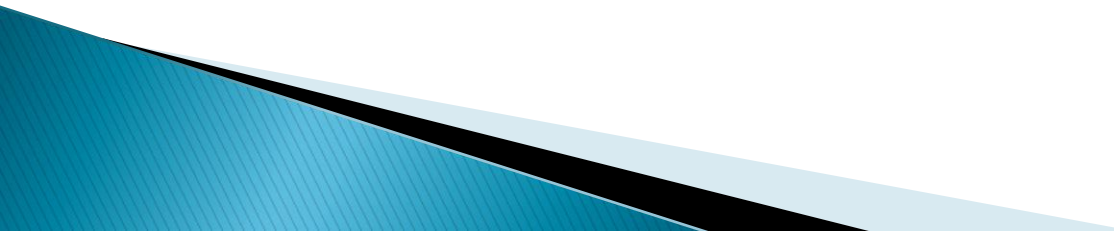


# **ASPERGILLUS**

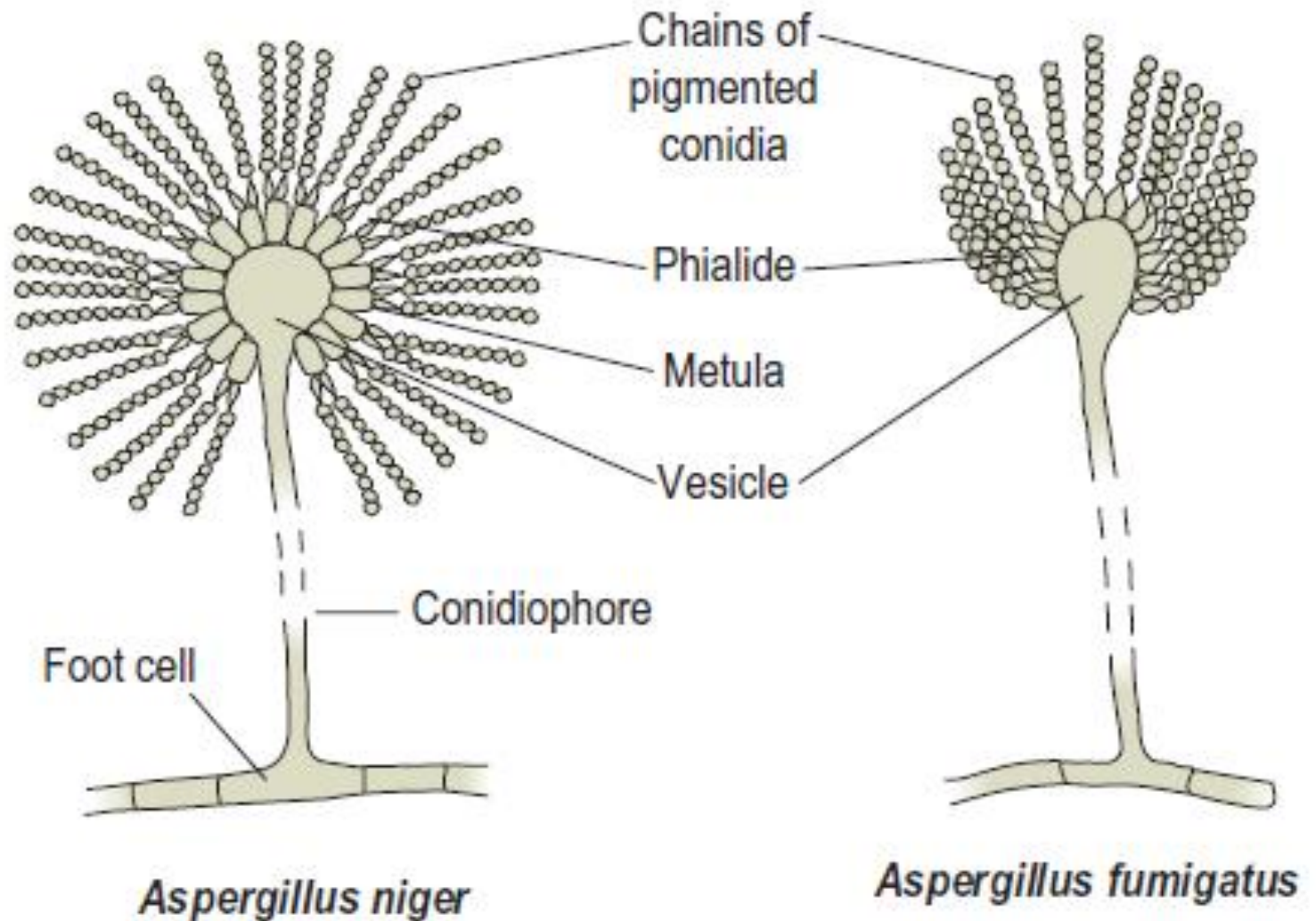
Dr. Poonam Shakya

# INTRODUCTION

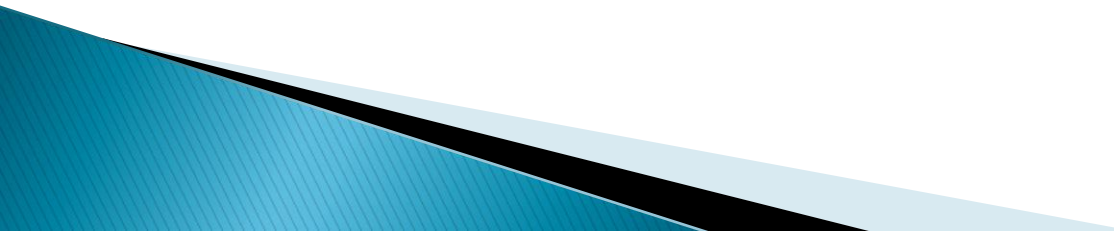
- ▶ More than 190 species
  - ▶ Most are harmless saprophytes
  - ▶ *Aspergillus fumigatus* is responsible for 90–95% of aspergillosis infections in animals
  - ▶ Other *Aspergillus* species that occasionally cause infections are *A. niger*, *A. flavus*, *A. terreus*, *A. deflexus*, *A. flavipes* and possibly *A. nidulans*
  - ▶ *Aspergillus flavus* is more commonly involved in aflatoxicosis
- 

- ▶ Rapidly growing, aerobic moulds with septate hyphae
