio Trabasso
			Larissa Ortolani Levi
			Cibele Aparecida Trararam
			Angelica Zaninelli-Schreiber
			Antonio José de Almeida Meirelles
	FUNCAMP	Campinas	Mariah Grimaldi e Cruz
	Universidade Federal de Mato Grosso do Sul (UFMS)	Campo Grande	Marcia Melhem
			James Venturini
	Instituto Adolfo Lutz (IAL)	Sao Paulo	Marcia Melhem
			Lucas Xavier Bonfietti
			Juliana Possatto Fernandes Takahashi
	Fundacão Oswaldo Cruz (FIOCRUZ)	Rio de Janeiro	Luciana Trilles
			Wieland Meyer
	Universidade Federal do Triangulo Mineiro	Uberaba	Mario Leon Silva Vergara
			Kennio Ferreira Paim
	Universidade Federal do Piauí	Teresina	Liline Maria Soares Martins
Argentina	Universidad Buenos Aires	Buenos Aires	Norma Beatriz Fernandez
	Universidad Nacional del Nordeste	Resistencia	Gustavo Giusiano
Venezuela	Insitituto Rafael Rangel	Caracas	Maribel Dolante
			Victor Alarcon Fernandez

Peru - Chile

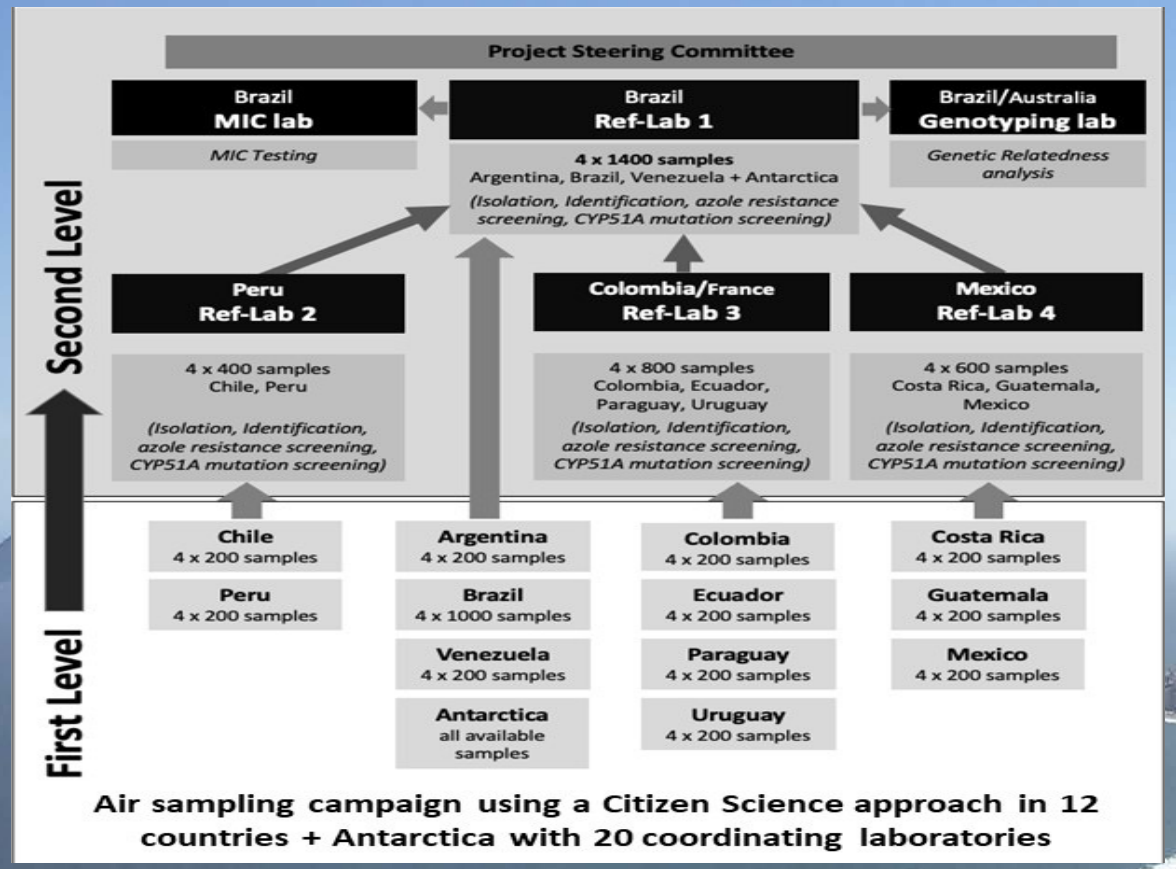
Peru	Universidad Peruana Cayetano Heredia (UPCH)	Lima	Beatriz Bustamante
	Universidad Peruana Cayetano	Lima	Edgar Neyra
Chile	Universidad de Chile	Santiago de Chile	Eduardo Andres Alvarez Duarte
	Universidad Austral de Chile	Valdivia	Patrício Godoy

