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UNIVERSITY OF MINNESOTA

College of Veterinary Medicine

VETERINARY CONTINUING EDUCATION



ST. PAUL, MINNESOTA
UNITED STATES OF MINNESOTA

Practical Laboratory Techniques For Identifying Mastitis-Causing Organisms

**Peggy Karmaoui, BS
Ralph J. Farnsworth, DVM, MS**

Introduction

The process of identification of mastitis organisms can be very complicated if an attempt is made to take identity to genus and species for all the organisms which are involved in bovine intramammary infections. However, the identification of genus and degree of species necessary for working with herd problems can be accomplished relatively easily with a reasonable degree of accuracy using fairly simple laboratory techniques which are practical for the practice laboratory. This paper is an attempt to cover what the authors consider to be the most practical methods which still allow reasonable accuracy and economy.

Sampling

Samples

- iced or refrigerated for short-term
- long-term or refrigeration in question
- frozen

This may increase numbers of organisms isolated. Cannot do cell counts of frozen samples, but in many instances, cell counts are not needed.

Composite/Cow Samples vs. Quarter Samples

- probably a little less accurate but better economy
- can overcome accuracy problem somewhat by volume plated

Primary Organism Isolation

Blood agar derived from:

- sheep blood
- bovine too young or wrong sex to have mastitis

Advantage of blood vs differential & selective medias:

- more organisms grow
- in colony characteristics are more uniform and typical

Volume plated

- the more the better (can get up to .2 ml on a standard blood plate)

- In general, it is harder to achieve a high degree of accuracy without going to more involved numerous testing.
- MacConkeys media is differential and selective for coliform organisms
- Probably can differentiate about 75% of the E. coli, enterbacter., pseudomonas, and klebsiella
- In general, E. coli is dark pink; klebsiella is wet and mucoid looking with light pink colonies; pseudomonas is lactose (-) translucent or yellow and can be confirmed with the oxidase test;
- To go further, you can go into biochemical test or API kits

Actinobacillus or Coryne Bacterium

- Small colonies take 48 hours to grow on blood agar

Yeast

- Grow on blood agar but look like staph species - tend to grow slower
- Best method of isolation is to use Saburauds dextrose agar containing antibiotics
- Can easily confirm that these are yeast and not resistant bacteria by use of the microscope

Mycoplasma

- Very complicated - require special media, CO₂ incubation, and microscopic reading. Not very practical for practitioner/laboratory, plus mycoplasma is so rare in Minnesota. Its probably best to send them in.
- Need to keep in mind that the sample where bacteria doesn't grow is more likely to be a coliform than mycoplasma

Bulk Tank Culturing

- Most practical method is to use differential and selective media and put milk on in dilutions
- Can use blood agar and pick colonies for identification but is laborious
- We use blood, TKT, Staph Camp and MacConkeys media
- Frozen samples are most practical
- More than one day's tank sample increases accuracy
- Amount plated also increases accuracy
- We use .2 ml on a plate as the first dilution which equals a 1-5 dilution
- Kits are also available with media and spreader rods
- Automatic pipettes with disposable tips are also quite helpful

