

NTproBNP ELISA



N-terminal pro-B type Natriuretic Peptide (ProBNP)

- · In clinic test to distinguish cardiac from respiratory dyspnea
- Validated in dogs JACVIM January 2008
- <210 pmol/L more likely respiratory disease
- · >210 pmol/L more likely cardiac disease
- · Falsely elevated by increased creatinine
- Helpful in distinguishing cardiac from respiratory dyspnea when creatinine is not elevated
- · Most helpful in dogs with airway disease and a murmur
- Often more helpful in cats whose thoracic rads can be more ambiguous
- · VetBLUE® ultrasound has made it less important

Cardiac Troponin



Cardiac Troponin I (cTnl)

- · Marker for myocardial damage
- Not in skeletal muscle 100% specific for the heart
- cTnl a more sensitive marker than cTnT
- We are still learning how to use this test clinically
- Elevated cTnl can distinguish healthy dogs from dogs with significant heart disease
- Low predictive value for DCM in dogs (NTproBNP better)

Thyroid Testing



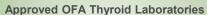
- T3 is mostly intracellular, so T3 blood tests are rarely recommended
- TSH, TT4, fT4 most common screening panel for dogs
- TT4, fT4 most common screening panel for cats
- Add TAb <u>+</u> fT4 by ED when you suspect hypothyroidism, but TT4 not low, or when ruling out NTIS

Thyroid Testing

OFA Thyroid Panel

- free T4 by equilibrium dialysis (RIA)
- TSH (ChL)
- TgAA = TgAb (ELISA)
- Ask for OFA Thyroid panel at TVMDL
 - \$53 cost, plus accession and shipping
 - Results delivered electronically to OFA
 - OFA bills you \$15 when they get the results, if not paid yet
 - Total clinic cost \$74 plus shipping

Thyroid Testing



- Antech
- Animal Health Laboratory Guelph
- Cornell Diagnostic Endrocrinology Lab
- Endocrine Diagnostic Center U Mich
- Idexx
- TVMDL
- UC Davis VTH Clin Path





Thyroid Testing

TSH (Thyrotropin)

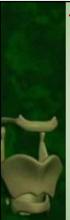
- · High with hypothyroidism
- · Human assays can not be used for dogs
- All commercial assays have poor sensitivity for canine hypothyroidism
 - Many false negatives
 - Up to 40% of hypothyroid dogs have normal TSH
- · Specificity is 92%+
 - 8% of normal dogs have high TSH
- TSH high = likely hypothyroid
 - Either clinical now or will be in the future



Thyroid Testing

Low TSH not clinically significant

- Commercial tests cannot distinguish between low normal and low values
- Effective reference range should go down to zero



Thyroid Testing

TT4

- Lower in dogs (normal 1.0-3.5 mcg/dl) than in people (normal 4-10 mcg/dl)
- Labs use RIA (radioimmunoassay) or CLIA (chemiluminescent immunoassay)
- In House ELISA
 - Helpful, but not as accurate
 - If in doubt, send sample to outside lab for confirmation
- If assay will not occur within 5 days:
 Best practice is to spin, freeze plasma/serum and send on ice in plastic tube. Otherwise, spin & ship on ice.



Thyroid Testing

TT4

- Hyperlipidemia and hemolysis do not interfere with TT4 RIA
- Overlap in reference ranges between euthyroid and hypothyroid
 - "borderline" reference range
- Most common reasons for falsely low TT4 are:
 - Euthyroid sick
- Inaccurate in house ELISA

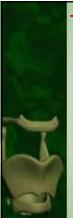


Thyroid Testing

TT4

- Different reference ranges for breeds
 - Sight hounds & Nordic breeds have lower TT4 and fT4 (T3 normal)
 - · Greyhound, Italian Greyhound, Whippet
 - Saluki, Borzoi, Sloughi, Afghan, Basenji, Pharoah Hound, Rhodesian Ridgeback
 - · Deerhound, Wolfhound
 - Husky, Malamute, Norwegian Elkhound, Samoyed
- Canine athletes have lower baseline TT4

 studies in Alaskan sled dogs



Thyroid Testing

fT4

- fT4 by ED is the gold standard T4 assay
 - T3Ab and TGAb do not interfere
 - The best single thyroid test, but it is by no means perfect
- fT4 by ED is 86-93% accurate
- TT4 is 75-85% accurate
- TT4 assays for humans *can* be used in dogs
- fT4 by ED assays for humans *cannot* be used in dogs



Thyroid Testing

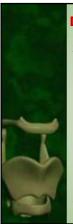
Thyroid Antibodies

- 15% of hypothyroid dogs have AntiT4
 These don't interfere with TT4
- 33% of hypothyroid dogs have antiT3
- · Also can have antiTG
- AntiT3 & antiTG can cause spuriously increased, normal or decreased TT4
 - Depends on the assay
 - Falsely increased more common
- Positive TAb is more significant than negative TAb
- If in doubt run a fT4ED to remove all interfering Ab



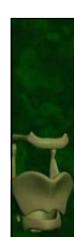
Diagnosis of K9 Hypothyroidism

- 1. If non-thyroid illness and low TT4
 - Treat illness and recheck TT4
- 2. If signs of hypothyroidism and no apparent non-thyroid illness
 - CBC, panel, TSH, TT4, fT4
 - High TSH, low TT4 & fT4 Eureka!
 - High TSH & low fT4
 - likely hypoT4, regardless of TT4
 - · Confirm with TAb + fT4ED
 - Normal TSH, low TT4 & fT4
 - Likely hypoT4



Diagnosis of K9 Hypothyroidism

- 2. If signs of hypothyroidism and no non-thyroid illness
 - Normal TSH & fT4, low TT4
 - · consider "euthyroid sick"
 - Investigate non-thyroid conditions that mimic hypothyroidism – other endocrinopathies, allergies, etc.
 - Thyroxine trial if no non-thyroid illness found
 - Normal TSH, TT4, fT4
 - · Hypothyroidism ruled out
- 3. If in doubt, do fT4ED + T3Ab, TGAb or recheck in 6 months



Factors Affecting Tests

Most common are

- 1. Concurrent illness
- Glucocorticoids Drugs or hyperadrenocorticism
- 3. Random fluctuations of thyroid hormones

Others:

- · Age, Breed, Athletic training
- Gender and OHE status
- Environmental and body temperature
- Body Condition & Nutritional Status