Colombia – Ecuador – Paraguay - Uruguay

Colombia	Pontificia Universidad Javeriana (PUJ)	Bogota	Claudia M. Parra-Giraldo (Lead)
	Université de Nantes, France	Nantes	Patrice P LePape
	Universidad Nacional de Colombia	Bogota	Carlos Alvarez-Moreno
	Institute National de Salud	Bogota	Elizabeth Castaneda
	Universidad del Rosario	Bogota	Sandra Carolina Firacative Ropero
			Beatriz Lucia Gomez-Giraldo
	Universidad Metropolitana Barranquilla	Barranquilla	Maria Clara Noguera Angarita
Ecuador	Pontificia Universidad Catolica del Ecuador	Quito	Lucia Jeannete Zurita-Salinas
			Andrea Lopez
Paraguay	Instituto Regional de Investigacion en Salud	Caaguazu	Gladys Mercedes Estigarriba-Sanabria
Uruguay	Ministerio de Salud	Montevideo	Andres Puime

Mexico – Costa Rica - Guatemala

Mexico	Universidad Nacional Autónoma de México (UNAM)	Mexico City	Laura Castanon-Olivera
			Edith Sanchez-Paredes
			Carolina Segundo-Zaragoza
	Universidad Autonoma de Noevo Leon	Noevo Leon	Gloria Maria Gonzales-Gonzalez
Costa Rica	Universidad de Costa Rica	San Jose	Norma Teresa Gross
			Daniela Jaikel-Viquez
Guatemala	Universidad de San Carlos	Guatemala	Anneliese Moller Sundfeldt
			Blanca Samayoa Herrera



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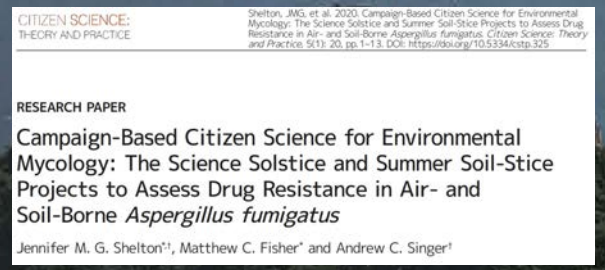


