

IDENTIFICATION OF UNKNOWNNS

In this exercise, you will draw upon many of your new skills to identify unknown bacteria. You will be given three bacterial cultures and asked to identify each to the level of genus. The organisms selected include types you have studied this semester and some new genera. Figure 1 presents a flow chart for identifying the unknown bacteria. You need to employ tests learned during this semester and a few new techniques to characterize morphological, nutritional, physiological and biochemical characteristics of your unknowns.

The procedure for identifying bacteria is similar to the game "Twenty Questions." A question is asked, for example "Is the organism Gram-negative or Gram-positive?" The answer to this question directs you to the next question, e.g., "Is the organism a coccus or bacillus?" Once again the answer directs you to the appropriate next question, and step by step you narrow your possible identification to a single genus. What fun!

Obviously, you cannot complete the identifications in a single lab period. You should anticipate completing the exercise during the next two weeks. You should not do tests unwarranted for a given organism, since this would be wasteful of supplies and needlessly time-consuming.

Because this exercise will extend over several periods, you will need to prepare stock cultures for each unknown. Do this by transferring the unknowns to slants of Trypticase Soy Agar, incubating at 37°C for 24 to 48 hours, and then **storing them in your drawer**. You will need to use this stock culture as a source of your unknown during the exercise. No replacement cultures will be provided! It wouldn't be a bad idea to save your original cultures in case of an emergency, but these may not remain viable for very long.

Many tests in this exercise have been included in earlier exercises. Since you have had previous opportunities to perform and interpret these tests, you will be on your own in this exercise; i.e., do not ask the instructor for help interpreting your results. You are encouraged to draw upon other knowledge that you have acquired during the semester to aid in your identification. Thus, the colony characteristics, pigmentation, mobility of cells, etc., may provide clues to their identification, and should be included on the unknown identification sheet. You are required to complete steps shown in Figure 1 that are necessary for the identification of each unknown, and not to perform unnecessary procedures. You should turn in your results when you have completed ALL of the unknowns.

Expectations

Each student will perform the Unknowns Lab exercise independently. You will receive three unknowns to identify. Your grade will reflect your ability to select and perform the necessary procedures and interpret the results.

- You will not be given new TSA slants, so Gram stains must be done promptly.
- You should only perform those tests that are required to identify a particular unknown.
- You will not receive media to repeat tests – so do it right the first time.
- You need to complete the initial test indicated on the chart during the first week.

Grading

30 points total: 3 unknowns x 10 points each

For each unknown there are three possible grades

- Gram-rx is wrong but shape is correct: grade = – 5pts;
- Gram-rx & shape are incorrect: grade = – 2
- Gram-rx & shape are correct, mistake occurs in subsequent step: grade = – 3 pts

When to turn in results

You should turn in the identification sheets for your 3 unknowns after you have completed all 3 unknowns, **which must be no later than the last lab period.**

A New test

Urease Test

Certain bacteria can degrade the compound urea by means of an enzyme called **urease**. Ammonia is released as a byproduct of urea metabolism, causing an increase in pH of the medium. Urea Broth medium contains urea and the pH indicator phenol red.

Procedure

1. Inoculate a tube of Urea broth with the test organism from a slant culture
2. Incubate at 37°C for 24 - 48 hours.
3. Record results.

Interpretation

The color of the medium is orange at neutral pH, but changes to cerise (cherry red) if urea is metabolized.

***** Media to be used to run the tests *****

These media should be obtained from the instructor as required.

Oxidase, endospore & catalase tests: use bacteria from your slant cultures

Citrate agar – Blue cap

MR-VP – Violet cap

Coagulase – Pink cap

Purple glucose broth – Yellow cap

Esculin (BEAA) – Plate

TSA + 7.5% NaCl – White cap

Lactose broth – Red cap

Urea broth – Brown cap

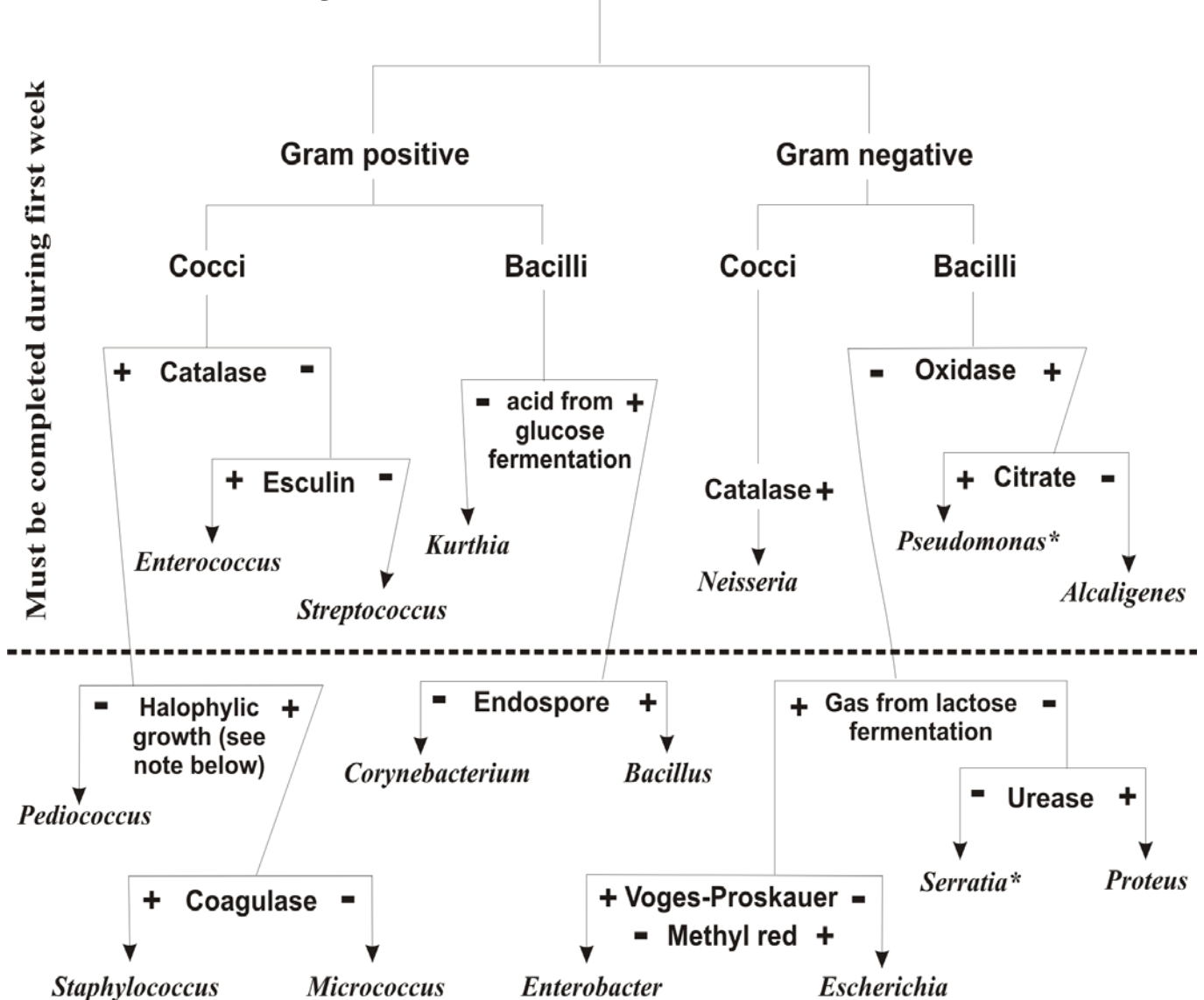
These test reagents and supplies will be available on the side bench

H₂O₂ Methyl red reagent

10 N KOH

α-Naphthol reagent

Figure 1. Flow chart for identification of unknowns.



* Non-pigmented strains of *Pseudomonas* and *Serratia* may be used

Halophytic growth: use TSA + 7.5% NaCl; growth on medium is positive result

Unknowns – 4

Name: _____

IDENTIFICATION OF UNKNOWN No. _____

REMEMBER, YOU SHOULD DO ONLY THOSE TESTS REQUIRED TO IDENTIFY EACH UNKNOWN.

Gram Reaction:

Characteristics of Cells

Shape:

Arrangement:

Test results (+/-)

Tests done using cells grown on TSA slant

Oxidase: _____

Endospore production: _____

Catalase reaction: _____

Media you need to obtain

initials

_____ Citrate agar (blue cap) Citrate metabolism: _____

_____ Coagulase plasma (pink cap) Coagulase test: _____

_____ Esculin (BEAA) (plate) Esculin metabolism: _____

_____ Lactose Broth (red cap) Gas from lactose: _____

_____ MRVP (violet cap) Methyl Red: _____
Voges Proskauer: _____

_____ Purple Glc Broth (yellow cap) Acid from glucose: _____

_____ TSA + 7.5% NaCl (white cap) Halophylic: _____

_____ Urea Broth (brown cap) Urea metabolism: _____

Additional Observations:

Genus _____

Unknowns – 5

Name: _____

IDENTIFICATION OF UNKNOWN No. _____

REMEMBER, YOU SHOULD DO ONLY THOSE TESTS REQUIRED TO IDENTIFY EACH UNKNOWN.

Gram Reaction:

Characteristics of Cells

Shape:

Arrangement:

Test results (+/-)

Tests done using cells grown on TSA slant

Oxidase: _____

Endospore production: _____

Catalase reaction: _____

**Media you need to obtain
initials**

_____ Citrate agar (blue cap) Citrate metabolism: _____

_____ Coagulase plasma (pink cap) Coagulase test: _____

_____ Esculin (BEAA) (turqu. caps) Esculin metabolism: _____

_____ Lactose Broth (red cap) Gas from lactose: _____

_____ MRVP (violet cap) Methyl Red: _____
Voges Proskauer: _____

_____ Purple Glc Broth (yellow cap) Acid from glucose: _____

_____ TSA + 7.5% NaCl (white cap) Halophylic: _____

_____ Urea Broth (brown cap) Urea metabolism: _____

Additional Observations:

Genus _____

Unknowns – 6

Name: _____

IDENTIFICATION OF UNKNOWN No. _____

REMEMBER, YOU SHOULD DO ONLY THOSE TESTS REQUIRED TO IDENTIFY EACH UNKNOWN.

Gram Reaction:

Characteristics of Cells

Shape:

Arrangement:

Test results (+/-)

Tests done using cells grown on TSA slant

Oxidase: _____

Endospore production: _____

Catalase reaction: _____

Media you need to obtain

initials

_____ Citrate agar (blue cap) Citrate metabolism: _____

_____ Coagulase plasma (pink cap) Coagulase test: _____

_____ Esculin (BEAA) (turqu. cap) Esculin metabolism: _____

_____ Lactose Broth (red cap) Gas from lactose: _____

_____ MRVP (violet cap) Methyl Red: _____
Voges Proskauer: _____

_____ Purple Glc Broth (yellow cap) Acid from glucose: _____

_____ TSA + 7.5% NaCl (white cap) Halophylic: _____

_____ Urea Broth (brown cap) Urea metabolism: _____

Additional Observations:

Genus _____