Factors Affecting Tests

NTIS – non-thyroid illness syndrome aka "euthyroid sick"

- Illness decreases protein binding of T4
- T3 more suppressed than T4, but tissue T3 levels are difficult to assay
- TT4 more affected by NTIS than fT4
- · No response to thyroxine trial

Usual pattern:

- · Low TT4, Normal TSH & fT4
 - fT4 can be high in cats with NTIS
- TSH rarely elevated by NTIS (<8%)
 Euthyroid sick dogs can have TT4 <0.5
- Hypothyroid dogs almost never have fT4 by ED > 1.5 mcg/dl, unless there are interfering antibodies



Factors Affecting Tests

Causes of NTIS

- · Any systemic illness, surgery or trauma
- Inadequate calorie intake
- Severity of illness is proportional to severity of suppression
- Dermatopathies and osteoarthritis are unlikely to cause NTIS
- It is nearly impossible to diagnose hypothyroidism in a significantly ill dog, unless illness is due *only* to hypothyroidism
 - thyroid scan or therapeutic trial may be required

Factors Affecting Tests Drugs • Glucocorticoids decrease TT4, fT4, T3, often into hypothyroid range - No corresponding increase in TSH - Topical can have same effect as internal - Glucocorticoids withheld 4-8 weeks prior to thyroid testing • Anticonvulsants - Phenobarbital decreases TT4 and fT4 into hypothyroid range • Also phenytoin, primidone, diazepam - Bromide could potentially interfere with iodide uptake by the thyroid



OF C

Factors Affecting Tests

Drugs

- Sulfonamide antibiotics
 - Truly suppress the thyroid
 - Interfere with T4 & T3 synthesis
 - TT4 can decrease to hypothyroid range within 1-2 weeks
 - TSH can increase within 2-3 weeks
 - Clinical signs can result, chronic therapy can result in goiter
 - Thyroid function returns to normal in 1-12 weeks
 - I avoid sulfonamides in hypothyroid dogs



Factors Affecting Tests

Drugs

- NSAIDs aspirin, carprofen, phenylbutazone
 - Decrease TSH, T4, freeT4 & T3
 - Only Etogesic caused clinical signs -KCS
- Tricyclic antidepressants
 - Inhibit T4 and T3 synthesis
 - Clomipramine, amitriptyline
 - Be sure these drugs are not used when assessing thyroid panel in dogs with behavior problems



Factors Affecting Tests

Drug

- Other drugs that decrease T3 & T4
 - Amiodarone (T3), propranolol, dopamine, nitroprusside, furosemide, henarin
 - Androgens
 - Imidazoles methimazole
 - Mitotane, propylthiouracil
 - Penicillin
 - Phenothiazines
 - Contrast agents iodide, ipodate (T3)



Factors Affecting Tests

Drugs

- Drugs that increase T3 & T4
 - Amiodarone (T4)
 - Estrogens
 - 5-fluoruacil
 - Halothane
 - Insulin
 - Narcotic analgesics
 - Contrast agents ipodate (T4)
 - Thiazide diuretics



Factors Affecting Tests

Age, Breed, Athletic Training

- Progressive decline of TT4, fT4 & T3 with age
 - Puppies <1 yr high end of normal range
 - Geriatrics at low end of normal range
 - Geriatrics have 30% less than puppies
- Older dogs have higher TSH and blunted TSH response
- Higher TAb in older dogs
- Smaller dogs (<10kg) have higher TT4
- Giant dogs (>30kg) have lower TT4



Factors Affecting Tests

OHE status for females

- Progesterone (diestrus), estrus and pregnancy increase T4 and T3
- Hypothyroidism is more common in spayed than intact females

Environmental and body temperature

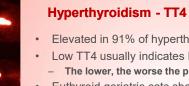
TT4 and fT4 increase in January and fall in outdoor dogs



Factors Affecting Tests

Body Condition & Nutritional Status

- Obese dogs have higher T3 and T4
- Fasting >48 hours decreases T3
- Starving dogs can have very low TT4
- Hypoalbuminemia results in low TT4
- Excess dietary iodine suppresses TT4



- Elevated in 91% of hyperthyroid cats
- Low TT4 usually indicates NTIS in cats
 - The lower, the worse the prognosis
- Euthyroid geriatric cats should be in the lower half of normal range
- Geriatric cats with TT4 in upper half of normal range, with signs of hyperT4
 - treat concurrent illness and recheck TT4
 - If no concurrent illness, can try a methimazole trial
 - Or if signs minor, can recheck thyroid panel in 6 months



- fT4ED sensitivity 98%, specificity 93% for feline hyperthyroidism
- 6-12% of NTIS cats have elevated fT4 but TT4 not elevated (low end of normal)
 - Recommend against fT4 alone in sick cats
 - Combine fT4 with TT4 in cats
- High fT4 + high TT4
- High fT4 + high-n TT4
- High fT4 + low or low-n TT4
- Normal fT4 + TT4
- Low or low-n fT4 + TT4

Iohexol Clearance - GFR

Michigan State U (Toxicology lab):

- 1. 12 hour fast from food (not water)
- 2. Make sure patient is well hydrated
- 3. lohexol 300 mgIE/kg (order from MSU)
- 4. 3-4 ml blood at 2, 3 and 4 hrs post
 - Record blood draw to the nearest minute
 - Clot tube or SST
 - Decant serum to plastic vial (need 1.2 ml)
 - Ship on ice in insulated container
- 5. Submit to lab include patient weight and iohexol dose (form)
- 6. Results provided as ml/min/kg clearance and comparison to normal

Diagnosing & Monitoring Adrenal Disease

Screening Tests

Urine creatinine:cortisol

Diagnostic Tests

ACTH Stimulation Test Low Dose Dexamethasone Test (Combined ACTH stim – LDD)

Differentiating Tests

Abdominal Ultrasound Atypical ACTH stim (Endogenous ACTH) (High Dexamethasone Test - HDD)

Diagnosing & Monitoring Adrenal Disease

Monitoring Tests

Baseline Cortisol ACTH Stimulation Test Electrolytes, kidney panel

Cortisol Assay Samples

- EDTA-plasma, serum or urine
- Centrifuge ASAP
- · Plasma will give you greater volume
- Be consistent with each patient (all samples plasma, or all samples serum)
- Ship on ice packs for delivery in 1-2 days
- If testing will be delayed, freeze immediately after spinning and store in plastic vials

