Fluid and Cytological Analysis

"No fluid, no cells, no diagnosis"

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Disclaimer:

- I am first a general practitioner, and second a diplomate of the ABVP, certified in Feline Practice. I am not a board certified clinical pathologist
    - If your clinical acumen does not support what you think you see, then you should always yield to the expertise of ACVP Diplomate.
    - My goal is to excite you, to encourage and empower you to look more, rather than getting you in over your head.
- I am a bit old school.
    - I cut my teeth in medicine when veterinarians flailingly vaccinated dogs with feline distemper and mink enteritis vaccines in our attempts to save lives.
    - In that era one needed to be a 'jack of all trades' as there was not a referral center on every corner.
    - I will not suggest that any of you perform any skills beyond what was taught in veterinary school (CVM '86).
        - Any skill must be used to maintain them, "use or lose".

What is fun about looking at fluid and cytology?

- In health, the body is composed of predictable collections of cells and fluid.
- In disease, things change. Vessels exude fluid and cells. Chemotaxis initiates an ongoing march of inflammatory cells. Neoplasia causes a proliferation of abnormal cells.
- These cells and fluids are there for your diagnostic pleasure; you simply need to ask the right questions.
- Simple, inexpensive sampling can provide your client with your ability to prognosticate, to prescribe a treatment plan, to cure and/or alleviate suffering in your chosen patient.
- Taking advantage of these sampling techniques saves your patient's time spent suffering in referral limbo. It minimizes your client's expense when you can give them much needed answers, sooner.
- Collectively, those of us in the trenches have the opportunity to see and recognize much that has not been reported by those in the referral settings.
    - We see 99% of the iceberg, while the 'dips' get to see 1%
    - We need to pay attention to what our patients show us.
    - Avoid taking presentations at face value and keep an open mind.
    - When something speaks to you, then dig deeper. Post it on VIN. Ask Dr. Google. Keep thinking about it. Get more samples.

Supplementary in-house laboratory services, as an adjunct to your outside lab?

- Adds value to your patient care
    - Takes very little time

- Takes up very little space, 7ft of counter space if you don't count the sink and the workstation which you probably already have
- You can charge for it, and it costs next to nothing[1]
    - Not counting labor, income is 5x expense for in-house services.
    - Despite the reality that the author finds reasons for doing things for free (in the interests of staff or personal CE and 'fun').
- Often allows you to intervene or start provisional pharmacological therapies sooner[2].
- Your pathologist will know you by name
    - Your succinct history, assessments and additional information will make cases more interesting, more real.
    - Your samples will become more time consuming, but more fun The pathologist will become more engaged in cases, knowing you want more.
- Utilizes your staff, enabling them to use skills they previously learned
    - Requires engaged team approach
- Empowers your staff to know they make a difference
- Provides an ongoing source of CE for doctors and technical staff
- Fends off practice burn out.

Reasons to consider in-house cytology & fluid analysis

It is an opportunity to establish a clinical diagnosis, relearn what you once knew, reinforce what you already know, and contribute to the CE of your coworkers (doctors, technicians, and even the receptionists).

You will miss more by **not** looking, than you will by not knowing.

Technicians maintain their skills and interests by routinely examining cytology and fluid samples. It is easier, if it is routine.

You will know beforehand if your samples are acceptable, or if you need additional samples. (Be sure to label all slides.)

It guides requests for additional stains and/or cultures.

Your pathologist and microbiologist will know you by name when you send in your samples with a tentative diagnosis already established. If you send them images of your clinical samples, they may even send you photomicrographs of their examinations in return.

Alert your laboratory about risks of infectious materials so they can insure proper infectious control protocols are used.

Cytologic examination may reveal the need for tissue biopsy now, rather than later. Many neoplasms can't be confirmed as such, without seeing architecture (e.g. LSA).

Cytology may reveal the need for immediate, aggressive care (exploratory surgery in the case of septic peritoneal effusions).

In-house cytology leads to definitive care sooner, rather than later, avoiding unnecessary morbidity and client expense.

