MMTN
MEDICAL MYCOLOGY TRAINING NETWORK

10 common mistakes in laboratory/basic mycology

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ASIA FUNGAL WORKING GROUP
an ISHAM working group

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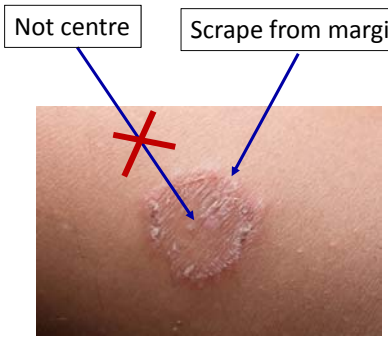
Errors in any laboratory investigation

- Pre-analytical errors
  - Sample collection
  - Transport of sample
- Analytical errors
  - Microscopy
  - Culture
  - Antifungal susceptibility testing
  - Serodiagnosis, molecular techniques, TDM
- Post-analytical errors
  - Interpretation of test results
  - Real-time communication

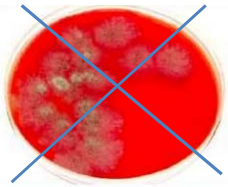
Mistake 1: improper collection and processing

- Sample collection: for efficient laboratory diagnosis sample collection is most important.
- Superficial fungal infections: dermatophytes / mycetoma– collect from active edge, collect granules



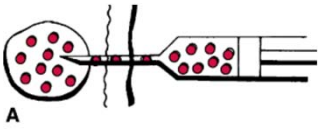
- **Hunt for granules!**
- In serosanguinous sinus discharge
- Under surface of the **Scab** covering sinus
- Adherent to the under surface of **Dressing**
- In the **Biopsy tissue / container**
- purulent material in saline, rinse repeatedly, allow to settle - Search for granules ! (black, pale yellow, white, pink)

- Keratitis- collect and inoculate at bedside

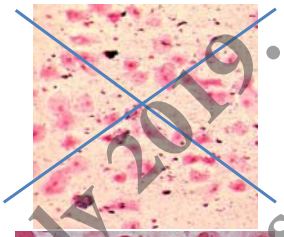


- Collect tissues in saline & not in formalin for fungal isolation
- FNAC's collect good amount for KOH & inoculate at bed side
- CSF –
  - Always culture 10-30ml of CSF
  - May collect sample even after start of therapy as fungi can be isolated till 3rd day of therapy

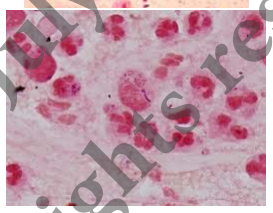
Proper sample collection



Deep abscess, CSF



Sputum, BAL, urine



Mistake 2: Transport condition

Specimen	Transport condition
Sputum	Sterile Screw capped container
Bronchoscopy Fluid	Sterile Screw capped container
CSF	If delay anticipated, specimen should be left at room temperature
Urine	If delay beyond 2hrs is anticipated, refrigerate at 4°C
Blood	Biphasic agar broth bottles designed especially for fungal cultures Or in automated culture bottle
Tissue biopsy	the specimen should not be frozen or allowed to dehydrate prior to culture

Swab is not a good specimen; try to avoid it

Criteria for specimen rejections

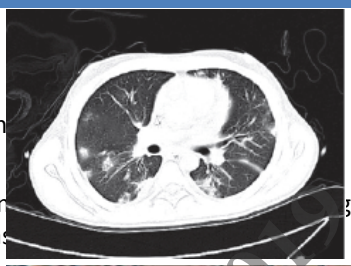
1. Absence of patient identification on the container or discrepancy between the information
2. Sputum specimen with >25 squamous epithelial cells as per low power field
3. A dried out swab or if the material collected is insufficient
4. The sample submitted in an improper container
5. The 24hr sputum or urine specimen for fungal culture is received

