UK NEQAS *for Microbiology*

Improving the stability of microbiology EQA specimens:

Fungal spore suspensions

Overview

Part 1 - Mycology

- Fungi / specimens distributed
- New specimen format development
- Results from pilot distribution
- Experience to date

Part 2 - Bacteriology

- EQA specimen design
- Stability results – lyophilised bacteriology specimens
- Open discussion

UK NEQAS Mycology Schemes

- Mycology Identification
  - Identification of filamentous fungi and yeasts, including dermatophytes and fungi associated with infection in the immuno-compromised
  - 4 specimens distributed 3 times per year
- Antifungal susceptibility
  - Identification and assessment of antifungal susceptibility testing including: Amphotericin B, Fluconazole, Flucytosine, Itraconazole, Voriconazole and Caspofungin
  - 2 specimens distributed 3 times per year

Core	Advanced	Genus only
Aspergillus flavus complex	*Myocladus corymbifera*	*Acremonium* species.
A. fumigatus complex	*Aspergillus candidus*	*Alternaria* sp.
A. niger	*A. glaucus*	*Bioplaris* spp.
A. terreus	*A. nidulans*	*Cladosporium* spp.
Candida albicans	*A. versicolor*	*Curvularia lunata*
C. dubliniensis	*Blastoschizomyces capitatus*	*Exophiala* spp.
C. glabrata	*Geotrichum candidum*	*Geotrichum* spp.
C. kefyr	*Candida guilliermondii*	*Fusarium* spp.
C. krusei	*C. lipolytica*	*Mucor* spp.
C. parapsilosis	*C. lusitaniae*	*Penicillium* spp.
C. tropicalis	*Chrysosporium keratinophilum*	*Phoma* spp.
Cryptococcus neoformans	*Cunninghamella bertholletiae*	
Epidermophyton floccosum	*Microsporum audouinii*	
Microsporum canis	*M. persicolor*	
M. gypseum	*P. lilacinus*	
P. variotii	*Phialophora richardsiae*	
Saccharomyces cerevisiae	*Rhizomucor pusillus*	
Scopulariopsis brevicaulis	*Rhizopus arrhizus*	
Trichophyton interdigitale	*R. microsporus*	
T. mentagrophytes	*Scedosporium apiospermum*	
T. rubrum	*S. prolificans*	
T. tonsurans	*Neoscytalidium dimidiatum*	
	N. hyalinum	
	Sporothrix schenckii	
	Trichophyton erinacei	
	T. terrestre	
	T. verrucosum	
	T. violaceum	
	Trichosporon beigelii	
	T.mucoides	

Participant performance (% correct) – mycology EQA non-dermatophyte fungi

Non-dermatophyte fungus	Correct results	Genus	Not identified
Scytalidium dimidiatum	87	4	9
Cunninghamella bertholletiae	84	5	11
Trichosporon mucoides	78	12	10
Aspergillus fumigatus group	96	3	1
Trichophyton soudanense	38	58	4
Cladosporium cladosporioides	15	72	13
Aspergillus candidus	82	10	8

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Participant performance (% correct) mycology EQA dermatophyte fungi

Dermatophyte fungus	Correct	Genus only	Not identified
Trichophyton interdigitale	66	32	2
Epidermophyton floccosum	86	0	14
Trichophyton soudanense	38	58	4
Microsporum fulvum	11	84	5
Trichophyton rubrum	74	24	2

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Participant performance (% correct) mycology EQA antifungal susceptibility

Organism	Amphotericin B	Fluconazole	Flucytosine	Itraconazole	Voriconazole
C. parapsilosis	91	93	94	74	87
C. tropicalis	90	93	96	30	91
C. krusei	90	61	23	44	100
R. mucilaginosa	95	100	97	91	22
C. neoformans	100	86	44	89	100
C. albicans	93	98	98	98	96
Overall (%) concordance by agent	**93**	**89**	**75**	**71**	**83**

