Introduction

In recent years the frequency of fungal infections is on the rise throughout the world. The exact data on the burden of fungal infections in India is not clear. One reason for this may be the presence of very few good diagnostic mycology laboratories in our country. Even the existing laboratories are not accredited. To have quality report from any laboratory, accreditation is absolutely essential. National autonomous body - NABL is taking an effort in this direction. For accreditation external quality assurance program (EQAS) is an important component. In India, though Indian Association of Medical Microbiologists (IAMM) is conducting an EQAS in the field of bacteriology, there is no such effort in the field of mycology. The Society for Indian Human and Animal Mycologists (SIHAM) has asked us (Mycology Division, at Department of Medical Microbiology, PGIMER, Chandigarh) to initiate the EQAS in medical mycology for our country. It would not be out of context to mention that our laboratory has been approved by Indian Council of Medical Research as ‘Center for Advanced Research in Medical Mycology’, and WHO has approved this center as ‘WHO Collaborating Laboratory for Reference and Research for Fungi of Medical Importance’.

Objectives

1. To assess the laboratories for its capabilities in diagnostic mycology in the following areas:

2. Processing of specimens for the diagnosis of fungal infection, and identification of pathogenic fungi (yeasts or moulds) isolated and interpretation of results obtained from the clinical specimen.

3. Serological tests performed for the diagnosis of fungal infections (by in –house methods or with commercial kits)

4. Antifungal susceptibility testing of the yeast and moulds isolated from the clinical specimen.

Participants: Any clinical diagnostic laboratory involved in the processing of the clinical specimens for the diagnosis of the fungal infections and performing the antifungal susceptibility testing is eligible to participate in this program.
**Frequency of quality assessment:** Each of the participating laboratories will be provided with a kit containing materials as specified below with specific instruction once every six months (June and December). The participating laboratory has to report the results within 60 days of delivery. Assessment will be made on the basis of responses of the participating laboratory.

**Kit Materials:** During the quality assessment each laboratory will be provided with the following materials to conduct testing or processing

1. A set of five fungal cultures (yeasts and moulds) along with the clinical histories of the patients from whom the fungi were isolated (The participants are expected to identify the fungi to species level and provide interpretation of its significance)

2. One serum sample along with the clinical details to perform the appropriate serological test/s.

3. (Will be started in the later years)

4. Antifungal susceptibility of one/two culture/s as specified. (The participants are expected to perform susceptibility by the method/s that is/are routinely followed in the laboratory for reporting.)

5. Standard quality control strains [as per Clinical Laboratory Standards Institute (CLSI) protocol] to perform the antifungal susceptibility testing (Standard strains will be provided only once. The participating laboratory is supposed to maintain the quality control strains in their laboratory for future use)

6. Simulated clinical samples for diagnosis of fungal disease (Will be started in the later years)

**Participation fee:** The participating laboratory has to pay Rs 2000/- annually in the form of Demand draft drawn in the name of the ‘Director, PGIMER, Chandigarh’ and that would cover all charges including material, postages etc. The draft should be posted to Dr Arunaloke Chakrabarti, Prof and Head Department of Medical Microbiology PGIMER, Chandigarh-160012.

**Dispatch of next batch of sample:** January and July

**Last date of registration by the participant:** Two month before dispatch of sample
Instructions to participants:

Specimen provided

Five sets of freeze dried/active cultures for the identification.

Two vials of standard strains to use as controls for the antifungal susceptibility testing.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample name / code No.</th>
<th>Clinical history</th>
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<tr>
<td></td>
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<td>Age/Sex</td>
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<tr>
<td>1</td>
<td>EQMM 1</td>
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<td><em>Candida parapsilosis</em></td>
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</table>

Precautions

The specimen provided may contain dangerous pathogens and should be handled carefully in the biological safety cabinet (level II) and only by the persons trained in handling the pathogenic microorganisms. The laboratory should follow standard biosafety norms.

After use, the laboratory should dispose the specimens according to the safe disposable methods used for pathogenic microorganisms like autoclaving.

Storage

If storage is required, the samples may be stored at 2-8°C. However fungi like zygomycetes may die at such temperatures. Therefore it would be preferable to process the samples immediately after receipt.

Replacement

If the container is broken, contaminated, damaged, opened and leakage is noticed, contact us immediately for the dispatch of the fresh samples. No fresh sample will be provided after one month of first date of delivery.

The safe delivery of the specimen in good condition should be acknowledged immediately after receiving.
Instruction for processing of specimen

The entire specimen should be handled and processed in the laboratory by the persons who routinely perform this kind of testing and in similar manner as routine samples are tested.