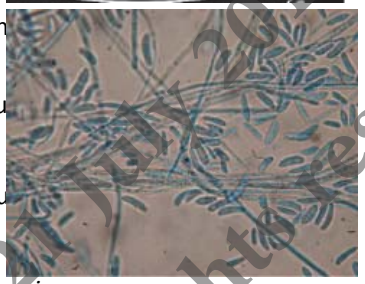
Mistake 3: awareness & suspect

- 20y/male with ALL, precursor B cell type rece y - partial remission in the induction course
- Could not finish the consolidation course due to prolonged neutropenia
- Later, due to relapse received modified chemotherapy with FLAG-IDA (idarubicin, fludarabine, cytarabine and G-CSF)
- During the second course of chemotherapy, the patient received 400 mg oral fluconazole daily as an antifungal prophylaxis.
- Febrile neutropenia two days later.
- Antibiotic treatment with imipenem (500 mg, i.v., every 6 h), vancomycin (1,000 mg, i.v., every 12 h) & micafungin (100 mg, i.v., daily)
- After four days, multiple skin lesions, starting from the legs and spreading to the face and upper extremities were identified. The lesions exhibited necrotic centers surrounded by spreading erythema
- Blood culture grew mycelial fungi – considered contamination - discarded

Case

- Lesions worsened & the antifungal treatmen
  caspofungin (100 mg, i.v., daily)
- Biopsy of the skin lesions showed the preser
  the vascular space - diagnosed as angioinva
- Voriconazole (200 mg, i.v., every 12 h) was in
  caspofungin with poor response.
- HRCT scan of the lungs found multiple specu
  densities in the lungs





- After 1 weeks, the sputum and skin tissue cu
- We were contacted after another week
- Morphological identification – suspected *Fusarium oxysporoum*
- Confirmed identification of *Fusarium oxysporoum* by both MALDI & sequencing
- **Patient succumbed to the disease before modification of antifungal**

Lesson learnt from the case

- **All septate hyphae are not *Aspergillus***
- **All mycelial fungi on blood culture are not lab contaminants – it can be *Fusarium* & *Scedosoporium***
- **Need of interaction with clinicians**
  - Any skin lesion
  - Antibiotic/antifungal use
  - Immunosuppression etc.

Dermatologic manifestation of invasive fungal infections

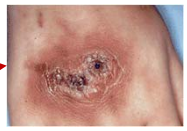
- Primary skin infection – tinea infection
- Primary skin infection by opportunist fungi – localized/invade
- Secondary skin infection from dissemination

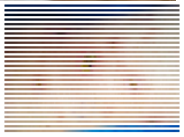
Aspergillosis at the cannula site

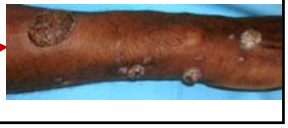
Skin lesion in disseminated fusariosis

Cutaneous presentation of IFI

Aspergillosis	Erythematous papules & plaques, necrotic or hemorrhagic echar, pustule, nodule, ulcer, cellulitis
Cryptococcosis	Abscess, pustule, papule, plaques, purpura, ulcer, cellulitis, sinus tract
Mucormycosis	Necrotic eschar with surrounding erythema, cellulitis with necrosis, macule, nodule, plaque
Fusariosis	Painful papule, violaceous nodule with ulcerated centre covered with eschar, necortizing lesions
Phaeo-hypomycosis	Nodule, cysts, cellulitis, plaques, eschar, ulcer

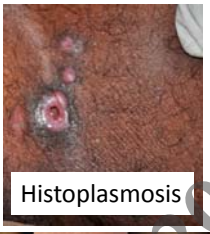






Can you distinguish skin lesions in AIDS patients?