Things That Increase Cortisol

- · Stress, excitement, chronic disease
- Recent administration of glucocorticoids
 - Dexamethasone does not cross react
- Estrogen
- Hyperadrenocorticism
- · Improper handling, storage, shipping

Things That Decrease Cortisol

- Chronic glucocorticoid administration
- Progesterones
- · Progesterone secreting adrenal tumor
- Hypoadrenocorticism
 - Primary, secondary, atypical
- Acute critical illness

Urine creatinine:cortisol (UCC)

- · A good screening test
- Negative (normal) result rules out HAC
- Positive (increased) result means the dog is sick or otherwise stressed
- 76% of dogs hospitalized with non-adrenal illness have elevated UCC
- Have owner collect urine at home to eliminate stress (non-absorbent litter for cats)
- Not a reliable test for monitoring therapy
- · Little data available on reliability in cats
 - UCC 3x higher in hospitalized cats vs. at home
 - Sick cats have UCC much higher than well cats

Urine creatinine:cortisol (UCC)

- Audrey Cook recommends collecting urine 3 days in a row (also for UPC)
- Proteinuria and cortisoluria have some variation from day to day
- Collect at home to minimize stress, and refrigerate until brought to the clinic on the day of the third collection – label with date
- · Request 3 separate assays

ACTH Stimulation Test

Tests the capacity of the adrenal gland to secrete cortisol under stress

Advantages

- Takes 1-2 hours (much shorter than LDD)
- Only 2 blood draws for dogs, 2-3 for cats
- Sensitivity 80-85% for PDH in dogs
- · Creates baseline for therapeutic monitoring

ACTH Stimulation Test

Advantages

- Fewer false positives due to stress than LDD
 - Only 14% of dogs with non-adrenal disease have elevated ACTH stim
- · Best test for identifying iatrogenic HAC
- Can also test for hypoadrenocorticism
- · Can be used to monitor therapy
- Less affected by glucocorticoid therapy than

ACTH Stimulation Test

Disadvantages

- Cosyntropin much more expensive than dexamethasone
- (ACTH gel hard to find, and must be compounded)
- 15-20% False negatives in dogs with HAC
- Sensitivity only 50% for ADH in dogs
- Sensitivity only 50% for all HAC in cats
- Can not distinguish between PDH and ADH

ACTH Stimulation Test

Dog Protocol 1 - Low Dose Cosyntropin

- · 12 hour fast baseline sample
- Administer 1-5 mcg/kg cosyntropin IV
 - 5 mcg/kg for diagnosis, 1-5 mcg/kg for monitoring
- 1 hour post-cosyntropin sample
- Split leftover reconstituted cosyntropin into plastic syringes and freeze
- No loss of activity for at least 6 months in the freezer, or 4 months in the refrigerator

ACTH Stimulation Test

Dog Protocol 2 - High Dose Cortrosyn

- · 12 hour fast baseline sample
- Administer 1 vial (250 mcg) cosyntropin IV or IM (I prefer IV)
- 1 hour post-cosyntropin sample
- Possible increased risk (still very low risk) for adrenal necrosis if dog is taking Trilostane

ACTH Stimulation Test

Dog Protocol 3 - ACTH gel

- 12 hour fast baseline sample
- Administer 1 mg/lb ACTH gel IM
- · Max out at 50 units per dog
- 2 hour Post-ACTH sample

ACTH Stimulation Test

Cat Protocol 1 - Cosyntropin

- · 12 hour fast baseline sample
- Administer 5mcg/kg cosyntropin IV or IM
 - IV is recommended, because ACTH levels are significantly higher, but all cats may not tolerate it.
- If given IM:
 - 30 minute post-cosyntropin sample
- 1 hour post-cosyntropin sample
- I given IV one sample at 60 minutes

Challenge with giving any ACTH IM is that if given intrafat, no stimulation takes place

ACTH Stimulation Test

Cat Protocol 2 - ACTH gel

- 12 hour fast baseline sample
- Administer 1 mg/lb ACTH gel IM
- 1 hour Post-ACTH sample
- 2 hour Post-ACTH sample

ACTH Stimulation Test

Results

- Hypoadrenocorticism
 - Pre less than 5
 - Post less than 5, and less than 2-3x pre
 - Pre and post are often <2 for primary
 - Post value on secondary Addison's can be as high as 3-4 ug/dl, but always less than 5 ug/dl
- Normal
 - Pre 0.1-6
 - Post <20 and >3x pre

ACTH Stimulation Test

Results

- · latrogenic Cushing's
 - Pre <6
 - Post 5-10 and less than 2x pre
- · Borderline stress, sick, early HAC
 - Pre 0.1-6 or more
 - Post 20-30
- Hyperadrenal or Severe stress
 - Pre >10-15
 - Post ->30 or more

ACTH Stimulation Test

Quiz

- Case: 3 year old SF Land Shark with PU-PD, SAP 315 and normal derm
 - Pre 12, Post 29
 - Could go either way look elsewhere first, come back to LDD if the dog still looks Cushingoid
- Case: 4 year old Yorkie with PU-PD and chronic relapsing GI upset
 - Pre 0.5, post 1
 - Hypoadrenocorticism

ACTH Stimulation Test

Quiz

- Case: 11 year old Boston Terrier with PU-PD, endocrine alopecia, SAP 1800, ALT 200
 - Pre 6, Post 6
 - Suspect latrogenic Cushing's check the medical record for glucocorticoids
 - Intrafat injection check route of administration
- Case: 9 year old Cairn terrier with PU-PD, endocrine alopecia, and who is fat and blue
 - Pre 12, Post 55
 - Probably Hyperadrenocorticism confirm with ultrasound

ACTH Stimulation Test

Quiz

- Case: 11 year old Persian with poorly regulated diabetes mellitus
 - Pre 8, 30 minute 42, 1 hour 19
 - Hyperadrenocorticism likely
- Case: 9 year old Labrador retriever with PU-PD and hepatomegaly
 - Pre 1, Post 6
 - Probably Normal pursue other diagnoses first

ACTH Stimulation Test

Quiz

- Case: 16 year old MN unregulated diabetic cat whose skin fell off when someone scruffed him
 - Pre 10, 30 minute 12, 60 minute 19
 - Don't Give Up Yet ACTH Stim 50% false negatives in cats, do LDD and ultrasound
- Case: 13 year old Schnauzer who presented for sudden blindness, red eyes and PU-PD
 - Pre 3, Post 66
 - Possible SARDs recheck 90 days