Cytology is fun, it is exciting, and your clients will appreciate your extra efforts and happily pay for your services.

Back to Basics

- The tools you need you already have.
    - Microscope-standard light microscopy 10, 40, and 100X
        - Being able to attach a camera makes it even more fun.
    - Refractometer
    - Microhematocrit centrifuge
    - Stain:
        - Romanowsky stain: Diff-Quik is a common commercially available stain
        - Methylene Blue: most commonly used for reticulocyte counts.
    - Cell Counters:
        - Total Nucleated Cell Count (TNCC):
            - Absolute numbers of cell types are important

        - You must know the TNCC of the blood or fluid for the cytology differential to make sense.
        - When you send samples out, be sure to ask for a full 'fluid analysis', rather than just 'cytology'
- Automated bench top analyzers (laser or impedance flow cytometers) can do this. The results you get will usually be adequate.
    - Check with your instrument manufacturer to find out if you can harm your equipment
    - Most manufacturers will not suggest you use their point of care (POC) units for anything other than whole blood
        - We need to know if there are < 500 cells, 1,500 cells, 5, 10, 50, or 100,000 cells. This does not need to be perfect, just close.
    - Clusters, clumping, and sheets of cells, and variable cell size will effect accuracy, but some POC equipment does a good job with many effusions[3].
    - Lastly, some microorganisms can be confusing to these units[4,5].
    - Many of us grew up using manual Unopette Systems. They are no longer available.
        - Leuko-TIC Test Kit for WBC (Unopette Replacement)[i]
            - 0.02ml of whole blood or fluid
            - 0.38ml of stained diluent (1:20 dilution)
            - Stains the WBC for ease of counting
            - MedixCorp.com
            - Cost is about $1.40 per test
        - Hemocytometer
            - Dilutions of hemolyzed blood or fluid samples are pipetted into the counting chambers.
            - Simple math. Each side of the counting chamber holds 0.9uL.
            - Typically, you have a standard dilution and a standard factor to multiply by.
- Selected serum and/or fluid biochemical analysis
    - I-STAT and EPOC:
        - Electrolytes, renal values, glucose, lactate, and pH
    - Bench top and hand held analyzers can assess routine serum chemistries, including:
        - Albumin, creatinine, triglycerides, glucose, and more.
    - Beta Hydroxybutyrate (Precision Ultra) for serum ketones
    - Coag Dx (SCA2000 is the old version) for PT and PTT.

Obtaining Samples:

- Venipuncture tips:
    - In addition to routine collection methods, consider using heparinized insulin syringes for obtaining smaller samples from your cats. Easier on the cat and their veins.
        - Note: the smaller diameter of the barrel means it is MUCH harder to mix the blood as it settles. For example, have you tried to 'mix' the blood in a spun PCV tube? You can't. Mixing blood in an insulin syringe barrel is a bit like shaking down a thermometer. Introduce a small air bubble and make sure it goes end-to-end, several times.
    - Tip for jugular draws: you can turn the syringe around and insert the needle in the direction of the blood flow (toward the heart). Often this can salvage a 'bad' vein and keep you from starting another site.
    - Be aware that anesthesia will decrease the serum proteins as well as the PCV[6-9].
- Paracentesis techniques:
    - Hypodermic needles
    - Butterfly catheters (not preferred, they slice 'n dice)
    - Through-the-needle jugular catheters
    - Four quadrant techniques
    - Diagnostic Peritoneal Lavage
    - The syringe is not your friend
    - Red Rubber (fenestrated) catheters
    - Commercial indwelling fenestrated catheters
    - Fenestrated (multi-holed) catheters (preferred)
        - Standard over-the-needle IV catheters can be quickly modified (in seconds) for paracentesis
        - If the cavity should be continuous (the peritoneum or hemothorax) then there is no need to use ultrasound to look for 'pockets'
    -
- FNA of solid tissues:
    - Minimal risks[10]
    - Excellent sensitivity and positive predictive value[11]
    - Neoplastic, vs. inflammatory vs need a surgical biopsy?
    - If ultrasound can show you a nodule ~ 1cm, you should be able to perform FNA.
    - Some samples will do great with a traditional FNA 'aspirate' (e.g. MCT).
    - Other tissues do better with a gentler 'woodpecker' or non-aspiration technique[12]
    - Some tissue and fluid samples can be smeared (pulled) like a blood smear.
        - Other will do better squashed
        - Others (some LSA) will cause cell rupture if touch in any manner. Better to look at the edge of a sprayed preparation.
    - A note about FNA and cytology of the liver
        - Some emphasize that results don't always correlate