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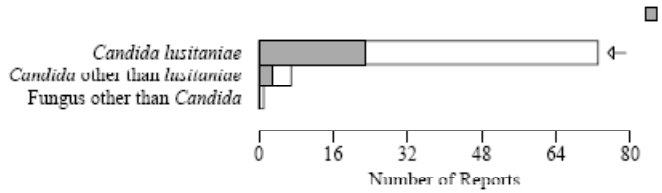
Participant performance – mycology EQA antifungal susceptibility

pathogen - specimen 8863
Intended result *Candida lusitaniae*

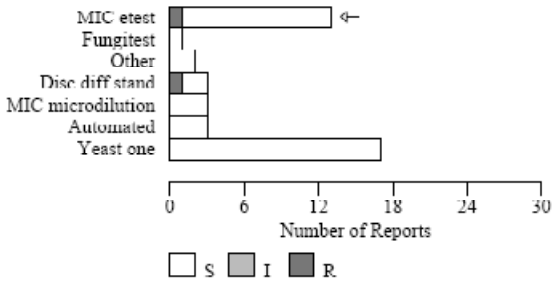
Blood culture isolate. The identity and antifungal susceptibility of the isolate was queried. The specimen contained *Candida lusitaniae*



Incorrect species:
C. famata 0(1)
C. guilliermondii 1(2)
C. parahaemolyticus 0(1)
C. sake 0(1)
C. tropicalis 2(2)

Incorrect genus:
Cryptococcus neoformans 0(1)

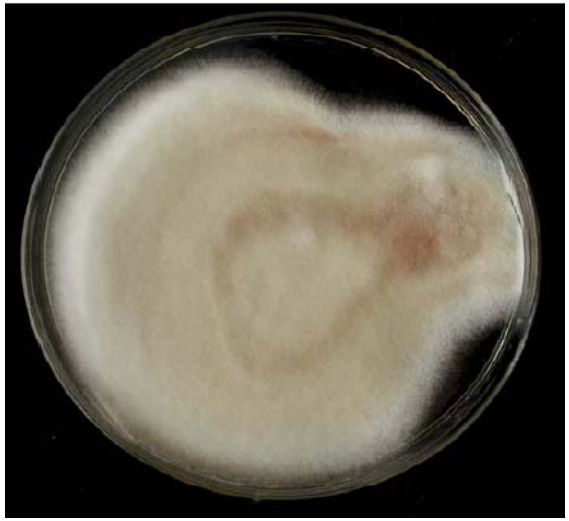
amphotericin B - specimen 8863
Intended result susceptible
Your Report : susceptible



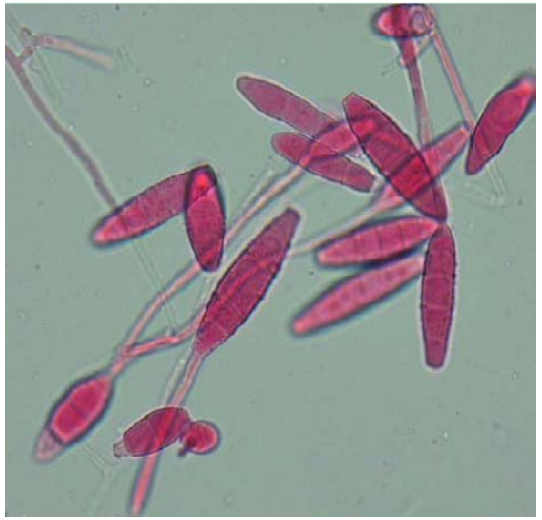
n	MIC in mg/L
14	0.25
10	0.125
9	0.5
5	0.38
5	0.064
4	0.06
4	1
3	0.03
3	0.032
3	0.047
2	0.016
2	≤0.25
1	0.002
1	.19
1	≤=0.25
1	0.094
1	0.19
1	0.512

	S	I	R	% concordance
score	2	1	0	
All	74	0	2	97.4
UK	23	0	0	100.0

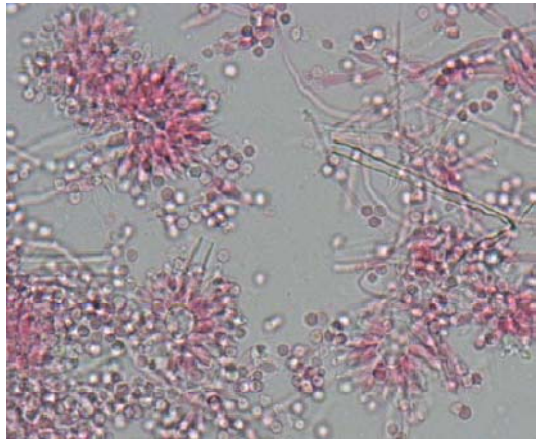
Developing new specimens



M. gypseum



A. versicolor



Review of specimen options

- Lyophilisation
- Under mineral oil
- Drying on silica gel
- Soil storage
- Spore suspensions in water