LatAsp

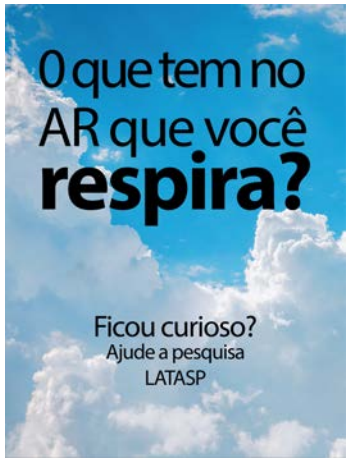
WM

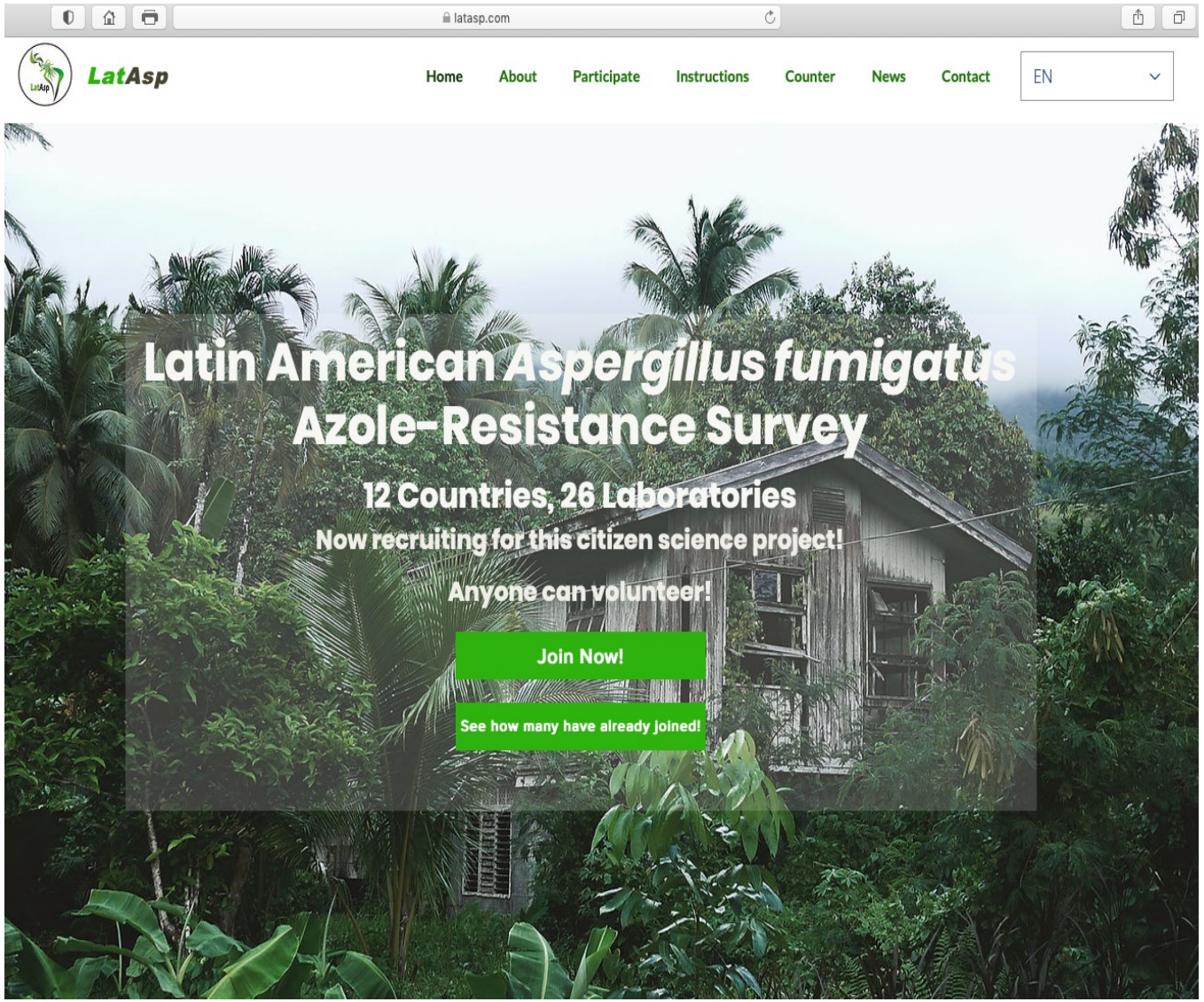
Citizen Science Project – Participant Recruitment

Social Media Campaign + www.latasp.com

- Advertises the project
- Searches Citizen Science Campaign Participants
- Recruits 100 participants per country

+ students
+ coworkers in institutes





Wend alive November 2021

latasp.com

Registration

LatAsp

Home About Participate Instructions Counter News Contact EN

Latin American Aspergillus fumigatus Azole-Resistance Survey

Latin American Research on Aspergillus fumigatus - Survey

You are invited as a volunteer to participate in the citizen science project of the LatAsp study to be carried out in 12 Latin American countries.

This project involves passive air sampling at four times during this year to get an idea of the presence of possible fungi in the environment.

Your participation will help to raise awareness among governments about the potential risk of fungal infections and to develop better treatments for these infections.

Your participation does you no harm, as we only collect fungal spores that are always present in our usual environment. After you've completed the form, you will receive an email confirming that we've received your expression of interest.

Volunteers will be selected to represent, if possible, all parts of each participating country and equal rural and urban areas. If you are selected, you will receive an email from lataspergillus@gmail.com confirming your participation in the project.

The survey will take place as follows: two weeks before the four pre-set times for air sampling (to be announced) you will receive an envelope containing:

- two air samplers,
- an instruction sheet on how to perform the sampling
- a newsletter about the project
- a short questionnaire related to sampling conditions and
- a prepaid mailing envelope for returning the material.

One day before the scheduled pick-up date, you will receive a reminder via SMS. If you do not receive the material within a week before the scheduled date, please send an email to lataspergillus@gmail.com

All personal data collected will be kept confidential and their locations will be made anonymous.