        - What results you do get rarely mislead.
            - If you see fatty liver cells, a suppurative response (+/- bacteria), a lymphoplasmacytic infiltrate, or neoplastic cells then this is probably 'real'.
- Swabs: multipurpose
    - Cotton tipped applicators
    - Micro brushes minimize trauma to cells
- Touch impression
    - Touch impressions from excisional biopsy specimens
    - Touch or rolled impression from core biopsy samples
    - Impressions and/or smears from scrapings
    - Impressions of all/most surgical excisional samples
        - Pick the surface you want to 'see'
        - Cut surface of GIT biopsy?
        - Mucosal surface?
        - Deep surface of suspected deep pyoderma biopsies.
    - Impression of in situ lesions
- Artifacts
    - Ultrasound gel obscuring field of view[13]
    - Ultrasound gel causing cell changes, and lysis[14]
    - Glove powder
    - Formalin[12]

Criteria of malignancy (basically happy & homogenous vs ugly & angry)[1,10,15]

- Macrocytosis
- Pleomorphism
- Anisocytosis
- Anisokaryosis
- Prominent/multiple nucleoli
- Increased N:C
- Variation in the N:C ratio
- Multiple nuclei
- Abnormal mitotic figures

Routine hematology: the manual CBC (< 10 minutes, 0.06ml of blood)

- RBC's
  - Remember, the PCV is a gold standard for measuring RBC volume
  - Be confident in your equipment & staff, work with them often.
  - 10,000 RPM for five minutes (or more, for less)[16]
  - Small volume (0.03ml of whole blood, Stat Spin)
  - Remember the buffy coat is typically 50% WBC and 50% platelets
    - Leukocytosis, thrombocytosis, metarubricytosis can all make the buffy coat interesting
  - HCT is always a calculated value, use it cautiously
  - RBC indices from commercial labs, in cats, don't over interpret them
  - Remember the PCV varies with age
  - Plasma Protein (total protein, total solids)
  - Reticulocytes
    - New Methylene Blue Stain
    - Absolute numbers are best (just like WBCs)
    - To calculate, you need
      - The % of aggregate reticulocytes
      - The total RBCs/ul will need to be estimate if you only have a % PCV % from a spun tube.

1) Use a ratio from this patient's past:

$$X = \frac{\text{Prior RBC count} * \text{Current PCV}}{\text{Prior PCV}}$$

2) Multiply PCV x 215,000 for an estimated RBC: ($20 \times 215{,}000 = 4.3 \times 10^9$ RBC/ul)

- WBC (or TNCC)
  - The basis of enumerating the differential count
  - Automated Systems are good, but you should manually evaluate the differential
  - Leuko-TIC Test Kit for WBC (see above)
  - Hemocytometer (see above).
- Differential cell count (the Diff)
  - Similar processes for fluid (blood) and tissue
  - Diff-Quik (a Romanowsky stain)
  - The 'extreme feathered edge' is overlooked and very important
    - Thrombocytopenias in cats are over-diagnosed

- Look for grossly visible platelet (and WBC) clumping
- Some platelets are activated by EDTA
- Draw fresh samples into heparinized or citrated insulin syringes

