Diagnostic Cytology of Body Cavity Fluids in Dogs and Cats

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Vet Education Pty Ltd
A REVIEW OF

DIAGNOSTIC CYTOLOGY & BODY CAVITY FLUIDS

Webinar Notes

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# DIAGNOSTIC CYTOLOGY

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Objectives

The notes and presentation is a refresher, designed for you to:

(1) collect and prepare cytological specimens for analysis

(2) recognise the features of cytopathological change, specifically inflammation and neoplasia, in samples collected from body cavities and solid tissue.

(3) recognise cellular features of malignancy.

(4) classify a neoplasm cytologically as a round cell tumour, or of mesenchymal (sarcoma) or epithelial (carcinoma) origin.

(5) classify a fluid effusion, and determine its possible pathogenesis based primarily on cellular content.

References


DIAGNOSTIC CYTOLOGY

Introduction

Cytopathology involves the examination of individual cell populations collected from a patient, in order to determine the underlying pathophysiological process.

The purpose of cytology is to provide a diagnostic method which will allow the clinician to determine if a lesion (solid tissue mass, body cavity fluid, or wash) is primarily inflammatory or neoplastic; and provide further information on the possible cause (aetiological diagnosis) and chronicity of the lesion, or the type of neoplasm and whether it is benign or malignant.

Hyperplastic, non-neoplastic and cystic lesions are possible. Occasionally the cytological interpretation may be inconclusive, in which case surgical biopsy may be required.

Sample Collection and Preparation

a) BODY CAVITY FLUID - Fluids from peritoneal, pleural cavities, synovial, cerebrospinal and pericardial fluids and aqueous humour are preferably collected into EDTA anticoagulant to preserve cell morphology. Sediment smears are made after gentle centrifugation (1500 rpm, 10 min); direct smears can be made if cell count exceeds 10 x10^9/L. Body cavity fluids requiring biochemical analysis or bacterial culture are collected into a plain serum tube as EDTA inhibits bacterial growth.

b) URINE - Fresh urine sediments can be examined for the presence of neoplastic or inflammatory cells and infectious agents such as bacteria, fungi or yeasts.

c) WASH - Using sterile saline, cells can be encouraged to exfoliate from surfaces such as bronchi, prostatic gland, nasal cavity and uterus (mare). Sediment smears are made. If sample contains macroscopic floccules, squash preparations of these are desirable. Collect into EDTA anticoagulant.

d) BRUSH/SCRAPE - Flat surfaces, in situ, are amenable to these procedures e.g. colon, vaginal wall, conjunctiva and nasal cavity. Cells are directly transferred onto slides. Cytobrushes with nylon bristles are preferable to cotton swabs for cytological brush collection, as cells cling to cotton fibres and distort when transferred to slides. Cotton swabs are suitable for microbial culture.

e) IMPRESSION/IMPRINT SMEARS - This technique is best for cut surfaces from excised lesions. It is not particularly useful for erosive surfaces as only superficial inflammatory cells may be obtained. The freshly cut surface is blotted dry onto an absorbant tow el to absorb excess fluid and blood, then gently rolled onto a slide. Suction artefacts are avoided by rolling the tissue.

f) FINE-NEEDLE ASPIRATION BIOPSY (FNAB) - Used for 3-dimensional tissue masses, which can be internal or external and palpable. For non-palpable masses, ultrasound guided collection helps to ensure the needle is sampling the correct site. Use a 22 gauge needle and 10 ml syringe and pass the needle in at least 3 directions in the mass before releasing vacuum. Collect cells into the needle only. Remove the needle and place air into the syringe before expelling the sample; smear to make monolayers of the sample. Stop the procedure if blood enters the syringe, as clot formation ruins the sample. Speed is required when transferring cells to slides. Syringe is often omitted for splenic aspirates to avoid haemorrhage. For the cytological diagnosis to be reliable, a basic premis of cytology is that the sample collected must be totally representive of the lesion, and several smears should be submitted from each lesion.

g) DRILL OR CORE BIOPSIES - Firm connective tissue and muscle do not readily yield cells on aspiration, and in these cases an air drill or larger needle (Vim-Silverman) may be required. Cell Block Preparations may also be made from these samples or from FNAB.
Slides and Staining

It is best to make several smears (at least 3) as special stains such as Gram’s, Oil Red O (for neutral fat) or Perl’s Prussian Blue (for haemosiderin) stain may be required. Two methods of smear preparation are possible for aspirate samples, and are selected according to the fluid nature of the aspirate. For thick, very cellular samples, gentle ‘spread preparations’ are preferred; for more fluid samples smears made like a blood smear are recommended.

