Dr Philippa Ravens

BSc BVSc MVS MANZCVS FANZCVS (Veterinary Dermatology)

Basic dermatological cytology

Getting the most from your skin and ear cases

Outline

- Inventory
- Sample collection and staining techniques
- Basic microscopy
- Interpretation of samples
  - Normal
  - Abnormal/ potentially significant
- Questions

Inventory

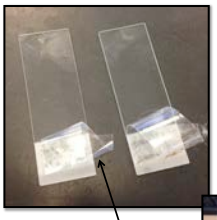
- Microscope
  - Have microscope serviced every 6-12 months
  - Cover scope to minimise dust
  - Microscope oil
- Windex
  - For cleaning microscope lens and stand after oil spills
- Kimwipes
- Glass slides
  - Frosted on one end for labeling
- Pencils

Inventory

- Sticky tape
  - Wide
  - Clear
  - Sticky
- Cotton buds
- Scalpel
- Paraffin oil
- Cover slips
- Stain
  - Diff quik
  - Change weekly
  - Separate tubs for 'dirty' samples

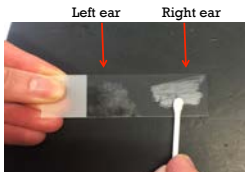
Collection technique – skin Sticky tapes

- Sticky tapes
  - Press several times to skin
  - Puncture pustule and apply tape to contents
  - Pick off scabs and apply tape to exposed area underneath
  - Fasten one end of tape to frosted edge
  - Curl other end of tape back over so sticky side out and fasten this end to frosted end of slide, slightly skewed



Collection technique - ears

- Ears
  - Cotton bud
  - Sticky tape for pinnae
  - Both ears on one slide
    - Left ear towards frosting



Collection technique – skin Impression smear

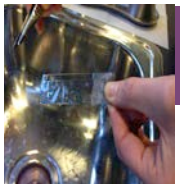
- Press slide to gently to skin
- Good for moist or waxy lesions

Staining technique – Sticky tapes

- Diff quik
  - Only the pink and purple stains
  - NO fixative
    - The fixative will make the tape cloudy and lose its adhesiveness

1. Rinse off stain gently under tap

2. Unfurl tape



3. Lie tape down flat against the slide, sticky side down

4. Place slide between handtowel

5. Firmly squeeze out any water between tape and slide

6. The finished slide

Skin scrapes

Traditional method

- Blunted scalpel blade
- Deep scrape
  - Paraffin oil applied to skin, scalpel and slide
  - Moderate pressure, scrape in direction of hair growth
  - Collect all material and apply to slide
  - Use coverslip
  - No staining necessary

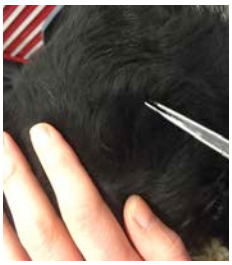
'Sticky tape squeeze' method

Pereira AVJ 2012

- Apply tape to skin
- Squeeze skin several times into the tape
- Lie tape on slide, sticky side down
- No staining necessary

Hair plucks

- Squeeze skin first if looking for mites
- Pluck using forceps in the direction of hair growth
- Place hair on slide in a pool of paraffin to secure



Basic microscopy

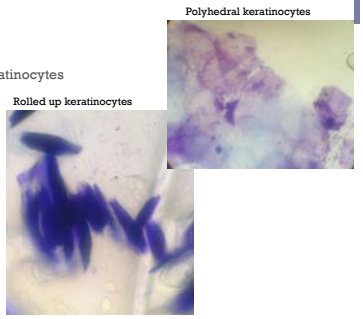
- 4x objective
  - Use to scan slide
  - Look for clumps of neutrophils to zoom in on
  - Mites
- 10x objective
- 40x
  - Yeast
  - Most 40x objectives not suitable for oil
- 100x
  - Oil objective
  - Bacteria and yeast

- Clean lenses using window cleaner and Kimwipes
- Keep microscope covered
- Service yearly

What you should be able to identify

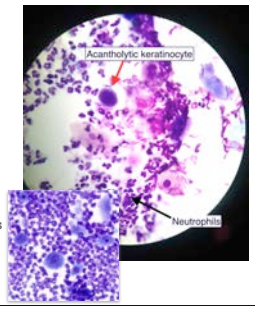
Keratinocytes

- Anucleate keratinocytes
  - Polyhedral
    - flattened
    - pale
  - Rolled up
    - dark

