SI Microbiology - Full Discipline Demo

Identification of an Unknown Microbe

Final Report - Answer Guide

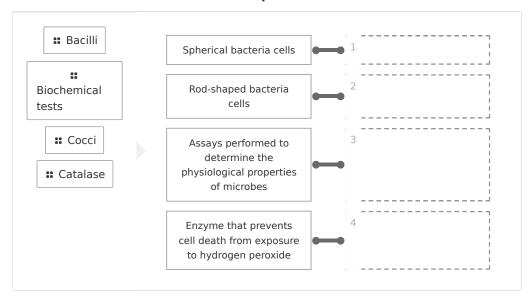
Institution Science Interactive University

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Instructor Sales SI Demo

Test Your Knowledge

Match each term with the best description.



Correct answers:

1 Cocci 2 Bacilli 3 Biochemical tests 4 Catalase



Categorize each statement as true or false.

::

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

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

::

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

::

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

True	False
r	
1	2
1	1
I	I

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.

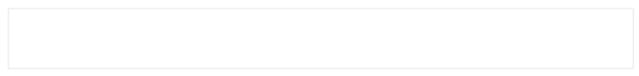
Exploration

Gram positive bacteria cells appear in color.	
green	
○ pink	
o 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/o metabolic pathways utilized by bacteria cells.	r
○ True	✓
○ False	
is used to perform catalase testing.	
Motility agar	
O lodine reagent	
Hydrogen peroxide	~
Gram stain	
EXECUTE Were cell and colony morphological observations sufficient for identifying the un nicrobe in this exercise? Reference your results recorded in Data Table 1 and the hart 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 ₂ 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			

Microbe colonies growing on agar plates differ in	
color	
form	
margin	
All of the above	~
Rod-shaped bacteria cells are called	
bacilli	~
o cocci	
spirilla	
● loci	
Gram staining is used to differentiate the shape and structure of the wall.	cell
○ True	~
False	
Catalase positive bacteria generate when exposed to hydrogen peroxide.	
 H₂S precipitate 	
color changes	
rapid bubbling	~



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 $\rm H_2S$ 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	Bacillus	Enterococcus	Shigella	Citrobacter	Proteus vulgaris	Klebsiella
Test	megaterium	faecalis	flexneri	freundii	Froteus vulgaris	pneumoniae
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 ₂ S	(-)	(-)	(-)	(+)	(+)	(-)
Starch hydrolysis	(+)	(-)	(-)	(-)	(-)	(-)

Bacillus	megaterium
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- 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	Bacillus megaterium	Enterococcus faecalis	Shigella flexneri	Citrobacter freundii	Proteus vulgaris	Klebsiella pneumoniae
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 ₂ 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.