Two methods of smear fixation are possible, air-dried or wet-fixed (alcohol or formalin) smears. However, each requires different staining procedures. Air-dried smears are suitable for routine Romanowsky blood stains (Wright’s Diff Quik, May-Grunwald-Giemsa) and provide adequate information in most cases. The advantages of alcohol-fixed smears, which require Papanicolaou, Haematoxylin & Eosin or trichrome stains is that, because of the effects of cytoplasmic clearing by alcohol, nuclear/nucleolar details are accentuated and dense cell clumps resolved; this is helpful in diagnosing neoplasia. Another exception is in vaginal smears when a cell maturation score (Eosinophilic Index) is needed, thus requiring trichrome staining. These latter stains need regular maintenance and are more suitable for larger, specialized laboratories.

The requirements for a good cytological preparation:

1) adequate cellularity
2) monolayer
3) intact cells
4) minimal blood contamination
5) submit several smears
6) the sample is representative of the lesion

*If blood contamination is unavoidable, collect concurrent CBC for comparison

Principles of Cytological Interpretation

Principles of cytological interpretation differ from those of classical histopathology where architecture of the tissue is emphasized. In cytology, individual cells provide the information, and these are examined at a sub-cellular level. The observations to be made on a cytological sample are listed below, and preferably reported in this order:

<table>
<thead>
<tr>
<th>OBSERVATIONS</th>
<th>INFLAMMATION</th>
<th>NEOPLASIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CELL POPULATIONS (%)</td>
<td>Segmented neutrophils, Macrophages (eosinophils, plasma cells)</td>
<td>Monomorphic</td>
</tr>
<tr>
<td>2. CELL MORPHOLOGY (nucleus and cytoplasm)</td>
<td>Inflammatory cells: Well-preserved or degenerate (karyolysis, pyknosis, cytoplasmic basophilia)</td>
<td>Nuclear criteria of malignancy</td>
</tr>
<tr>
<td></td>
<td>Resident cells: Reactive; retroplastic</td>
<td></td>
</tr>
<tr>
<td>3. BACKGROUND</td>
<td>Protein, mucin, bacteria, fungi, cholesterol crystals</td>
<td>Cytoplasmic products</td>
</tr>
</tbody>
</table>
Inflammatory lesions are classified initially on the basis of the predominant cell type found in the lesion as: **neutrophilic** (suppurative, purulent, acute; septic or non-septic), **mixed cellular** (PMNs & macrophages; pyogranulomatous), and predominantly mononuclear cells, **macrophages** (chronic) or **granulomatous** (with multinucleate giant cells; Table 1). Other lesions may reflect allergic responses and contain many eosinophils. A search for foreign organisms is made in most inflammatory lesions. Bacterial infections are likely when PMNs exceed 85% and the PMNs appear degenerate; fungal, chronic & foreign body infections are possible when macrophages are > 15%.

**TABLE I: CYTOLOGY OF INFLAMMATION**

<table>
<thead>
<tr>
<th>Neutrophilic inflammation:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Septic (bacterial):</strong></td>
<td>PMNs predominate with &gt; 85% neutrophils</td>
</tr>
<tr>
<td></td>
<td>Degenerate nuclear change (karyolysis, nuclear swelling) of PMNs</td>
</tr>
<tr>
<td></td>
<td>Bacteria or other organisms seen intra- and extra-cellularly.</td>
</tr>
<tr>
<td></td>
<td>Proteinaceous background.</td>
</tr>
<tr>
<td></td>
<td><strong>Marked toxic process:</strong> karyolysis, karyorrhexis, foamy</td>
</tr>
<tr>
<td></td>
<td>basophilic cytoplasm, toxic granulation, (Döhle bodies).</td>
</tr>
<tr>
<td></td>
<td><strong>Non-toxic process:</strong> (slow cell death) karyorrhexis, pyknosis,</td>
</tr>
<tr>
<td></td>
<td>hypersegmentation, cytoplasmic changes not as pronounced.</td>
</tr>
<tr>
<td><strong>Nonseptic:</strong></td>
<td>Mixed cellular response with &gt; 70% nondegenerate neutrophils</td>
</tr>
<tr>
<td></td>
<td><strong>Immune-mediated process:</strong> PMNs (± lymphocytes and plasma</td>
</tr>
<tr>
<td></td>
<td>cells)</td>
</tr>
<tr>
<td></td>
<td><strong>Eosinophilic:</strong> PMNs plus eosinophils (&gt; 10%), plasma cells, lymphocytes.</td>
</tr>
<tr>
<td></td>
<td>Eosinophilic inflammation can occur in allergies, hypersensitivities, some</td>
</tr>
<tr>
<td></td>
<td>neoplasms, mast cell tumour, lymphomas, migrating parasitisms, etc.</td>
</tr>
</tbody>
</table>