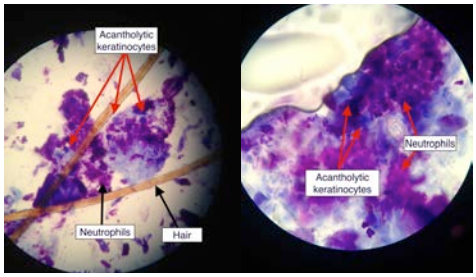


Abnormal/potentially significant findings - keratinocytes

- Nucleated keratinocytes
  - Reflect ↑ KC turnover or early detachment from epidermis
- Polyhedral
  - Common, nonspecific
- Acantholytic keratinocytes
  - Look like 'eyeballs'
  - Pemphigus
  - Infections
    - Bacteria, dermatophytes



Acantholytic keratinocytes



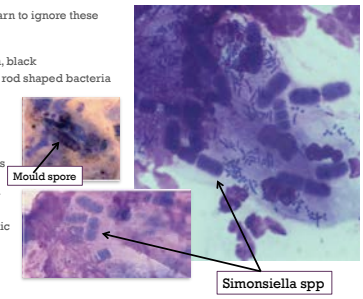
40x objective

100x oil objective

What you should be able to identify

Red herrings – learn to ignore these

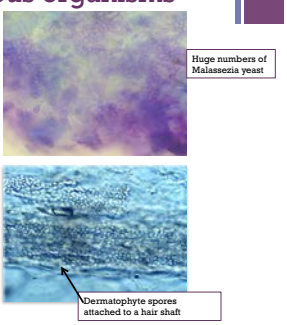
- Melanin
  - Yellow, brown, black
  - Can look like rod shaped bacteria
- Pollen
- Mould spores
- House dust mites
- Simonsiella spp.
  - Oral bacteria
  - Not pathogenic



Abnormal/potentially significant findings – Infectious organisms

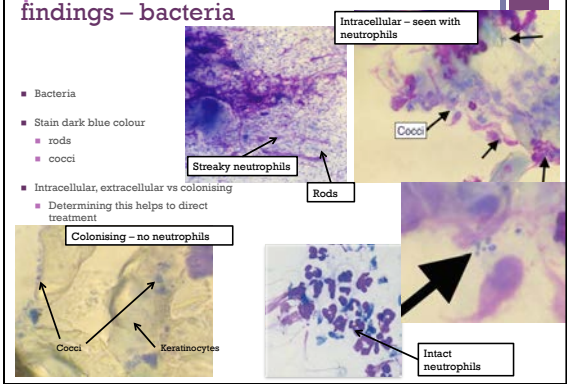
Fungi

- Yeast
  - Malassezia spp.
  - How many is significant?
  - Depends on clinical signs
  - Associated with neutrophils, likely significant
- Dermatophytes
  - No KOH prep
  - Routine Diff Quick



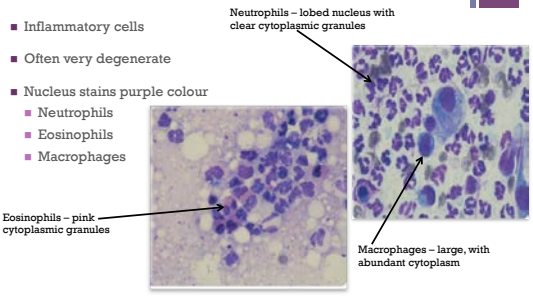
Abnormal/potentially significant findings – bacteria

- Bacteria
- Stain dark blue colour
  - rods
  - cocci
- Intracellular, extracellular vs colonising
  - Determining this helps to direct treatment



Abnormal/potentially significant findings – inflammatory cells

- Inflammatory cells
- Often very degenerate
- Nucleus stains purple colour
  - Neutrophils
  - Eosinophils
  - Macrophages



Abnormal/potentially significant findings – demodex

- Dogs
  - Demodex canis
    - Normal bodied demodex
    - Hair follicles, ear canals
    - Deep scrapes
    - Squeeze tapes
  - Demodex injai
    - Long bodied
    - Sebaceous glands
    - Multiple deep scrapes
    - Greasy dorsal topline terriers
  - 'Demodex cornei'
    - Short bodied
    - Stratum corneum
    - Morphological variant of D. canis
    - Tapes, scrapes

Demodex canis

Demodex injai

'Demodex cornei'

Abnormal/potentially significant findings – demodex

- Cats
  - Demodex cati
  - Demodex gatoi
  - 'Demodex felis'

D. cati

D. gatoi

Abnormal/potentially significant findings – Sarcoptes, Otodectes

- Sarcoptes
  - Numerous broad superficial scrapes

- Otodectes
  - Swab ear discharge with cotton bud
  - Spread out on slide with paraffin

Questions?