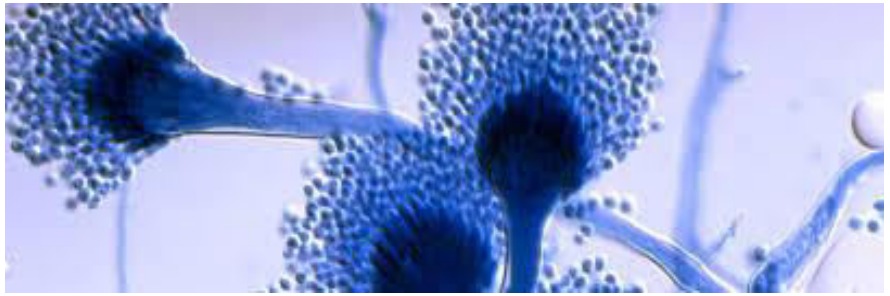
Processing of Specimens in Mycology Lab



Specimens in mycology lab include:

- Skin scraping
- Nail
- Hair
- Biopsy tissue
- Blood
- CSF
- Urine
- Body fluids
- Sputum
- Bronchial brush
- Abscesses
- Bone marrow
- Vaginal swap

Processing of clinical specimens:

1- Macroscopic examination

2- Microscopic examination

3- Culture

4- Biochemical reactions

5- Serology

Macroscopic examination:

- Proper collection of specimen (proper container and should be in plastic bag, no leaking, labeled)
- Good quantity of specimen
- Color of the specimen
- Consistency
- Presence of blood, mucus or pus

Direct microscopic examination (direct mount):

- 10% Potassium hydroxide (10% KOH) stain
- Periodic acid–Schiff (PAS) stain
- Giemsa stain
- Gram stain
- Ziehl–Neelsen (ZN) stain
- Hematoxylin and eosin (H & E) stain
- Silver stain(GMS stain)
- Negative stain (India ink stain, Nigrosin stain)

Collection and Processing of Specimen

Skin scraping

Collection:

- Clean the site with alcohol swap
- Then scrap the lesion by sterile glass slide
- Collect the specimen in sterile petri dish then take it to the lab for processing.

Skin scraping

1. **DM examination:** use 10% KOH or PAS stain
2. **Culture:** on SDA and SDA CC at 30°C
3. **Microscopic ex. from culture:** use LPCB
4. **API 20C** (if yeast)

Suspected fungi??

Hair Specimen

Collection:

- Take the infected hair shaft with its base
- The infected hair can be selected by using Wood's lamp (UV light)>> green flourcens with the infected hair
- Collect the specimen in sterile petri dish then take it to the lab for processing.

Hair Specimen

1. **Macroscopic ex.:** see if there's any nodules
2. **DM ex.:** use 10% KOH or PAS stain
3. **Culture:** on SDA, SDA CC and BHI at 30°C
4. **Microscopic ex. from culture:** use LPCB
5. **API 20C** (if yeast)

Suspected fungi??

Nail Specimen

Collection:

- Clean the site with alcohol swap
- Then scraped deeply to obtain invaded nail tissue (get far back as possible)
- The initial scrapping should be discarded
- It is better to take from several nails

Nail Specimen

1. **DM ex.:** use 10% KOH or PAS stain
2. **Culture:** on SDA and SDA CC at 30°C
3. **Microscopic ex. from culture:** use LPCB
4. **API 20C** (if yeast)

Suspected fungi??

Tissue Biopsy

First of all grind the tissue with sterile saline than start identification.

1. **DM ex.:** use 10% KOH or PAS stain and H&E stain

2. **Culture:** on SDA, SDA CC, modified SDA, BHI, BA at 30°C & at 37 °C

3. **Microscopic ex. from culture:** use LPCB

4. **Serology:** ID or C.I.E.

Urine Specimen

Collection:

- Urine should be collected in a sterile container as a first morning, mid stream urine
- 25-50 ml

1. Macroscopic ex.: see the color, clear or cloudy, bloody

2. Centrifuge the urine

N.B: for any body fluid (urine, CSF… etc) we have to centrifuge the specimen.

