

**P1654**

**Paper Poster Session**

**Fungal diagnosis: from culture to molecular techniques**

**Evaluation of viability and stability of pathogenic mould and yeast species by using three different maintenance methods over a 12-year period**

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**Background:** The serious mycological work requires a reliable source of cultures, which is ensured by the safe long-term storage of such collections. In this study, a total of 1,186 clinical fungal isolates consisting of molds (20 species in 11 genera) and yeasts (21 species in seven genera) maintained in water, under mineral oil at room temperature and freezing at -80°C for periods ranging from 1 year to 12 years were evaluated for viability and stability.

**Material/methods:** The strains were subcultured onto either Sabouraud dextrose agar or potato dextrose agar to determine the viability and purity. The stability of the dermatophytes was confirmed using urease test medium, *Trichophyton* agar test and morphological examination. The stability of yeasts was evaluated by microscopic morphology and was validated by determining the antifungal susceptibilities of random samples of yeasts (n=120). Additionally, 365 strains (dermatophytes, n=115; yeasts, n=250) were further characterised by "Matrix-assisted laser desorption ionization-time of flight mass spectrometry" (MALDI-TOF-MS).

**Results:** After 12 years of preservation, survival rates with three different preservation techniques, namely in water, under mineral oil and by freezing, were assessed as 94.7%, 82.0% and 97.4%, respectively. Viability was found generally unrelated with the duration of storage. More stable and consistent growth was achieved after storage in water and freezing compared to under mineral oil preservation (Table 1).

**Conclusions:** Our results show that the procedure for maintaining fungal cultures in water is a simple and inexpensive method which together with freezing method can be reliably used for long-term preservation of most fungal isolates.

**Table 1.** Viability of isolates retrieved from storage in water, under mineral oil and cryopreservation for 1 to 12 years (no viable isolates/no tested)

Species	Water-stored	Mineral oil-stored	Cryopreservation
<b>Yeast*</b>			
<i>Candida albicans</i>	239/239	235/239	239/239
<i>Candida dubliniensis</i>	2/4	1/4	3/4
<i>Candida glabrata</i>	78/79	67/79	78/79
<i>Candida guilliermondii</i>	5/5	3/5	5/5
<i>Candida kefyr</i>	28/28	25/28	28/28
<i>Candida krusei</i>	27/27	21/27	27/27
<i>Candida lipolytica</i>	0/1	0/1	1/1
<i>Candida lusitaniae</i>	16/16	8/16	16/16
<i>Candida parapsilosis</i>	62/63	47/63	62/63
<i>Candida pelliculosa</i>	11/11	8/11	11/11
<i>Candida tropicalis</i>	70/71	68/71	71/71
<i>Candida zeylanoides</i>	3/3	2/3	3/3
<i>Cryptococcus neoformans</i>	3/3	2/3	3/3
<i>Cryptococcuszbekistanensis</i>	1/1	1/1	1/1
<i>Geotrichum candidum</i>	1/1	1/1	1/1
<i>Blastoschizomyces capitatus</i>	1/1	0/1	1/1
<i>Rhodotorula mucilaginosa</i>	3/3	3/3	3/3
<i>Saccharomyces cerevisiae</i>	1/1	0/1	1/1
<i>Trichosporon asahii</i>	3/3	2/3	3/3
<i>Trichosporon cutaneum</i>	6/6	5/6	6/6
<i>Trichosporon mucoides</i>	1/1	1/1	1/1
<b>Total yeast* (%)</b>	<b>561/567 (98.9)</b>	<b>500/567 (88.2)</b>	<b>564/567 (99.5)</b>
<b>Molds</b>			
<b>Moniliaceous molds</b>			
<i>Aspergillus clavatus</i>	2/2	1/2	2/2
<i>Aspergillus flavus</i>	1/1	1/1	1/1
<i>Aspergillus fumigatus</i>	11/14	10/14	14/14
<i>Aspergillus nidulans</i>	1/1	1/1	1/1
<i>Aspergillus niger</i>	5/6	6/6	6/6
<i>Fusarium</i> spp.	1/2	2/2	2/2
<i>Mucor</i> spp.	2/2	1/2	1/2
<i>Rhizopus</i> spp.	1/2	0/2	2/2
<i>Scopulariopsis brevicaulis</i>	2/3	1/3	3/3
<b>Dematiaceous molds</b>			
<i>Alternaria</i> spp.	2/2	1/2	1/2
<i>Curvularia</i> spp.	1/1	0/1	0/1
<i>Fonsecaea pedrosoi</i>	1/1	1/1	1/1
<b>Dermatophytes</b>			
<i>Epidermophyton floccosum</i>	2/6	1/6	4/6
<i>Microsporum audouinii</i>	0/1	0/1	1/1
<i>Microsporum canis</i>	16/19	10/19	19/19
<i>Microsporum gypseum</i> complex	0/1	0/1	1/1
<i>Trichophyton interdigitale</i>	144/151	133/151	146/151
<i>Trichophyton rubrum</i>	321/350	263/350	334/350
<i>Trichophyton tonsurans</i>	48/51	40/51	50/51
<i>Trichophyton violaceum</i>	1/3	0/3	2/3
<b>Total molds (%)</b>	<b>562/619 (90.8)</b>	<b>472/619 (76.3)</b>	<b>591/619 (95.5)</b>
<b>General survival rate (%)</b>	<b>1123/1186 (94.7)</b>	<b>972/1186 (82.0)</b>	<b>1155/1186 (97.4)</b>

\*Yeast and yeast like organism