Evaluation of the viability of pathogenic filamentous fungi after prolonged storage in sterile water & review of recent published studies on storage methods

Borman *et al*. Mycopathologia (16) June 2006

- Evaluation of the survival and potential morphological alterations of 45 species of pathogenic filamentous fungi that had been stored in sterile water following Castellani's method in the National Collection of Pathogenic Fungi (NCPF), UK
- Storage duration varied from 2 months to over 21 years
- Ninety percent of stored organisms were shown to be viable
- Viability was largely independent of the duration of storage, but did apparently vary to some degree in an organism-specific manner
- This was especially marked for several isolates of dermatophytes, where storage resulted in loss of recognisable colonial features, and overproduction of sterile mycelium with aberrant or no conidia
- These findings suggest that while Castellani's method remains an easy and inexpensive method for long-term preservation of most fungi, water storage should be supplemented by a second storage method to increase the chances of retaining both viability and morphological stability over long periods

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Pilot - Dermatophytes selected

Trichophyton rubrum

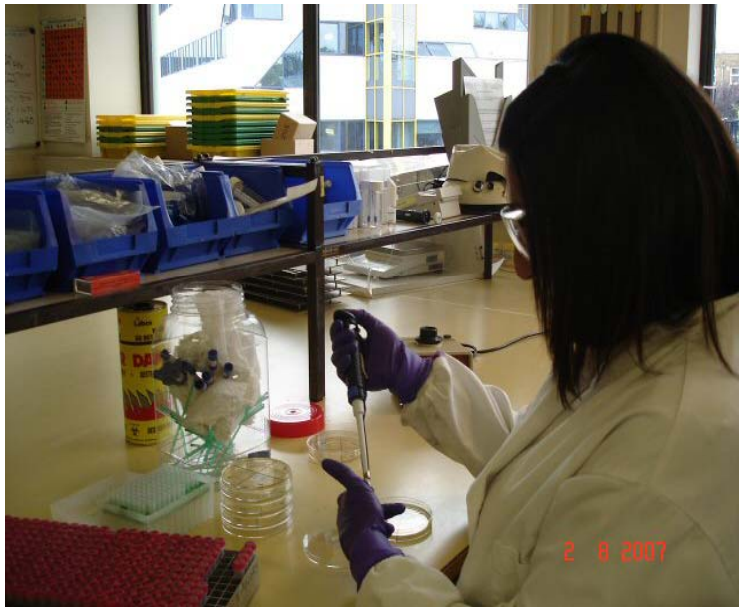
Trichophyton tonsurans

Pilot - Dermatophytes selected

Trichopyton interdigitale

Epidermophyton floccosum

Preparation of spore suspensions



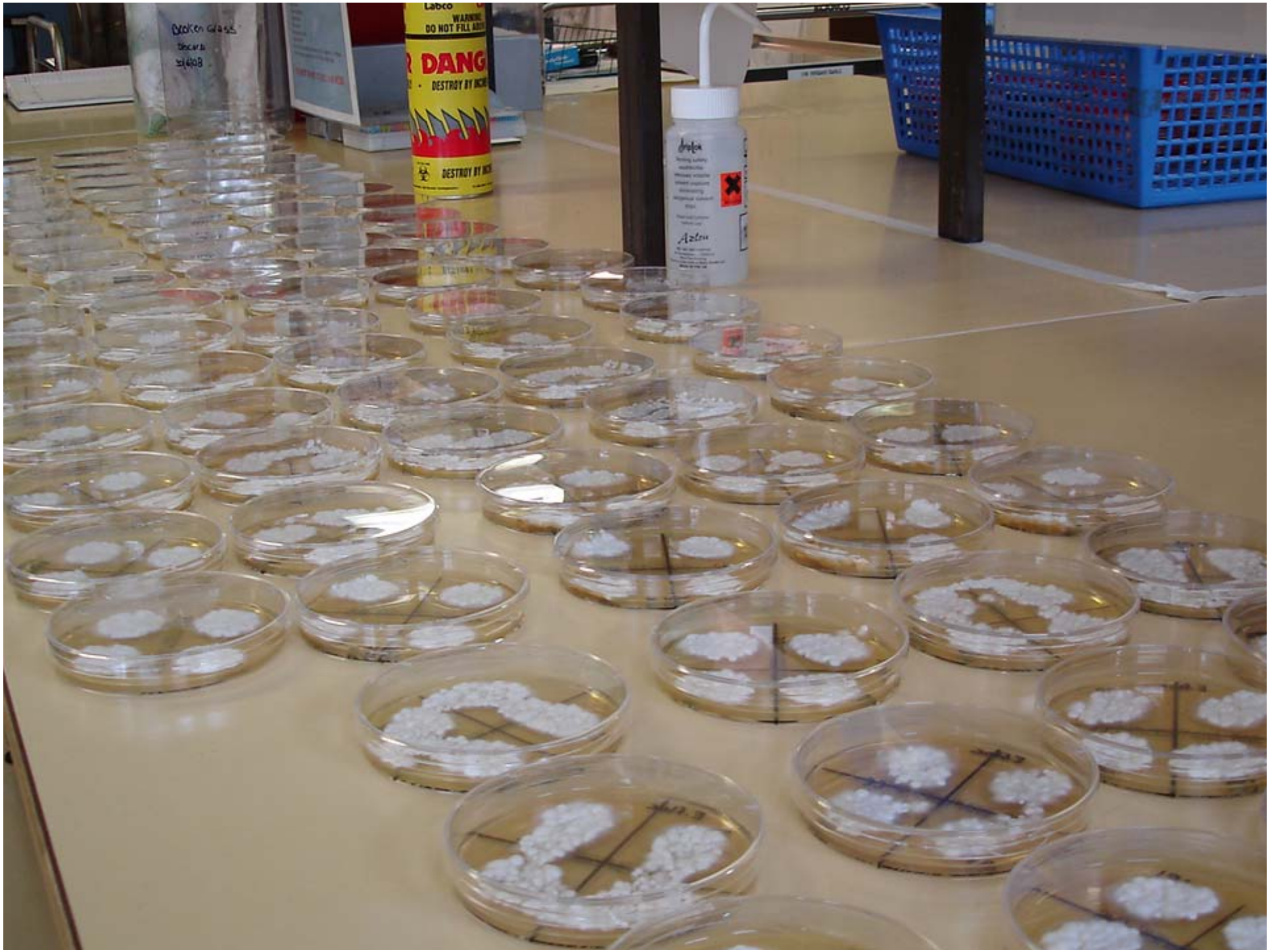
- **Flood a single colony on the SABM plate with 5mL distilled water**
- **Scrape the surface of the colony to release the spores**