SARDS and HAC

SARDS

•Sudden Acquired Retinal Degeneration Syndrome

Adrenal Tests Look Cushingoid
Apparent HAC rarely needs treatment
Usually resolves on its own
Schnauzers

Low Dose Dexamethasone Test

Tests the integrity of negative feedback

Advantages

- Takes a full 8 hours have to plan ahead
- Dexamethasone much cheaper than cosyntropin or ACTH gel
- More sensitive than ACTH stim will identify 95-98% of dogs with HAC
- Can sometimes distinguish between PDH and ADH

Low Dose Dexamethasone Test

Disadvantages

- 3 blood draws for dogs and 5 for cats
- More false positives due to stress
 - 40-50% of dogs with non-adrenal disease had inadequate suppression at 4 and 6 hours
- · No baseline for therapeutic monitoring
- Not a good test for identifying iatrogenic HAC and cannot detect hypoadrenocorticism
- Phenobarbital will cause false positive

Low Dose Dexamethasone Test

Protocol - Dog

- 12 hour fast baseline sample 8-9am
- Administer 0.01-0.015 mg/kg dexSP IV
- Diluting dexSP with saline may make dosing more accurate for small patients
 - 3 mg/ml dexamethasone
- 4 hour post-dex sample8 hour post-dex sample

Low Dose Dexamethasone Test

Protocol - Cat

- It can be helpful to place jugular catheter the day before if cat resents venipuncture
- 12 hour fast baseline sample 8-9am
- · Administer 0.1 mg/kg dexSP IV
- 2 hour post-dex sample
- · 4 hour post-dex sample
- · 6 hour post-dex sample
- 8 hour post-dex sample

Low Dose Dexamethasone Test

Results - Dogs

- Suppression cortisol falls below 1.4, or 50% of baseline
- Suppression at 4 and 8 hours is normal
- May not suppress fully until 8 hours if stressed
- Suppression at 4 hours, and then "escape" back to baseline at 8 hours suggests PDH or stress
- Lack of suppression at all means either advanced PDH or ADH – confirms HAC

Low Dose Dexamethasone Test

Quiz

- Case: 14 year old SF Dachshund with polycythemia, lung disease and endocrine alopecia
 - Pre 7, 4 hour 1.2, 8 hour 10
 - PDH
- Case: 10 year old SF biting Cocker Spaniel with bilateral ruptured cruciates, SAP 2500, ALT 400
 - Pre 12, 4 hour 10, 8 hour 1.0
 - Normal look for other causes

Low Dose Dexamethasone Test

Quiz

- Case: 7 year old Sheltie with hyperlipidemia, SAP 2500, ALT 1890, and skin disease
 - Pre 7, 4 hour 0.4, 8 hour 1.3
 - Normal look elsewhere for cause, do ACTH stim or repeat LDD or ACTH stim in 6 months
- Case: 10 year old MN Blue Heeler with PU-PD, endocrine alopecia and highly regenerative anemia
 - Pre 12, 4 hour 10, 8 hour 10
 - HAC PDH or ADH, do differentiating test (US Abdomen first)

High Dose Dexamethasone Test

Advantages

- Distinguishes between PDH and ADH 70-75% of the time
 - 25% of PDH do not suppress
- Can therefore characterize multiple adrenal nodules
- Much easier sample handling than Endogenous ACTH

High Dose Dexamethasone Test

Disadvantages

- Doesn't always distinguish between ADH and severe PDH
- Takes all day have to plan ahead
- · Have to take 5 samples from a cat

High Dose Dexamethasone Test

Protocol - Dog

- 12 hour fast baseline sample 8-9am
- · Administer 0.1 mg/kg dexSP IV
- · 4 hour post-dex sample
- 8 hour post-dex sample

High Dose Dexamethasone Test

Protocol - Cat

- It can be helpful to place jugular catheter the day before
- 12 hour fast baseline sample 8-9am
- Administer 1 mg/kg dexSP IV
- 2 hour post-dex sample
- 4 hour post-dex sample
- 6 hour post-dex sample
- 8 hour post-dex sample

High Dose Dexamethasone Test

Poculto

- Suppression on HDD but not LDD confirms PDH in dogs and cats
- Lack of suppression on both LDD and HDD suggests ADH, but can also be severe PDH in dogs
 - not particularly helpful
- · Rarely done in practices with US

Endogenous ACTH

PDH - High ACTH - >40-45 pg/ml

ADH - Low (undetectable) - <20 pg/ml

20-40 - nondiagnostic

- · Diagnostic 75% of the time in dogs
- 4% of results are incorrect in dogs
- Technically difficult and expensive to ship
 - Spin and separate plasma immediately
 - Add protease inhibitor aprotinin
 - Freeze (<10 min) & ship THAT DAY overnight frozen in plastic tube
 - must be frozen until assay
 - To Michigan State (consult lab before sending)

Baseline Cortisol

Screening for hypoadrenocorticism

- >2 $\mu g/dL$ hypoadrenocorticism highly unlikely if not receiving prednisone
- <2 μg/dL little information obtained

Monitoring trilostane treatment for hyperadrenocorticism – having symptoms

- ≥1.3 µg/dL excludes excessive suppression in dogs undergoing trilostane therapy
- <2.9 µg/dL excludes inadequate control in dogs undergoing trilostane therapy
- 1.3-2.9 µg/dL or 50% of the pretreatment baseline cortisol acceptable control in dogs undergoing trilostane therapy
- >2.9 μg/dL hard to say, need ACTH stim

Baseline Cortisol

Screening for hyperadrenocorticism

- latrogenic hyperadrenocorticism <6 μg/dL
- Normal 6-18 μg/dL
- 18-24 μg/dL suggestive of spontaneous hyperadrenocorticism
- >24 µg/dL strongly suggestive of spontaneous hyperadrenocorticism

Extended ACTH Stim

- · Indications:
 - Animal looks Cushingoid, but ACTH stim and LDDex are not diagnostic
 - Unexplained endocrine alopecia
- Assays (6): cortisol, aldosterone, estradiol, androstenedione, 7-hydroxyprogesterone, progesterone
- Follow the usual ACTH stim protocol, collect serum
- Available at U of Tennessee
 - Allow to clot, spin ASAP and ship on ice

Aldosterone

Indications:

- · Hyponatremia, hyperkalemia, normal ACTH stim
 - To identify hypoaldosteronism (selective mineralocorticoid Addison's)
 - UTenn extended ACTH stim
- · Normal electrolytes, hypoadrenal ACTH stim
 - Distinguish primary atypical and secondary Addison's
 - Predict need for mineralocorticoids in the future
- To assess hormonal function of an adrenal mass
- Hypokalemia, hypernatremia, hypertension
 - To identify hyperaldosteronism



Fructosamine

Diabetic rechecks

- · Weight and exam
- If still showing clinical signs of DM, do glucose curve without fructosamine
- If clinical signs under control, do Fructosamine (FRA)
 - Resolution of PU-PD and weight stabilization are key
- · If FRA high or low, do glucose curve
- · If FRA acceptable, no curve needed
 - send home on that dose and schedule recheck

Fructosamine



glycosylated serum protein

- Averages blood glucose levels over the past 1-3 weeks
- Not affected by acute stress hyperglycemia
- Decreased by
 - Hypoproteinemia, Hemolysis
 - Hyperlipidemia
- Azotemia
- Prolonged storage at room temperature
- Hyperthyroidism, thin body condition
- · Increased by
- Hyperglobulinemia (acromegaly, chronic Ag stim)
- Hypothyroidism
- In house HESKA, Idexx, Abaxis
- Or send out (freeze until shipped)

Fructosamine

Low (<200)

- · Mild to moderate hypoglycemia
- Not enough to cause Somogyi (glucose 65-80)
- Decrease insulin by 10-20%

Normal range (200-350)

Good diabetic control (350-450)

Fructosamine High (>450 mcmol/L)

- >600 danger zone
- · Significant periods of hyperglycemia
- Not enough insulin, or too much insulin
 - Hypoglycemia, rebound hyperglycemia

Low FRA with poor glycemic control – check T4 Remission is defined as normal FRA w/o insulin



Glycosylated Hemoglobin

- Average blood glucose over past 2-4 months
- HbA_{1c} alone is used in diabetic people
- "A1c" alone is not helpful in dogs there are 2 other fractions (HbA_{1a}, HbA_{1b}) that must be
- Need all three fractions in dogs
- In house assays for people are all A1c alone Must send out - not widely available
- 1-4% normal (ave dog 3.3, ave cat 1.8)
- 4-6% good diabetic control
- >7% poor control
- >8% danger zone



Glycosylated Hemoglobin



- A1c only dogs and cats
- \$99 MSRP
- Overnight results
- Need the in house test kit let dry for at least 3 hours prior to shipping
- Supporting literature

IGF-1 Assay

- Acromegaly, Pituitary Dwarfism
- IGF-1 levels more stable than pulsatile GH
- Approximates GH secretion over 24 hrs
- Available at MSU Endocrine lab \$58 cost
 - serum -- refrigerate or freeze and ship on ice
- Affected by numerous variables
 - Age higher in younger dogs & cats
 - Body Size lower in small dogs
 - Falsely low early in acromegaly, starvation, diabetic dysregulation (60 days)
 - Falsely high new diabetics, high insulin doses, hypothyroidism

IGF-1 Assay

- >1,000 ng/ml is diagnostic for acromegaly in cats
- >2-3x normal is diagnostic for acromegaly in dogs

Plasma Free Metanephrines

- · Can measure plasma free metanephrines or fractionated urine metanephrines
- · Much higher in people and dogs with pheochromoctyomas
- · Requires special handling of the sample contact the lab
 - Add acid, refrigerate, protect from light
- · Phenoxybenzamine, corticosteroids and tricyclic antidepressants can falsely increase metanephrines

Marshfield Labs

Insulin & Glucose

- · Must be evaluated from the same serum/plasma sample
- Glucose must be <50-60 mg/dl
- Insulin:
 - high insulinoma likely (73% of insulinomas)
 - upper half reference range insulinoma possible (21% - time will tell)
 - lower half reference range insulinoma unlikely (6% of insulinomas)
 - low insulinoma ruled out

Make sure assay is validated for cats

Amended Insulin: Glucose

Serum insulin (mcU/ml) x 10 Blood glucose (mg/dl) - 30

- Factor of 30 serum insulin undetectable when blood glucose less than 30 mg/dl
- When blood glucose <30 mg/dl, use "1" as divisor rather than the negative number
 - AI:G > 30 is diagnostic for Insulinoma
- Hepatic tumors and sepsis are common false positives
- Fructosamine will be low



DNA Testing

- <u>www.dog-dna.com</u> appears to be out of business
- www.vetgen.com
 - Breed analysis
 - Designer dog confirmation
 - Purebred confirmation
 - Paternity testing
 - Genetic trait tests coat color, length, furnishings, curl, parti-color, piebald, extreme white
 - OFA registration



DNA Testing

- www.vetgen.com
 - Genetic disease testing
 - Cardiology DCM
 - Dermatology albinism, hyperkeratosis
 - Endocrine pituitary dwarfism
 - Hematology cyclic neutropenia, hemophilias, thrombasthenias, macrothrombocytopenias, thrombopathias, PK and PFK deficiencies (anemias), von Willebrands Dz



DNA Testing

- www.vetgen.com
- Genetic disease testing
 - Musculoskeletal muscular dystrophies, dwarfisms, osteogenesis imperfecta, exercise induced collapses
 - Neurology Husky encephalopathy, hereditary ataxia, DM, vertebral malformations, collapse syndromes, neurodystrophies, hypomyelination, Malinois behavior, narcolepsy, cerebellar malformations
 - Ophthalomology retinopathies, dry eye, juvenile cataracts, lens luxation
 - Urinary amino acidurias, hereditary nephritis



DNA Testing

- www.vetgen.com
 - Genetic disease testing
 - Systemic catalase deficiency, cobalamin malabsorption, copper storage disease, lysosomal storage diseases, connective tissue diseases, immunodeficiency syndromes



DNA Testing

- www.optigen.com
- 39 congenital eye disease tests
 - Retinal degeneration, cataracts, glaucoma, lens luxation
- Dermatology coat and skin colors, coat curliness, skin defects
- Hematology Leukocyte disorders, PK and PFK deficiency (anemias)
- Immune mediated dermatomyositis
- Musculoskeletal CMO, collapse syndromes, muscular dystrophies, dwarfisms



