Practical Bovine Clinical Pathology

Dr. Ed Shacklady, *Presiding*

E.H. Coles, *D.V.M., Department of Laboratory Medicine College of Veterinary Medicine, Kansas State University, Manhattan, Kansas 66506*

I. Introduction

A. Why use a clinical laboratory?

1. Requirments for a diagnosis - information from
   a. History
   b. Physical examination
   c. Laboratory data
   d. Radiology
   e. Biopsy
   f. Exploratory surgery
   g. Response to treatment
   h. Necropsy
   2. No single activity provides the diagnosis — need "logical sequence"
      a. History
      b. Physical findings
         (1) With the history — dictates needed additional data
      c. Laboratory findings
         (1) Selective tests — base on history and physical findings
         (2) Results may suggest need for additional or follow-up data.
      d. Other information collected as dictated by early findings.

II. Common laboratory procedures

A. Hemograms
   1. Helpful if cause of illness not immediately obvious
      a. Determine disease severity
      b. Determine disease duration
      c. Assist in prognosis
      d. Evaluate the response to treatment

B. Urinalysis
   1. Detection of renal disease
   2. Evaluation of extrarenal disease
   3. Prognostically and to evaluate treatment

C. Blood chemistries
   1. To detect abnormal organ function
   2. Detect unsuspected metabolic disturbances
   3. To evaluate therapy
   4. As a basis for prognosis

D. Cytology
   1. When there is fluid accumulation
      a. Thorax
      b. Abdomen
      c. Joint
      d. Subcutaneous or intramuscular
   2. Used to identify and differentiate among
      a. Infections (exudates)
      b. Inflammation (transudates)
      c. Neoplasia
   3. Cutaneous lesions
   4. As a part of the necropsy examination

E. Microbiology
   1. To confirm a diagnosis of bacterial or fungal disease
      a. Frequently submitted to a central laboratory
      b. Simple tests can be done in the practice laboratory
   2. To determine antibiotic sensitivity

F. Parasitology
   1. A part of most routine examinations (herd health)
   2. In any "poor doing" animal
   3. Skin diseases
   4. Enteric diseases

G. Cerebro-spinal fluid
   1. In animal with CNS disturbance

III. Abnormal vs. Normal data

A. What is "normal"? What is "abnormal"?
   1. Must standardize within practice and for the laboratory
   2. Published values usually serve as a base.
      a. Hematology
         (1) Leukocyte values well standardized for bovine
         (2) Erythrocyte values depend somewhat on locality
      b. Urinalysis
         (1) Well established — little influenced by environment or lab.
      c. Chemistries
         (1) Well standardized for most tests
         (2) Enzyme values variable according to the technique used and the unit basis for the test technique.
   3. Normal listings usually reported as 2 standard deviations from the mean.
      a. Interpret with caution.
   4. Evaluation of data may prove system or organ is normal or diseased.
      a. Abnormal values = obvious disease

b. Normal values = base that suggests normal function or that tests are not sufficently sensitive to detect abnormalities
(1) Just as important as abnormal findings.

IV. Collection of laboratory data

A. *Sample collection*

1. Hematology
   a. Use a good anticoagulant-EDTA is the best
   b. Standard collection tubes are the best (vacutainer or equivalent)
   c. Prepare smear for differential count if blood must be held for some time or blood parasites are suspected.
2. Urinalysis
   a. Use a clean container
   b. Catheterized specimen best if possible
   c. Fresh sample needed — provides most reliable results
3. Chemistry
   a. Serum is the best
      (1) Remove cells as soon as possible-failure to do so will alter results.
      (2) Anticoagulants will interefere with some test results
      (3) Most commercial laboratories cannot do tests on plasma as it cannot be used in their automated equipment.
4. Cytology
   a. Always collect aseptically
5. Bacteriology
   a. Always collect as aseptically as possible
   b. Refrigerate or freeze specimens as soon as possible.
   c. Specimens representative of the disease process
   d. Collect before use of animicrobial drugs if possible
   e. Examine as soon as possible after collection
6. Parasitology
   a. Collect feces from rectum if possible
   b. Examine soon
   c. If must hold — refrigerate or preserve with 10% formalin
7. Cerebro-spinal fluid
   a. Collect from cisterna magna
   b. Collect with surgical asepsis

B. *Therapy and laboratory data*

1. Collect first specimen prior to therapy
   a. Drugs may alter results even though patient condition is unchanged
   b. Even with knowledge of drugs used, results difficult to interpret
   c. Some drugs interfere with absorbance peaks in spectrophotometers
   d. Drug metabolic products may interfere
2. Wrong data — worse than no data
3. Solution to drug interference problem
   a. Prevent by withholding treatment (not always a good choice)
   b. Wait until effects of drug reduced or gone (often too late then)
   c. If animal needs immediate treatment and you want lab data or even think you may want it — collect samples before treating
      (1) Collect both clotted and unclotted samples.
      (2) Sometimes advisable to subsample if further tests are indicated by the original results.

C. *"Do it yourself" vs commercial laboratory*

1. "Do it yourself"
   a. Advantages
      (1) Tests done quickly
      (2) Cost may be less
      (3) Familiar with quality and reliability of own laboratory
   b. Disadvantages
      (1) May not have as much capability
      (2) Increases overhead
      (3) Difficult to predict quantity of tests done — outdated chemicals
      (4) Experienced technical help may be required
      (5) Personnel changes may influence results
2. Commercial laboratory
   a. Advantages
      (1) Capable of doing most tests needed
      (2) Good reproducibility
      (3) Good quality control
      (4) May be less costly per test
      (5) Permanent record available
      (6) May have a resident pathologist
      (7) If used by many veterinarians = good normals established
   b. Disadvantages
      (1) Human labs may not understand veterinary problems
      (2) "Stat" requests may mean extra charges
      (3) Delay in analysis may affect results
      (4) Delay in getting results may invalidate need for test
      (5) Some tests useful in man not reliable or useful in bovine
      (6) Normal bovine values may be enough different than man that automated system cannot handle
      (7) Enzyme units not standardized = each laboratory a different normal
      (8) Some labs not willing to modify techniques to accommodate bovine samples.

D. *The hospital laboratory*

1. Tests available in every bovine practitioners laboratory
   a. Microhematocrit and hemoglobin

b. Total leukocyte count
c. Differential leukocyte count
d. Platelet estimation
e. Total protein and fibrinogen (gamma globulin estimation)
f. Parasitology
g. Cytology
h. Chemistries
(1) Urea nitrogen (Creatinine)
(2) Glucose
(3) Chloride(particularly dairy practices)
(4) Total CO_2
i. Urinalysis

2. Tests that would be "nice to have"
a. Bacteriology and sensitivity testing
b. Chemistries
(1) Sodium and potassium
(2) Calcium and phosphorous
(3) CPK
(4) SGOT or SDH
(5) Blood gases, pH, and HCO_3
(6) Serum albumin

3. Office diagnostic systems for chemistries
a. MUST HAVE ABILITY TO VARY WAVELENGTH
b. Procedures of "cook-book" type
c. Should have quality control program
d. Reagents with long shelf life
e. Test method useful on bovine blood
f. Reasonable per test cost
g. Available service for unit.

4. Semi-quantitative screening tests
a. Have a real place in bovine practice
(1) Quick
(2) Inexpensive instrumentation
(3) Easy method for following course of an illness
b. Must use with caution as
(1) May get false results
(2) Absolute accuracy impossible — only drastic changes detected
(3) May require visual matching = poor reproducibility.

V. Hematology

A. *Evaluation of anemia*

1. Degree
a. Must have hemoglobin and packed cell volume
b. May also require a total erythrocyte count

2. RBC characteristics
a. Mean corpuscular volume (MCV)
(1) Requires PCV and total RBC
(2) Macrocytic = often is regenerative anemia
(3) Microcytic = usually Fe deficiency
(4) Normocytic = frequently non regenerative
b. Mean corpuscular hemoglobin concentration (MCHC)
(1) Requires hemoglobin and PCV
(2) Less than normal = hypochromia = lack of Fe
(3) Normal = normochromia = normal Hb = seen in non regenerative anemias
(4) Greater than normal = cannot occur = may indicate intravascular hemolysis or presence of abnormal substance in plasma that makes Hb read higher than it should be.
c. Staining characteristics
(1) Polychromasia (some RBC grey or blue) = reticulocytes in blood = seen in regenerative anemias
(2) Hypochromia (light staining cells) = Fe deficiency
(3) Basophilic stippling (RBC with dots) = often regenerative sign
(4) RBC parasites
d. Morphology
(1) Anisocytosis (variation in size) some is normal in bovine if excessive may indicate regeneration with macrocytes present
(2) Poikilocytosis (variation in shape) = abnormal RBC destruction or production

3. Regenerative vs. non-regenerative anemia
a. Regenerative
(1) Blood loss
(2) Hemolytic
b. Non-regenerative
(1) Nutritional
(2) Hypoplastic marrow
(3) Chronic disease
c. Characteristics of regenerative
(1) MCV = usually increased
(2) MCHC = variable but frequently decreased
(3) Staining characteristics
(a) Polychromasia
(b) Basophilic stippling
(4) RBC morphology
(a) Anisocytosis usually increased
(b) Microcytes may be present
(5) Reticulocytosis present
d. Characteristics of non-regenerative anemia
(1) MCV = usually normal (occasionally may be decreased)
(2) MCHC = usually normal (occasionally decreased)
(3) No polychromasia or basophilic stippling
(4) No reticulocyte response
(5) Anisocytosis usually absent or within normal limits for bovine

B. *Evaluation of leukocyte response*

1. Total leukocyte counts
2. Differential leukocyte counts
a. Absolute vs. relative values

(1) Impossible to interpret differential with % distribution only.

3. What is normal? What is abnormal?
   a. Leukocytosis = total WBC greater than 12,000
   b. Leukopenia + total WBC less than 4,000
   c. Neutrophilia = greater than 4,000 total neutrophils including bands
   d. Neutropenia = less than 1,000 (Schalm says 600)
   e. Left shift = more than 200 bands or younger cells of neutrophil series
   f. Lymphocytosis = more than 7,500 lymphocytes
   g. Lymphopenia = less than 3,000 (Schalm says 2500)
   h. Monocytosis = more than 850
   i. Eosinophilia = more than 1,500
4. Typical bovine response
   a. Frequently not a great increase in total WBC's
   b. Inversion of lymphocyte to neutrophil ratio, frequently with more neutrophils than lymphocytes
   c. Cattle have a small marrow reserve so acute infection frequently have a neutropenia with a left shift
   d. Any significant stress reaction will reduce lymphocytes and eosinophils as well as monocytes while the number of neutrophils is increased.
5. Use of fibrinogen to evaluate the degree of inflammation
   a. Fibrinogen increases with inflammation
   b. To differentiate absolute increase from increase due to dehydration calculate the total serum protein to fibrinogen ratio (TP/F)
   c. TP/F of 15:1 or greater = Normal
   d. TP/F 10:1 or less = absolute fibrinogen increase

VI. Clinical Chemistries

A. *Renal profiles*
1. Indications for
   a. Polyuria and polydipsia
   b. Suspected urethral obstruction and/or ruptured bladder
   c. Routine part of physical examination in patient with non specific clinical signs or in older patients.
   d. Hematuria or hemoglobinuria.
2. Should include
   a. Minimal
      (1) Urea nitrogen
      (2) Urinalysis
      (3) CBC to include fibrinogen
   b. Additional tests
      (1) Creatinine
      (2) Serum electrolytes
3. Urinalysis
   a. Occult Blood
      (1) Detects intact RBC
      (2) Detects free hemoglobin
      (3) Ascorbic acid inhibits
   b. Ketostix
      (1) Also use for Blood (Read in 15 seconds)
      (2) False negative with bacterial destruction
   c. Protein
      (1) False positive if
         (a) High alkaline urine
         (b) Highly buffered urine
         (c) Contaminated with Quaternary ammonia
   d. Bilirubin
      (1) False negative-sunlight exposure
      (2) False positive-phenothiazine type tranquilizer
   e. pH
      (1) Altered by bacterial action
      (2) Bilirubin pigments may mask
   f. Refractometer for specific gravity
      (1) Usually accurate
      (2) Cheap units frequently are not temperature corrected
      (3) In cloudy urines readings may be too high
         (a) Centrifuge to remove and redo on the supernatant.
4. Rapid methods for BUN
   a. Azostix (Ames) useful range of 10-60 mg/dl
      (1) False low from too much washing, delayed color matching, excess anticoagulants, unknown reasons.
      (2) False high from ammonia contamination
      (3) Useful in detecting unsuspected elevations
      (4) Use more sensitive technique if clinical signs suggest renal disease
   b. Urograph (BUN-O-Graph) useful range to 75 mg/dl
      (1) False low — strips too old.
      (2) False high — Ammonia or smoke contamination
      (3) Drafts may influence
      (4) Useful if UN not too high or as initial test to detect reduced renal function.

B *Liver profiles*
1. Indications for
   a. Unexplained icterus
   b. Edema and emaciation
   c. Suspect photosensitization
   d. Abdominal pain
   e. Suspect "fat cow syndrome"
2. Should include
   a. Minimal
      (1) Total and direct reacting bilirubin
      (2) CBC to include fibrinogen
      (3) Total protein an albumin
      (4) Urine bilirubin (urinalysis)

b. Additional tests
(1) Serum enzymes (GOT or preferably SDH)
(2) BSP clearance — determine T½

3. Serum globulin screening test
a. Sodium sulfite precipitation test.
(1) 3 solutions sodium sulfite — 14, 16 & 18%
(2) Put 1.9 ml of each concentration ito individual tube
(3) Add 0.1 ml serum to each tube
(4) Let stand 1 hour at room temperature
(5) Interpretation

Ig Concentration	Sodium Sulfite Concentration (%)		
	14	16	18
<500 mg/dl	–	–	+
500-1000 mg/dl	–	+	+
>1500 mg/dl	+	+	+

Deficient = less than 500 mg/dl

C. *Digestive disturbances profiles* for
1. Indications for
a. Clinical signs compatible with a disease of the digestive system to include abomasal displacement, ruman impaction, bloat etc.
2. Should include
a. Minimal
(1) CBC to include fibrinogen
(2) Total CO_2 or HCO_3
(3) Chloride
(4) Total protein
(5) Urinalysis (at least the chemical protion)
b. Additional tests
(1) Blood pH and PCO_2
(2) Cytology on peritoneal fluid
(3) Urea nitrogen
(4) Sodium and potassium
(5) Fecal occult blood
(6) Serum enzymes (GOT or SDH)
(7) BSP clearance

D. *Metabolic disturbances* — profiles for
1. Indications for
a. Clinical signs compatible with such diseases as parturient paresis, "downer cow", fat cow syndrome, in herds with reproductive problems, acetonemia, etc.
2. Should include
a. Minimal (selection of one or more of these tests may depend on the clinical signs)
(1) Calcium and phosphorus
(2) Glucose
(3) Ketonemia (usually urine ketone test and milk ketones)
(4) Liver function profile
b. Additional tests
(1) GOT or CPK as prognostic tests in "downer cow"
(2) Serum electrolytes
(3) CSF examination

VII. Cytology

A. *Introduction*
1. Useful in:
a. Differentiation between inflammation and suppuration
b. Detection of infectious agent
c. Diagnosis of neoplasia
2. Advantages
a. Gives rapid assessment
b. Easily done with minimum, equipment and time invested
c. Will often provide a definitive diagnosis
d. Provides information as to type of tissue reaction
(1) Inflammatory or noninflammatory
(2) Allergic
(3) Neoplastic
e. May identify a potential zoonotic disease

B. *Specimen collection*
1. Always done as aseptically as possible
2. Should be representative of the lesion
a. If pyogenic infection collect from edges of lesion
b. May require more than one specimen collection
3. Fluids
a. Abdominal cavity
(1) Use midline approach — 1-2 inches caudal to xyphoid cartilage
(2) Use needle long and large enough (14-16 ga. 2 in.)
(3) As exudtes may clot use EDTA tube
(4) Introduce needle with syringe attached and use gentle suction — may have to rotate needle
b. Thoracic cavity
(1) Auscultate thorax to determine fluid level line
(2) Introduce needle (14-16 ga 2-3 in) between ribs
(3) If fluid does not flow use gentle suction with syringe
c. Synovial fluid
(1) Use needle with stylet and short bevel (18 ga 1-2 in)
(2) Using aseptic techniques introduce needle, remove stylet and attach syringe — remove 5-10 ml fluid

C. *Methods for slide preparations* (Prepare as soon as possible)
1. All slides should provide a thin film

2. Most smears are prepared from unconcentrated material except for some fluids.
   a. Centrifuged sediment may be used to concentrate particulate matter but it negates ability to estimate numbers of cells or microorganisms
   b. Centrifugation may distort cells.
3. Swabs
   a. If cultural examination indicated, complete media inoculation first
   b. Gently but firmly roll swab over surface of clean glass slide.
      (1) If material thick and tenacious place a drop of saline on slide
4. Fluids
   a. Prepare thin fluids as for a blood film
      (1) Microorganisms tend to accumulate in the "feathered edge"
   b. Viscid fluids may present a problem
      (1) Spread material slowly using a "pusher" slide
      (2) If very viscid make a "squash" preparation
   c. Fluids containing fibrin necessitate a "squash" preparation
5. Impression smears
   a. From necropsy and biopsy tissues
      (1) If surface moist or bloody remove by gently touching surface to absorbent paper
      (2) Gently touch tissue to surface of clean glass slide
      (3) Several touch preparations can be made on single slide
      (4) If only small fragments of tissue (as in punch biopsy) a "squash" preparation is usually made
   b. From hair and skin scrapings
      (1) If bacterial disease suspected suspend material in saline on slide
      (2) If mycotic infection suspected place material on slide in a few drops of 10% potassium hydroxide, add coverglass, heat gently, and examine.

D. *Staining and examination*
1. Fixation — will depend on the staining technique used
2. Commonly used stains
   a. New methylene blue
   b. Wright's or other Romanowsky stain (dip stains are of this type)
   c. Gram
   d. Acid-fast
   e. Modified acid-fast
3. Microscopic examination
   a. Scan with low power to get overall impression
   b. Use oil-immersion objective for detailed examination
   c. Examine feathered edge carefully
4. Evaluation report should include:
   a. Predominant cell types
   b. Morphology of any organisms observed
   c. Staining characteristics of any organisms (if Gram stain used)
   d. Estimate as to the number of organisms
   e. Tentative identification of infecting agent if present
   f. Presence of inclusions

E. *Interpretation* — microscopic observation
1. Purulent or draining lesions
   a. Leukocytes
      (1) If induced by toxin producing agent-cells are frequently degenerate
      (2) Nonseptic lesions- leukocytes appear relatively normal
   b. If chronic lesions-lymphocytes, plasma cells, macrophages and possible epitheloid cells if granulomatous
   c. Microorganisms
      (1) Note morphology of individual cells and the arrangement of the cells
      (2) If Gram stain used note reaction
2. Fluid accumulations
   a. Leukocytes- same characteristics as in purulent or draining lesions
   b. Note presence of unusual cells and the number-describe the cell
   c. Note presence of microorganisms and describe
   d. Indicate whether exudate or transudate
3. Suspected neoplasia = biopsies or impressions
   a. Describe cell types present and indicate estimate of malignancy
4. Nasal or tracheal lavages or lavages from other areas
   a. Cell type important
      (1) Leukocytes well preserved = nontoxic
      (2) Leukocytes distorted = probably septic
      (3) Eosinophils predominate = allergy
      (4) Microorganisms noted and morphology given.
5. Milk
   a. Note cell type and numbers
   b. Note microorganism present
      (1) Staphylococci
      (2) Streptococci
      (3) Rods
         (a) If very small, plomorphic, arranged in clusters = corynebacteria
         (b) If small, slender = probably Gram-negative
         (c) If large and club shaped = Clostridia
         (d) If large, sporulated, parallel side = Bacillus
   c. Gram stain may be helpful but getting good one

on milk is difficult

6. Synovial fluid
   a. Normal fluid transparent, pale yellow, free of flocculent material
   b. With degenerative disease fluid frequently flocculent
   c. Blood may appear — need to differentiate between traumatic tap and joint hemorrhage
      (1) Traumatic = usually blood streaked
      (2) Hemorrhage into joint = usually evenly bloody
      (3) Centrifuge— yellow supernatant usually indicates joint hemorrhage and cell breakdown
   d. Normal joint fluids and from degenerative joint diseases usually do not clot, acute to subacute and chronic septic or infectious arthritis usually clot
   e. Total cell counts and differential cell counts should be done
      (1) Traumatic arthritis = transudative effusion in which predominant cell is lymphocyte or monocytes
      (2) Septic arthritis = large cell count with majority of cells neutrophils
   f. Chemical examination
      (1) Mucin clot
         (a) Add one ml of synovial fluid to 4 ml of distilled water to which had been added 0.1 ml of 7N glacial acetic acid
         (b) N = tight ropy clump in clear solution
         Fair = soft mass in slightly turbid solution
         Poor = a small mass in a turbid solution
         Very poor = a few flecks in a turbid solution.
         (c) Mucin clot usually normal with trauma or degenerative diseases
         (d) Abnormal clot with infectious arthritides

VIII. Microbiology

A. *General considerations*
   1. Collection
      a. Prior to treatment with antimicrobial agents
      b. From area where microorganisms most likely to be found
      c. Use sterile containers
      d. Provide laboratory with clinical information to guide their activities

      e. Specimens for anaerobic culture must be handled differently
         (1) Must reduce lethal effect of oxygen by filling container completely and sealing, OR use of vacutainer for liquids, OR immediate use of tube half filled with prereduced anerobically sterilized transport medium

B. *Handling specimens in laboratory*
   1. Plate or culture immediately on receipt
   2. Refrigeration acceptable for some specimens
      a. Wound, urogenital, throat, rectal swabs or fecal samples can be refigerated 2-3 hours without loss of pathogens
      b. Urine specimens can be held for 24-36 hours if refigerated
   3. After plating make smear for direct microscopic examination if such a procedure is indicated.

C. *Direct microscopic examination*
   1. Advantages
      a. Provides rapid evaluation and may establish a diagnosis or suggest a regimen for treatment
      b. Great time saver in some circumstances

D. *Selection of media and tests for identification*
   1. Blood agar plates (5% bovine or ovine blood)
      a. Inoculate to insure separated colonies
      b. Interpretation
         (1) Hemolysis will vary
            (a) No action
            (b) Complete (beta)
            (c) Incomplete (gamma) cells modified, discoloration around colony
            (d) Double zone combination of 2 and 3-target pattern
         (2) Additional characteristics as colony size, shape, color and consistency are also evident
   2. MacConkey agar — a selective and differential medium
      a. Inoculated along with blood agar plate
      b. Interpretation
         (1) No growth — bile salts prevent growth of Gram positive and some Gram negative microorganisms
         (2) Slow growth
         (3) Rapid growth
         (4) Colorless colonies = no lactose fermentation
         (5) Red to pink colonies = lactose fermentation
         (6) Colonies other colors = pigment produced by bacteria

E. *Reactions used for identification*
   1. Staining — Gram reaction
      a. Used standard ram stain — can purchase prepared
      b. Gram reaction using 3% KOH
         (1) Place 1 drop of 3% KOH on clean microscope slide
         (2) Mix a large loopful of the microorganisms in KOH
         (3) Gram positive bacteria will either clump or form a uniform suspension
         (4) Gram negative bacteria will form a

viscuous solution that strings out when teased with the loop.

2. Determination of morphology
   a. Shape, arrangement and internal structure are noted
3. Triple sugar iron agar — a screening biochemical medium
   a. Inoculate by streaking slant and stabbing butt
   b. Interpretation of reaction (slant/butt)
      (1) No change/no change (NC/NC)
      No growth or no action on substrates
      (2) Alkaline/no change (ALK/NC)
      Amino acid degradation on slant surface
      (3) Alkaline/acid (Alk/A)
      Glucose fermentation with amino acid degradation on surface
      (4) Alkaline/acid with H_2S (ALK/A + H_2S)
      Same as (3) but with H_2S From sulfur containing amino acids blackens the medium
      (5) Acid/acid (A/A)
      Lactose and/or sucrose and glucose fermentation
      (6) Acid/acid = gas (A/A = Gas)
      Same as (5) but CO_2 or other gas produced from sugars
      (7) Acid/acid +H_2S(A/A+H_2S) Same as (5) but H_2 additionally produced.
4. Catalase production
   a. Reagent = 3% hydrogen peroxide
   b. Method — a few drops of H_2O_2 on TSI slant
   c. Positive reaction = bubbling off of O_2
5. Coagulase production
   a. Reagent = rabbit plasma or horse plasma
   b. Rapid slide test
      (1) 1 drop of plasma on slide
      (2) Loopful of culture
      (3) Positive reaction = clumping
   c. Tube test
      (1) 0.5 ml of plasma in a tube
      (2) Loopful of culture
      (3) Incubate at 37°C for 4-12 hours
      (4) Positive reaction = coagulation
6. Indole production
   a. Medium = 1% tryptone broth
   b. Regents, xylene, or chloroform plus Ehrlich's reagent
   c. Inoculate broth and incubate then add solvent and shake add Ehrlich's reagent to layer between medium and solvent
   d. Positive reaction = red color in rign of Ehrlich's
7. Degradation of urea
   a. Medium — urea broth
   b. Positive reaction — urea degraded to ammonium ions with alkaline reaction and appearance of brilliant red color
8. Utilization of citrate
   a. Medium — sodium citrate as sole source of carbon — slant
   b. Inoculate by streaking and stabbing butt
   c. Positive reaction — citrate utilized, sodium released with production of an alkaline reaction = from green to blue
9. Motility determination
   a. By examining wet mount microscopically
   b. Use motility medium and stab
   c. Motility = growth out from the stab
10. Casein hydrolysis
    a. Medium contains 10% powdered milk
    b. Inoculate a slant heavily
    c. Positive reaction = clearing of the medium
12. Growth in 6.5% sodium chloride broth
    a. Growth of streptococci indicates fecal origin

F. *Bacteriological examination of mastitic milk*
   1. Streak on blood agar and MacConkey agar plates
   2. Do direct microscopic examination
   3. Identity of isolated bacteria
      a. Streptococci
         (1) Colonies are characteristically small, glistening, to rough, transparent to pale white
            (a) Hemolysis on blood agar is variable from none through alpha to beta
         (2) CAMP test
         (3) Edward's medium containing esculin
      b. Staphylococci
         (1) Colonies are characteristically medium size, white to yellow or orange, opaque, glistening
         (2) Hemolysis usually complete, occasionlly double zone, sometimes no hemolysis
         (3) Confirm by using coagulase test
      c. Gram negative rods — frequently coliforms
         (1) On blood agar — medium sized colonies that are translucent, white to light grey, appear wet and hemolysis is variable
         (2) On MacConkey agar — if coliforms colonies are pink to red
         (3) Identify biochemically using TSI, indole, catalase, urea degradation
      d. Gram positive rods
         (1) Corynebacterium — colonies on blood agar very tiny glistening, white, usually hemolytic if pathogenic.
         (2) Appear as small coccobacillary rods arranged in palisades
         (3) Identify biochemically *(C. pyogenes)* catalase negative, casein hydrolysis positive
         (4) Other diphtheroids can be confused with *C. pyogenes*
         (5) Bacillus — usually a contaminant — very large colonies that are dry, rough, and some

very hemolytic — should usually be ignored

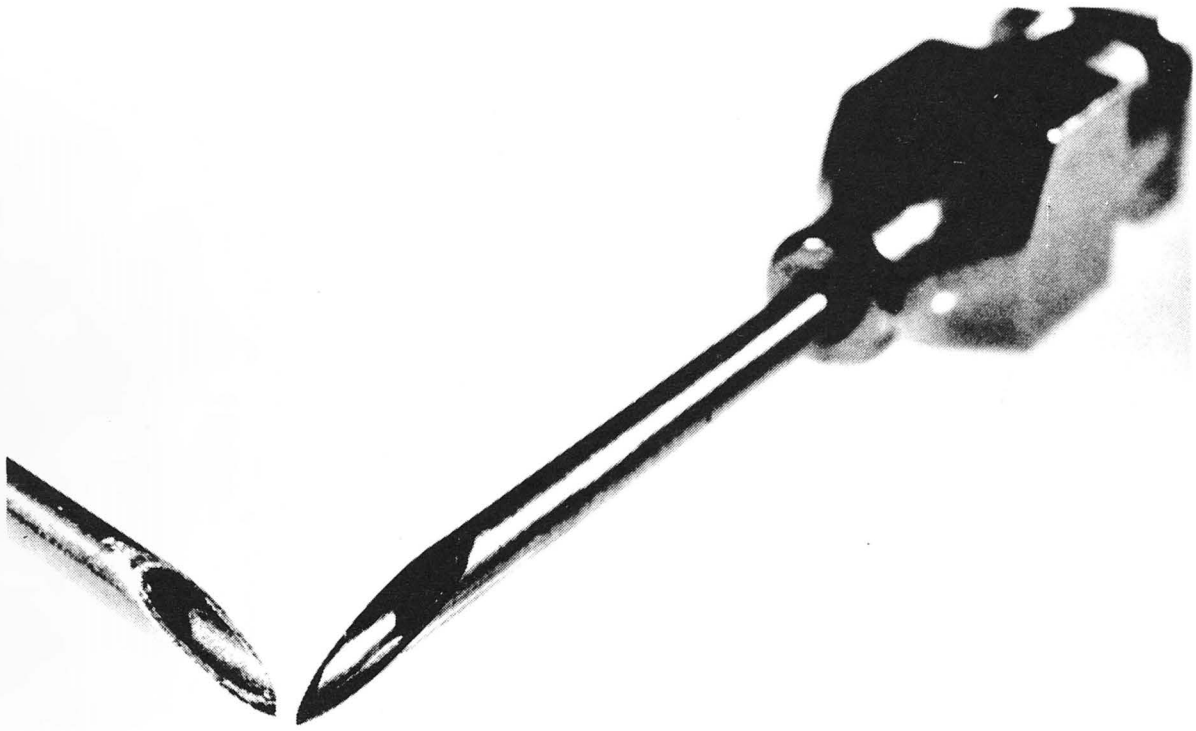
G. *Bacteriological examination for enteric diseases*
   1. Direct smear of feces may be indicated
      a. If clostridial type enterotoxemia almost a pure culture of club-shaped gram-positive large rods
      b. If coliform or Salmonella infection will have small, slender sometimes coccobacillary gram-negative rods.
   2. Culturing feces
      a. May be done directly on MacConkey agar
      b. May want to use enrichment medium as selenite or tetrathionate broth
   3. Coliforms
      a. Produce pink to red colony on MacConkey
      b. Identify by biochemical tests
   4. Salmonella
      a. Produce clear colorless colony on MacConkey
      b. Identify by biochemical tests or by serology

H. *Bacteriology — urinary tract infections*
   1. Sample before beginning any antibacterial therapy
   2. Collect urine as aseptically as possible
   3. Spread on ml of urine over sufrace of blood agar plate and do the same with a MacConkey agar plate
   4. Incubate, count colonies and note colony characteristics on both plates
   5. If coliforms must decide if they are contaminant or associated with renal disease.
   6. If Corynebacterium will have very small, shiny, colonies on blood agar plate
      a. May require more than 24 hours for them to become apparent
      b. Morphology characteristic of corynebacteria
      c. Identify as to species with urea degradation test
         (1) *C. renale* = rapid degradation of urea

I. *Antibiotic sensitivity testing*
   1. Must be done on a pure culture
   2. Best is by the Kirby-Bauer technique
      a. Use standard plates of Meuller-Hinton agar 6 mm deep
      b. Standardize broth or saline suspension of organisms from surface of agar
         (1) Should have same optical density as standard prepared by adding 0.5 ml of 1% sulfuric acid
      c. Dip sterile swab into standardized culture and wring out on lip of tube
      d. Swab surface of agar plate going in all directions
      e. Allow plate to dry
      f. Dispense antibiotic sensitivity discs and press them onto surface of agar
      g. Invert plate and incubate overnight at 37°C
      h. Measure size of zone of inhibition around disc and compare to standard table to evaluate.

The Hypodermic Needle that surpasses all others!!!!

The cislak *Smooth-point*® *Husky Hub*® Needle



Compare the **cislak** *Smooth-point*® with the competitor's needle
(unretouched photograph)

The stainless steel smooth-point needle with the chrome-plated, brass hub features:

Gentle, smooth point

Husky Hub - easy to grasp - will not slip

Fits Luer lock and Luer slip adaptors

Lower priced to save you money

Get your **cislak** *Smooth-point*® *Husky Hub*® NEEDLE from your favorite veterinary supplier. It is sold in popular gauges and lengths.

Names of Suppliers can be obtained from:

F.W. Cislak Manufacturing & Engineering Co., Inc.
5768 West 77th Street, Burbank, Illinois 60459

Telephone: 312—458-6163

® Trademark