Suggested Techniques and Clinical Pathology Information for the Veterinary Practitioner

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Medi-Chem*
Calcium/Magnesium Titer Set

Materials Required:

1. Micro-buret or serological pipette calibrated in 0.01 ml.
2. Pipettes: 0.5 ml volumetric. 1, 2, and 5 ml serological.
3. 50 ml beaker or flask.

Step-by-Step Procedure:
FOR CALCIUM:

1. Add 0.50 cc serum to a small Erlenmeyer flask or small beaker.
2. Add 5.0 cc distilled water.
3. Add 1.0 cc NaOH reagent (Sodium Hydroxide 0.75 N).
4. Add 1.0 cc indicator reagent (Eriochrome Blue SE 0.01% w/v). Solution turns a wine-red color.
5. Titrate using the EDTA solution reagent (EDTA Acid Disodium 0.0372% w/v) to a violet end-point. Note the buret reading. Save this solution if a Magnesium determination is desired.

FOR MAGNESIUM:

1. Refill the buret to a reference starting point with more EDTA solution.
2. To the solution from Step #5 of the Calcium determination, add 1.0 cc HCL reagent (Hydrochloric Acid 0.6 N) and 2.0 cc of the buffer reagent (Ammonium Chloride 0.83% w/v; Ammonium Hydroxide 12% v/v). The solution will again turn a wine red.
3. Titrate again to a violet end-point. Note the buret reading.

Sources of Error:

1. All glassware should be rinsed with distilled water free of metal ions.
2. Titrate with constant stirring using a white background.
3. It is advisable to titrate the standard provided with the set before attempting an unknown. This will accustom the technician to the end-point color changes.
4. Keep all solutions at room temperature with the caps tightly sealed.
5. If series determinations are to be done, do not wait longer than five minutes between the addition of the NaOH reagent and the beginning of the titration.
6. If serum is highly colored, it may be necessary to double the volume of indicator used in the procedure.

The manufacturer recommends that a lyophilized control serum be run with the above procedure as an additional measure of quality control. Control sera can be obtained from Metrix (registered trademark of Metrix Division of Armour Pharmaceutical Co., Chicago, Illinois) (Normal and Abnormal Clinical Chemistry Control). Following the given procedure carefully, the suggestions listed above, and good laboratory practice should result in reasonable accuracy and reproducibility.

Medi-Chem*
Wright's Dip Stat

Materials Required:

1. Microscope.
2. Slides and cover glasses.
3. Immersion oil.

Step-by-Step Procedure

1. Immerse a dried blood smear in Fixative (Methyl Alcohol, Absolute, Acetone-free) for 5 seconds in order to "fix" the smear. A longer period of time in the methanol does no harm.
2. Remove the "fixed" smear and immediately transfer to Eosinate Stain (Eosin Y 0.1% w/v; Methyl Alcohol 7.5% v/v; buffered) for approximately 6 seconds.
3. Remove the stained smear and immediately rinse with Rinsing Solution (Distilled Water pH 6.4-6.8).
4. Immediately immerse the washed smear in Polychrome Stain (Polychrome Methylene Blue

*Medi-Chem Division
Medical Chemical Corporation
1713 - 20th Street
Santa Monica, CA 90404
(213) 829-4304

0.1% w/v; Methyl Alcohol 7.5% v/v; buffered) for approximately 6 seconds.

5. Remove stained smear and rinse as in Step #3. Air-dry and examine under immersion oil. Label clearly.

Bendixen's Key for Classifying Cows Relative to Lymphosarcoma Based on Age and Absolute Lymphocyte Count*

Age in Years	Normal	Suspect	Positive
0-1	<10,000	10,000-12,000	>12,000
1-2	< 9,000	9,000-11,000	>11,000
2-3	< 7,500	7,500- 9,500	> 9,500
3-4	< 6,500	6,500- 8,500	> 8,500
4	< 5,000	5,000- 7,000	> 7,000

*Schalm, Oscar W. Veterinary Hematology. 1965. Second Edition. Lea & Febiger, Philadelphia. Page 488.

Reinsch Test for Heavy Metals** (Arsenic, Mercury, Antimony, Bismuth)

Materials and Reagents:

1. Macerated liver or urine containing heavy metal standards for positive control; and a negative macerated liver or urine for a blank control.
2. Hydrochloric acid (conc.).
3. 10% potassium cyanide.
4. 5% sodium sulfite (Na_2SO_3).
5. 15% nitric acid.
6. Bismuth test reagent: 1 gm of quinine sulfate is dissolved in 100 ml of 0.5% nitric acid; then dissolve 2 gm potassium iodide into solution.
7. Test tubes and rack, flasks, hot plate or water bath.
8. Copper - sheet 5 x 10 mm or copper wire (spiral).

Procedure:

1. Ten ml of macerated liver or kidney (kidney is best for mercury) or urine, gastric contents, etc., is placed in a small Erlenmeyer flask. At the same time, set up a positive and negative urine for control comparison.
2. Two ml of concentrated hydrochloric acid and a small copper sheet (5 x 10 mm) are added. The copper must be shiny clean to show clearly a contrast of a deposition of a heavy metal, when positive. If sheet copper is not available, a small copper wire spiral may be used.
3. Cover the flask with a watch glass and heat gently for about 1 hour; boiling is to be avoided to prevent too rapid evaporation. If large amounts (over 0.100 mg) are present, deposition will take place in less than 1 hour. In one hour, deposition will then demonstrate the small traces.

Heavy	Appearance of Copper	Sensitivity of Test (at least)
Mercury	shiny-silver deposit	0.050 mg/10 ml
Arsenic	dull black deposit	0.010 mg/10 ml
Bismuth	shiny black deposit	0.020 mg/10 ml
Antimony	dark purple sheen	0.020 mg/10 ml

Report amount present as: Negative, small, moderate, or large.

To differentiate the dark deposit from each other:

a. Mercury: Silver-self evident.
b. Arsenic: Dull black-place copper in a small test tube and add about 15 drops of 10% potassium cyanide. The dark deposit due to arsenic dissolves; large amounts of arsenic will require more cyanide to dissolve. Antimony or bismuth dark deposit *does not* dissolve. This test for arsenic is very sensitive; less than 0.010 mg can be detected.
c. Bismuth: Shiny black-place the copper and deposit into a small test tube and add about 15 drops of 5% sodium sulfite and 1 ml of 15% nitric acid. The deposit due to bismuth dissolves. Arsenic or antimony do not dissolve. To the dissolved bismuth solution add 1 ml of water and 1 ml of bismuth test reagent-orange turbidity. This test is specific and sensitive to 20 micrograms of bismuth.

   Bismuth originally takes a little longer to deposit on the copper than the others.
d. Antimony: Purple-shiny deposit-unchanged by both of the above treatments.

Test for Parathion**

1. Steam-distill 10 ml of vomitus or gastric contents and collect 10 ml of distillate.
2. Add 2 pellets of sodium hydroxide to this distillate and gently heat on a water bath.
3. A yellow color is positive for p-nitrophenol (a metabolite of parathion).
4. Report as negative, small, moderate, or large amounts.
5. This test is very sensitive to small, early exposure.
6. Early dramatic remission of signs and symptoms in response to atropine therapy is an excellent, sensitive biologic test.
7. Can place flies in container with rumen contents.

Tissues Saved for Examination

Bovine Fetus.

1. Lung, liver, and adrenal gland: a small piece is frozen for fluorescent antibody examination for IBR.
2. Kidney: saved for culturing.
3. Abomasal contents aspirated with a sterile syringe. A darkfield examination may be performed as well as bacterial culturing.

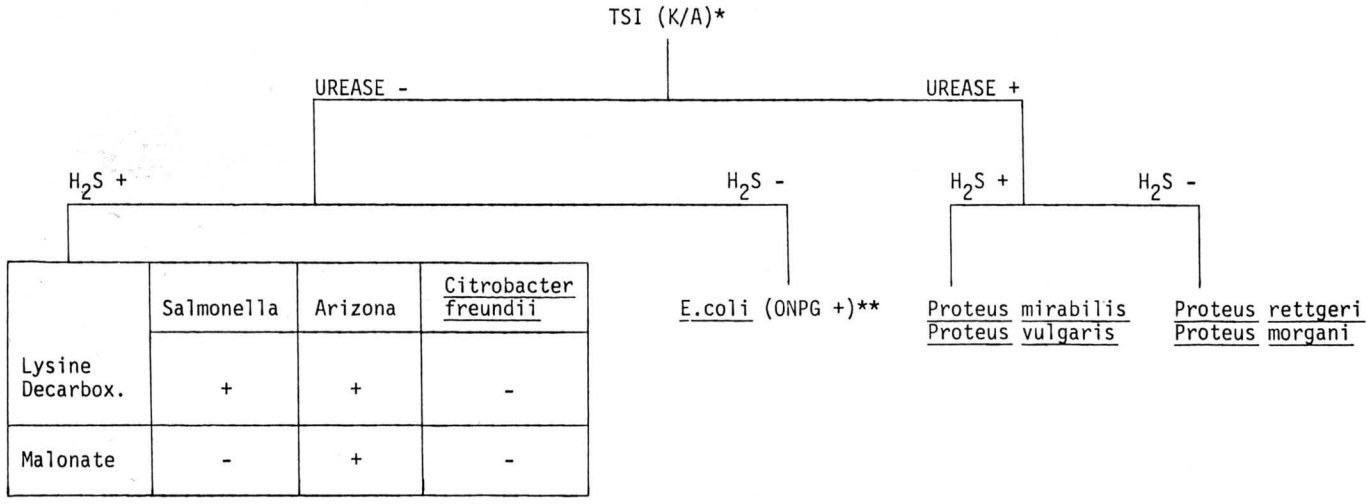
NOTE: Duplicate blood agar plates should be inoculated. One plate should be incubated in a 10% CO_2 environment, while the other may be incubated in a vented jar, the air being evacuated and replaced with a mixture of 80% nitrogen, 10% CO_2, and 10% O_2.

Calves Under Two Weeks of Age.

1. Kidney and spleen: saved for culturing.
2. Small intestine: Culturing for enteric pathogens.

**Kaye, Sidney. Rapid, Simple, Reliable Tests for Poisons. Laboratory Medicine. May 1972. pp. 28-41.

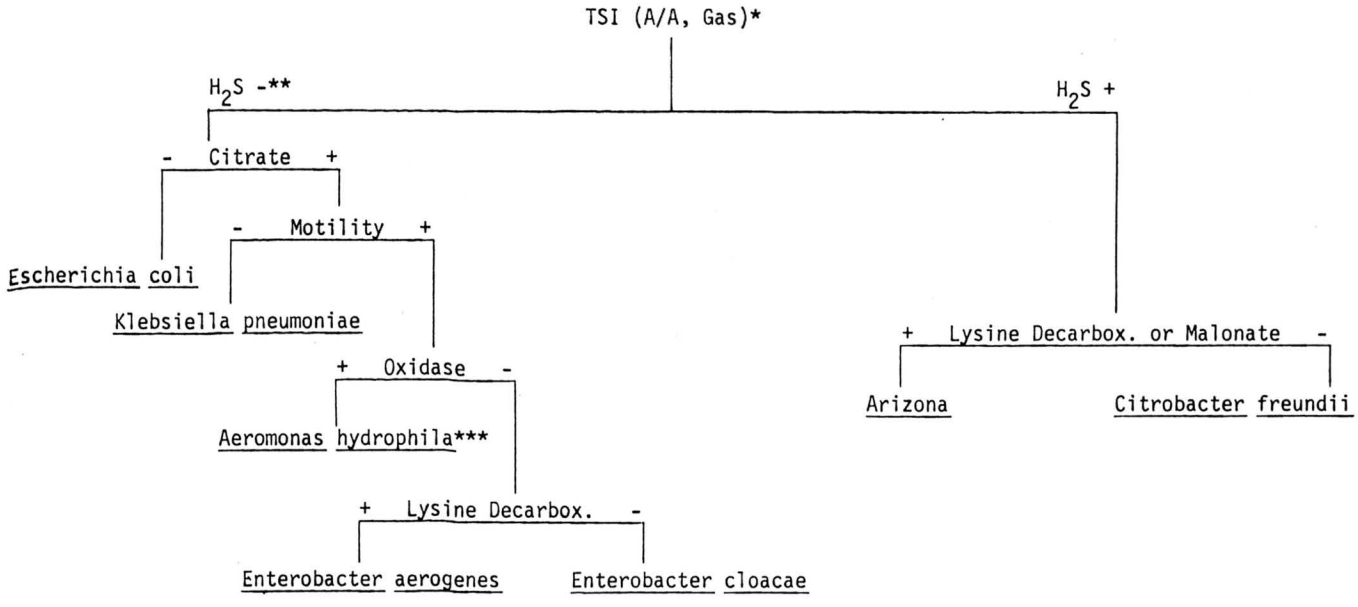
Identification of Selected Enterobacteriaceae



*K = Alkaline reaction (red) in "slant" portion of Triple Sugar Iron Agar tube.
A = Acid reaction (yellow) in "butt" portion of Triple Sugar Iron Agar tube.

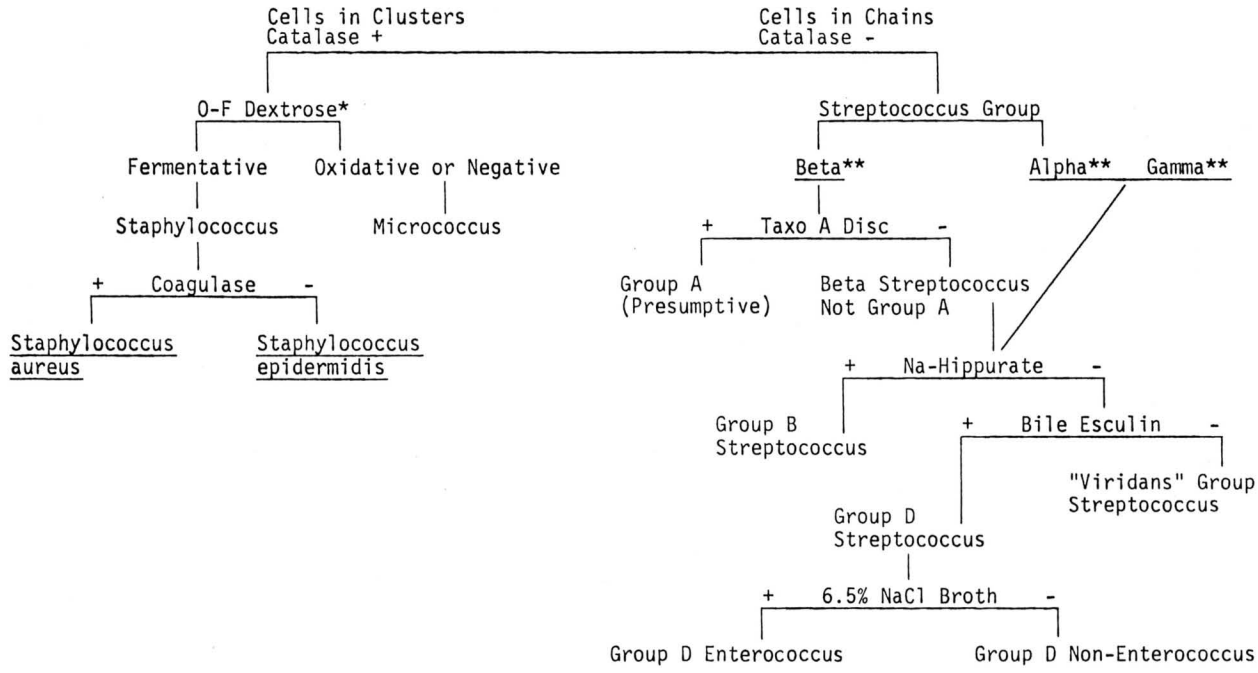
**ONPG = A test for the production of the enzyme "lactase." It is sometimes masked because of a deficiency in a second enzyme "permease" which allows the substrate to enter the bacterial cell.

Identification of Selected Enterobacteriaceae



* A = Acid reaction (yellow) throughout Triple Sugar Iron Agar tube.
** H_2S = Appears as a black precipitate in butt portion of TSI.
*** May resemble members of Enterobacteriaceae, although it does not belong to this group.

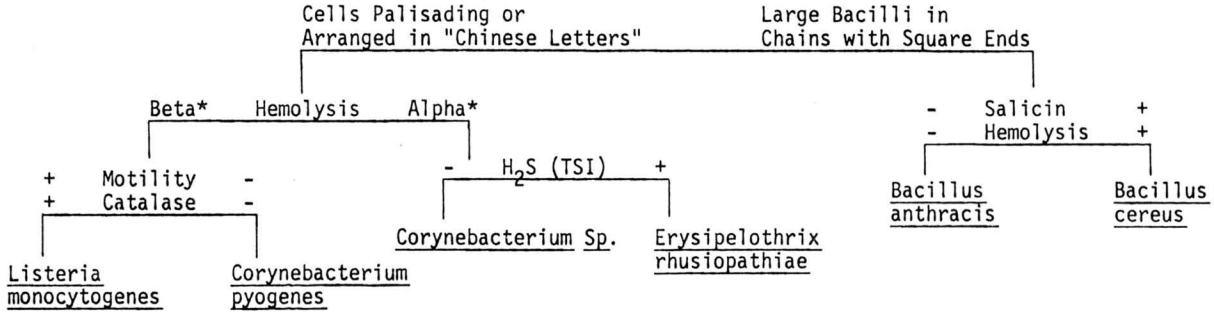
Identification of Aerobic Gram-Positive Bacilli



*Oxidative-Fermentative medium of Hugh and Liefson.

**Beta = Complete lysis of red blood cells around the colony.
Alpha = A partial hemolysis of red blood cells as evidenced by a green zone around the colony.
Gamma = Indicates no hemolysis.

Identification of Aerobic Gram-Positive Cocci



*Beta = Complete lysis of red blood cells around the colony.
Alpha = A partial hemolysis of red blood cells as evidenced by a green zone around the colony.

An intestinal smear is also made for FA examination for the Reo calf scour virus (CSVI).

3. Large intestine: Culturing for enteric pathogens. A small section of the large intestine is frozen for thin-sectioning and FA examination for the Corona calf scour virus (CSVII).

NOTE: Intestinal contents may be cultured on an anaerobic blood agar plate if necropsy findings indicate the possibility of enterotoxemia. Also, intestinal contents should be inoculated into a potentiating medium such as tetrathionate or selenite if Salmonellosis is suspected.

Normal Values*

		Cattle		
Test	Units	Newborn Range	Yearling Range	Adult** Range
Ca	Mg%	(10.7) 9.7-11.7	(10.7) 9.5-10.7	(8.9) 8.1-9.8
In. P	Mg%	(6.9) 5.3-8.5	(6.7) 5.8-7.6	(6.7) 5.4-8.0
Glu.	Mg%	(67) 40-112	(87) 65-108	(81) 61-102
BUN	Mg%	(19) 4-26	(13) 8-18	(20) 16-25
Uric Acid	Mg%	(1.6) 0.9-2.6	(1.2) 0.9-1.5	(1.2) 0.9-1.5
Chol.	Mg%	(47) 33-61	(103) 83-123	(118) 87-149
T.P.	Gm%	(5.7) 4.0-7.4	(7.4) 6.9-7.9	(8.3) 7.6-8.9
Alb.	Gm%	(2.3) 1.9-2.8	(3.5) 3.1-3.9	(3.1) 2.4-3.7
T.B.	Mg%	(1.4) 0.2-2.7	(0.27) 0.1-0.4	(0.45) 0.2-0.7
Alk. Phos.	mU/ml	(416) 151-681	(152) 84-220	(82) 28-227
L.D.H.	mU/ml	(712) 483-941	(1155) 960-1350	(694) 486-903
SGOT	mU/ml	(114) 50-178	(113) 83-143	(103) 85-103
Na	Meq/L			(142) 132-152
K	Meq/L			(4.8) 3.9-5.8
Cl	Meq/L			(104) 97-111
Hgb.	Gm%	11.9	11.7-14.0	(12.9) 11.0-14.0
Hct.	%	35.7	32-38	(35.3) 32-38
RBC	mm^3			(7.67) 6.5-8.5
WBC	mm^3	10.2	7.6-13.0	(10.3) 7.5-13.0

*Intermountain Laboratories, Veterinary Pathology Service, Salt Lake City, Utah.
**Non-lactating range cattle.

Quick Gram Stain

Reagents:

1. Crystal Violet: 1% aqueous solution.
2. Iodine Solution: 1 gm Iodine crystals; 2 gm KI; made up to 300 ml with distilled water.
3. Acetone-Alcohol Decolorizer: equal parts acetone and alcohol.
4. Safranin: 2% aqueous solution of Safranin O.

Procedure:

1. Make a smear of the bacteria and heat-fix.
2. Flood slide with Crystal Violet and allow to stand for 10 seconds.
3. Wash off Crystal Violet with Iodine solution. Let Iodine stand for 10 seconds.
4. Wash off Iodine with acetone-alcohol until no more Crystal Violet flows off slide (requires about 0.5-1.0 ml). Allow slide to dry (requires about 10-15 seconds).
5. Flood slide with Safranin solution. Allow to stand for 10 seconds (1-2 minutes for Campylobacter).
6. Wash slide in water, dry, and read. NOTE: This is the only time water is used in the procedure.

Negative Stain for Treponema

Reagents:

1. 1% aqueous solution of Congo Red.
2. Acid-Alcohol solution (97 ml of 85% ETOH; 3 ml of concentrated HCl).

Procedure:

1. Place 1 drop of Congo Red solution on a clean glass slide.
2. Make smear of intestinal contents in the drop of Congo Red. Allow to air-dry.
3. Flood slide with acid-alcohol solution. Continue to flood slide until color change (red to blue) is complete. NOTE: Organisms will appear white against a blue background. Avoid making the original smear too heavy.

New Methylene Blue***

New Methylene Blue Powder	0.5 gm
Potassium Oxalate	1.6 gm
Distilled Water	100.0 ml

Staining Procedures:

I. 1. Mix equal parts of whole blood and stain in a small test tube and allow to react 15-20 minutes.
   2. Prepare a film on a slide.
   3. Examine.

II. 1. Make smear of tissue and let air-dry.
   2. Add a drop of stain.
   3. Cover slip and examine.

***Coles, Embert H. 1974. Veterinary Clinical Pathology. 2nd Edition. W. B. Saunders Company.