- Assess RBC, thrombocytes, and 5 nucleated cell types
- Inclusions and or free organisms:
  - Organisms:
    - Bacteria:
      - Most bacteria are Diff-Quik positive
        - Mycobacterium sp. (not reported hematologically) have a thick, waxy (hydrophobic) cell wall and they do not stain with routine stains[17].
      - The sensitivity of (tissue) cytology is comparable to bacterial culture[2].
      - Bacteremia:
        - Sepsis and bacteremia can be cause by many organisms
        - Anaplasma sp.
    - Protozoa:
      - Cytauxzoon felis[18]
      - Babesia[19]
      - Hepatozoon[20]
      - Trypanasoma sp.[21]
      - Toxoplasma gondii[22-24]
    - Fungi:
      - Histoplasma capsulatum (man & dog)[25,26]
      - Candidiasis (man)[27]
      - Not uncommon in man, are we missing it?
    - Nematodes, filarial:
      - Dirofilaria immitis
      - Brugia malayi (tropics)[28].
  - Metabolic & genetic disease inclusions
    - Birman & Siamese Cats[29,30]
    - Mannosidosis[31]
    - Mucopolysaccharidosis[31]
    - Gangliosidosis[32]
    - Chediak Higashi Syndrome[33]

In-house cultures: bacterial

- Mostly used for urine.
  - Included as part of our routine urine analysis
- Controversial

    - BSL2 facility is required for handling cultures including:
        - Bacterial, dermatophytes (fungal), and parasites
        - Urine, tissues, hair (DTM), fecal (Tritrichomonas) and other cultures
        - Positive culture plates (all plates) should be autoclaved in special bags when discarding
- In-expensive
    - Disposable calibrated loops (10-ul)
        - Allows for quantitative fluid cultures.
    - Blood agar plates (inexpensive)
    - No flames
- Effective
    - Positive cultures are evident in 6 to 8 hours
    - More than 95% of urine cultures are negative
    - No longer 'fear' the negative urine culture
    - Can be used screening test in young cats as well
    - Recommend morphotyping with Diff-Quik stain
    - Gram staining is discouraged, unless processing multiple samples daily
    - Catalase reaction +/- is helpful when you have cocci bacteria
        - Staph sp. vs enterococcus or streptococcus
    - Osborn's chart of significance[34,35]
- Versatile aerobic culturing
    - Urine culture
    - FNA of any tissue (liver)
    - Gallbladder-bile
    - Fluid culture
    - Blood culture (a drop of blood spread on a plate) if suspecting septicemia
    - Impressions from surgical sample (liver, pancreas)
    - Deep surface of skin punch biopsy samples

Fluid analysis (same as for a CBC)

- You need:
    - Fluid
        - Volume, color, clarity, viscosity
    - Total Protein / Fluid Specific Gravity
        - 2.5 g/dl above and below
        - Find a table of your choosing, but none are perfect
    - TNCC
    - Cytology
    - Fluid Culture (+/-)
    - Selected biochemistries (+/-)

How to make a fenestrated catheter

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A fenestrated catheter is a practical and indispensable diagnostic tool that facilitates establishing a diagnosis. The fashioning of a home-made, over-the-needle, multi-holed "fenestrated" catheter is a simple technique often employed in emergency and critical care practice. The cost to create the catheter is limited to the cost of the over-the-needle IV catheter, a scalpel blade, and less than 60 seconds of effort. While the catheters can be fashioned aseptically without them, a gloved technique is recommended to avoid possible contamination. These catheters can be fashioned intraoperatively for centesis of cystic lesions. Alternatively, they could be premade, repackaged, and ethylene oxide sterilized for later use. Commercially made catheters are similarly available and can used as well (but what fun is that?).

A variety of lengths and diameters can be used. Most commonly in our practice 16 to 18 G, 1 to 2" catheters are used. These catheters are suited for paracentesis of the thorax, pericardium, peritoneum, and retroperitoneum, as well as hepatic, renal, perirenal cysts. Paracentesis techniques may be diagnostic or therapeutic.