- **Take up the water from the plate, now containing the released spores and transfer to a 5mL bijou**
- **Vortex the spore suspension for one minute to break any mycelium fragments**
- **Inoculate 100µL of the suspension onto five SABM plates. Spread the inoculum around the surface of the agar plate using a plastic spreader**
- **Incubate the SABM plates for 14 days.**

Bulk preparation

- After incubation confirm the purity of the organism
- Perform a spore count using a counting chamber The numbers of spores/mycelial fragments found should concur by species with the guidelines provided by the Mycology Reference Laboratory on spore forming filamentous fungi
- Prepare a bulk specimen

Spore production characteristics of some common fungi

Fungus	Heavily sporing	Moderately sporing	Poorly sporing
Dermatophytes	*Microsporum gypseum*	*Epidermophyton floccusom*	*Microsporum canis*
	Microsporum fulvum	*Trichophyton soudanense*	*Trichophyton rubrum*
Non-dermatophytes	*Chrysosporium keratinophilum*	*Acremonium* spp	
		Fusarium spp	
Ascomycetes	*Aspergillus fumigatus complex*	*Acremonium* spp	
	Aspergillus flavus	*Alternaria* sp	
	Paeciliomyces variotti	*Phialophora* spp	
	Paeciliomyces lilacinus	*Scedosporium apiospermum*	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Pilot specimens - results

Trichophyton rubrum, T. interdigitale T. tonsurans, Microsporum canis, M audounii

- Every vial in the pilot batch (n=400) produced viable fungal colonies demonstrating typical morphology

Epidermophyton floccosum

- First batch 50% failed to grow; second batch revealed 100% viability with typical morphology

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Bacteriology specimens – homogenity and stability

EQALM Microbiology Working Group Discussion

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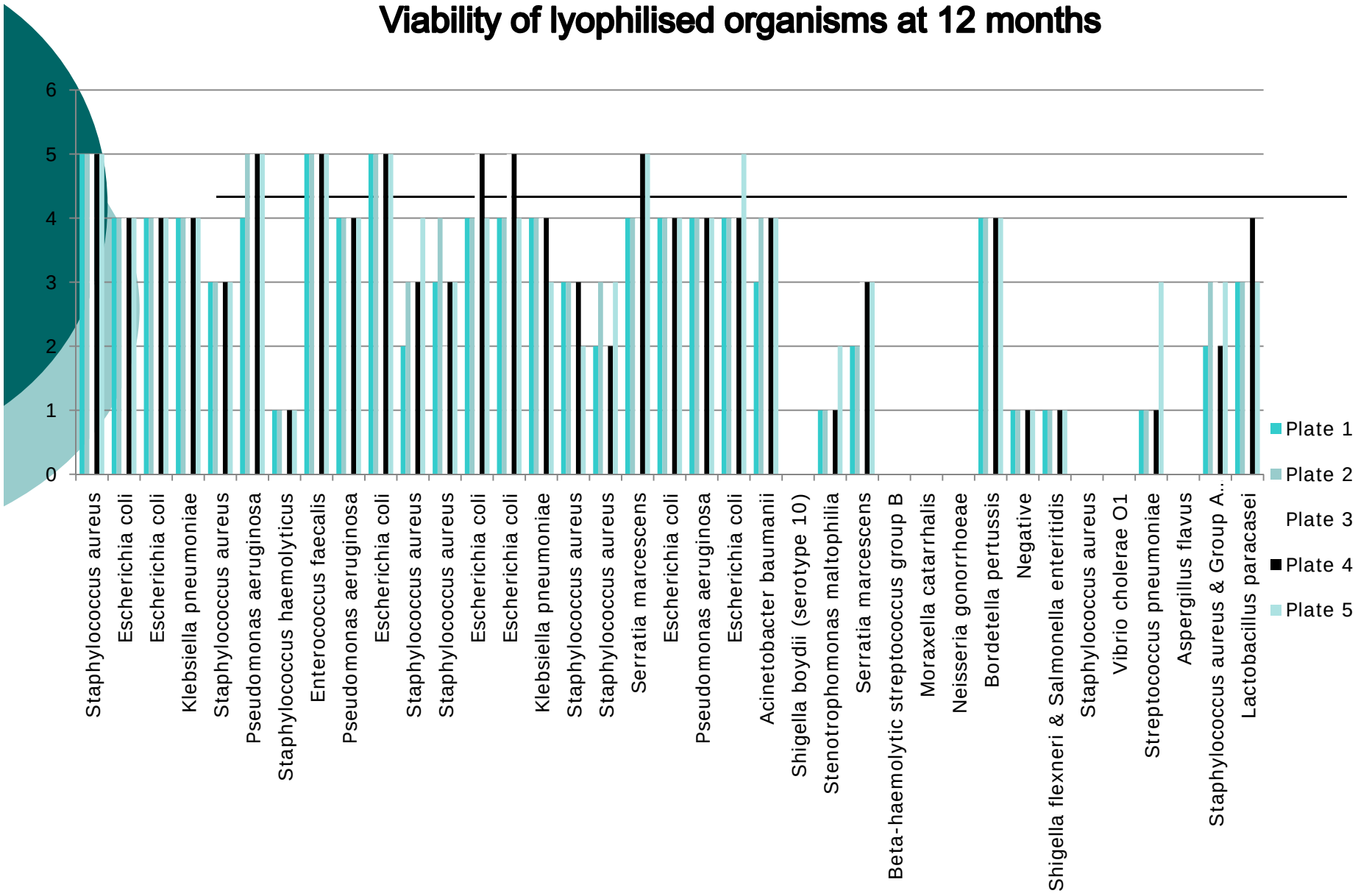
What are the design criteria for EQA schemes?

- Clinically relevant
- Homogeneous specimens
- No matrix effect
- Stable specimens
- Adequately characterised
- Measurement and assessment of performance is possible

Viability of lyophilised organisms at 12 months



12th October 2010

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Viability of lyophilised organisms at 24 months