**Mixed cell inflammation:**

| Subacute inflammation:     | 50% or more neutrophils  |
| 30-50% mononuclear cells:  | monocytes, active macrophages,  |
| some lymphocytes, occasional plasma cells & mast cells.  |
| Chronic:                   | > 50% mononuclear cells.  |
| Active macrophages predominate.  |
| Viral:                     | Often a predominance of mononuclear cells, with lymphocytes and  |
| plasma cells  |
| Granulomatous:             | Epithelioid cells, giant cells, active macrophages & PMNs.  |
| Foreign material e.g. crystals, plant material or specific organisms.  |
**Morphology of Normal and Neoplastic Cells**

In observing sub-cellular detail, the basic principle of cytological interpretation is that:

| a) | the **nucleus** indicates the **state of growth activity** of the cell (e.g. euplastic, proplastic, retroplastic and neoplastic); |
| b) | the **cytoplasm** indicates the **lineage** of the cell and its **functional differentiation** (e.g. chondrocyte, mast cell, fibrocyte). |

The state of growth activities recognisable morphologically (by nuclear features) are:

**EUPLASIA** - A cell with normal growth activity has regular, smooth cytoplasmic and nuclear margins and an even, bland chromatin pattern.

**PROPLASIA** - A cell with increased growth activity, in response to hormonal stimulation, injury or repair has a slightly higher nuclear/cytoplasmic ratio, an apparent nucleolus and a denser chromatin pattern.

**RETOPLASIA** - A cell with decreased growth activity may occur in injury, aging or starvation. The nucleus will by pyknotic and the cytoplasm more basophilic than normal.

**DYSKARYOSIS** - A cell with an abnormal nucleus is called 'dyskaryotic'. The change may be a pre-neoplastic change or result from a toxic insult. These cells present the greatest difficulty in interpretation to distinguish them definitively from neoplastic cells. Histological examination may be required.

**NEOPLASIA** - A cell which has undergone neoplastic change, will show distinct nuclear and nucleolar irregularities. There is marked variation in size, shape and number of nucleoli, with dense chromatin reflecting increased ploidy of the cell, and usually a high nuclear to cytoplasmic ratio. (see Table 2). These features vary with the type and degree of malignancy of the neoplasm and whether it is of epithelial or mesenchymal origin.

Nuclear features and chromatin patterns change through the cell cycle; nuclei have a fine sieve-like (cribriform) chromatin pattern in resting (G zero; figure A), a stippled chromatin pattern with a nucleous during rapid growth (proplasia; figure B), and coarse clumped chromatin pattern in the pre-mitotic phase (figure C). Chromatin may darken in malignant cells due to polyploidy, nucleoli become more prominent and variable, and many malignant cells are found in G zero phase.

In diagnosing neoplasia a number of cytologically characteristics of malignant cells must apply and generally a monomorphism of cell type is present. Mitotic figures alone are not necessarily useful unless mitoses themselves are abnormal or are in large numbers. The frequency of mitoses is often reported.

Cytoplasmic morphological features initially help to define the lineage of cells in a neoplasm. However poorly differentiated blastic cells are more challenging, and immunophenotyping using immunocytochemistry is often recommended to detect antigenic markers which may be in the cytosol or cytoplasmic membrane.

Molecular based diagnostic tests are increasingly available to characterise neoplastic cell populations, by identifying mutations of oncogenes (e.g., c-kit gene), chromosomal abnormalities and clonality in lymphoma and leukemia cells to distinguish reactive from neoplastic lymphocytes. Tests such as PCR for antigen receptor rearrangements (PARR) identify homogeneity in gene rearrangements of neoplastic lymphoid cells. However despite variations in survival times found with various subsets of lymphoma types, histology & flow cytometry for immunophenotyping is still recommended to help establish prognosis in lymphoma patients.
TABLE 2: CYTOLOGICAL FEATURES OF MALIGNANCY

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Criteria</td>
<td></td>
</tr>
<tr>
<td>Macrocytosis</td>
<td>extremely large cells up to 2 times larger in diameter than normal size. (Size should be compared to the erythrocyte).</td>
</tr>
<tr>
<td>Hypercellularity</td>
<td>increased cell exfoliation due to decreased cell adherence (this varies with the type of tumour)</td>
</tr>
<tr>
<td>Pleomorphism and anisocytosis</td>
<td>variable size and shape of the cells of the same family (except lymphoid tissue)</td>
</tr>
<tr>
<td>Nuclear Criteria</td>
<td></td>
</tr>
<tr>
<td>Nucleus</td>
<td>anisokaryosis (variation in nuclear size)</td>
</tr>
<tr>
<td></td>
<td>high nuclear/cytoplasmic ratio</td>
</tr>
<tr>
<td></td>
<td>nuclear deformation by other nuclei</td>
</tr>
<tr>
<td></td>
<td>macrokaryosis (&gt; 10 microns diameter)</td>
</tr>
<tr>
<td></td>
<td>multinucleation (particularly if variable in size)</td>
</tr>
<tr>
<td></td>
<td>nuclear moulding (deformation of nuclei by other nuclei)</td>
</tr>
<tr>
<td>Nuclear membrane</td>
<td>extreme variations in thickness</td>
</tr>
<tr>
<td></td>
<td>sharp angularity and irregularities in outline (esp. carcinomas)</td>
</tr>
<tr>
<td></td>
<td>close parallelism of nuclear and cytoplasmic margins over extended distances</td>
</tr>
<tr>
<td></td>
<td>sharply defined inner and outer surfaces</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>marked variations in size, shape and number (≥ 5)</td>
</tr>
<tr>
<td></td>
<td>irregular shapes with sharp angularity</td>
</tr>
<tr>
<td></td>
<td>Micronucleoli</td>
</tr>
<tr>
<td>Chromatin</td>
<td>dense, but chromacentres variable, clear parachromatin areas</td>
</tr>
<tr>
<td>Mitoses</td>
<td>abnormal mitotic figures. (mitoses are rare in normal tissue)</td>
</tr>
<tr>
<td></td>
<td>binucleate cells containing different sized nuclei</td>
</tr>
</tbody>
</table>