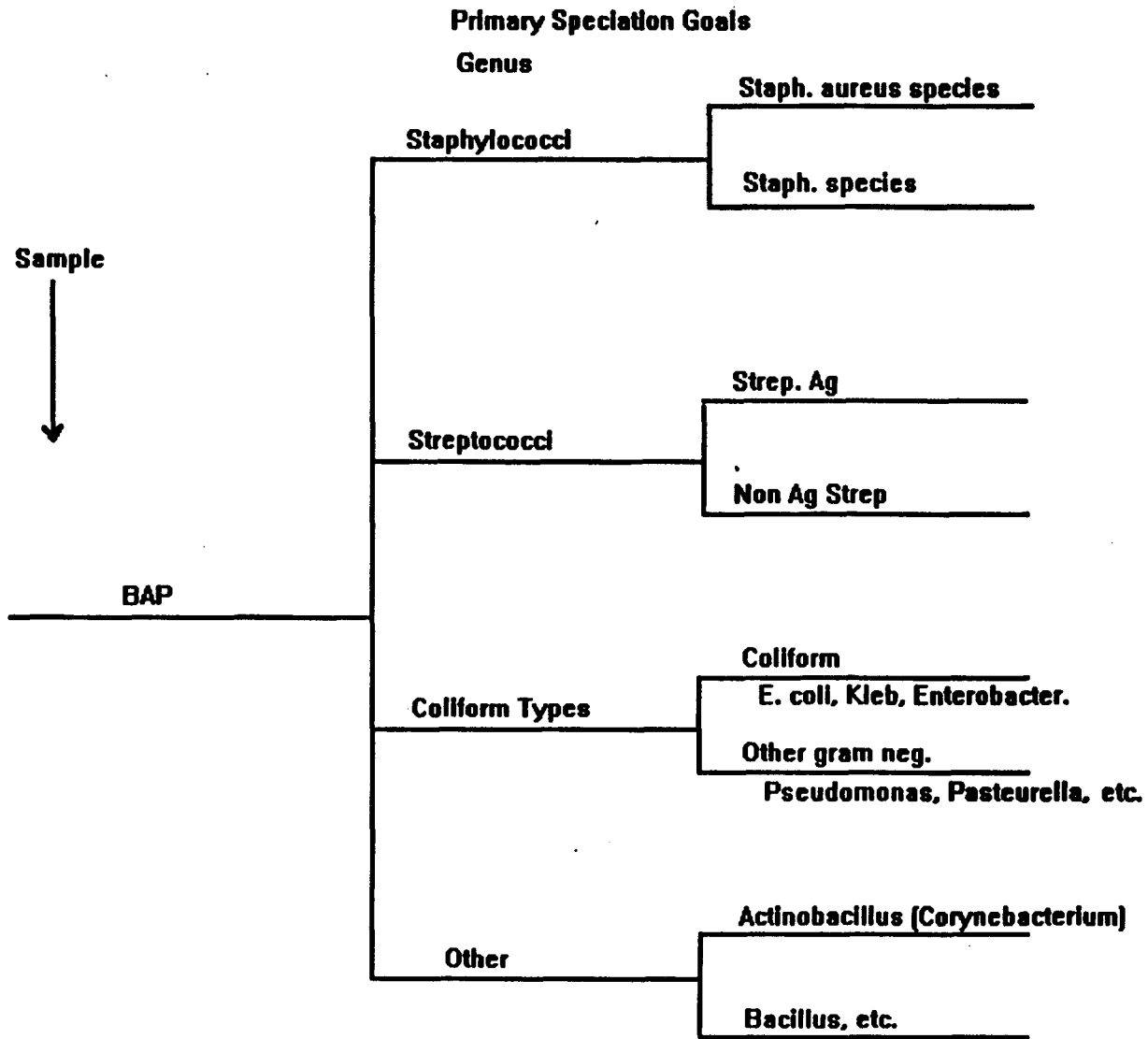


Figure 1

**Colony Characteristics on Primary Plate
and Secondary Plates**

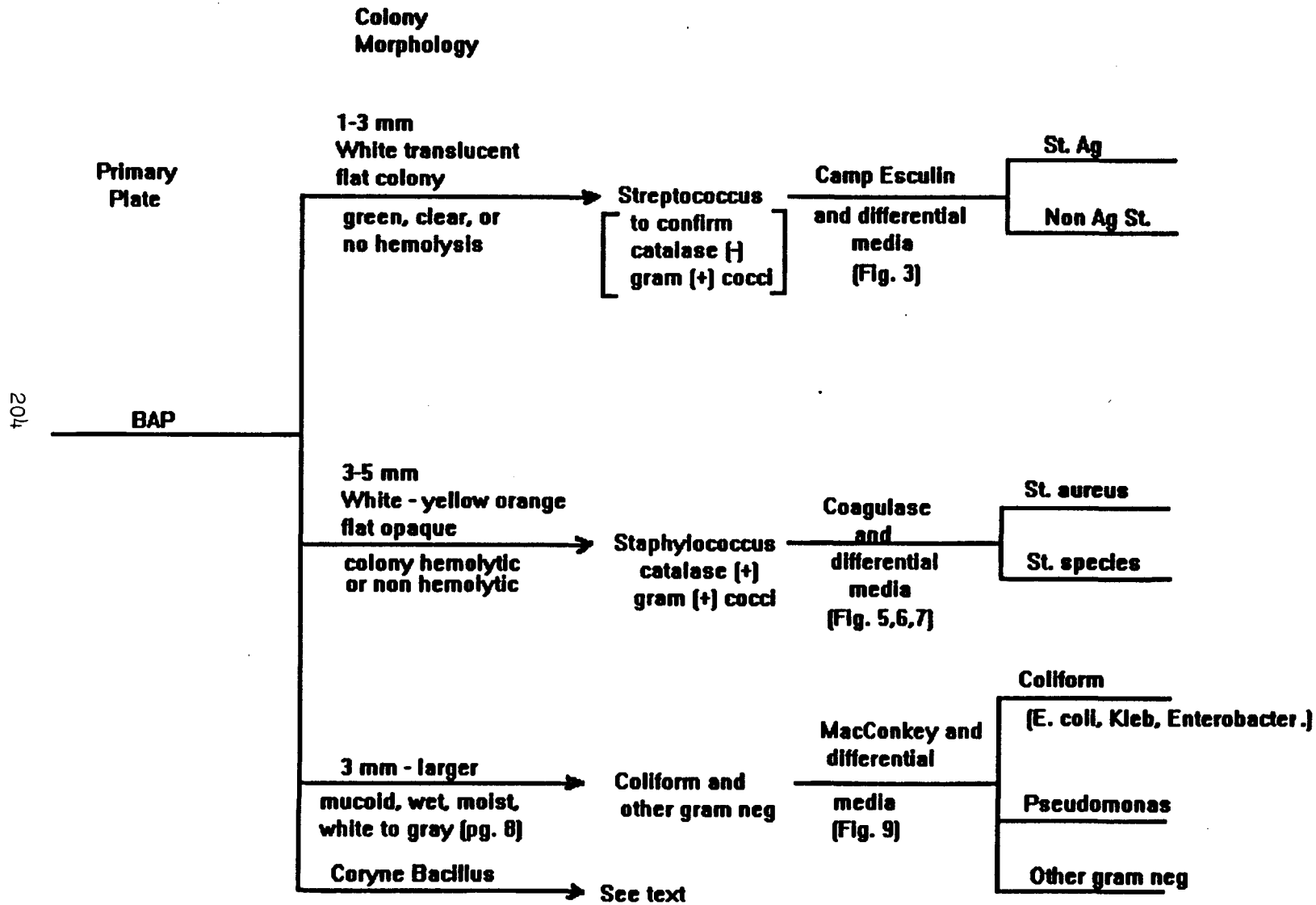
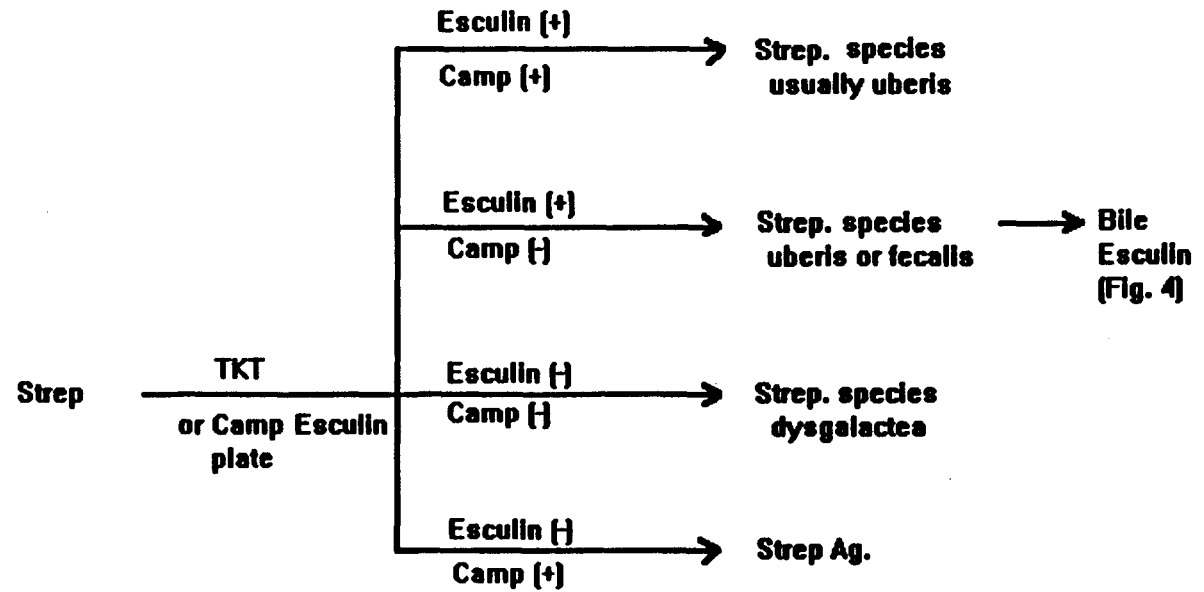


Figure 2

Strep. Identification



Esculin (+) brown to black colony
can be darkening of media around colony

Camp (+) clear ring of hemolysis

Strep. Rapid Strep. (Api) Kit. → 20 biochemical and enzyme test reactions are compared to enclosed chart