- ▶ Highly coloured colonies that range from bluish-green through yellow to black due to the profuse production of pigmented spores (conidia)

- ▶ The chains of small (2–3  $\mu\text{m}$ ) oval or spherical conidia are borne from the tips of phialides radially positioned over the surface of the swollen tip (vesicle) of the aerial hypha (conidiophore) which develops at right angles from specialized hyphal foot cells



**Figure 39.1** Comparison of the fruiting heads of *Aspergillus niger* and *Aspergillus fumigatus*.

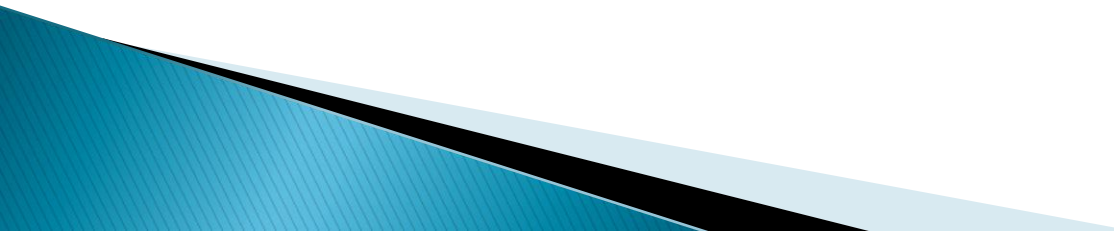
- ▶ Aspergillus species can cause disease in several ways
  - ▶ They can be invasive
  - ▶ Cause mycotoxicoses
  - ▶ Allergic reactions
- 