The neoplasm should be classified into one of 3 groups on the basis of lineage, as epithelial, mesenchymal (spindle cell) tumours, and round cell tumours.

- **Epithelial neoplasms** are characterised by cell clusters or sheets with obvious close cytoplasmic attachment (desmosomes), abundant cytoplasm and 1-3 prominent nucleoli. They include squamous cell carcinoma, adenocarcinoma, etc. Squamous cells are expected to have a central nucleus and may have a moderate amount of clear cytoplasm. Adenocarcinomas are characterized by having eccentric nuclei and may appear in papillary, acinar or tubular structures depending on their tissue of origin; they may be secretory and have cytoplasmic cystic inclusions.

- **Mesenchymal neoplasms** contain cells with fusiform (spindle-shaped) to polyhedral cytoplasm and round to oval nuclei. The cytoplasmic margins are often indistinct. They include fibrosarcoma, haemangiosarcoma, osteosarcoma and chondrosarcoma, haemangiopericytoma, etc. Histopathology is often required to accurately classify mesenchymal neoplasms.

- **Round cell tumours** contain individual, discrete round cells and include lymphosarcoma, histiocytoma, mastocytoma, plasmacytoma, melanoma (can appear round or spindle) and transmissible veneral tumour (TVT). TVT can be further subtyped cytologically as lymphoid (with > 60% round cells having central nuclei), plasmacytoid (with > 60% ovoid cells with eccentric nuclei) and mixed type
containing both lymphocytoid and plasmacytoid cells with neither exceeding 59% of the total cells (Florez et al 2012, VCP 41(1) 4.

Another classification includes a fourth group, of cells which readily lose their cytoplasm on smearing, leaving bare nuclei and making their lineage hard to determine. These need to be distinguished from cells of poorly made smears with damaged cells. Bare nuclei neoplasms are commonly endocrine or neuroendocrine tumours. They include tumours of thyroid gland, islet cells, paragangliomas, chemodectomas, carcinoids, etc.

Lipomas are one of the most common benign neoplasms diagnosed and are often poorly cellular and contain oily lipid and very large vacuous adipocytes with a very small nucleus and a very low nuclear to cytoplasmic ratio. Contrasted to this, liposarcomas are quite cellular, may often contain cells which are very poorly differentiated and contain very little free fat either within the cell or outside the cell.

Non-Inflammatory and Non-Neoplastic Lesions

This category includes cysts, transudates and hyperplastic lesions. Samples from these lesions are usually a simple accumulation of residential normal cells, e.g. epidermal cysts contain primarily anucleate epithelial squames and cholesterol crystals, although they can occasionally be accompanied by an inflammatory infiltrate; salivary cysts contain mucinous aggregates, some phagocytes and salivary epithelial cell clusters. The diagnosis of benign hyperplasia (e.g. in a lymph node) is a difficult one cytologically as the cells are not abnormal and show only euplasia or proplasia; they may show increased proportions of intermediate sized lymphoid cells. It is a diagnosis which has to be made with the knowledge of tissue/organ enlargement and with the assurance that infiltrating cells such as in a metastatic neoplasm have not been missed in sampling.

Limitations

Cytopathology is an adjunct to clinical diagnosis. It can give a rapid and reliable result if handled with skill, but it does not replace histopathology. In some instances, an answer of inflammation vs neoplasia is all that is possible; but in other cases a more definitive diagnosis can be given depending on the experience of the person reading the slides. In commencing this study, it is desirable to examine parallel cytology and histology preparations. Samples may also provide misleading information if not properly collected and handled. A positive cytological diagnosis of malignancy is meaningful, but a negative finding does not necessarily rule out malignancy. Additional or alternative tests may be required. However, the initial expense of cytological examination is minimal and the requirements for sample collection are minimal.
LYMPH NODE CYTOLOGY