Emergomycosis due to *Er. africanus*

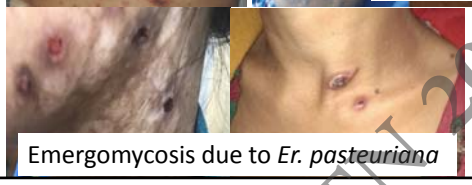


Histoplasmosis

Talaromycosis

Sporotrichosis

Histoplasmosis

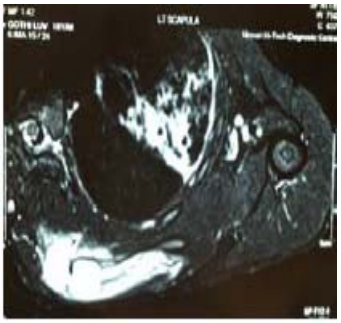


Emergomycosis due to *Er. pasteuriana*

George AA *et al*. Am J Trop Med Hyg 2015; 93: 1125
Schwartz IS, *et al*. OFID, 2017
Malik R, *et al.* Mycoses 2016; 59: 127
Pei, *et al.* Am J Trop Med Hyg 2008

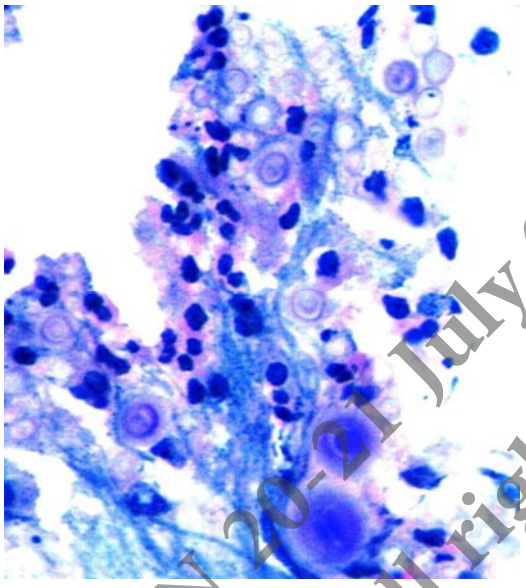
Case

MRI shoulder



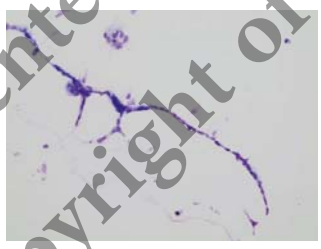
MRI ankle

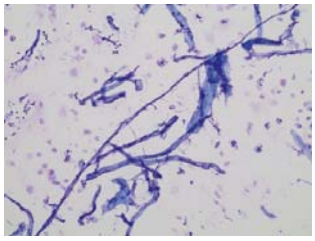
ZN stain



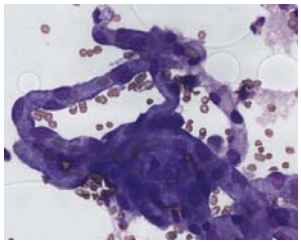
Mistake 4: expertise

Artefacts mimicking like fungal hyphae



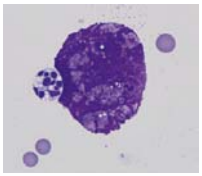


Hyphae/pseudohyphae?

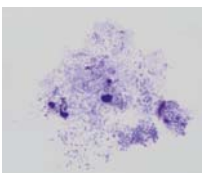


Mucormycosis?
Capillary vessel with water droplets

Contamination of stains

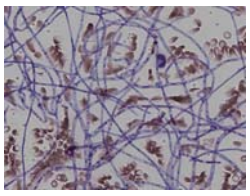


Histo?