Host	Disease	Comments
Cattle	Mycotic abortion	Sporadic occurrence, thickened, leathery placenta and raised plaques on skin of foetus
	Mycotic mastitis	Chronic, progressive infection associated with use of contaminated intramammary antibiotic tubes
	Intestinal aspergillosis	Described occasionally in calves
	Mycotic pneumonia	Rare, in housed calves
Horses	Guttural pouch mycosis	Sporadic, often unilateral, necrotizing infection of guttural pouch. May involve carotid blood vessels and glossopharyngeal nerve
	Nasal granuloma	Presents as nasal discharge and interference with breathing
	Keratomycosis	May follow on from ocular trauma
	Intestinal aspergillosis	Described occasionally in foals

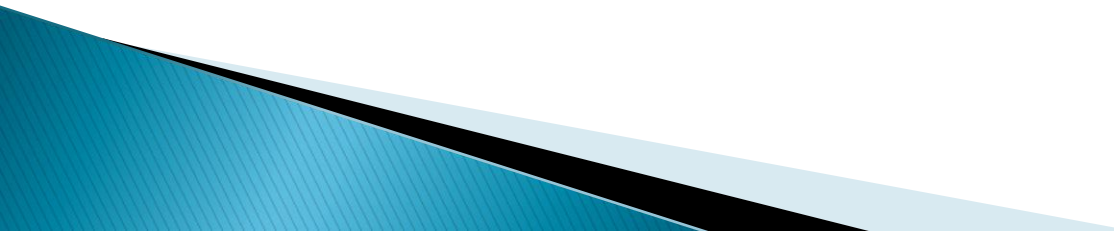
Dogs	Nasal aspergillosis	Sporadic occurrence, usually in young to middle-aged dolichocephalic breeds. Persistent nasal discharge. May involve turbinate bones and paranasal sinuses
	Otitis externa	Occurs as part of mixed infection
	Systemic aspergillosis	Rare, recorded in German Shepherd breed particularly. Osteomyelitis is often a feature
Birds	Brooder pneumonia	Respiratory infection that occurs in newly hatched chicks exposed to high numbers of spores in incubators
	Aspergillosis	Pneumonia and air sacculitis in birds exposed to aerosols of spores from contaminated litter or feed. May occur in outbreaks where birds are stressed, for example, penguins kept in warm environments. Infection may become disseminated to other internal organs