DNA Testing

- www.optigen.com
 - Neurology/Behavior acral mutilation, narcolepsy, cerebellar ataxias, juvenile epilepsy, DM, collapse syndromes, degenerative neuropathies
 - Oncology histiocytic sarcoma
 - Respiratory primary ciliary dyskinesia
 - Urinary aminoacidurias, familial nephropathy
 - Systemic Lysosomal storage diseases
 - Wisdom Panel breed analysis



DNA Testing

- www.healthgene.com \$48-78 per test
- Testing Schedules
 - Small Animal Congenital Disease Tests
 - Small Animal Infectious Disease Tests
 - Small Animal Infectious Disease Profiles
- Client Handouts
 - Canine Family Protection Testing
 - Feline Family Protection Testing
 - Avian Family Protection Testing
- · Submission Form (small animal)



DNA Testing

- TVMDL
 - TickPath Layerplex (12 organisms) \$60
 - Ehrlichia canis, E. chaffeensis, E. ewingii
 - · Rickettsia rickettsii
 - Anaplasma phagocytophilum
 - Borrelia species (B. burgdorferi, B. hermsii, B. parkeri, B. turicatae)
 - Babesia species (B. gibsoni, B. canis, B. caballi)

Borrelia burgdorferi DNA is not typically detected in peripheral blood , even when Lyme disease is the cause of clinical signs. Submit joint fluids, CSF, etc.



DNA Testing

- TVMDL \$30 per test
- Canine Single Tests
 - Canine Distemper Virus (CDV)
 - Canine Herpesvirus 1 (CHV1)
 - Canine Parvovirus (CPV)
 - Campylobacter jejuni



DNA Testing

• TVMDL - \$30 unless otherwise stated

Canine Panels

- Babesia panel (B. gibsoni, B. canis, B. caballi)
- Borellia panel (B. burgdorferi, B. hermsii, B. parkeri, B. turicatae)
- Cryptosporidium/Giardia Panel \$40
- Canine Respiratory Panel 7 (Bordetella bronchiseptica, CAV-2, CDV, CHV-1, CIV (H3N8 & H3N2), CCoV, CPIV -\$70 – no mycoplasma
- Rickettsial panel (Ehrlichia canis, E. chaffeensis, E. ewingii, Rickettsia rickettsii, Anaplasma phagocytophilum)



DNA Testing

- TVMDL \$30 unless otherwise noted Feline
 - Chlamydia spp.
 - Feline Calicivirus (FCV)
 - Feline Herpesvirus1 (FHV-1)
 - Mycoplasma hemofelis
 - Tritrichomonas foetus
 - Feline respiratory panel 6 (Bordetella bronchiseptica, FHV, FCV, influenza A, Chlamydophila spp, and Mycoplasma spp. - \$70



DNA Testing

TVMDL - \$30 per test

Canine and Feline

- Cryptosporidium spp.
- Giardia spp
- influenza type A (matrix gene) H3N8 & H3N2
- Leptospira spp
- Mycoplasma spp.
- Neospora caninum



DNA Testing

Auburn Molecular Diagnostics

- · Anaplasma phagocytophilum / platys
- · Feline Immunodeficiency Virus
- · Babesia gibsoni / canis
- · Feline Infectious Peritonitis Virus mRNA
- · Bartonella henselae
- Influenza A Virus
- · Borrelia spp.
- · Hepatozoon americanum / canis
- · Canine Distemper Virus
- · Leptospira spp. (pathogenic)



DNA Testing

Auburn Molecular Diagnostics

- · Chlamydia spp.
- Listeria monocytogenes
- · Dirofilaria immitis / repens
- Mycoplasma haemofelis / haemocanis
- Ehrlichia canis / chaffeensis / ewingii
- Pseudomonas aeruginosa
- Escherichia coli 0157:H7
- Salmonella spp.
- First test \$80, each additional \$70
- · PCR sample buffer kits



DNA Testing

- Wisdom Panel Health Screening direct marketed to clients
 - www.wisdompanel.com
 - Basic Genetic Health Panel MDR1 and EIC
 - Advanced Genetic Health Panel 150 genetic conditions
 - Projected adult weight

TAMU GI Panel

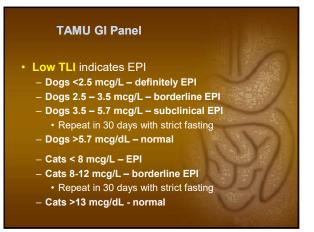
B12 and Folate

- B12 produced by bacteria in the gut
- Intrinsic factor binds to B12 to prevent its digestion, and allow its absorption
 - Intrinsic factor made by the pancreas in the cat and the stomach in the dog
- B12 complex is absorbed in the ileum, folate in proximal SI
- About one third of dogs with chronic diarrhea will have low B12
- Diarrhea won't get better until this is corrected
 B12 deficiency is more common in cats with chronic
 GI disease, as compared to dogs
- B12 <200 carries worse prognosis
- Abnormal folate is an indicator to investigate intestinal disease not as often supplemented as B12

TAMU GI Panel

- Low B12 (TAMU GI Lab Form) DDx
 - Distal small intestinal disease

 - Dysbiosis in upper small intestine in dogs
 - Pancreatitis in cats
- Low B12 not usually due to dietary insuff
- · High B12 not clinically significant
- · Low Folate proximal intestinal disease
- · High folate proximal intestinal dysbiosis



TAMU GI Panel

- Increased TLI trypsin like immunoreactivity
 - >50 mcg/dl in the dog, >100 mcg/dl in the cat
 - acute pancreatitis (30-40%) short lived
 - malnutrition
 - Minimal elevation in renal disease
- Normal PLI <200 mcg/L (dog), <3.6 mcg/L (cat)
- High PLI Canines
 - 200-400 mcg/L borderline
 - Recheck 2-3 weeks if chronic, 1-2 days if acute
 - >400 mcg/L pancreatitis
 - Send out for a number for monitoring

TAMU GI Panel • High PLI Felines - 3.6-5.3 mcg/L - borderline • Recheck 2-3 weeks for chronic disease - ≥5.4 mcg/L - likely pancreatitis • False positives for diabetes, IBD, hepatitis • Margie Scherck says this test is useless in cats • Dr. Suchodolski et al probably don't agree • I have found it clinically useful