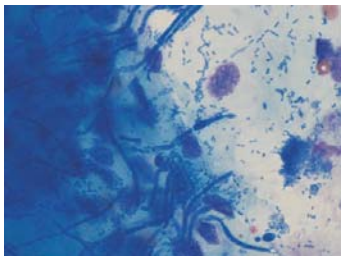
Stain precipitate

Bacterial contamination

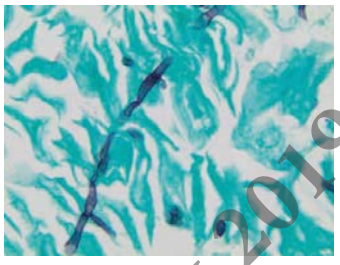


Fungal contamination of stain

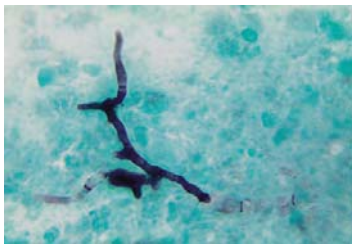
Microscopy challenges



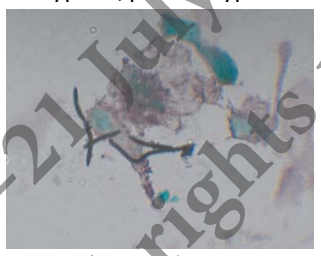
Hyphae/pseudohyphae?



Hyphae/pseudohyphae?



Aspergillus or Mucor?



Aspergillus? Bacterial rods

Mistake 5: Analytical - Blood Culture

Conventional methods

- Broth culture / biphasic media

  - blood is inoculated into a broth or biphasic media

  - subculture on solid media after 1,2,3,7 days of incubation/ examine macroscopically for evidence of growth after similar incubation, make a smear and subculture on solid media if smear is positive

  - incubate the solid media overnight

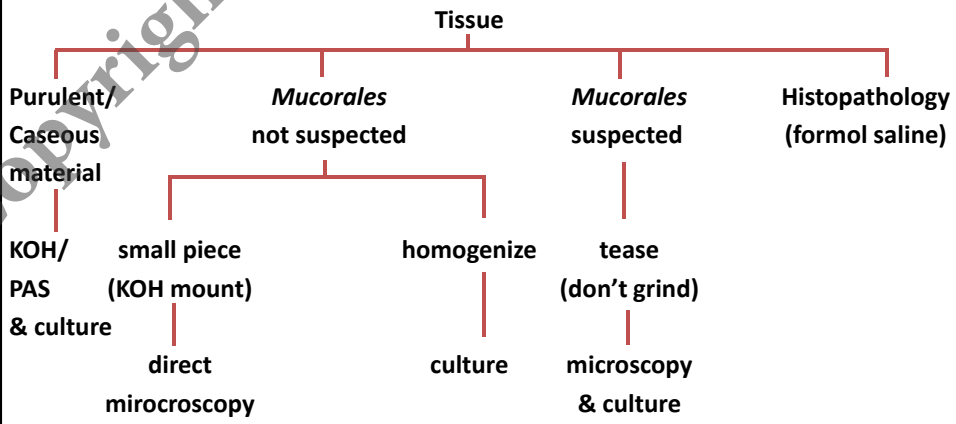
Blood Culture

Conventional methods

- Broth culture / biphasic media
  - blood is inoculated into a broth or biphasic media
  - subculture on solid media after 1,2,3,7 days of incubation/ examine macroscopically for evidence of growth after similar incubation, make a smear and subculture on solid media if smear is positive
  - incubate the solid media at least for **48 hours**
  - examine everyday macroscopically for yeast growth(initially it may be very tiny, pin point colony)

Tissue specimen

Isolation fails in >50% mucormycosis cases



Mistake 6: Identification

Over-dependence on commercial system

Method	Comment
API-20C	Identify as *Rhodotorula glutinis, Candida sake, Saccharomyces cerevisiae*
Vitek - 2	Identify as *Candida haemulonii, Candida famata* (updated database may able to identify)
BD Phoenix	Identify as *Candida haemulonii*
Microscan	Identify as *C. famata, C. guilliermondii, C. lusitaniae, C. parapsilosis*
MALDI	**Can identify *C. auris* after improvement of data base** Before improvement – we updated the data base on our own (Ghosh *et al.* Clin Microbiol Infect. 2015; 21: 372-378)
DNA sequencing	**D1-D2 domain** of large subunit can identify correctly