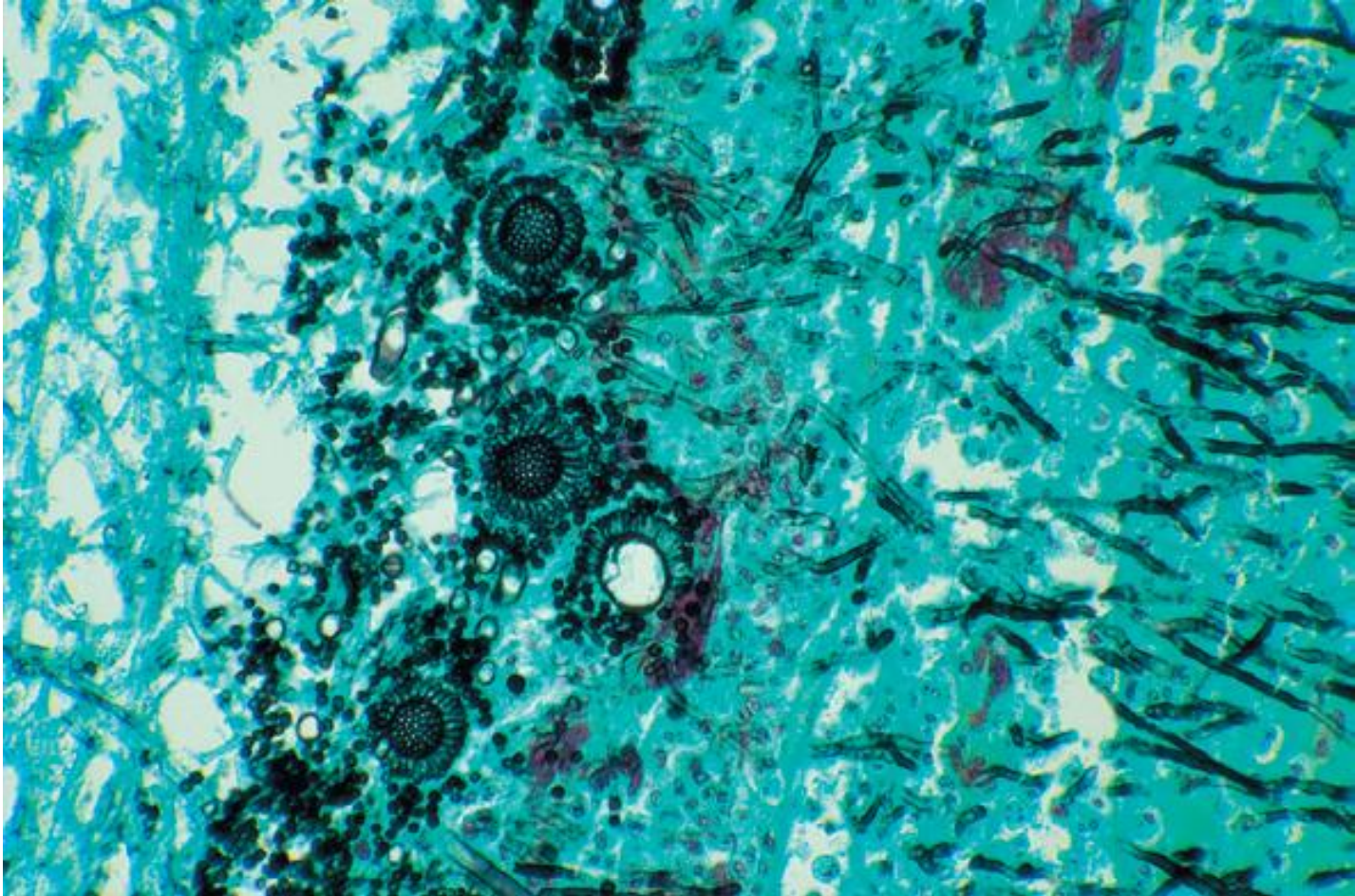
# Natural Habitat

- ▶ Ubiquitous
  - ▶ Can be isolated from soil, air & decomposing organic matter
  - ▶ Worldwide in distribution
  - ▶ Common laboratory contaminants due to the presence of their spores in dust and air
- 

# Pathogenesis

- ▶ Opportunistic pathogen
  - ▶ Infection by inhalation or ingestion
  - ▶ *Aspergillus fumigatus* produces haemolysins, proteolytic enzymes and other toxic factors, in particular gliotoxin, a mycotoxin which has immunosuppressive properties
  - ▶ In pulmonary infections, following spore inhalation, suppurative exudates accumulate in the bronchioles
- 

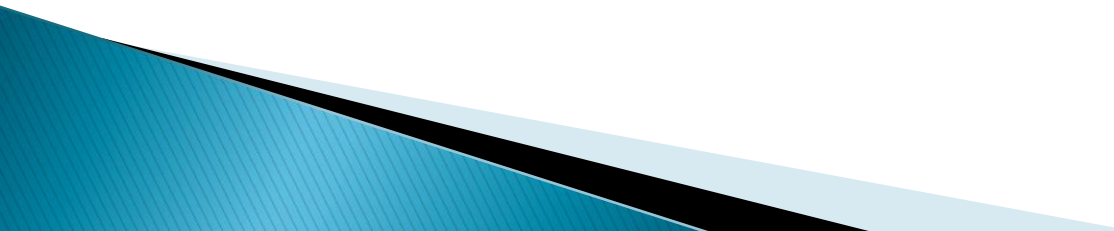
- ▶ Spores are small enough to be carried to the terminal parts of the bronchial tree. Mycelial growth may extend into blood vessels, leading to vasculitis, thrombus formation and dissemination to other parts of the body.
- ▶ Granulomas can develop in many body organs and are visible as yellowish-grey nodules
- ▶ If *A. fumigatus* breaks out into an air space in the body, such as the air sacs in chickens, distorted fruiting heads can be formed