Figure 3

Strep. Identification

**Colony
from
TKT plate**

**St. uberis
St. fecalis**

Bile Esculin

No growth

St. uberis

Growth

fecalis

Figure 4

**Staph Identification
(Secondary Media)**

Staph Coagulase Test

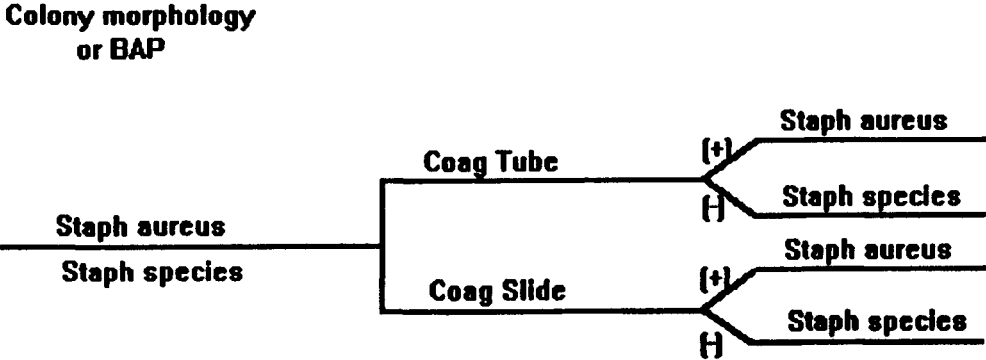


Figure 5

**Staph Identification
(Secondary Media)**

**Colony Morphology
on BAP**

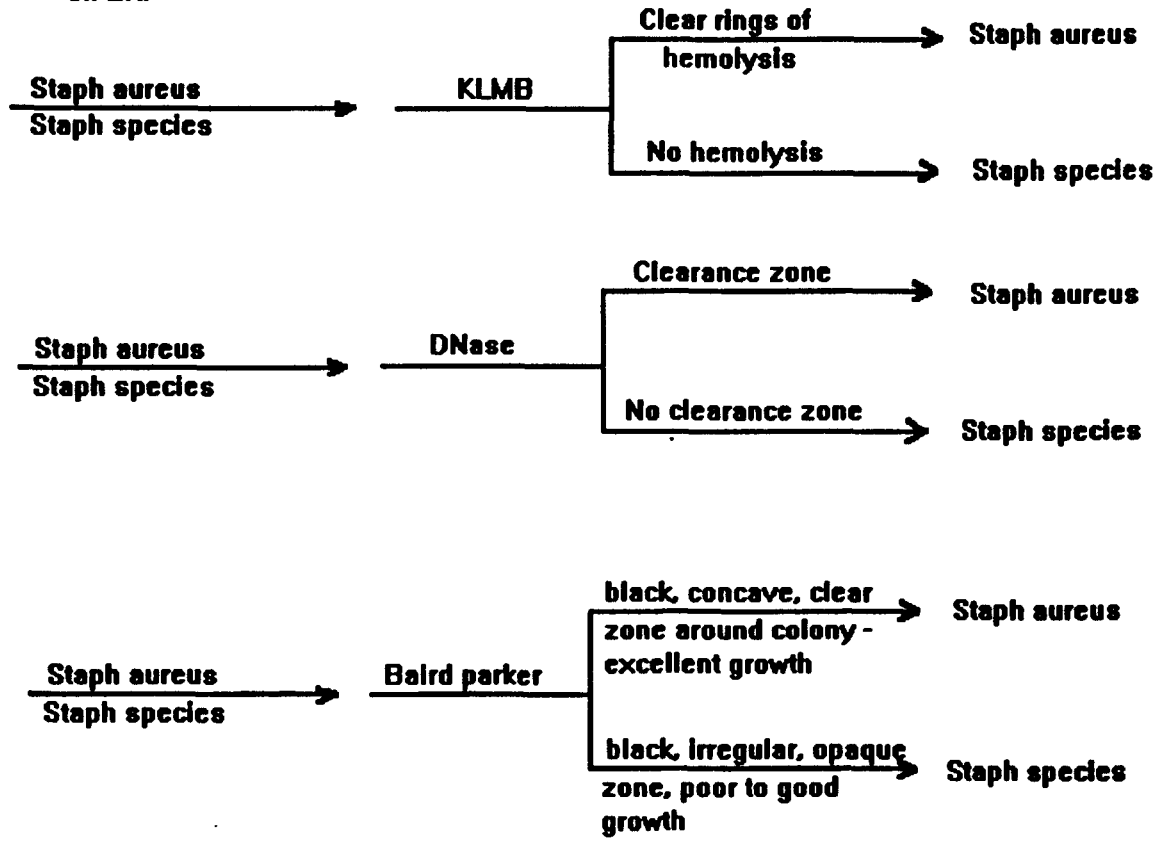


Figure 6

**Staph Identification
(Secondary Media)**

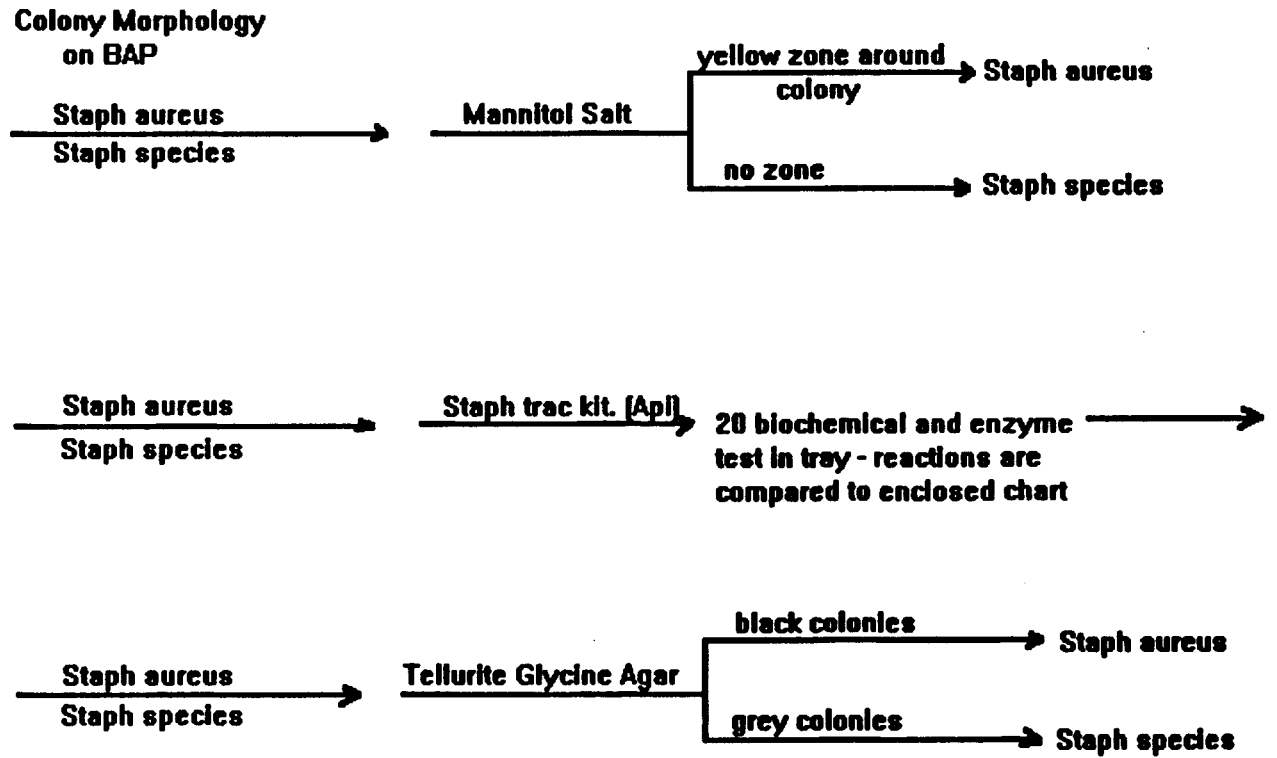


Figure 7

Coliform & Other Gram Neg Identification