TAMU GI Panel

Dysbiosis Index (handout) - 1g feces, ship on ice overnight

- PCR based assay that quantifies the abundances of 8 bacterial groups and summarizes them in one single number.
- The individual microbial profile can predict normal or abnormal conversion of fecal bile acids (i.e., lack of conversion of primary to secondary bile acids)
- Both interpretations will be listed on the results form.
- Assesses need for treatment of dysbiosis
- Probiotics (Nutramaxx® Proviable)
 - Antibiotics
 - Fecal transplant

TAMU GI Panel Gastrin Spin serum as soon as possible, freeze in plastic tube Ship on dry ice – degrades in 4 hrs at room temp Do not ship samples on dry ice on Friday Normal gastrin level is undetectable Elevated in: chronic renal failure (liver failure) gastric outlet obstruction, GDV small intestinal resection immunoproliferative enteropathy of Basenji dogs administration of proton pump inhibitors gastrinoma (usually pancreatic neoplasia)

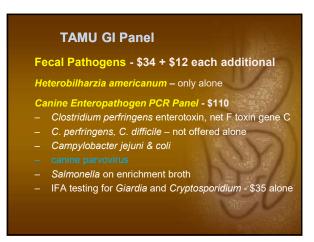
TAMU GI Panel

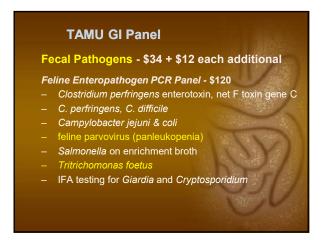
Bile acids, triglycerides

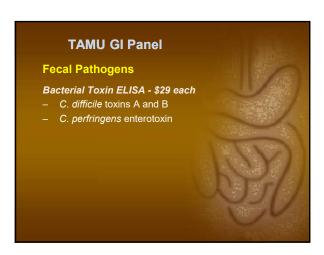
C Reactive Protein (canine)

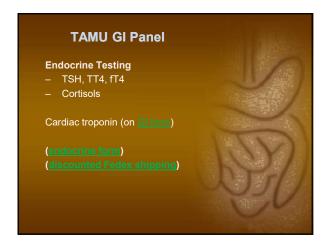
- Nette be enforced with Bretei
- Mortto be confused with Protein C
 Marker of systemic inflammation acute phase
- Helps prognosticate inflammatory intestinal disease
- Monitors response to therapy for IBD and lymphangiectasia
- Spin serum ASAP and ship frozen on dry ice

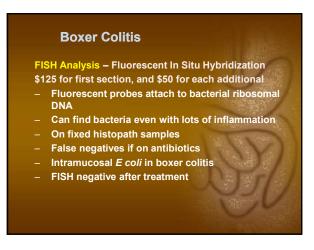
TAMU GI Panel MMA (methylmalonic acid) - can diagnose cobalamin deficiency in patients that have a low normal serum cobalamin concentration - Cobalamin is an essential cofactor for the enzyme methylmalonyl CoA mutase - Falsely increased by renal failure - Spin serum ASAP and ship frozen on dry ice Canine fecal alpha proteinase 1 inhibitor - 3 fecal samples (test kit 15 for \$25) - Freeze ASAP and ship on dry ice - Test for PLE, more specific than albumin or ATIII (not digested)











Colorado State Lymphoma Testing

- When do you run flow cytometry or PARR? PARR = Lymphoma PCR
- When not responding to therapy for either IBD or lymphoma and you want to confirm
 - Cats with lymphoma can have IBD pathology
- PARR shows 35% false negatives
- Flow cytometry
 Homogenous cell size indicates lymphoma
 Heterogenous cell size indicates IBD
- CSU Submission Form CSU Lymph Guide

INFECTIOUS DZ TESTING

Mira Vista - fungal antigen detection

- Aspergillus spp
- Histoplasma spp
- Coccidioides
- · Blastomyces
- Cryptococcus
- BD glucan

INFECTIOUS DZ TESTING

Pan American Vet Laboratories

• Pythium blood test (titer) - \$50

www.pavlab.com

• Pythium vaccine

Dr. Hansen used to be at Pan American Labs

- (405) 826-6710
- email rhansen@solidtechah.com

IMMUNOLOGIC TESTING

Protein Electrophoreses

Indication: globulins are high, but there is no known chronic antigenic stimulation (especially if hyperviscosity)

Two steps:

- 1. Protein electrophoresis quantitative
 - · monoclonal or polyclonal gammopathy?
- 2. Immunoelectrophoresis qualitative
 - which class presents in the monoclonal gammopathy? • Bence Jones protein in the urine (multiple myeloma)
 - Alpha, beta, and/or gamma immunoglobulin clones with other neoplasias

IMMUNOLOGIC TESTING

Protein Electrophoreses

- Ruling out: polyclonal gammopathy
 - neoplasia
 - infection (viral, chronic bacterial, parasitic, fungal)
 - · Immune mediated disease
 - Most common in cats

 - Chronic skin disease
 - Fungal infection
 - (FeLV, FIV, heartworm disease)
 - Most common in Dogs
 - · cutaneous parasites, pyoderma
 - heartworm disease
 - · Ehrlichiosis

BOX 12-2. CAUSES OF HYPERGLOBULINEMIA IN D Systemic lupus erythematosus (SLE), including glomerulonephritis, immune-mediated hemolytic anemi (IMHA) and thrombocytopenia (IMT), and polyarthritis* IMHA, IMT (not because of SLE)* Pemphigus complex, bullous pemphigoid* Rheumatoid arthritis*

POLYCLONAL

Pyoderma Bacterial endocarditis

Bacterial endocarditis
Viral
Feline infectious peritonitis (FIP)¹
Feline immunodeficiency virus (FIV)
Feline leukemia virus (FELV)
Feline leukemia virus (FELV)
Feline leukemia virus (FELV)
Fungal³
Systemic fungal infections (e.g., blastomycosis,
Netopiasmosis, coccidioidomycosis)
Rickettisil³
Ehrlichiosis
Parastic
Dirofilariasis ⁴
Dirofilariasis ⁴
Demodicosis
Scabies

Immune-Mediated Disease Infections (immune complex) Dirofilariasis*-† Feline cholangitis/cholangiohepatitis

Infection Ehrlichiosis^{1,1} Leishmaniasis

Leishmaniasis**
FIP (rare)
Idiopathic^{†,‡}
Benign monoclonal gammopathy

Miscellaneous Cutaneous amyloidosis Plasmacytic gastroenterocolitis

*Mild (4 to 5 g/dl). †Moderate (5 to 6 g/dl). ‡Severe (>6 g/dl). NOTE: Effect of age sho