*Aspergillus fumigatus*: infected air sac of a swan showing septate hyphae and fruiting heads.  
(Methenamine silver stain,  $\times 400$ )

- ▶ Spores of *Aspergillus fumigatus* present in organic stable dust may act as an allergen and have been associated with cases of chronic obstructive pulmonary disease in horses

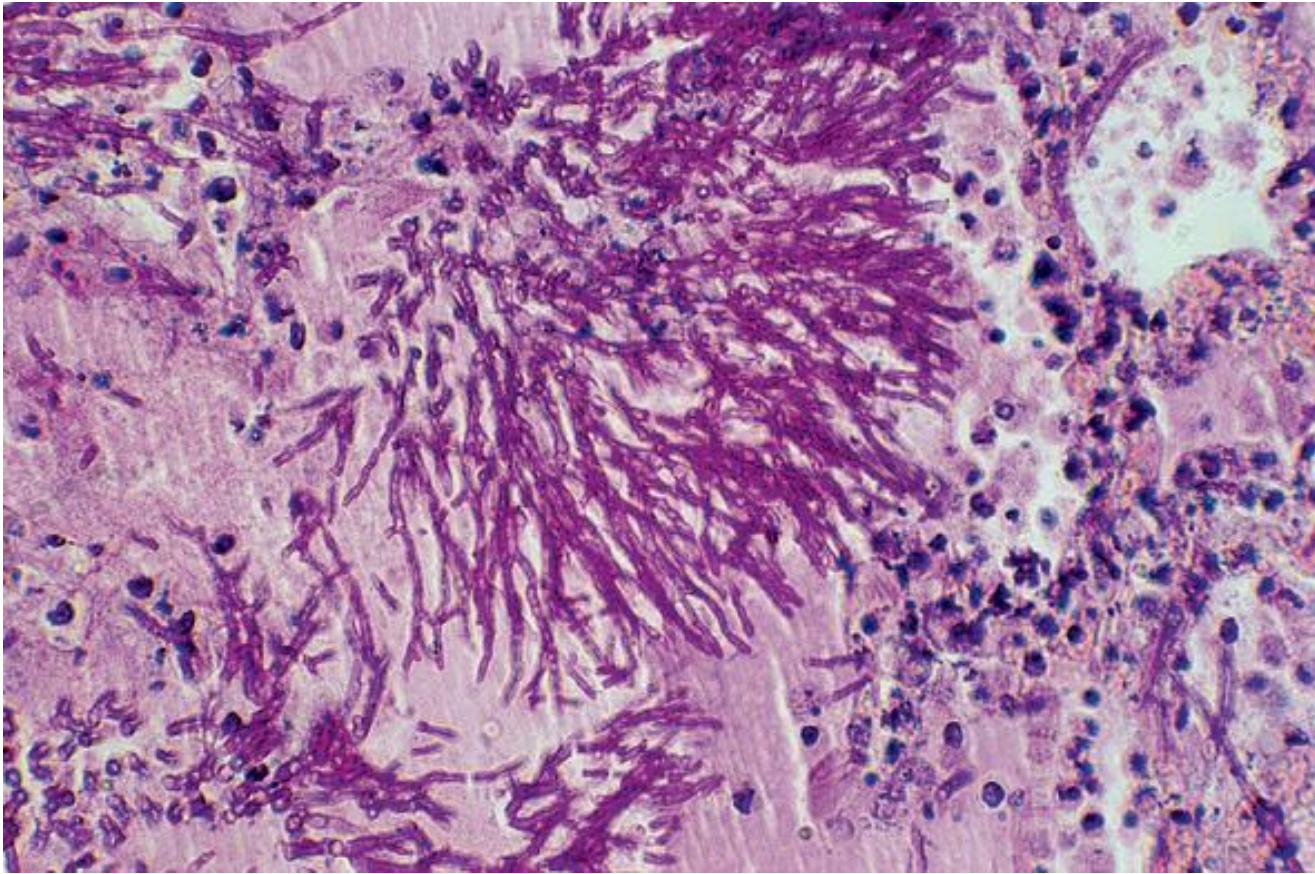
# Laboratory Diagnosis

- ▶ **Specimens**
  - ▶ Pneumonic lung
  - ▶ Granulomatous nodules
  - ▶ Centrifuged mastitic milk
  - ▶ Foetal lesions
  - ▶ Foetal stomach contents, cotyledons
  - ▶ Ear swabs, skinscrapings and biopsies from nasal granulomas
  - ▶ Fungal plaques in the guttural pouch
- 