Cytological Categories
Lymphadenitis (non-septic & septic) - contains increased inflammatory cells
  - Neutrophilic lymphadenitis (> 5% neutrophils)
  - Eosinophilic lymphadenitis (> 3% eosinophils)
  - Pyogranulomatous lymphadenitis (neutrophils & macrophages)
Immunoreactive hyperplasia (> plasma cells; > 15% medium and/or large lymphoid cells)
Lymphoma - classify type (> 50% medium or large lymphoblastic cells; there are exceptions)
Metastatic neoplasia
  - (Inconclusive) - i.e. cellular changes not definitive for lymphoma

Causes of Error in Diagnosis
Inadequate sample - insufficient cells; blood contamination
Sample not representative of entire lesion - e.g. early metastatic neoplasm

Normal lymph nodes are difficult to aspirate but contain a heterogeneous population of lymphoid cells of various stages of maturation, with a predominance of well-differentiated small and medium sized lymphocytes (approx 90%). There are some prolymphocytes containing a single nucleolus and a scattering of large lymphoblasts containing several nucleoli; medium and large lymphoid cells may constitute < 5 to 10% of the cell population. There may be an occasional plasma cell and mast cell, while neutrophils are very rare in normal lymph nodes. The background should be clear of cell debris and may be lightly proteinaceous.

Lymphadenitis is deemed septic if organisms can be observed on smears. Fungal hyphae may only show by negative staining on Romanowsky stains, and PAS stain may be required. Bacteria, fungal spores, Cryptococcus and Aspergillus sp. may be found. Aspirates may be directly cultured onto agar plates if sepsis is suspected. Inflammatory cells are increased (neutrophils and macrophages), and some plasma cells may be noted; medium sized lymphocytes may be mildly increased. Neutrophils may show degenerative karyolytic change if foreign organisms are present in the node. Purulent lymphadenitis (or abscessed node) may be diagnosed if neutrophils are excessive. Chronic lymphadenitis may be diagnosed if macrophages are plentiful, and granulomatous lymphadenitis contains inflammatory giant cells.

Eosinophilic Lymphadenitis involves an inflammatory reaction in the node with a higher proportion of eosinophils with occasional mast cells, along with a mixed lymphoid population. This may be associated with hypersensitivity reactions, pruritic skin disease such as flea allergy dermatitis, chronic seborrhoeic dermatitis, generalized allergies and some rare fungal infections. The background may contain plentiful fine particulate debris in dermatopathic lymphadenopathy.

Immunoreactive Lymphoid Hyperplasia consists of predominantly small mature lymphocytes with increased medium lymphocytes and plasma cells indicating antigenic response. The node may also be oedematous.

Lymphoma. In lymphoma an homogenous population of lymphoid cells generally replaces the normal mixed population, and immature (blastic) cells predominate with > 50% medium or large lymphoid cells. In dogs, the lymphoblastic form is commonly seen. Full categorisation of the type of lymphoma is best achieved using a combination of morphology, immunocytochemistry and antigenic markers using flow cytometry, but this is not readily available in Australia at present. Lymphoblasts and prolymphocytes are often fragile, and as cells rupture, cytoplasmic fragments form as tiny spherical, blue-staining bodies, “lymphoglandular” bodies. They are frequently found in the background of lymphoma smears. Macrophages with lymphophagia may be seen, with several nuclear remnants in the cytoplasm.

Metastatic Neoplasia. Basically this shows a mixed lymphoid population with scattered foreign malignant cells and some inflammatory cells. Metastatic squamous cell carcinoma and adenocarcinoma are the common types seen; metastatic melanoma and mast cell tumours may also be diagnosed. Metastatic sarcomas, haemangiosarcoma and poorly differentiated leukemias may be more difficult to diagnose.
BODY CAVITY FLUIDS

Mechanisms for Effusion Formation

1. Decreased plasma oncotic pressure (e.g. plasma albumin < 10 g/L). This results in a bland (pure) transudate.

2. Increased vascular hydrostatic pressure (e.g. congestive heart failure, hepatic arterio-venous fistula, portal vein hypertension, cirrhosis of the liver).

3. Lymphatic obstruction & leakage (e.g. neoplasia).

4. Increased capillary permeability (e.g. inflammation, vasculitis, thromboemboli, ischemia).

5. Haemorrhage (e.g. coagulation defect, infarction, trauma, thrombocytopenia, neoplasia).

6. Rupture — (e.g. uroperitoneum, bile peritonitis, ruptured bowel)

Collect fluid sample into EDTA for cytology, and a plain tube for culture and biochemical analysis. Make direct and sediment smears for cytological evaluation.