In addition to the terminal opening of the catheter lumen, four side holes increase the likelihood of successful fluid collection[36]. A #15 or #12 scalpel is used to cut the wall of the catheter against the side of the stylet. The holes in the catheter should be positioned at 0, 90, 180, and 270° and each approximately 1-3mm long, 1-1.5mm wide, and spaced end-to-end at least 3mm apart. No holes should be cut within 1 cm of the hub as these will be in (or outside) the body wall. If the holes are too larger or spaced too close together they may weaken the shaft of the catheter and lead to buckling during insertion or breaking during extraction. Similar to whittling a stick, the side holes are cut with a smooth, flowing movement, and a flick of the wrist to finish.

When cutting the holes into the catheter this author's technique is to aim the catheter toward myself and cut "sharp edge to sharp point." The intent is to have no burrs remain, but if a small burr is created it should be on the leading edge of the side-hole in the catheter. This facilitates a smooth entrance of the catheter into the body cavity. Any burrs created should 'catch' during a slow, controlled withdrawal of the catheter from the body wall. If resistance, the 'catch', is felt, the catheter should be rotated along its long axis to facilitate extraction and reduce the likelihood of breaking off a portion of the catheter in the body cavity.

Hair should be clipped and the site surgically prepped as you would for IV catheter placement. Depending on the patient, local anesthesia, sedation, and/or general anesthesia are appropriate considerations. Once the catheter and stylet are inserted into the body cavity, the stylet should remain fixed while the catheter is advanced to its hub. Resistance to advancement indicated that the catheter is not into the body cavity, or there is a burr that is catching on the tissues.

While the catheter is in the cavity it may be possible to attach a closed system for removal of large volumes of fluids. Fluid will flow by capillary action and collect in the hub of the catheter. When only small amounts of fluid are present, the fluid can be aseptically drawn from the hub into an appropriately sized syringe with needle attached. Do not attach the hub of a syringe to the catheter and create negative pressure as this will cause omentum, tissues or debris to lodge in the side holes and interfere or stop the flow of fluid altogether. If there is enough volume of fluid that it drips from the hub, then the fluid can be collected directly into a blood collection tube. If the volume is large you may be able to attach a syringe or extension tubing directly to the hub and aspirate in a conventional manner. If the fluid is a hemorrhagic fluid, the syringe or collection tube may be heparinized to prevent ongoing clot formation.

PARACENTESIS

Thoracocentesis

- Generally, the patient is kept in sternal recumbency during thoracocentesis
- Don't count ribs unless placing an indwelling thoracostomy tube
- There is no need to use ultrasound to look for pockets as each hemithoracic cavity should be a contiguous space (potential space).
- You may elevate parts of the chest to get the fluid to pool dependently during thoracocentesis.
- Aseptically prep a very small area.
- Use a local block or general anesthesia.
- An IV extension set is used so the syringe is not held against the chest.
- The three-way stop-cock is added time and expense
- If there is an effusion, rarely is there enough negative intrathoracic pressure to actually suck air in, particularly if there is an extension set attached.
- Grossly, when you collect a pleural effusion you should have a visual suspicion if the collected fluid is a: transudate, chylous, exudate, pyogranulomatous, neoplastic or hemorrhagic. Based on your suspicion ask these questions:
    - What volume should be removed?
    - Should this cat have an echocardiogram to evaluate the LA and myocardium?
    - Will furosemide help, or hurt this patient.
    - What biochemical analyses are warranted?
        - Fluid triglycerides vs serum?
        - Fluid glucose or lactate?
    - What is expected cytologically, and should the smear to be looked at concurrent with abdominocentesis (takes two minutes)?
        - Are cultures (aerobic and anaerobic) warranted based on cytology?

        - Can provisional pharmacological decisions be based on the morphology of visible organisms?
    - Should the client be given a poorer prognosis based on suspicion of neoplastic cells?
    - Is post-centesis radiography warranted?
    - If the fluid is hemorrhagic, should it be collected aseptically for possible autotransfusion?