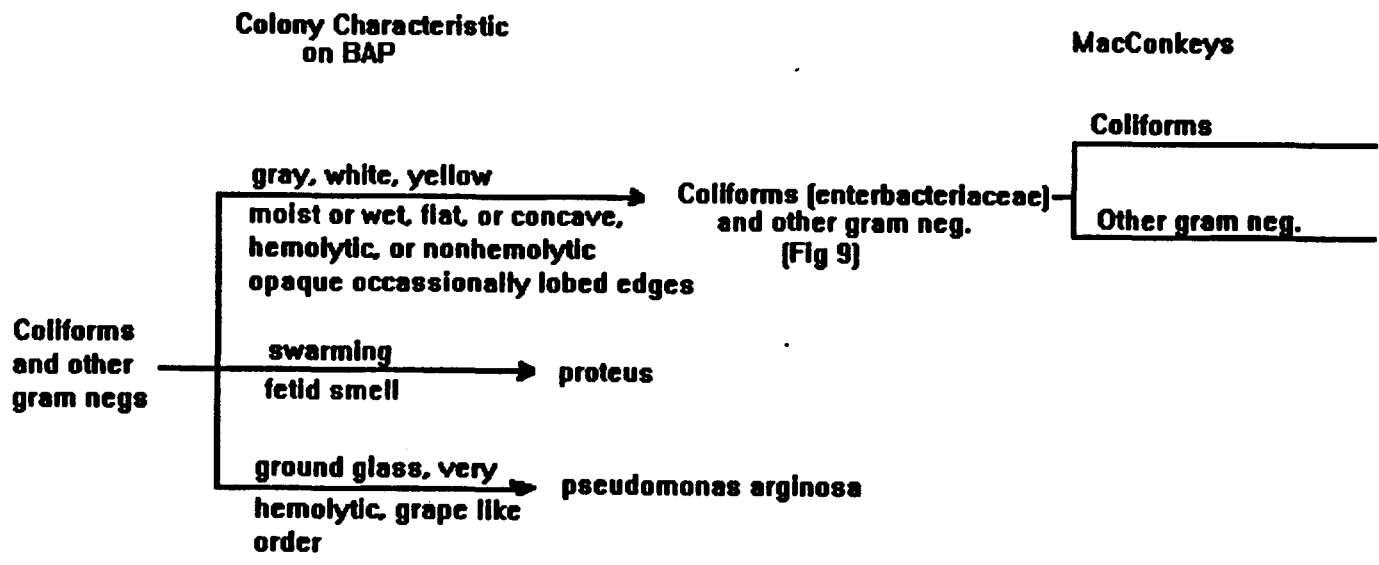


Figure 8

**Gram Negatives
(Secondary Media)**

Colony Morphology

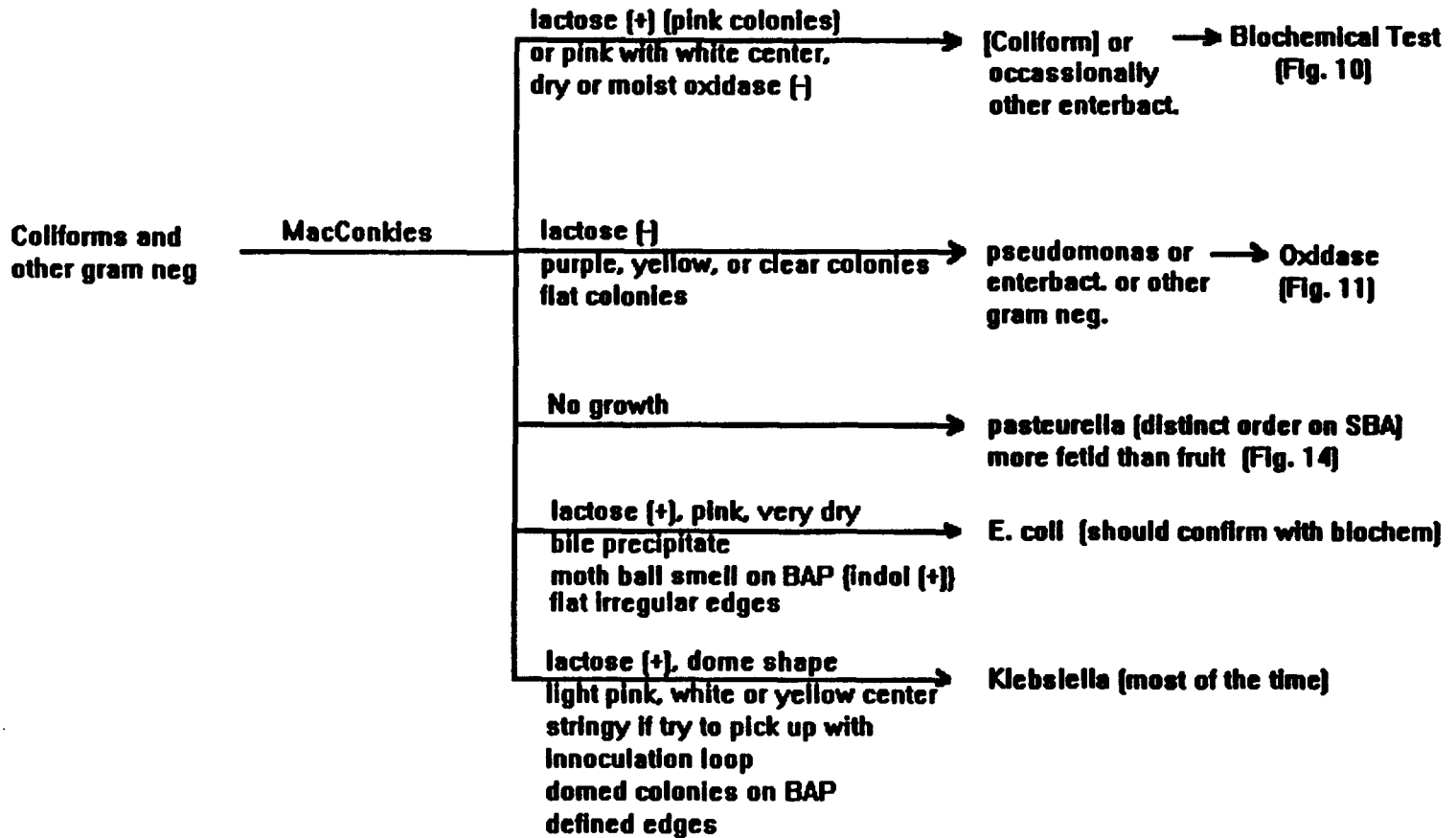


Figure 9

Gram Negative Identification

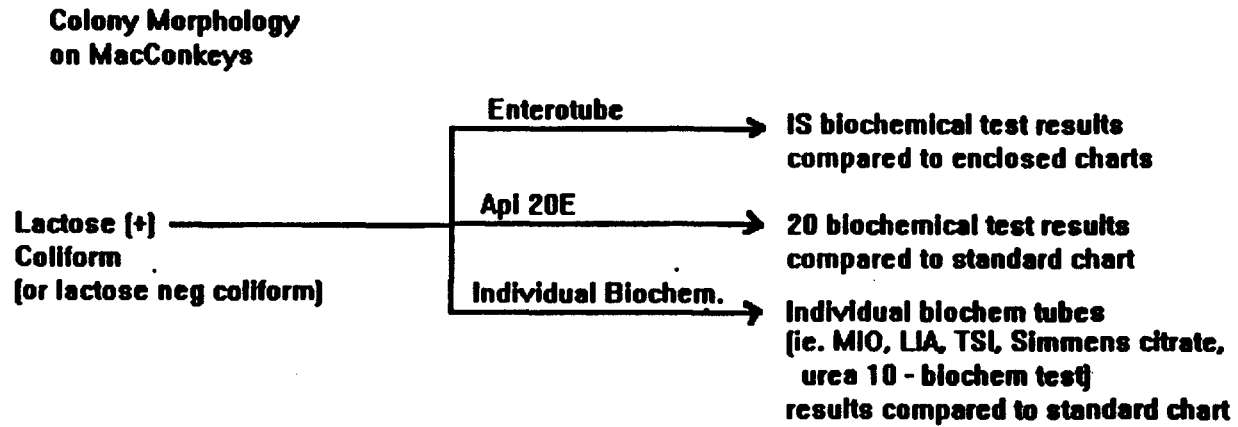


Figure 10

Gram Negative Identification

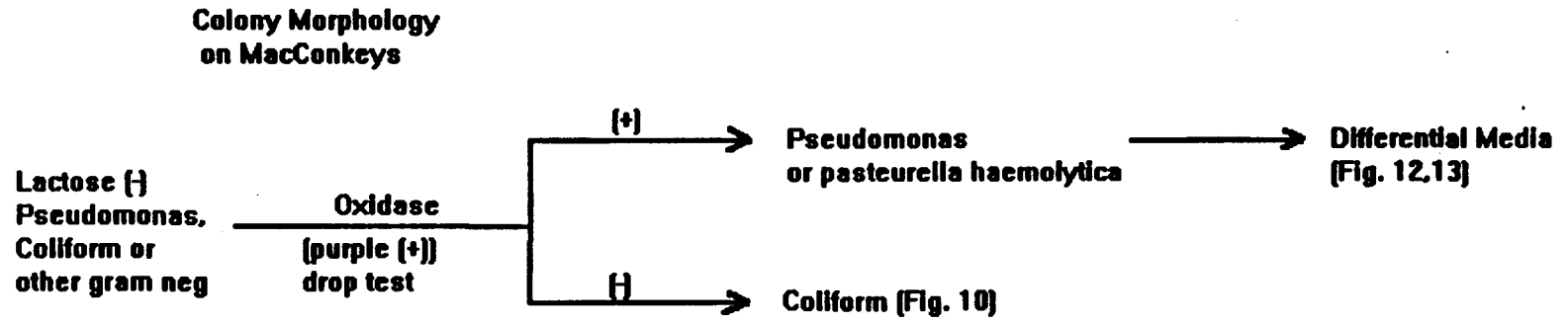


Figure 11

Gram Negative Identification

**Morphology
on MacConkey**

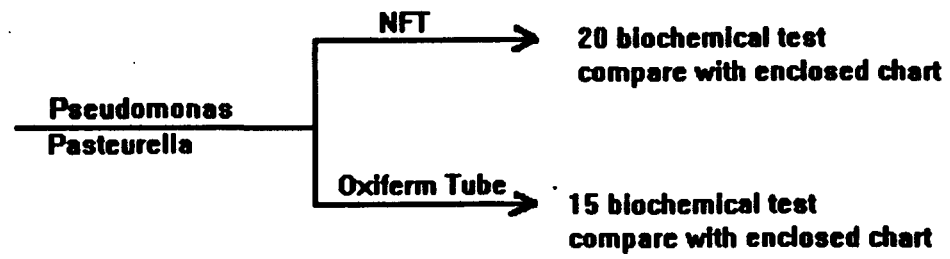


Figure 12

Gram Negative Identification

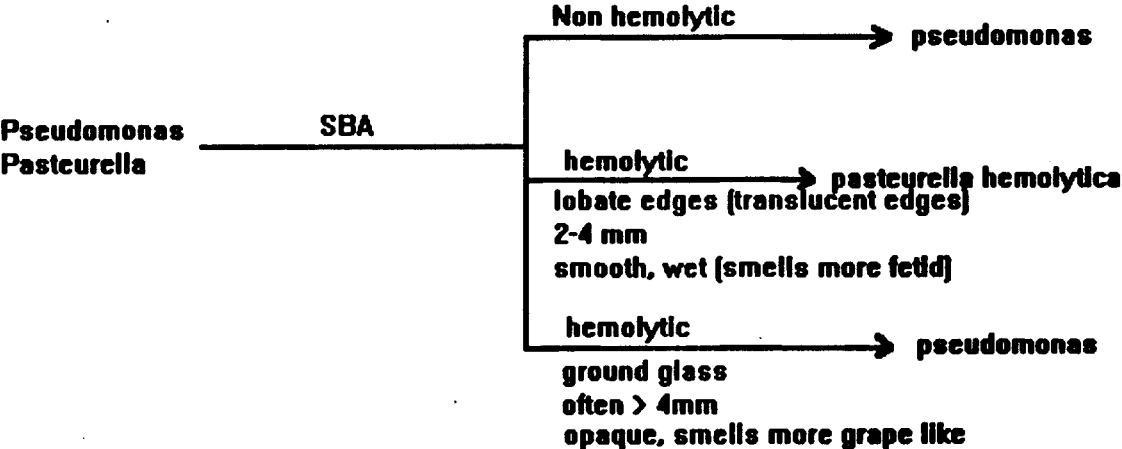