The samples provided are freeze-dried or active cultures on Sabouraud dextrose agar/malt extract agar slants/plates. Revive the cultures accordingly to the method mentioned below

Procedure to revive the active cultures

Subculture the organisms, within a day or two after receiving, onto Sabouraud dextrose agar/malt extract agar slants/plates and incubate at 30°C and inspect every day. Proceed for further testing after obtaining good growth (Note: time taken to obtain good growth may vary from 2 days to 15 days depending on the growth rate of the given culture). Test should be carried out as per the methods commonly employed in your laboratory.

Procedure to revive Freeze-dried cultures

Care should be taken while opening the ampoule as the contents are in vacuum.

Mark the ampoule around the middle of the ampoule using the sharp file.

Wipe the ampoule around the marking using alcohol

Wrap the ampoule with cotton wool and break the ampoule at the marked area.

Gently open the pointed tip of the ampoule and remove the cotton plug slowly and carefully add 0.3-0.4 ml of sterile distilled water and let it stand for 20 minutes to make a suspension of the culture. Avoid frothing or creating aerosols.

Streak a few drops of the suspension onto the Sabouraud dextrose agar in a petriplate. Rest of the suspension may be transferred on to the Sabouraud dextrose broth in a test tube.

Incubate the cultures at 30°C preferably in a BOD incubator till there is good growth (the growth may take even 15 days for some isolates)

All the remains of the original ampoule should be treated as infected and autoclaved before discarding.
**Antifungal susceptibility testing**

Specimen coded as + AFST is meant for carrying out antifungal susceptibility testing additionally. If any laboratory does not perform antifungal testing, it should be mentioned clearly in the report.

**Procedure to carry out antifungal susceptibility testing**

1. Perform disk diffusion or MIC determination for the isolate as per the procedure followed routinely in your laboratory.

2. For disk diffusion technique (follow CLSI protocol, document M 44A) mention the zone inhibition diameter or as susceptible (S), intermediate (I), resistant (R) or susceptible dose dependent (S-DD) as per CLSI recommendation (Refer Table given at the end of the document).

3. If any other method of disk diffusion technique is followed indicate the method followed with the reference and write the zone inhibition diameter and interpretation as S, I, R, and S-DD.

4. If Minimum inhibitory concentration is determined, indicate the exact MIC as =, ≤, > for each antifungal agent tested.

**Reporting instruction**

All completed reports should be sent by post to the undersigned within 60 days from the date of dispatch of the kit. Any delay in reporting should be intimated before the deadline to the undersigned. No report will be entertained, if received after 75 days of dispatched date of the kit.

The participating laboratory should retain a copy of the completed report.

**Confidentiality**

The report sent to us will be a confidential document and will not be disclosed to any other source. Only pooled result of all laboratories may be disclosed. The performance of each laboratory will be conveyed to the in-charge of the laboratory only.

**Scoring of performance**

The performance of the each laboratory will be assessed and graded as

- Excellent (>80%)
- Good (80-60%)
- Satisfactory (60-50%)
- Below average (<50%)
Result form

Name:                                   Date:

Name of the laboratory:

Name of the Institute

City

Contact details: E mail:

Telephone:

Fax:

Mold or Yeast identification

<table>
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<tr>
<th>Sl no</th>
<th>Sample No</th>
<th>Identification</th>
<th>Interpretation</th>
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Antifungal susceptibility testing

Disk diffusion method for Yeast

Test method used

☐ As per CLSI Document M 44 protocol

☐ Other method Specify the method (with Reference)
<table>
<thead>
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<th>Antifungal agent</th>
<th>Conc. of disk</th>
<th>Inhibition zone</th>
<th>Interpretation</th>
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**Minimum inhibitory concentration for yeasts / moulds**

Test method used

- [ ] Broth macrodilution
- [ ] Broth microdilution
- [ ] E – Test
- [ ] Yeast one colorimetric microdilution
- [ ] Others Specify

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<th>MIC in µl/ml</th>
<th>Interpretation</th>
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Yeast identification method used

☐ Conventional method   ☐ API   ☐ IDS rapid system

☐ Minitek   ☐ Vitek

☐ Others (Specify)

Declaration

We, the undersigned declare that all the samples provided were handled as per the methods we handle the routine samples and as per bio- safety norms.

For any case of laboratory acquired fungal infection while handling the material/fungal strain in our laboratory, we shall be responsible. PGIMER, Chandigarh will not be responsible for such laboratory acquired fungal infection.

Lab In-Charge   (Signature)____________________________

(Name)________________________________

Testing Personnel 1. (Signature)_________________________

(Name)________________________________

Testing Personnel 2. (Signature)_________________________

(Name)________________________________