Abdominocentesis

- Generally, the author performs abdominocentesis with the cat in lateral recumbency.
    - Local block, or general anesthesia
    - Small aseptic clip and prep 3 cm cranial to the umbilicus
    - Especially when there is a small volume of effusion do not use a syringe.
        - "Syringes suck" for abdominocentesis.
        - Use a heparinized syringe and needle to draw fluid from the catheter hub.
- Grossly, when you collect an abdominal effusion you should have a visual suspicion if the collected fluid is a: transudate, suppurative exudate, pyogranulomatous, chylous, neoplastic, bilious, or hemorrhagic effusion. When should you suspect uroperitoneum?
- What volume should be removed?
- When should you consider cardiac disease and echo?
    - Will furosemide help, or hurt this patient?
- What biochemical analyses are warranted?
    - Fluid triglycerides vs serum?
    - Fluid creatinine vs serum?
    - Fluid glucose or lactate?
    - Fluid bilirubin vs serum bilirubin?
    - Albumin/globulin ratio?
- What is expected cytologically, and should the smear to be looked concurrent with the centesis (takes two minutes)?
    - Are cultures (aerobic and anaerobic) warranted based on cytology?
    - Bacteria in an abdominal effusion suggests treatment is prompt surgery rather than medical management.
    - Can you make provisional pharmacological choices based on morphology of visible organisms?
    - For hemorrhagic effusions, are you seeing platelets that suggest iatrogenic or ongoing hemorrhage?
- Should the clients be informed of a poorer prognosis based on strong suspicion of neoplastic cells?

- Is post-centesis radiography indicated?

Retroperitoneocentesis

- Can be based on US or intraoperatively
- Chylous
- Uroretroperitoneum
- Suppurative
- Others

Pericardiocentesis[37-42]

- Often associated with systemic disease and other effusions
- Diagnosis is usually based on radiographic signs of an enlarged rounded heart
- Ultrasound (concurrent) examination is highly recommended
  - Cytology, aerobic and anaerobic culture
  - Volume
    - 1.5 to 95ml, not correlated to clinical signs
  - Classification:
    - Transudate (hypoalbuminemia, volume overload).
    - Modified Transudate: CHF (30 to 75%?), PPDH
    - Biliary (PPDH): two reported cases
    - Suppurative: Septic (10%)
    - Eosinophilic: ECats case
    - Pyogranulomatous: FIP (>15%)
    - Neoplastic: LSA & others (>15%)
    - Hemorrhagic: coagulopathy (10%), neoplasia, L atrial tear

Cholecystocentesis[43,44]

- FNA of the gallbladder
- With US this is quick, easy
- Bile peritonitis and/or GB rupture is a rare sequela
- Make it routine for GIT cases

Airway Sampling

- Nasal swabs and exudates
  - Don’t discard that nose wipe!
  - Blind, otoscopic, or endoscopic
  - They don’t reveal all, but they don’t lie
    - Inflammatory cells
    - Neoplastic cells
    - Intracellular bacteria

        - Fungal disease
            - Pathogenic fungi
            - Rafts of saprophytic fungi
        - Eucoleus boehmi (dogs)[45]
        - Pneumonyssus caninum (nasal mite)[46]
- Nasopharyngeal flush[47]
    - Washes exudates and dislodges tissues
    - Impression smears and histopathology
    - Note about a special bacterium:
        - Simonsiella sp. belongs in the oral cavity.
        - It is distinctive and suggests oral contamination, or a very unusual infection.
- Bronchoalveolar Lavage[48]
    - Blind or endoscopically guided
    - Examine sample at the time, during the collection[49]
        - Homogenize, wet prep, stained smear
        - Assess cellularity
        - Assess cilia motility
- Pulmonary FNA[50]
    - Generalized and nodular disease
    - Blind, or planned based on radiographs and rib spaces
    - Ultrasound may help to localize peripheral masses

Arthrocentesis (joint aspirates)[51]

- Septic effusions
    - Mycoplasma is underappreciated, difficult to see and culture
    - In-house culturing helps
- Synovial Cysts[52-54]

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