Urine Specimen

3. **DM ex.:** use 10% KOH, PAS or Gram stain

4. **Culture:** on SDA, SDA CC and BHI at 30°C

5. **Microscopic ex. from culture:** use LPCB

6. **API 20C** (if yeast)

CSF Specimen

Collection:

- 3ml of CSF should be collected in sterile container

1. Macroscopic ex.: see if its bloody or viscous

2. Centrifuge: CSF should be centrifuged at 2000 rpm for 10 min

- Make microscopic ex. And culture from the deposit
- Make serology tests from the supernatant.

CSF Specimen

3. DM ex.: use India ink (3 smears are done before consider specimen as negative)

4. Culture: on SDA and BHI at 30°C

5. Microscopic ex. from culture: use LPCB

6. Serology: Ag analysis

Sputum Specimen

Collection:

- It should be collected as a first early morning sample after the patient teeth are brushed
- 5-10 ml in a sterile sputum cup.

1. **Macroscopic ex.:** see if its purulent or mucoid
2. **Add N-acetyle-L-cystine:** sputum is very viscous therefore we add N-acetyle-L-cystine to liquefy the sputum
3. **Centrifuge:** take the deposit and supernatant

Sputum Specimen

4. **DM ex.:** use 10% KOH or PAS stain

5. **Culture:** on SDA, SDA CC and BHI at 30°C

6. **Microscopic ex. from culture:** use LPCB

7. **Serology:** ID or C.I.E.

Blood Specimen

Collection:

- A strict aseptic technique must be used to collect the blood
- Blood specimen should be collected in a sterile anticoagulant tube
- It’s recommended to collect 2 blood specimens at different times
- blood specimen must be cultured immediately

Blood Specimen

1. **DM ex.:** use PAS, Giemsa or Gram stain
2. **Culture:** on diphasic media or BACTEC media
3. **Microscopic ex. from culture:** use LPCB
4. **Serology:** ID or C.I.E.

Blood Specimen

Diphasic media:

- It’s media that contain 2 phases:
  - Solid phase (agar) and
  - liquid phase (broth)

- This media provides:
  - Fast growth to the organisms and
  - Isolates wide range of pathogens

Blood Specimen

BACTEC media:

- BACTEC blood culture system is a fully automated system hat used to detect microbial growth from blood specimens

- BACTEC media is a very sensitive media that contain radioactive materials which can be detected by special sensors in the BACTEC machine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Serological Diagnosis

Serology

- In serology lab the specimen (usually serum or body fluid) is tested to detect the presence of Ag or Ab in the patient specimen.

- **Ag:** is a foreign substance that stimulate the immune system
- **Ab:** is a protein called immunoglobulin (Ig) that produce against specific Ag

- **The most common serological test :**

1. D.I.D
2. C.I.E
3. Latex agglutination

Duple Immuno Diffusion (Ouchterlony) Test (DID)

Principle:

- This technique depends on diffusion of Ag and Ab through semisolid medium which result in formation of precipitin reaction if they are specific to each other.

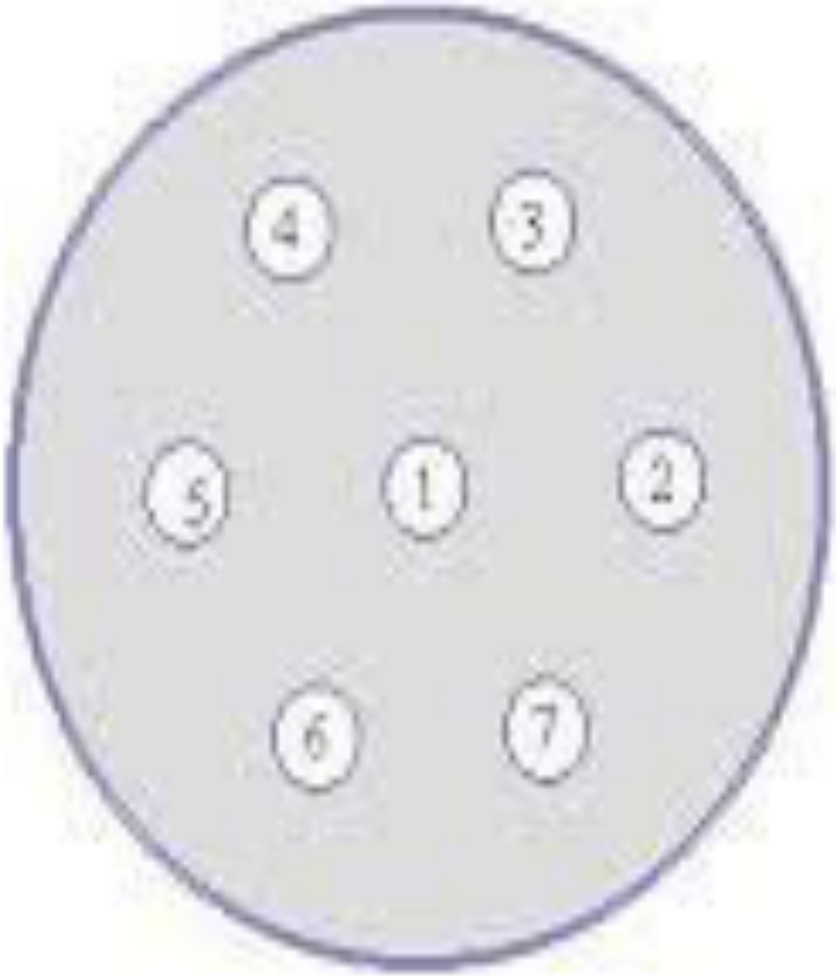
Uses:

- To detect fungal Ag in body fluid.

Duple Immuno Diffusion (Ouchterlony) Test (DID)

Procedure:

1. In small Petri dish, add 6 ml agar, and keep it to solidify
2. Make 5 wells in the agar
3. In the peripheral wells add 20ml of different Ag (20ml different patient specimen)
4. In the center well add 20ml known Ab
5. Incubate the Petri dish at RT for 24-72 hr to 1 week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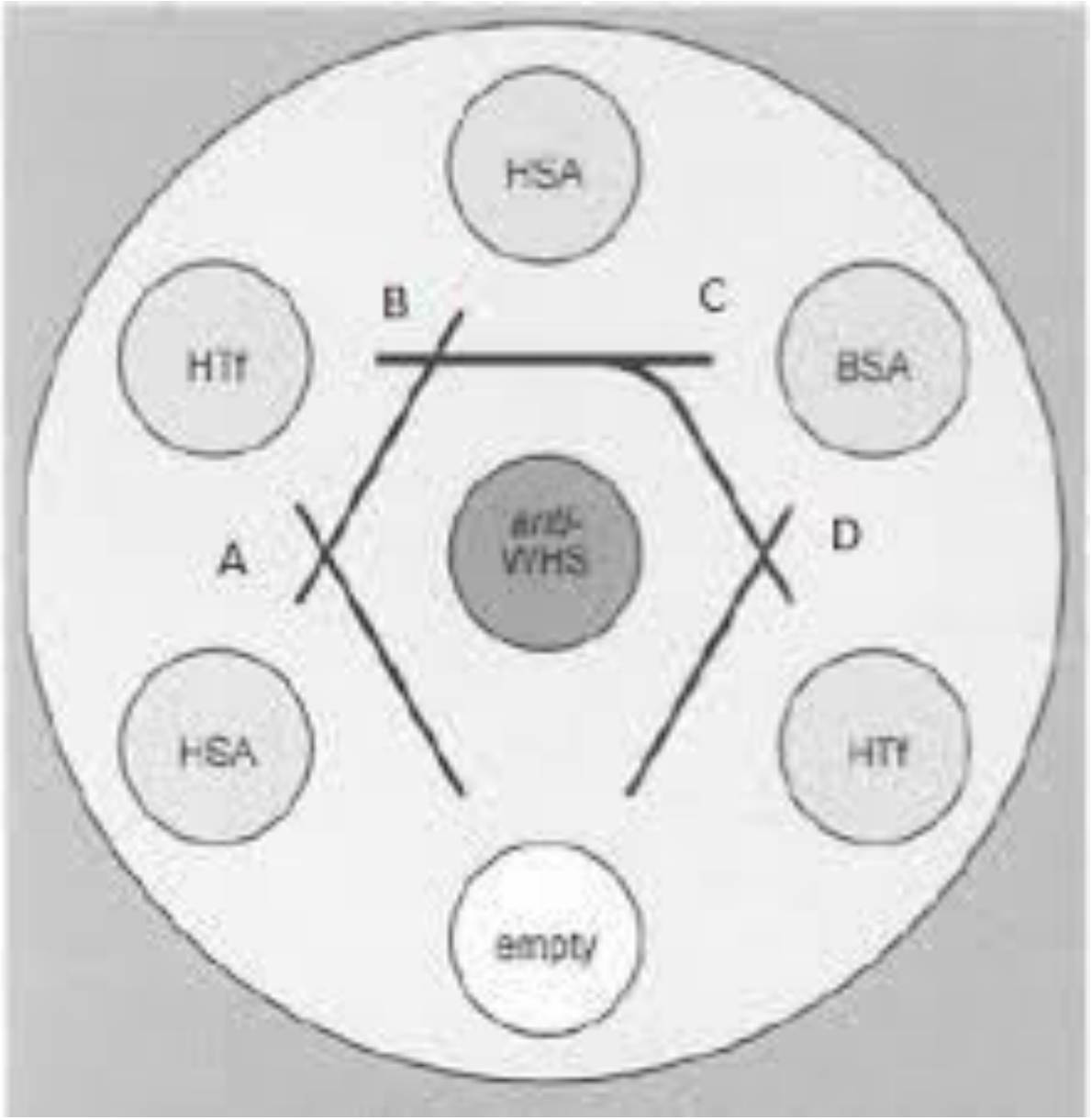
Duple Immuno Diffusion (Ouchterlony) Test (DID)

