

# SI Microbiology - Full Discipline Demo

## Identification of an Unknown Microbe

### Final Report - Answer Guide

|                    |                                        |
|--------------------|----------------------------------------|
| <b>Institution</b> | Science Interactive University         |
| <b>Session</b>     | SI Microbiology - Full Discipline Demo |
| <b>Course</b>      | SI Microbiology - Full Discipline Demo |
| <b>Instructor</b>  | Sales SI Demo                          |

### Test Your Knowledge

Match each term with the best description.

Terms to match:

- Bacilli
- Biochemical tests
- Cocci
- Catalase

Descriptions to match:

- Spherical bacteria cells
- Rod-shaped bacteria cells
- Assays performed to determine the physiological properties of microbes
- Enzyme that prevents cell death from exposure to hydrogen peroxide

Correct answers:

1 Cocci    2 Bacilli    3 Biochemical tests    4 Catalase

Categorize each statement as true or false.

|                                                                                                                                                         |                     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| <p>⚡</p> <p>Microbe colonies growing on agar plates differ in color, form, elevation, and margin.</p>                                                   |                     |
| <p>⚡</p> <p>Gram positive cells appear pink or light red in color after Gram staining.</p>                                                              |                     |
| <p>⚡</p> <p>Triple sugar iron (TSI) agar slants are used to differentiate bacteria based on the ability to ferment carbohydrates and reduce sulfur.</p> |                     |
| <p>⚡</p> <p>Starch hydrolysis testing is a biochemical test to determine if a microbe secretes catalase.</p>                                            |                     |
| <p><b>True</b></p>                                                                                                                                      | <p><b>False</b></p> |
| <p>1</p>                                                                                                                                                | <p>2</p>            |

Correct answers:

1

Microbe colonies growing on agar plates differ in color, form, elevation, and margin.

Triple sugar iron (TSI) agar slants are used to differentiate bacteria based on the ability to ferment carbohydrates and reduce sulfur.

2

Starch hydrolysis testing is a biochemical test to determine if a microbe secretes catalase.

Gram positive cells appear pink or light red in color after Gram staining.

## Exploration

**Gram positive bacteria cells appear \_\_\_\_ in color.**

- ☐ green
- ☐ pink
- ☒ purple ✓
- ☐ orange

**An unknown bacteria sample is first cultured on an agar plate so that colonies can be observed.**

- ☒ True ✓
- ☐ False

**Biochemical tests are used to determine the presence of enzymes and/or metabolic pathways utilized by bacteria cells.**

- ☒ True ✓
- ☐ False

**\_\_\_\_ is used to perform catalase testing.**

- ☐ Motility agar
- ☐ Iodine reagent
- ☒ Hydrogen peroxide ✓
- ☐ Gram stain

## Exercise 1

**Were cell and colony morphological observations sufficient for identifying the unknown microbe in this exercise? Reference your results recorded in Data Table 1 and the diagnostic chart in Table 1 in your explanation.**

Morphological observations of individual cells and colonies were not sufficient for identifying the unknown microbe. Both the colony morphology and cell morphologies recorded in Data Table 1 were present for many of the six microbes included in the diagnostic chart in Table 1: five were rod-shaped and three had circular colonies.

**Which of the biochemical and functional media test results from this exercise were most important for identifying the unknown microbe? Reference your results in Data Table 1 and the diagnostic chart in Table 1 in your explanation.**

The TSI slant results were key in identifying the unknown microbe. Only *Shigella flexneri* produces a red slant/yellow butt TSI result as recorded in Data Table 1 and provided in the diagnostic chart. When comparing the other results recorded in Data Table 1 to the diagnostic chart, more than one microbe produced negative results for starch hydrolysis, motility, and Gram staining, so these results alone would not permit the identification of the unknown.

**Data Table 1: Unknown Microbe Identification**  
(SAMPLE ANSWER BELOW)

| Test                        | Result                  |
|-----------------------------|-------------------------|
| Colony color and morphology | Cream, circular, convex |
| Cell morphology             | Pink rods               |
| Gram stain                  | (-)                     |
| Catalase                    | (+)                     |
| Motility agar               | (-)                     |
| TSI slant/butt              | K/A (red/yellow)        |
| TSI gas                     | (-)                     |
| TSI H <sub>2</sub> S        | (-)                     |
| Starch hydrolysis           | (-)                     |
| Unknown identity            | Shigella flexneri       |

## Competency Review

**The identification process for an unknown microbe frequently begins with observing cell and colony morphologies.**

☒ True

☐ False



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**Microbe colonies growing on agar plates differ in \_\_\_\_.**

- ☐ color
- ☐ form
- ☐ margin
- ☒ All of the above

✓

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**Rod-shaped bacteria cells are called \_\_\_\_.**

- ☒ bacilli
- ☐ cocci
- ☐ spirilla
- ☐ loci

✓

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**Gram staining is used to differentiate the shape and structure of the cell wall.**

- ☒ True
- ☐ False

✓

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**Catalase positive bacteria generate \_\_\_\_ when exposed to hydrogen peroxide.**

- ☐ H<sub>2</sub>S precipitate
- ☐ color changes
- ☒ rapid bubbling
- ☐ iodine

✓

**Turbidity throughout a soft gel tube is a positive test result for \_\_\_\_.**

- ☐ carbohydrate fermentation
- ☐ sulfur reduction
- ☐ starch hydrolysis
- ☒ motility



**TSI agar slant results are interpreted by \_\_\_\_.**

- ☐ color change
- ☐ gas production
- ☐ precipitate formation
- ☒ All of the above



**An unknown microbe appeared as pink rods after Gram staining; produced a motility agar tube with a defined stab line; produced a TSI agar slant with red slant, yellow butt, no gas, and no H<sub>2</sub>S precipitate; and produced an uniformly dark starch agar plate when flooded with IKI reagent. After comparing the results to the diagnostic table below, the unknown microbe should be identified as \_\_\_\_.**

| Test                 | <i>Bacillus megaterium</i> | <i>Enterococcus faecalis</i> | <i>Shigella flexneri</i> | <i>Citrobacter freundii</i> | <i>Proteus vulgaris</i> | <i>Klebsiella pneumoniae</i> |
|----------------------|----------------------------|------------------------------|--------------------------|-----------------------------|-------------------------|------------------------------|
| Colony morphology    | White, concave, smooth     | White, small, circular       | Cream, circular, convex  | Grey, smooth, convex        | Colorless, smooth, flat | Grey, circular, dome-shaped  |
| Cell shape           | Chain-forming rods         | Spheres                      | Rods                     | Rods                        | Rods                    | Rods                         |
| Gram stain           | (+)                        | (+)                          | (-)                      | (-)                         | (-)                     | (-)                          |
| Catalase             | (+)                        | (-)                          | (+)                      | (+)                         | (+)                     | (+)                          |
| Motility agar        | (+)                        | (-)                          | (-)                      | (+)                         | (+)                     | (-)                          |
| TSI slant/butt       | A/K (yellow/red)           | A/A (yellow/yellow)          | K/A (red/yellow)         | A/A (yellow/black)          | A/A (yellow/black)      | A/A (yellow/yellow)          |
| TSI gas              | (-)                        | (-)                          | (-)                      | (+)                         | (+)                     | (+)                          |
| TSI H <sub>2</sub> S | (-)                        | (-)                          | (-)                      | (+)                         | (+)                     | (-)                          |
| Starch hydrolysis    | (+)                        | (-)                          | (-)                      | (-)                         | (-)                     | (-)                          |

- ☐ *Bacillus megaterium*
- ☐ *Proteus vulgaris*
- ☐ *Klebsiella pneumoniae*
- ☒ *Shigella flexneri*



## Extension Questions

A microbiologist has been provided with a pure sample of an unknown microbe that is suspected to be *Bacillus megaterium* and must confirm its identity for the client. Use the diagnostic table below to suggest three key procedures that will allow the microbiologist to confirm the microbe as *Bacillus megaterium*.

| Test                 | <i>Bacillus megaterium</i> | <i>Enterococcus faecalis</i> | <i>Shigella flexneri</i> | <i>Citrobacter freundii</i> | <i>Proteus vulgaris</i> | <i>Klebsiella pneumoniae</i> |
|----------------------|----------------------------|------------------------------|--------------------------|-----------------------------|-------------------------|------------------------------|
| Colony morphology    | White, concave, smooth     | White, small, circular       | Cream, circular, convex  | Grey, smooth, convex        | Colorless, smooth, flat | Grey, circular, dome-shaped  |
| Cell shape           | Chain-forming rods         | Spheres                      | Rods                     | Rods                        | Rods                    | Rods                         |
| Gram stain           | (+)                        | (+)                          | (-)                      | (-)                         | (-)                     | (-)                          |
| Catalase             | (+)                        | (-)                          | (+)                      | (+)                         | (+)                     | (+)                          |
| Motility agar        | (+)                        | (-)                          | (-)                      | (+)                         | (+)                     | (-)                          |
| TSI slant/butt       | A/K (yellow/red)           | A/A (yellow/yellow)          | K/A (red/yellow)         | A/A (yellow/black)          | A/A (yellow/black)      | A/A (yellow/yellow)          |
| TSI gas              | (-)                        | (-)                          | (-)                      | (+)                         | (+)                     | (+)                          |
| TSI H <sub>2</sub> S | (-)                        | (-)                          | (-)                      | (+)                         | (+)                     | (-)                          |
| Starch hydrolysis    | (+)                        | (-)                          | (-)                      | (-)                         | (-)                     | (-)                          |

(SAMPLE ANSWER BELOW)

The microbiologist should Gram stain the unknown and then perform TSI agar slant and starch hydrolysis testing. If *Bacillus megaterium*, the microbe will appear as purple connected rods, unlike any of the other microbes on the chart. Similarly, only *B. megaterium* produces TSI slants with red slants and yellow butts. Lastly, *B. megaterium* is the only microbe on the chart that produces positive results for starch hydrolysis.