IMMUNOLOGIC TESTING

Protein Electrophoreses

- Ruling In:
 - Monoclonal gammopathy from lymphoproliferative disorder
 - Multiple myeloma Bence Jones protein in the urine
 - B-cell Lymphomas
 - macroglobulinemia
 - · Rarely immunodeficiencies

Ehrlichiosis, Leishmania spp or autoimmune disease can rarely cause a monoclonal gammopathy

IMMUNOLOGIC TESTING

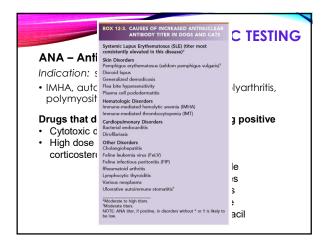
ANA - Antinuclear antibody

Indication: signs of SLE

• IMHA, autoimmune skin disease, ITP, polyarthritis, polymyositis, proteinuria, FUO, meningitis

Drugs that decrease ANA: Drugs causing positive

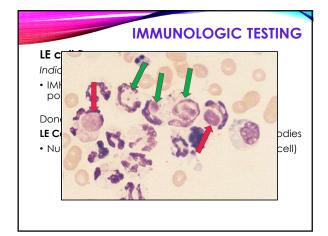
- Cytotoxic drugs
- · High dose or chronic corticosteroids
- ANA:
- Griseofulvin
- Hydralazine
- Procainamide Sulfonamides
- Tetracyclines
- Methimazole
- Propylthiouracil



IMMUNOLOGIC TESTING

ANA - Antinuclear antibody

- False positive due to systemic disease is often a low titer
- ANA titer due to autoimmune disease is often higher
- There is an area of overlap
- ANA must be paired with tissue sampling that supports auto-immune inflammation



IMMUNOLOGIC TESTING

LE cell Prep

Indication: signs of SLE

• IMHA, autoimmune skin disease, ITP, polyarthritis, polymyositis, proteinuria, FUO, meningitis

Done on blood, bone marrow or joint fluid

LE Cells – neutrophils with large, round purple LE bodies

- Nuclear proteins bound to ANAntibodies (dead cell)
- "ragocytes" have smaller inclusions
- 3-4 LE cells per slide are a positive
- 3 slides must be tested before pronounced negative
- Highly specific but low sensitivity for SLE
- False negative common when ANA is high titer

IMMUNOLOGIC TESTING

RF - Rheumatoid Factor

- low sensitivity and specificity for immune mediated arthritis
- Performed on serum



UC San Diego Neuromuscular Lab

- Muscle and nerve histopathology
- Immunohistochemistry
- 2M Ab Masticatory Myositis
- · Myasthenia Gravis titer
 - Acetycholine receptor Ab
- Lactate, pyruvate
- · Muscular dystrophy panel, immunoblot
- Myositis panel
- Sarcolemmal Ab, Antistiral Ab
- Video Consultation
- · DNA purification and storage

Submission Form

SUMMARY

PowerPoints

- vtaa.
- <u>.pdfs 1</u> and <u>6 slides</u> per page

Client Handouts

- Protecting the Immunocompromised Family Member
 - Avian Testing
 - Canine Testing
 - Feline Testing

SUMMARY

Laboratory Information

Auburn Molecular Diagnostics – <u>Submission Form</u>, <u>PCR</u> collection Kits

Colorado State U Immunology – <u>Flow Cytometry</u> <u>Collection</u>, <u>Submission Form</u>, <u>LSA Diagnostics Guide</u>

Cornell U FISH Analysis – <u>Electronic Payment</u>, Submission Form

HealthGene – <u>Congenital Dz Tests</u>, <u>Submission Form</u>, <u>Infectious Dz Tests</u>, <u>Infectious Dz Profiles</u>

Michigan State U Toxicology – <u>Client Information</u>, Submission Form, Test fee List

MiraVista Diagnostics (fungal) – <u>Submission Form</u>, <u>List</u> of Tests

SUMMARY

Laboratory Information

Optigen – Test Request Form, Vet Submission Form, Pricing, List of Tests

Pan American Lab – <u>Submission Form</u>

TAMU GI Lab – <u>Info Dysbiosis Index</u>, <u>Endocrinology</u> Submission Form, <u>GI Submission Form</u>, <u>Ship Instructions</u>

UC Davis Neuromuscular Lab - Submission Form

U of Tennessee Endocrinology – <u>Submission Form, List of Fees, Packaging Instructions, Electronic Payment Form, Submission & Test Protocols</u>

Category B Biohazard label

SUMMARY

Vet Handouts

- Blount Adrenal Testing in Dogs and Cats
- Baycom References on HbA1c Testing
- Willard Diagnostic Algorithm <u>Hyperglobulinemia</u>
- Willard Diagnostic Algorithm <u>Hyperthyroidism</u>
- Idexx Diagnostic Algorithm <u>SNAP TT4 Canine</u>
- Idexx Diagnostic Algorithm <u>SNAP TT4 Feline</u>
- U of Tennessee <u>Testing for Atypical Cushing's Dz</u>
- Willard <u>List of Recommended Outside Labs</u>

SUMMARY

Hidden Slides

Cardiac troponin

Test Protocol – High Dose Cortosyn
Test Protocol – ACTH stim with ACTH gel – dog, cat

Test Protocol – High Dose Dexamethasone Test

Test Protocol - Endogenous ACTH

HbA1c – diabetic control

IGF-1 Assay – Acromegaly, Pituitary Dwarf

Amended Insulin:Glucose – difficult insulinomas

Rheumatoid Factor

SUMMARY

Cases

- Greta Antithyroid antibodies
- Maddie thyroid breeder screen
- Eli hypothyroidism
- Benji Boy Hepatozoonosis

ACKNOWLEDGEMENTS

- **Richard Nelson.** Small Animal Clinical Diagnosis by Laboratory Methods, 5th Edition. Ch 12 Endocrine, Metabolic and Lipid Disorders. Eds. Michael Willard, Harold Tvedten.
- Mark C Johnson. Small Animal Clinical Diagnosis by Laboratory Methods, 5th Edition. Ch 12 – Immunologic and Plasma Protein Disorders. Eds. Michael Willard, Harold Tvedten.
- TAMU GU Lab http://vetmed.tamu.edu/gilab