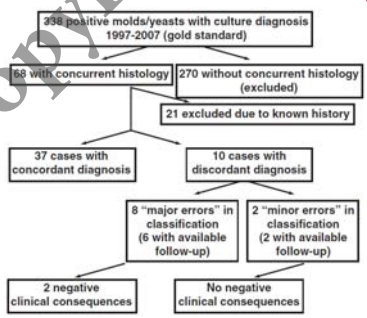
JCM Accepted Manuscript Posted Online 26 July 2017
J. Clin. Microbiol. doi:10.1128/JCM.00921-17

Identification – difference between H/P & microbiology

Am J Clin Pathol 2009;131:364-375

Challenges and Pitfalls of Morphologic Identification of Fungal Infections in Histologic and Cytologic Specimens

Ankur R. Sangoi, MD,[1] William M. Rogers, MD,[1] Teri A. Longacre, MD,[1] Jose G. Montoya, MD, Ellen Jo Baron,[2] PhD,[2] and Niaz Banaei, MD[1]



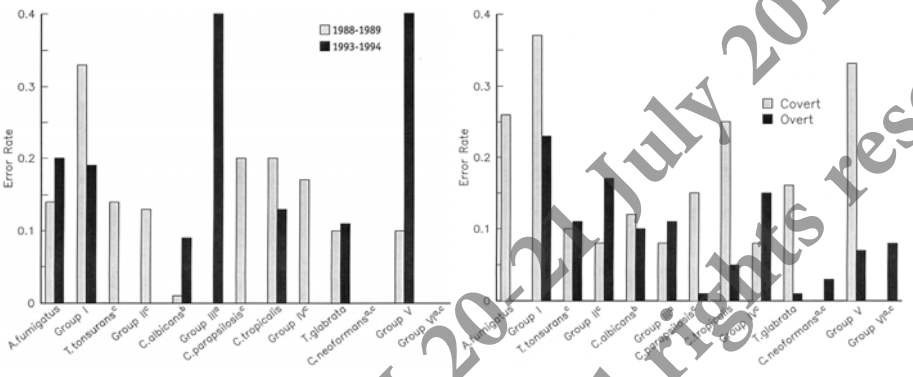
Surgical Pathology Diagnosis†	Surgical Pathology Comment	Special Stains	Culture Diagnosis	Defer‡	Treatment§
Consistent with Zygomycetes	NA	ND	*Rhizopus*	Yes	No
Consistent with *Rhizopus*‖	NA	ND	*Rhizopus*	Yes	Yes
Fungal organisms identified	***Aspergillus***	GMS	*Rhizopus*	Yes	Yes
Fungal elements present	**Suggestive of mucormycosis**	GMS	*Aspergillus niger*	Yes	Yes
Consistent with *Coccidioides*#	NA	GMS	*Aspergillus fumigatus*	No	No
Aspergillus	NA	PAS-D	*Fusarium*	No	Yes
Fungal hyphae	**Compatible with *Aspergillus***	ND	*Scedosporium apiospermum*	No	No
Consistent with *Aspergillus*	NA	GMS	*S apiospermum*	No	Yes
Compatible with *Cryptococcus*	NA	GMS	*Coccidioides immitis*	Yes	No
Yeast forms identified	**Resemble *Candida* spp**	GMS	*Histoplasma capsulatum*	Yes	No

Identification of fungi is a challenge even in best laboratories

JOURNAL OF CLINICAL MICROBIOLOGY, July 1999, p. 2297–2305

Evaluation of Mycology Laboratory Proficiency Testing

ANDREW A. REILLY,[1,2]* IRA F. SALKIN,[1,2] MICHAEL R. McGINNIS,[3] SALLY GROMADZKI, LESTER PASARELL,[3] MAGGI KEMNA,[1] NANCY HIGGINS,[1] AND MAX SALFINGER[1,4]