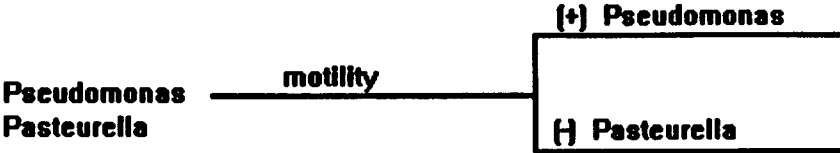


Figure 13

Gram Negative Identification

**Morphology
on MacConkeys**

No growth

Oxidferm

**15 biochemical test
compare to enclosed chart**

Rapid NBT

**20 biochemical test
compare with enclosed chart**

Figure 14

**Some Possible Sources of Materials for
Mastitis Laboratory Procedures**

Api Strips (Analytical Products)

Customer Service
Sherwood Medical Co.
1831 Olive Street
St. Louis, MO
USA - 1-800-645-0666

API 20E System
Rapid NFT System
Staph Trac System
Rapid Strep System

Di Med

2956 Yorktown Blvd
St. Paul, MN 55117
612-490-5350

Enterotube
Oxiferm
Spot test reagents
Blood agar plates
MacConkeys
TKT
KLMB
Biplates: BAP/MacConkeys
biochemical tubed media (MIO, LIA, TSI, etc.)

VWR

P.O. Box 66929
O'Hare AMF
Chicago, IL 60666
1-800-932-5000

Coagulase
biochemical tubed media (MIO, LIA, TSI, etc.)

Wolff Laboratories
9025 Penn Avenue So.
Minneapolis, MN 55431
1-800-642-9085/612-884-3113

Blood agar plate
TKT/FC agar plates
Triplate - mastitis - - BAP, MacConkey, TKT
Quad plate - mastitis - - BAP, MacConkey, TKT, Baird-Parker
Coagulase plasma

FISHER
1600 W. Glenlake Avenue
Itasca, IL 60143
1-800-766-7000

Gram stain

BAXTER
13505 Industrial Blvd.
Minneapolis, MN 55441
1-800-964-5227/612-553-1171

Enterotube II
Oxiferm tube
Spot test reagents (oxidase, indolkova, Vogues Prauskava)
Blood agar plates
MacConkeys
Coagulase
Saline
biochemical tubes (MIO, LIA, TSI, Simons Citrate, Urea, etc.)

National Mastitis Council, Inc.
1840 Wilson Blvd.
Arlington, VA 22201
(703) 243-8268

Laboratory and Field Hand