Instituto Nacional de Tecnología Industrial INTI
Ministerio de Industria Presidencia de la Nación
8th WORKSHOP PROFICIENCY TESTING IN ANALYTICAL CHEMISTRY, MICROBIOLOGY AND LABORATORY MEDICINE
Eurachem A focus for analytical chemistry in Europe

Developing of test items using native cultures microorganisms

1 Graciela O. Davel • 2 Gladys M. Mastromonaco • 1 Marta G. Mazza • 2 Fernando Kornblit • 1 Mariana Mazza • 1 Nicolás Refojo

1 Administración Nacional de Laboratorios e Institutos de Salud (ANLIS) "Dr. Carlos G. Malbrán", Buenos Aires, Argentina. 2 Instituto Nacional de Tecnología Industrial (INTI), Pdo. de San Martín, Argentina.

INTRODUCTION

Microbiological PTs for health, cosmetic, food or environmental areas requires test items from microorganism cultures. In that sense, native and regionally circulating microorganisms, allows to evaluate field laboratories through items of similar and comparable features as for routine samples.
The use of native cultures avoids the entrance of exotic cultures which could damage the natural flora, generate diseases focus or affect regional ecology.

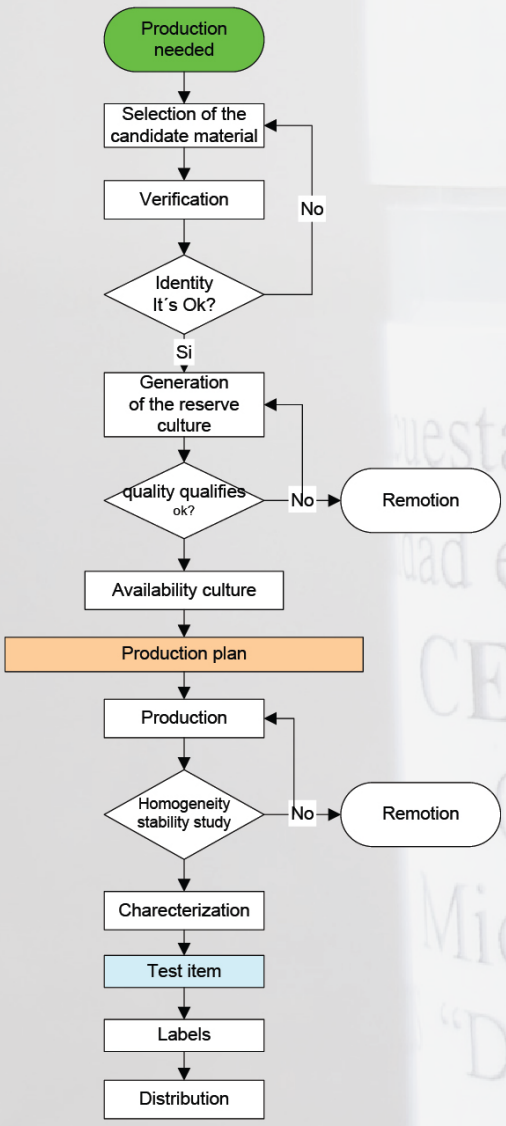
OBJECTIVE:

Develop test items by means of native microbial cultures with defined and traceable phenotypic and genotypic characteristics. In this project, two official institutes from Argentina (ANLIS and INTI), worked together in order to provide PT schemes to field laboratories in the country.