# Direct microscopy

- ▶ Tissue scrapings - 10% KOH
- ▶ Methenamine silver stain or PAS stain

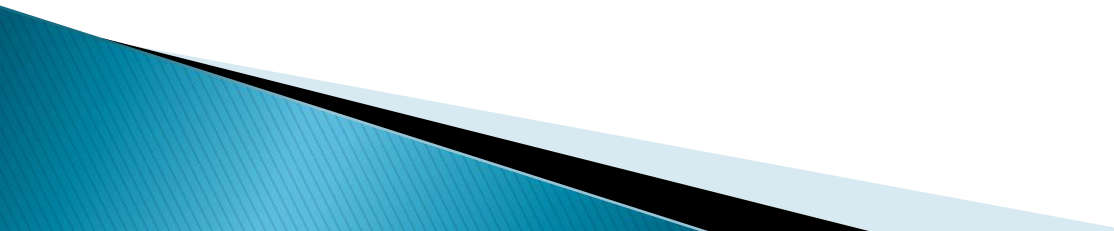




*Aspergillus fumigatus*: equine mycotic pneumonia. (PAS stain,  $\times 400$ )



# Isolation

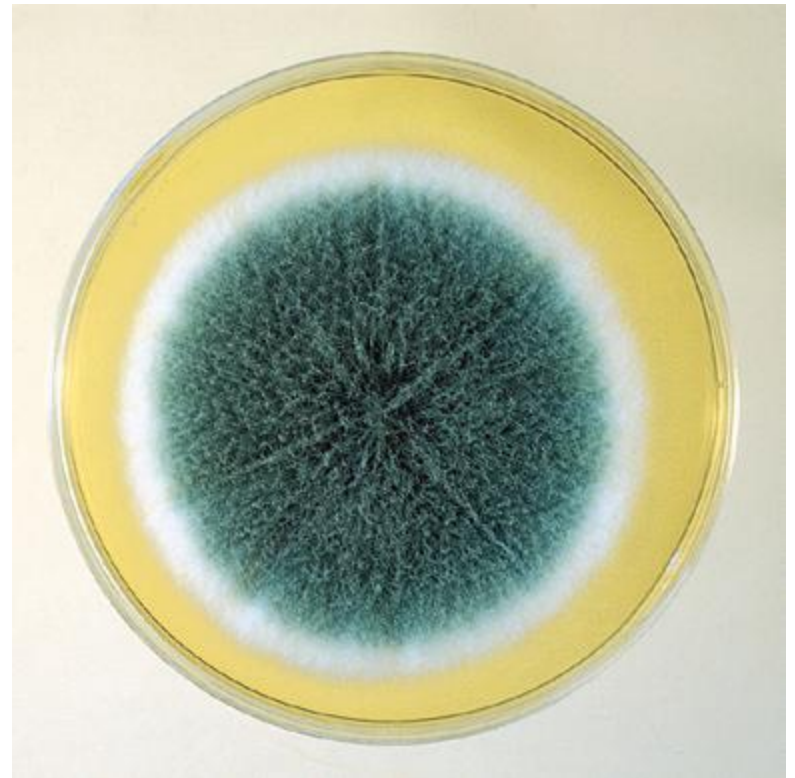
- ▶ Sabouraud dextrose agar, with and without 0.05 g/L chloramphenicol, is used
  - ▶ The surface of the agar should be crosshatched to a depth of about 2 mm in four to five well-separated areas on the plate.
  - ▶ Small pieces of tissue (about one-quarter the size of a finger nail) are placed on the cross-hatched areas and gently pushed into the agar.
  - ▶ Incubated aerobically at 37°C for up to five days
- 