Classification of Effusions

a) Types of Effusions

   Transudate – bland or modified, by the addition of protein or cells

   Exudate – non-septic or septic, with presence of foreign organisms, microscopically or cultured

   Chylous – true chylous or pseudochylous effusions

   Haemorrhagic – acute or ongoing (PCV in effusion >0.05 L/L)

   Neoplastic – contains neoplastic cells

b) Analyses of Body Cavity Fluid

   Appearance
   Turbidity
   Nucleated cell count
   RBC count
   PCV
   Protein content
   Microscopic sediment examination – cellular content
   (or make Buffy Coat smear if haemorrhagic effusion)
   Special chemistry – urea, creatinine, bilirubin, lactate, glucose, triglycerides, cholesterol
   Culture – aerobic & anaerobic
c) Measurable Characteristics

<table>
<thead>
<tr>
<th>Determination</th>
<th>Transudate</th>
<th>Modified transudate</th>
<th>Exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Clear</td>
<td>Straw, yellow, amber</td>
<td>Variable(cream brown, red)</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Clear</td>
<td>Variable</td>
<td>Turbid</td>
</tr>
<tr>
<td>Protein</td>
<td>&lt; 25 g/L</td>
<td>&gt; 25 g/L</td>
<td>≥ 30 g/L</td>
</tr>
<tr>
<td>Clots#</td>
<td>None</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>RBC</td>
<td>None to few</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Nucleated cell count*</td>
<td>&lt; 0.5 x 10⁹/L</td>
<td>≥ 3 x 10⁹/L</td>
<td>&gt; 3 x 10⁹/L</td>
</tr>
<tr>
<td>Dominant cell type</td>
<td>Few</td>
<td>Macrophages (+ others)</td>
<td>PMNs</td>
</tr>
</tbody>
</table>

* Normal nucleated cell count varies with the species & site:
  Peritoneal fluid
  Dog & Cat < 3 x 10⁹/L (often < 1 x 10⁹/L)
  Horses 9.0 x 10⁹/L (often < 5 x 10⁹/L)
  Cattle 6.0 x 10⁹/L (variable, increased post-partum)

# clots will form in fluid with a high fibrinogen content

d) Predominant Cell Types in Body Cavity Fluid

Lymphoid Cells

Note - if cells are blastic (with nucleolus) or mature
  - lipid or chylomicra present, or macrophages containing phagocytosed punctuate lipid droplets

Conditions - lymphoma
  - chylous effusion - [triglycerides high; chylomicra present – this may result from leakage from thoracic duct or intestinal lymphatics]
  - pseudochylous effusion - [cholesterol high; many PMNs; this results from chronic inflammation]
  - tuberculosis; viral infections (e.g. Distemper CSF)
  - thymoma

Neutrophils and Macrophages

Note - if cells are degenerative (karyolytic) or normal
  - identity of particles phagocytosed
  - foreign organisms
  - proportions of PMN to macrophages (acute, sub-acute, chronic)

Conditions - inflammation ± sepsis
**Eosinophils**

*Conditions*
- parasitic larval migration
- hypersensitivities
- trauma

A cytological diagnosis of *eosinophilic inflammation* may be made if eosinophils are plentiful.

**Mesothelial Cells**

*Note*
- if cells are reactive or neoplastic
- cells clustered

*Conditions*
- reactive hyperplasia
- haemopericardium
- low numbers in modified transudates
- mesothelioma

**RBCs**

*Note:*
- erythrophagia/haemosiderophagia
- PCV of fluid (compare to CBC)
- ± platelets

*Conditions*
- pathological haemorrhage and iatrogenic blood contamination

(i) Iatrogenic blood contamination (at time of collection) - resembles peripheral blood, with many platelets. PCV of fluid likely to be < 0.05 L/L.

(ii) Pathological haemorrhage within few hours - intact RBCs with fresh leukocytes and lysing platelets. PCV of fluid > 0.05 L/L in pathological haemorrhagic effusion.

(iii) Pathological haemorrhage within days - erythrophagia and early evidence of hemosiderin within macrophages. Neutrophils hypersegmented, some cytophagia. No platelets if haemorrhage has stopped.

(iv) Note that the mast cells normally found in body cavity fluids contain heparin which prevents clotting *in vivo* of haemorrhagic effusions in a body cavity.

**Foreign Cells** e.g. neoplastic cells; or intestinal epithelial cells if aspirate enters lumen of bowel

Neoplasms which exfoliate well include carcinomas, round cell tumours and mesotheliomas. Poorly exfoliative neoplasms are sarcomas & germ cell tumours. Most effusions (93%) with a pH greater than 7.0 (using urosticks) or 7.3 (using pH meter) were found to be non-inflammatory or neoplastic, whereas those with a lower pH were benign or non-neoplastic.

(i) Squamous cell carcinoma - pleomorphic squamous epithelial cells, with active inflammatory response (e.g. gastric carcinoma in horse).

(ii) Adenocarcinoma - cell aggregates of glandular or tubular epithelial cells, occasionally with secretory product.

(iii) Lymphoma – blastic or large lymphoid cells in abundance; may find lymphophagia (phagocytosis of lymphocytes).
Mesothelioma – usually extremely cellular, best seen on direct smears, with many large clumps of aberrant mesothelial cells showing criteria of malignancy. These must be distinguished from very reactive and dysplastic mesothelial cells seen in chronic inflammation.