Your name and address are only needed to send the sample packages, and we assure you that they will not be disclosed.

Only the locations you enter in the questionnaire will be assigned to the sample, for analysis of the geographic distribution of the fungi.

Your mobile number will only be used to send a reminder one day before the scheduled air collection date and will be removed later.

Providing your email address will be required as we will provide you with updates on project progress as well as news and updates. At no time will your personal data be shared with third parties and will only be kept registered during the period of this project.

You may opt-out of this project at any time by communicating your decision by email to lataspergillus@gmail.com; We will then delete all your data, including information relating to collections that you may have already carried out.

If you would like to receive detailed information about the project, go to: www.latasp.com
or follow the project on:

- Twitter: @lataspergillus1
- Instagram: @lataspergillus
- Facebook: Lat Asp
- Or contact us at: lataspergillus@gmail.com

Thank you, LatAsp Team.

To register as a volunteer please follow the steps below:

Comments:

1) If you are using a cell phone (iOS/Android), enable "Location" in the Setup;

2) For Safari users in iOS devices, disable the browser's "hide reader" option

Interested in joining this project? Complete the questionnaire!

Join now!

www.latasp.com

Part 1 - Identification

Personal Information

Full name*

What is your age range?*

-Please Select-

Your address:

Country*

-Please Select-

Address*

Number*

Neighborhood*

City*

-Please Select-

Village

Postal Code*

Address Comments

Please provide any further details or comments about your address here

E-mail address*

E-mail address (Confirmation)*

Cell phone number (+country code) (area code) (phone number)*

Part 2: Air Sampling Kit delivery/collection

Please indicate how you would like to receive your air sampling kit:*

Sent to my mailing address

I am part of an institutional/university sampling group; please select from below:

Institutional/University sampling group:

-Please Select-

Argentina - Universidad Buenos Aires

Argentina - Universidad Nacional del Nordeste

Brasil - Universidade Estadual de Campinas (UNICAMP)

Part 3: Air Sampling Locations

Sampling Location 1

Country*

-Please Select-

Address*

Number*

Neighborhood*

City*

-Please Select-

Village

Postal Code*

Address Comments

Please provide any further details or comments about the sampling address here

Probable sampling area*

-Please Select-

Sampling Location 2

Country*

-Please Select-

Address*

Number*

Neighborhood*

City*

-Please Select-

Village

Postal Code*

Address Comments

Please provide any further details or comments about the sampling address here

Probable sampling area*

-Please Select-

Urban

Rural

interest to participate in this project!

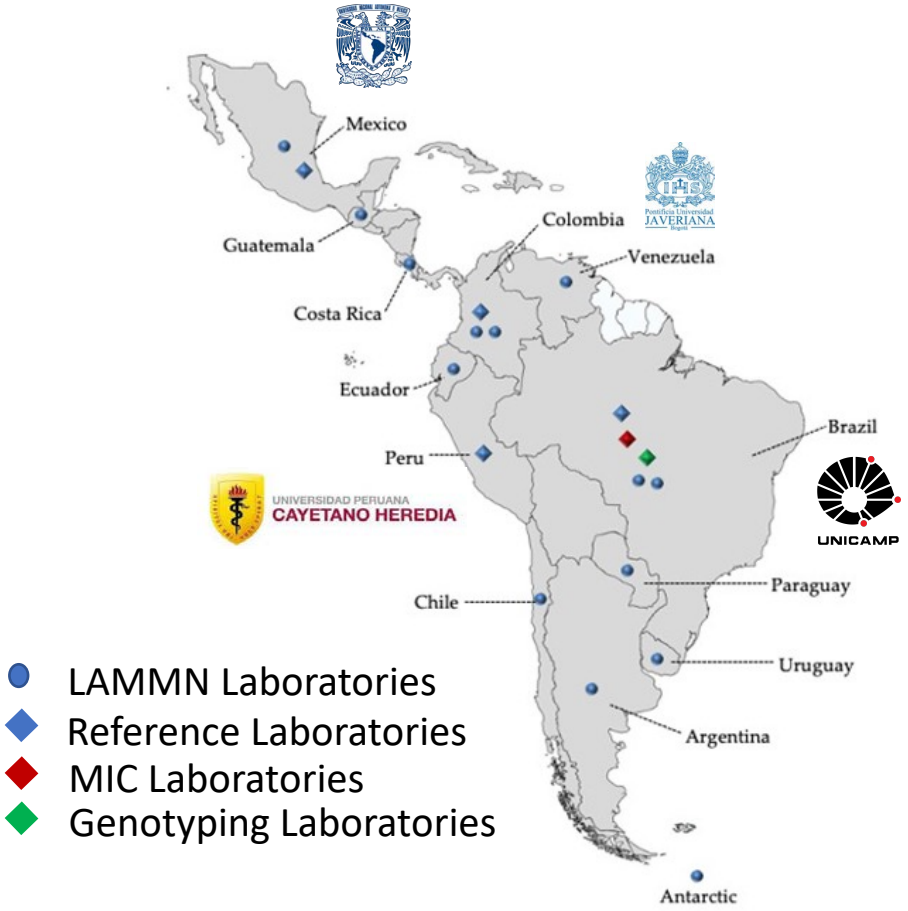
Citizen Science Project – Online Counter as per 21 June 2022

	Via LatAsp webpage	Via LAMMN Labs
Argentina:	100	
Brazil:	242	258
Chile:	44	56
Colombia:	41	59
Costa Rica:	29	71
Ecuador:	6	94
Guatemala:	45	55
Mexico:	17	83
Paraguay:	133	
Peru:	32	68
Uruguay:	17	83
Venezuela:	30	70
	736	**864**

www.latasp.com

Citizen Science Project

1. UNICAMP preparing sampling packs for all LAMMN Laboratories
2. LAMMN Laboratories send out or directly give individual sampling packs to all Citizen Science Participants
3. Individual Citizen Science Participants take two air samples
4. Individual Citizen Science Participants send back the air samples using a prepaid retune envelop to the corresponding LAMMN Laboratory
5. LAMMN Laboratories send the returned sampling packs to the respective Reference laboratories



CF

Air sampling pack preparation

4x 1600 sampling kits
-> 4x 3200 air samples
Total: 12,800 air samples

Each Sampling Kit contains:

- Objectives of the study
- Sampling Instructions
- Sampling Form
- 2 air sampler films
- 2x small envelopes
- Prepaid retune envelop

Citizen Science - Air Sampling Dates

1	June 2022	Sampling packs send out
	July 2022	**Air Sampling Week**
2	September 2022	Sampling packs send out
	October 2022	**Air Sampling Week**
3	December 2022	Sampling packs send out
	January 2023	**Air Sampling Week**
4	March 2023	Sampling packs send out
	April 2021 Air	**Sampling Week**

1. Air Sampling - Citizen Science Project

1 **Amostragem de *Aspergillus fumigatus***

Ver descrição detalhada no verso

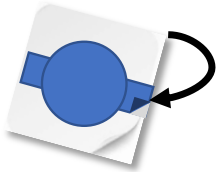
Amostragem de ar ➡ Em 2022: junho; setembro e dezembro. Em 2023: março

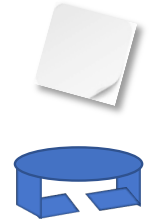
Registre-se no link: https://pt.latasp.com/

Conteúdo

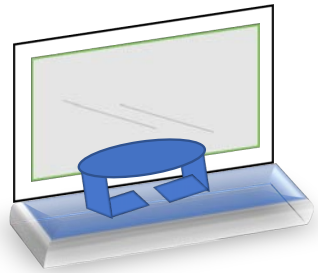
- 1 folha: Objetivos da amostragem
- 1 folha: Instruções (esta folha)
- 1 folha: Condições de amostragem
- 2 amostradores de ar
- 2 envelopes pequenos para os amostradores
- 1 envelope maior para devolução do material

Amostrador de ar

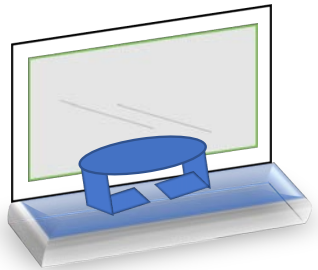




Descole (e conserve) a etiqueta branca

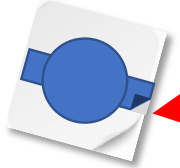


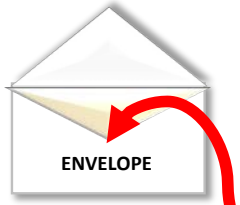
Coloque o amostrador de ar na **janela** do primeiro andar da casa ou local de trabalho.



Deixe o amostrador de ar com o lado pegajoso virado para cima durante **10 a 12 horas.**

Coloque-o de volta na etiqueta branca

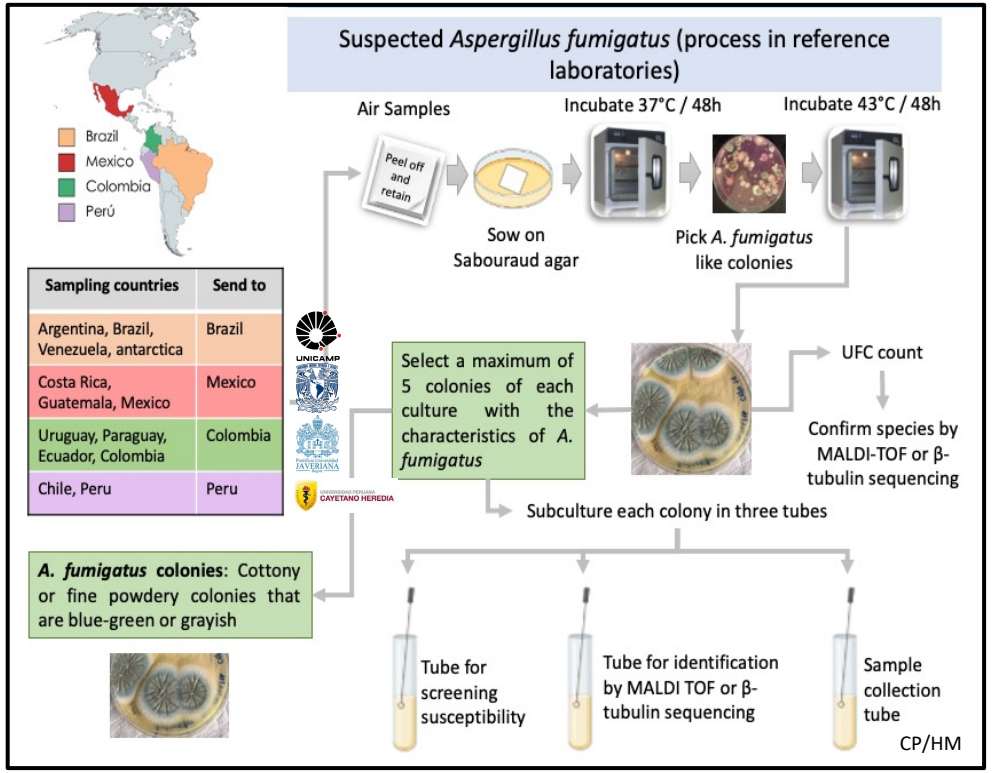




Enviar a:

Laboratório de referência

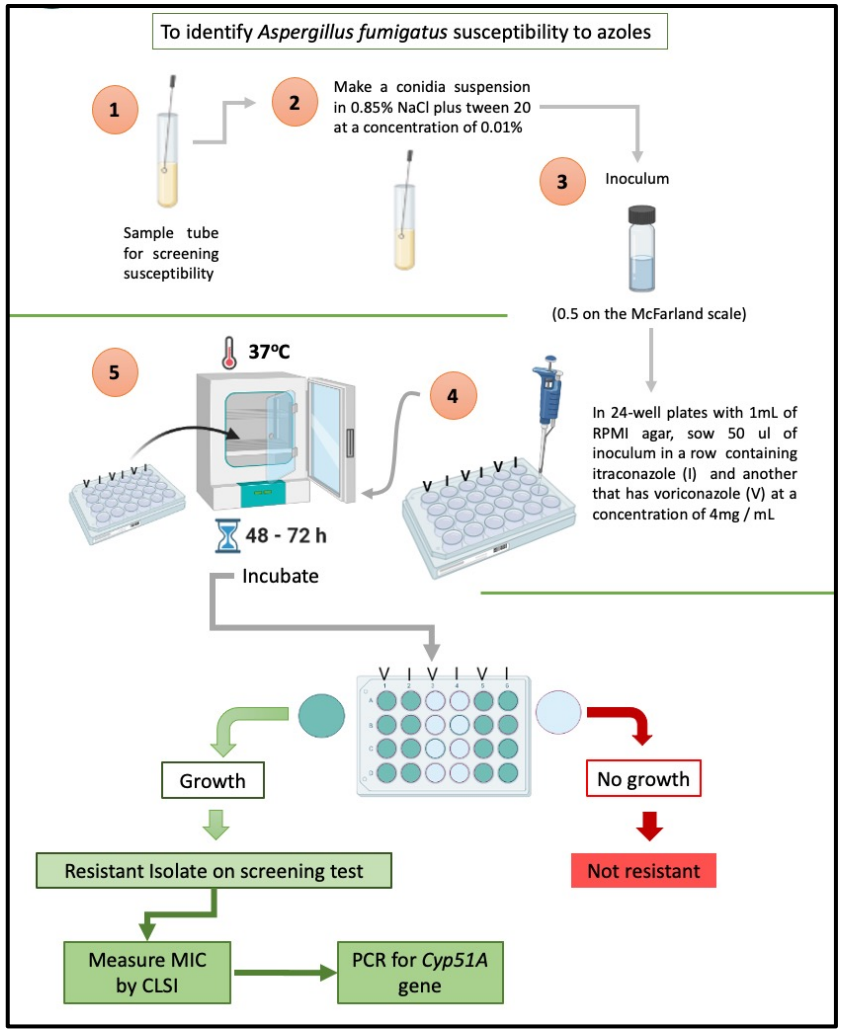
2. *A. fumigatus* Isolation



3. *A. fumigatus* ID

Ref Lab 1 Brazil: MALDI-TOF/*β-TUB* sequencing
Ref Lab 2 Peru: *β-TUB* sequencing
Ref Lab 3 Colombia: MALDI-TOF
Ref Lab 4 Mexico: *β-TUB* sequencing

4. Azole Resistance Screening



Ref Lab 1 Brazil
Ref Lab 2 Peru
Ref Lab 3 Colombia
Ref Lab 4 Mexico

5. MIC Testing

Antifungal susceptibility testing against Itraconazole, Voriconazole, Posaconazole, Miconazole and Isavuconazole will be performed according to CLSI M38-A2 protocol

In vitro susceptibility and resistance will be defined according to the recently published CLSI epidemiological cutoff values (ECVs) and clinical breakpoints for *A. fumigatus*.

Universidade Federal de Mato Grosso do Sul
Instituto Adolfo Lutz

6. *CYP51A* Mutation Detection

The *CYP51A* gene regions will be amplified from resistance isolates showing high MICs to the above tested azoles and sequenced.

University of Campinas
Université de Nantes

7. Genetic Relatedness Analysis

Microsatellite analysis (9 loci) will be performed

Fundacão Oswaldo Cruz
Université de Nantes
Curtin University

8. Agricultural Azole Usage Analysis

The LAMMN laboratories in each of the 12 countries will conduct an agricultural usage analysis.

Evaluate azole fungicide data collected from people around the air sampling points.

Evaluate data obtained from the relevant government offices and non-profit/non-governmental organizations.

Proposed Project Impact

- Provide baseline info on environmental *Aspergillus fumigatus* azole resistance
- Provide Latin American governments with data to identify links between antifungal usage in agriculture and emerging resistance in environmental *Aspergillus fumigatus* isolates
- Provide evidence to Governments to influence the usage of antifungals in agriculture
- Provide WHO with baseline info on *Aspergillus fumigatus* azole resistance to develop better treatment guidelines
- Enable decision making for clinical treatment of invasive aspergillosis between monotherapy with a first-line triazole or a combination therapy of a triazole with an echinocandin or liposomal amphotericin B, in case with >10% environmental triazole-resistance prevalence (Verweij P. *et al.* 2015)

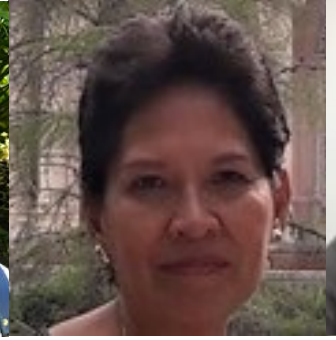
PI-1 Wieland Meyer

PI-2 Plinio Trabasso

PI-3 Marcia Melhem

PI-4 Maria Luisa Moretti

PI-5 Luciana Trilles



PI-6 Beatriz Bustamante

PI-7 Clauda Parra-Giraldo

PI-8 Laura Castañón-Olivera

Latin American Medical Mycology Network Laboratories

Funding: CDC contract BAA 75D301-21-R-71738

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Thank You Gracias Obrigado Merci to all Citizen Science Participants