Result:

- The Ags and the Ab will diffuse in the agar
- At optimal concentration if Ag is specific to Ab they will bind and make white line called **line of precipitaion.**
- If Ag is not specific to Ab they will not bind and there's no line of precipitation


HSA
B
C
HTf
BSA
anti-
VWHS
A
D
HSA
HTf
empty

Duple Immuno Diffusion (Ouchterlony) Test (DID)

Advantages:

- It’s specific test

Disadvantage:

- It’s not very sensitive because Ab and Ag will diffuse in all direction

Latex Agglutination Test

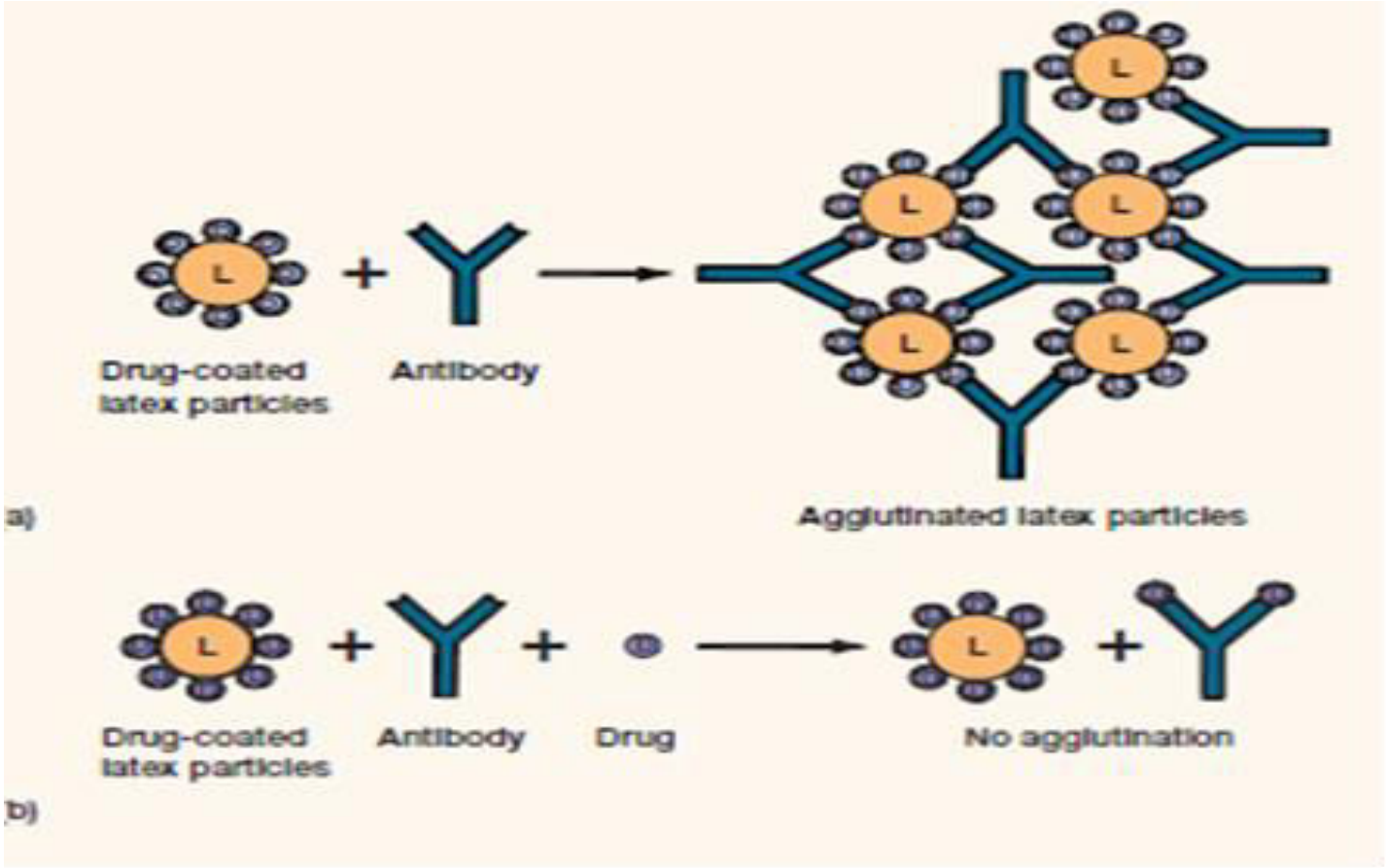
Uses:

This test used to detect the presence of Ag or Ab in patient body fluid

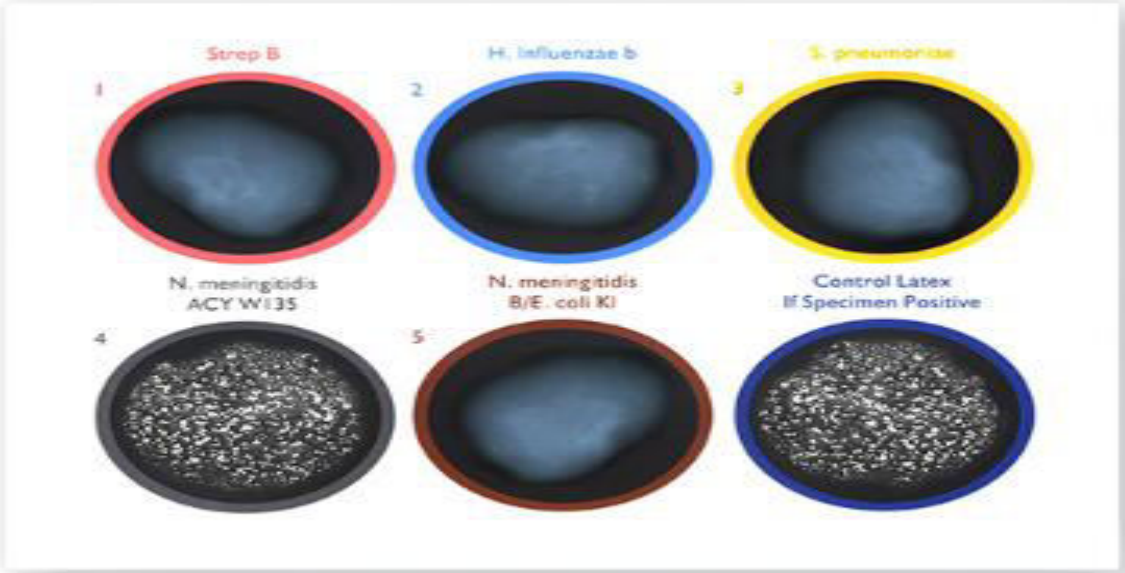
Procedure:

The sample is mixed with latex beads coated with a specific antibody or antigen. If the suspected substance is present, the latex beads will clump together (agglutinate).

Latex Agglutination Test



Latex Agglutination Test



Latex Agglutination Test

Advantages:

- Simple, sensitive and rapid

N.B: Latex agglutination for cryptococcal antigen detection is more sensitive than India ink staining and CSF culture.