Mistake 7: Antifungal susceptibility testing

- Majority use vitek system
- **But dis-correlation with standard CLSI/EUCAST system - >10%**
- **Standard micro-broth dilution (CLSI/EUCAST)**
  - Trailing growth: Certain *Candida* spp. at fluconazole concentrations above MIC show reduced but persistent growth. In absence of sufficient experience, these isolates are labelled as drug resistant isolates. Better result at pH 5.5
  - Testing media :*Cryptococcus*; supplementation with glucose. If the pH varies due to any reason, it can hinder the growth of fungi resulting in misleading results.
  - Incubation times: In case of *Cryptococcus*, 72 hours is the recommended. But, in case of fluconazole, reading beyond 24 hrs can dramatically increase the chances of misreading isolates showing trailing growth as resistant. Similarly, in case of filamentous fungi, echinocandins to be read at 24 hours & azoles and polyenes at 48 hrs. Any deviation can lead to misleading results.
  - Drug panels for various fungi: Norm of laboratories is to prepare microtiter plates with drug and media beforehand. In such cases, drugs which show no activity against certain genus of fungi are also put up. This can confound the technician reporting the susceptibility results. For eg, testing echinocandins for *Cryptococcus* and *Rhizopus* spp., *fluconazole* for Aspergillus spp.
  - Mixed yeast cultures used for susceptibility testing: leading to incorrect interpretation of susceptibility results.

Mistake 8: Galactomannan & beta-D-glucan

- Collection of blood (avoid contamination)
  - Exclusive collection in vacutainer
  - Use excess skin disinfectant in swab to avoid friction of cotton with skin
  - Do not collect blood from line – dilution error
  - At least 5-6 days after dialysis to reduce glucan interfering substances
- Serum separation
  - Avoid haemolysis, sufficient time to clot, use calibrated centrifuge at optimum speed
  - Serum transfer in biosafety cabinet
- Test
  - Performance of test only in bio-safety cabinet
  - Plastic wares, tips etc. sterile & pyrogen free
  - Accurate pipetting

Mistake 9: Therapeutic drug monitoring

- Inappropriate medical indication
  - Need proper medical reasoning
- Inappropriate sample collection
  - Sample should not be collected from i.v. lines
  - Peak or trough sample
- Inappropriate method
  - Chromatography better method
- Inappropriate interpretation
  - Correlate with clinical parameters

Mistake 10: Interpretation - *Candida* in respiratory tract & in urine

-Shall I report?

-Any use of count of *Candida*?

- **IDSA guideline, 2016 - Recommendation**
  - ***Candida* from respiratory secretions - usually indicates colonization** & rarely requires treatment with antifungal therapy (strong recommendation; moderate-quality evidence)
  - ***Candida* in urine – Treatment with antifungal agents is NOT recommended** unless the patient belongs to a group at high risk for dissemination; high-risk patients include neutropenic patients, very low-birth-weight infants (<1500 g), & patients who will undergo urologic manipulation (strong recommendation; low-quality evidence).
  - **Neutropenic patients & very low–birth-weight infants should be treated** as recommended for candidaemia (strong recommendation; low-quality evidence).
  - **Patients undergoing urologic procedures should be treated** with oral fluconazole, 400 mg (6 mg/kg) daily, OR AmB deoxycholate, 0.3–0.6 mg/kg daily, for several days before and after the procedure (strong recommendation; low-quality evidence)
  - **No significance of counting *Candida* in respiratory tract or urine**

Summary

- Knowledge of pre-analytical, analytical and post-analytical parameters of any test is essential
- Training of both mycologists & technical staff essential
- Participation in EQAS program helps in identifying gaps & improved reporting
- Interaction with clinicians very important
- Call alert is essential
- Molecular techniques help in improving analytical skill
- Though automation is difficult in mycology laboratory, research should be directed for POCT