Examples of Specific Processes in Body Cavity Effusions

1. Bland Transudate
   - hypoalbuminaemia (< 10 g/L)
   - pre-sinusoidal obstruction to portal blood supply in conjunction with reduced functional hepatic mass or chronic hepatic disease; excessive IV fluid administration

2. Modified Transudate
   (commonly associated with underlying changes in vascular hydrostatic pressure or lymphatic drainage, with leakage out of normal or non-inflamed vessels)
   - intrahepatic portal hypertension (some hepatic lymph)
   - ascites of congestive heart failure (protein 25-50 g/L)
   - lung lobe torsion, diaphragmatic hernia
   - neoplasm e.g. lymphoma, adenocarcinoma

3. Chylous and Pseudochylous Effusions
   (opaque milky fluids)
   - Chylothorax/chylous effusion (small mature lymphocytes predominate; chylomicra, triglycerides in fluid are 2 to 20 x that in serum. May be due to leakage from obstruction of thoracic duct, cardiomyopathy in the cat, cardiovascular disease, neoplasia (thymoma, lymphoma, lymphangiosarcoma), heartworm, trauma, diaphragmatic hernia, lung torsion, fungal granulomas, chronic cough, or idiopathic. Chylous ascites: neoplasia, steatitis, biliary cirrhosis, lymphatic rupture or leakage, ligation of thoracic duct. Chronic accumulation of chylous fluid leads to influx of inflammatory cells, large reactive macrophages show punctate cytoplasmic vacuolation of phagocytosed chylomicra.
   - Pseudochylous effusions (cholesterol is higher in fluid than serum; contains mixed cell population with inflammatory cells; usually due to chronic pleural disease). Rare condition; term now out of favour. Chyliform effusions in humans; tuberculosis is most common cause, rheumatoid lung disease, malignancies, etc – contains inflammatory cells & cholesterol from cell membranes.

4. Non-septic Exudate
   - uroperitoneum (creatnine and urea in fluid exceeds that in serum, odour; low serum Na:K)
   - gall bladder rupture, bile peritonitis (green, bile pigment phagocytosed)
   - intestinal infarct/torsion (RBCs)
   - sterile foreign body
   - acute pancreatitis (should contain lipid droplets, with concurrent mild lipoaemia)
   - feline infectious peritonitis effusive form (usually low cell count, high protein & globulins)

5. Septic Exudate
   - bacteria, fungi
   - intestinal rupture (may also follow volvulus, torsion, etc)
   - ruptured abscess

6. Haemorrhage (iatrogenic vs pathological)
   - coagulopathy (check coagulation profile)
   - trauma, ruptured organ (liver, spleen)
   - neoplasm (spleen)
   - infarct
   - thrombocytopenia
   - blood contamination at collection
SYNOVIAL FLUID

**Normal**

Gross Appearance: Clear or straw coloured, viscous, clear

Cell count: < 0.5 x 10^9/L; (some normal joints have up to 2 x 10^9/L)

Chemical analysis: Hyaluronic acid content can be measured directly. Alternately, the viscosity of the fluid can be assessed grossly and with the mucin clot test.

Protein content: usually < 25 g/L (or ref range 15 – 30 g/L)

Mucin clot test: Tests the polymerization of hyaluronic acid; this provides the viscosity of normal synovial fluid. Mix one part synovial fluid into 4 parts 2.5% acetic acid. Normal (good clot test) shows a tight dense clot; a poor clot reaction shows a diffuse precipitate or cloudy solution and indicated.

Microscopic: Cellularity - direct synovial smears have only 1 - 2 cells per x400 mag. Background – granular, highly proteinaceous (glycoanimoglycans) Cells - predominately small mononuclear cells, synoviocytes, small lymphocytes, less than 5% neutrophils

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cells</th>
<th>Mucin Clot Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious arthritis</td>
<td>Neutrophils, bacteria, fungi or foreign organisms</td>
<td>Poor</td>
</tr>
<tr>
<td>Non-infectious inflammation</td>
<td>Neutrophils</td>
<td>Variable</td>
</tr>
<tr>
<td>Degenerative joint disease</td>
<td>Mononuclear, &lt; 5 x 10^9/L (range 1 – 10) ± cartilage fragments &gt; 10% large vacuolated macrophages Multinucleate or binucleate cells</td>
<td>Good</td>
</tr>
<tr>
<td>Trauma</td>
<td>Some inflammatory cells, RBCs</td>
<td>Usually good</td>
</tr>
<tr>
<td>Haemarthrosis</td>
<td>Many RBCs, inflammatory cells, haemosiderophages, haematoidin crystals</td>
<td>Variable</td>
</tr>
</tbody>
</table>