DEVELOPMENT:

For this project a group of yeast cultures of: Candida albicans, C. krusei, C. parapsilosis, C. tropicalis, C. glabrata, Cryptococcus neoformans and C. gattii from clinical sources was selected. And preserved at -70°C in the culture collection maintained by the ANLIS Mycology Department (www.aam.org.ar/cultivos_microbianos). The cultures were identified at the genus and species level using the reference methods cited in [1].
They are based on biochemical, physiological and morphological tests, and sequencing of the region ITS1-5.8S-ITS2. The resulting sequences were compared with the public databases of the National Center for Biotechnology Information (NCBI) and with the Centraalbureau voor Schimmelcultures (CBS). Each culture was multiplied and inoculated in 600 steril Whatman paper disks Nº 1 with diameters of 0,7 cm. Then they were dried and packaged in groups of three units in sterile vials with thread covers. The homogeneity study was performed in two stages: first, a number of $3\sqrt[3]{n}$ selected disks were inoculated in a nutritive media of culture and morphological characteristics were observed. Then, they were spread in a differential media, for a macroscopic observation. Short and long term stability tests of relevant properties were determined on two disks taken from two randomly selected vials and preserved at different temperatures and times.

Test items production Flowchart



RESULTS

HOMOGENEITY:

100 % of the disks have fullfilled the requirements of Viability, Purity and Identity.

Figure 1: Test item production

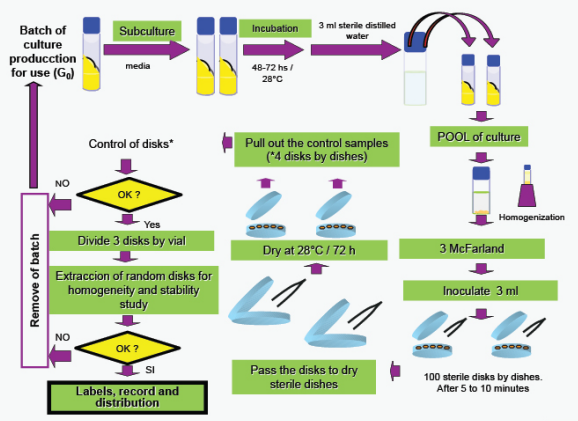


Figure 2: Design of the homogeneity/stability studies

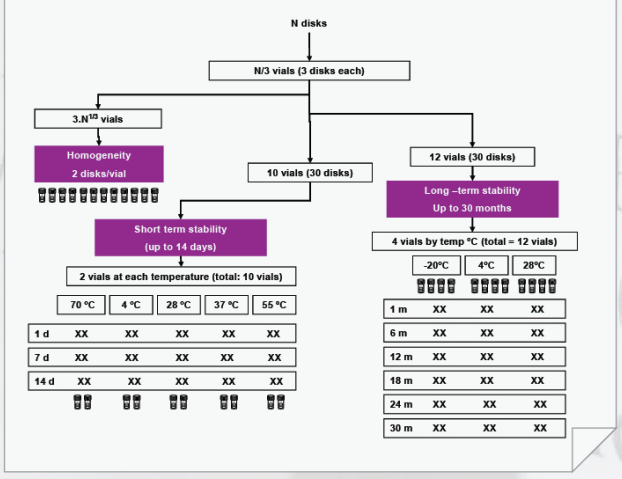


Table 2: Stability

LONG-TERM STABILITY	
STORAGE -70°C and 4°C	The results of identity, viability and purity are satisfactory in the 100 % of the disks, for during 14 days.
STORAGE 28°C and 37°C	The results of identity, viability and purity for **C. albicans, C. krusei, C. parapsilosis, Cr. gattii Cr. neoformans** are satisfactory in the 100 % of the disks, for during 14 days. **C. tropicalis, C. glabrata** survived only 7 days
STORAGE 55°C	**Neither of de culture could survive.**
SHORT-TERM STABILITY	
STORAGE 4°C	The 100 % of the disks of **C. albicans, C. krusei, C. tropicalis, C. glabrata, C. parapsilosis, Cr. gattii** and **Cr. neoformans**, resulted satisfactory in viability, purity and identity, for during 12 months.
STORAGE -20°C	The 100 % of the disks of **C. albicans, C. krusei, C. tropicalis, C. glabrata, C. parapsilosis, Cr. gattii** and **Cr. neoformans**, resulted satisfactory in viability, purity and identity, for during 6 months.
STORAGE 28°C	The 100 % of the disks of **C. albicans, C. krusei, C. parapsilosis Cr. gattii** and **Cr. neoformans**, resulted satisfactory in viability, purity and identity during a month. **C. tropicalis, C. glabrata** were not survive Only **Cryptococcus neoformans** and **C. gattii** resulted satisfactory in viability, purity and identity over 6 months

TABLE 1. Microbiological test for evaluation the homogeneity and stability of the material produced.

Microorganims	Media	Temp.	Time	Test	Acceptance Criteria
Candida albicans		28 ºC	3 -7 days	Colony characteristics on CHROMagar Candida	It grows and develop creamy colonies, green colour. Without contaminant
Candida tropicalis		28 ºC	3 -7 days	Colony characteristics on CHROMagar Candida.	It grows and develop creamy colonies, blue colour. Without contaminant
Candida krusei	CHROMagar Candida	28 ºC	3 -7 days	Colony characteristics on CHROMagar Candida	It grows and develop dry colonies, pink colour. Without contaminant.
Candida parapsilosis	CHROMagar Candida Malt Extract	28 ºC	3 -7 days	Colony characteristics on CHROMagar Candida. Characteristics of the micromorphology in Malt Extract	CHROMagar Candida: It grows and develops pink colour colonies. Without contaminant Malt Extract: Cells are ovoid with multipolar busing, cylindrical cells may be present and develops pseudohyphae.
Candida glabrata	CHROMagar Candida Fast test of Trehalose	28 ºC	3 -7 days	Colony characteristics on CHROMagar Candida. Fast assimilation of trehalose.	CHROMagar Candida: It grows and develops cream to mauve colonies. Without contaminant Fast test of Trehalose: positive
Cryptococcus neoformans	Caffeic acid agar Canavanine-glycine-bromothymol Agar (CGB)	28 ºC	3 -7 days	Development of colony caracteristics on caffeic acid agar. Growth on CGB	Caffeic acid agar: It grows and develops brown colonies. Without contaminant. CGB: Do not grow and the colour of the medium remains greenish-yellow.
Cryptococcus gattii	Caffeic acid agar Canavanine-glycine-bromothymol Agar (CGB)	28 ºC	3 -7 days	Development of colony caracteristics on caffeic acid agar. Growth on CGB	Caffeic acid agar: It grows and develops brown colonies. Without contaminant CGB: It grows and the medium turns blue.

Maldi-tof results

CEPA	DMic Number	Score	Identification
C. albicans	113874	2.141	C. albicans
C. tropicalis	113920	2.12	C. tropicalis
C. krusei	134341	1.992	C. krusei
C. glabrata	113884	1.998	C. glabrata
C. parapsilosis	113911	.045	C. parapsilosis
Cryptococcus gattii	073122	2.127	C. gattii
Cryptococcus neoformans	083456	1.966	C. neoformans

Meaning of Score Values

Range	Description	Symbols	Colors
2300 ... 3000	highly probable species identification	(+++)	green
2000 ... 2299	secure genus identification, probable species identification	(++)	green
1700 ... 1999	probable genus identification	(+)	yellow
0000 ... 1699	not reliable identification	(-)	red

CONCLUSIONS:

The description and application of this methodology will allow the design and organization of PTs responding to Argentine laboratories demands. This design may be applied to another microorganisms beyond native cultures. In addition this technic insures the purity and characteristic of these cultures from a reserve culture batch called "G_0". It is preserved in liquid nitrogen and in ultrafreezer, keeping its phenotipic and genotipic stability, as well as the traceability. The Maldi-tof technique is a rapid and sure tool for homogeneity and stability studies. The cultures have demonstrated to be homogeneous and stable at least by twelve (12) months at the conditions mentioned in Table 2. Once the culture is inoculated in paper disks they remain pure and stable for more than fourteen (14) days at room temperature, and therefore, they may be sent to the participants

REFERENCE: Kurtzman C. P. Fell, J. W. Boekhout, T. The Yeast, a Taxonomic Study 2011 Elsevier Science Publisher B. V. Amsterdam 5th edition.