# Identification

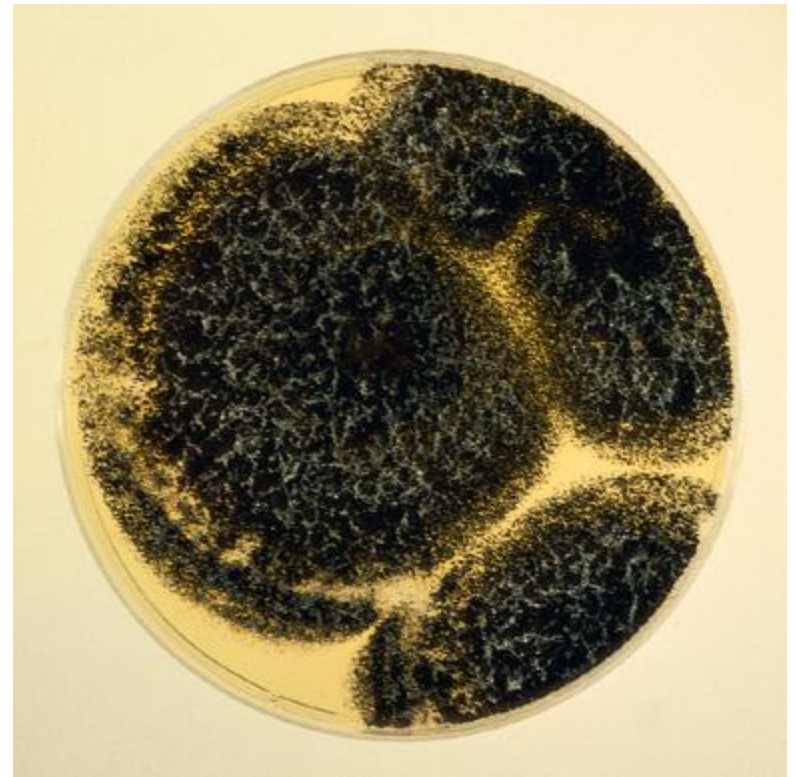
- ▶ On the basis of colony pigmentation; the size and length of the conidiophores; the shape and size of the vesicles; presence or absence of metulae; position of phialides; size, shape and appearance of the conidia; the length of the chains of spores and other criteria

# Colonial morphology

- ▶ *Aspergillus fumigatus*: white fluffy colony when it first appears, rapidly becoming velvety or granular and a bright bluish-green in colour



- ▶ *Aspergillus niger*: white at first when very young but soon developing a black pepper effect as the black conidia are produced



- ▶ *Aspergillus flavus*: cottony aerial mycelium when young but soon becomes a yellow-green with a sugary texture



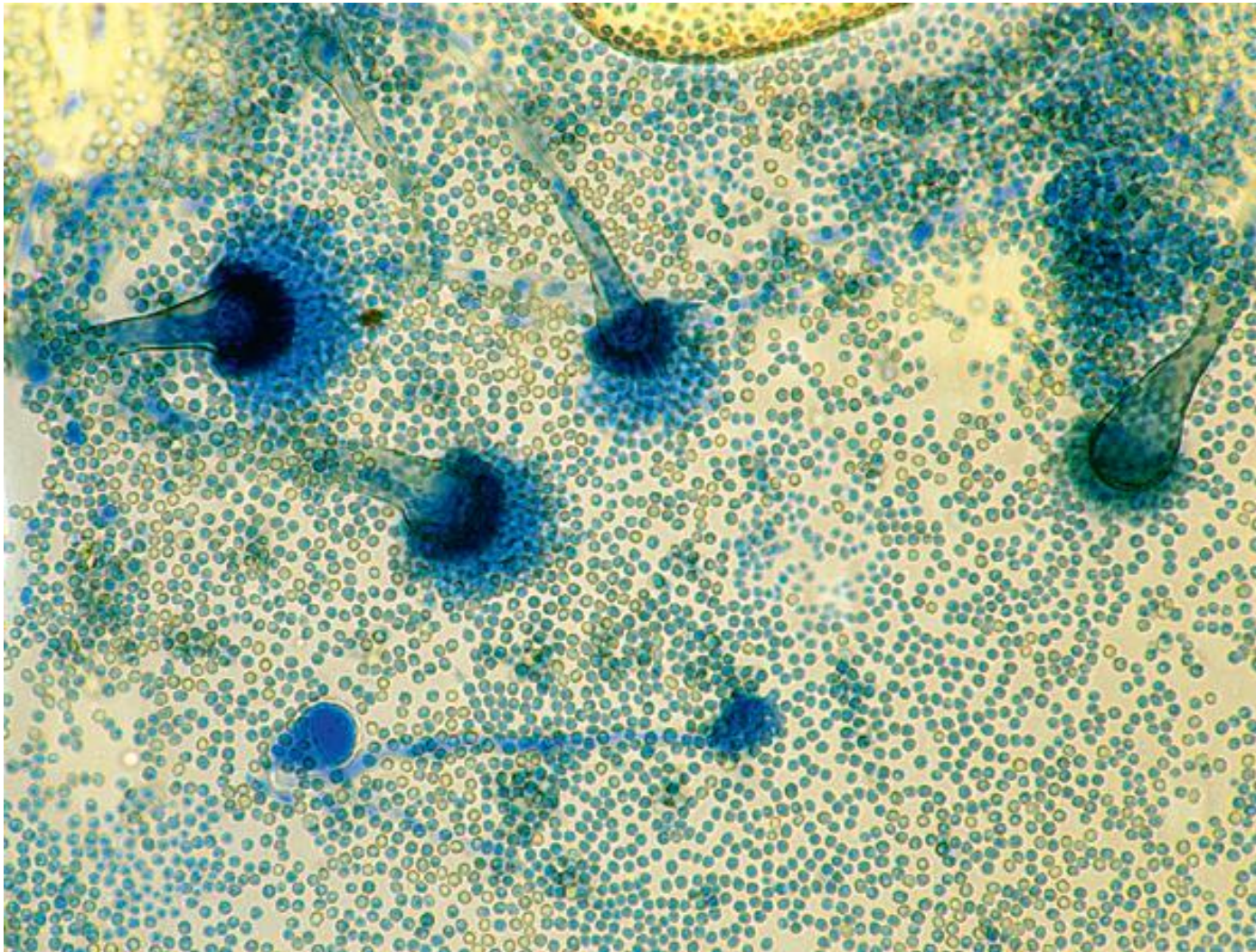
- ▶ *Aspergillus terreus*: white becoming a cinnamon-buff and sugary in texture as the profuse sporulation occurs

# Microscopic appearance

- ▶ Lactophenol cotton blue
- ▶ The characteristic fruiting heads indicate the genus but rather more experience is required for speciation

- ▶ *Aspergillus fumigatus*: conidiophores are moderate in length and have a characteristic ‘foot cell’ at their bases
- ▶ The vesicles are dome-shaped





*Aspergillus fumigatus*: conidiophores and conidia. Transparent adhesive tape preparation. (LPCB,  $\times 400$ )

- ▶ *Aspergillus niger*: this has very large fruiting heads that look like small black balls under the dissecting microscope

- ▶ *Aspergillus flavus*: the vesicles are round with sporulation over the entire surface
- ▶ The conidia are 3–5  $\mu\text{m}$  in diameter, yellowish, elliptical or spherical and become spiny with age

- ▶ *Aspergillus terreus*
- ▶ Vesicles are small and dome-shaped
- ▶ The conidia (2–3  $\mu\text{m}$  diameter) are elliptical.

# Serology

- ▶ ELISA, AGID and counterimmunoelectrophoresis

# Molecular techniques

- ▶ Techniques such as PCR have been developed for the rapid detection and identification of *Aspergillus fumigatus* in clinical specimens