Note: Lymphocytic synovitis and eosinophilic synovitis may also occur as inflammatory variants
BODY CAVITY FLUID CASES

1. **Case 460-69**

Peritoneal fluid from a 2-year-old dog involved in a motor vehicle accident 2 hours ago:

- **Colour**: red
- **Turbidity**: 2+
- **Supernatant**: yellow
- **Protein (TS)**: < 25 g/L
- **Sp. gravity**: 1.012

**RBC**: 110 x 10^9/L

**WBC**: 0.2 x 10^9/L

**Urea**: 98.3 mmol/L

**Creatinine**: 3570 µmol/L

**Sediment**: Only RBCs and scattered degenerate PMNs and macrophages, which are moderately vacuolated, but no evidence of cytophagia.

**Serum Biochemistry**: urea 22.4 mmol/L; creatinine 387 µmol/L

**Diagnosis**: __________________________________________________________________________

**Reasons for diagnosis**: __________________________________________________________________

2. **C-122**

Peritoneal fluid from a 5-month-old cat with anorexia for 2 weeks and abdominal swelling:

- **Colour**: dark yellow and viscous
- **Turbidity**: clear
- **Protein (TS)**: 75 g/L; albumin 14 g/L (18%); globulin 61 g/L (82%)
- **WBC**: 1.3 x 10^9/L

**Sediment**: Predominately non-degenerate PMNs (69%) with 30% moderately vacuolated reactive macrophages and occasional small lymphocyte. Highly proteinaceous background. Scant cytophagia. No foreign particles detected.

**Diagnosis**: __________________________________________________________________________

**Reasons for diagnosis**: __________________________________________________________________

**Note**: The globulins are expected to be high in FIP. If albumin is > 48% it is not likely to be FIP.
3. **C-61**

Peritoneal fluid from a 7-year-old male Border Collie who had been hit by a car 3 days previously. Only 2 ml of fluid was obtained.

- **Appearance**: dark brown and turbid
- **Nucleated cell count**: $15 \times 10^9$/L
- **Protein (TS)**: 45 g/L
- **Bilirubin**: 675 µmol/L

**Sediment**: Predominately degenerate PMNs with only a low proportion of non-degenerate PMNs and activated macrophages containing bile pigment. Free bile pigment was also evident on the smears.

**Diagnosis**: ____________________________________________
_________________________________________________________________________________________
_________________________________________________________________________________________

**Reasons for diagnosis**: ____________________________________________
_________________________________________________________________________________________
_________________________________________________________________________________________

4. **C-72**

Pleural fluid from a 7-year-old German Shepherd who had severe respiratory dyspnoea and exercise intolerance.

- **Appearance**: red, 3+ turbidity
- **Protein (TS)**: 40 g/L
- **Nucleated Cell Count**: $0.4 \times 10^9$/L
- **PCV**: 0.02 L/L

The fluid did not clot in serum tube.
* RBC count was not recorded.

**Sediment**: Many RBCs with reactive mesothelial cells. Many granular reactive macrophages, some non-degenerate PMNs, some mast cells. Erythrophagia is present. No neoplastic cells detected.

**Diagnosis**: ____________________________________________
_________________________________________________________________________________________

**Surgery**: The dog was found to have a unilateral diaphragmatic hernia with a twisted lobe of liver. The liver lymphatic & post-sinusoidal fluid was though to be the source of the high protein content. Recovery was good.
5. **C-73**

Peritoneal fluid from an 8-year-old male Cocker Spaniel with pale mucous membranes and lethargy. Haemangiosarcoma of the spleen was removed one week ago.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>dark red, turbidity 3+</td>
</tr>
<tr>
<td>Protein (TS)</td>
<td>65 g/L</td>
</tr>
<tr>
<td>Nucleated Cell Count</td>
<td>27.0 x 10⁹/L</td>
</tr>
<tr>
<td>PCV</td>
<td>0.17 L/L</td>
</tr>
</tbody>
</table>

**Sediment:** Some reactive vacuolated macrophages with recent erythrophagia and cytophagia of cells. Blood and some platelets present.

**Diagnosis:**

Further tests required:

6. **Box C-77**

Pleural fluid from an Abyssinian cat, 1½ years, male. Temp. 38.9ºC. Has been treated with Ampicillin and Lincomycin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>pale yellow, turbidity moderate (2+)</td>
</tr>
<tr>
<td>Protein (TS)</td>
<td>68 g/L</td>
</tr>
<tr>
<td>Nucleated Cell Count</td>
<td>55.4 x 10⁹/L</td>
</tr>
</tbody>
</table>

**Sediment:** Predominately degenerate PMNs (with moderate karyolysis) and some pyknotic PMNs – occasional macrophage and RBC. Low numbers of Gram positive cocci and Gram negative rods seen. No Nocardia organisms detected. Heavy proteinaceous background.

**Diagnosis:**

Further tests to assist in treatment: