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Introduction

Firstly, the lack of fever, swollen lymph notes or rashes inside the mouth indicates a non-systemic infection. Additionally, since the rash was only presented on the surface of the skin, around the mouth and nose, and not on the feet or hands, the most likely bacterial pathogens causing the rash are *Staphylococcus aureus* or *Streptococcus pyogenes*.

The only sample taken for laboratory testing was a swab of the rash surface.

Staphylococcus aureus

Staphylococci are Gram-positive, non-spore-forming, nonmotile bacteria which form spherical cells of 0.5 to 1.5 μm [1]. Under the microscope, they can be seen as single cocci, or forming pairs, tetrads or short chains of cocci. The Staphylococcus species are facultative anaerobes [1], meaning that they make ATP by aerobic respiration if oxygen is present, but if oxygen is unavailable, they can switch to anaerobic respiration or fermentation [2]. The genotypic characterization of the Staphylococcus genus is a G+C content of 30 to 39 mol% [1]. Additional characteristics that set *S. aureus* apart from other taxa of Gram-positive cocci can be seen in Table 1 below [1].

TABLE 1 Differentiation of members of the genus *Staphylococcus* from other Gram-positive cocci[a]

Genus and exceptional species	G+C content (molecular %) of DNA	Strict aerobe	Facultative anaerobe or microaerophile	Strict anaerobe	Tetrad cell arrangement	Strong adherence on agar	Motility	Growth on: 5% NaCl agar	Growth on: 6.5% NaCl agar	Growth on: 12% NaCl agar	Growth on: P agar in 18 h[b]	Growth on: Schleifer-Kramer agar[c]	Catalase reaction result[d]	Benzidine test result[e]	Modified oxidase test result[f]	Anaerobic acid production from glucose[g]	Aerobic acid production from glycerol	Resistance to: Lysostaphin (200 μg/ml)	Resistance to: Erythromycin (0.4 μg/ml)	Resistance to: Bacitracin (0.04 U)[h]	Resistance to: Furazolidone (100 μg)[i]
Staphylococcus spp.	30–39	−	*d*	−	*d*	−[j]	−	+	+	*d*	+	+	+	+	−	*d*	+	−	+	+	−

[a]Symbols and abbreviations: +, 90% or more species or strains positive; ±, 90% or more species or strains weakly positive; −, 90% or more species or strains negative; *d*, 11 to 89% of species or strains positive; ND, not determined. Parentheses indicate a delayed reaction.

[b]Growth on P agar is under aerobic conditions at 35 to 37°C. Positive growth is indicated for detectable formation of colonies of at least 1 mm in diameter; ± indicates detectable formation of colonies of between 0.5 and 1 mm in diameter. Growth on sheep or bovine blood agar is slightly greater but less discriminative of staphylococci and other genera.

[c]Growth is under aerobic conditions at 35 to 37°C for 24 to 48 h. Positive growth is indicated for a number of CFU on selective medium comparable to that on plate count agar and a colony of 0.5 mm in diameter; ± indicates a significant reduction in the number of CFU on the selective medium compared to that on plate count agar, and parentheses indicate a colony of pinpoint size to 0.5 mm in diameter.

[d]Sometimes a weak catalase or pseudocatalase reaction can be observed with certain strains of species designated catalase negative. In some species, catalase activity may be activated by hemin supplementation.

[e]Detects the presence of cytochromes. Some strains of benzidine test-negative species can synthesize cytochromes on aerobic media supplemented with hemin (140).

[f]Determined by the modified oxidase test using tetramethyl-*p*-phenylenediamine dihydrochloride-impregnated disks or strips to detect the presence of cytochrome *c* (140).

[g]Standard oxidation/fermentation test.

[h]A disk is used. + indicates resistance and no zone of inhibition. *Micrococcus*, *Kocuria*, *Kytococcus*, *Stomatococcus*, and *Aerococcus* spp. are susceptible and have an inhibition zone of 10 to 25 mm in diameter.

[i]A disk is used. + indicates resistance and no zone of inhibition or a zone of up to 9 mm in diameter. Susceptible species have an inhibition zone of 15 to 35 mm in diameter.

S. aureus is found on skin and mucous membranes of mammals and birds, and is considered the most common and important human pathogen among the staphylococci [1]. It is estimated that 50% of humans are either permanently or intermittently colonized by it [1], making it an opportunistic pathogen due to its virulence factors (ie. adhesins, enzymes, and toxins). In this case study, *S. aureus* would be a possible cause of impetigo [1] or psoriasis [3].

The characteristics that set *S. aureus* apart from all other species of *Staphylococcus* can be seen in Table 2 below [1].

TABLE 2 Differentiation of *Staphylococcus* species

Species	Characteristic[a]																																						
	Expression of:																								Acid production (aerobically) from:															
	Large colonies[b]	Colony pigmentation[c]	Anaerobic growth[d]	Aerobic growth[e]	Coagulase test result	Clumping factor[f]	Heat-stable nuclease	Hemolysins[g]	Catalase[h]	Oxidase[i]	Alkaline phosphatase	Arginine arylamidase	Pyrrolidonyl arylamidase[j]	Ornithine decarboxylase	Urease[j]	β-Glucosidase[j]	β-Glucuronidase[j]	β-Galactosidase[j]	Arginine utilization[j]	Acetoin production	Nitrate reduction	Esculin hydrolysis	Novobiocin resistance[k]	Polymyxin B resistance[l]	D-Trehalose	D-Mannitol	D-Mannose	D-Turanose	D-Xylose	D-Cellobiose	L-Arabinose	Maltose	α-Lactose	Sucrose	*N*-Acetylglucosamine	Raffinose
S. aureus subsp. *aureus*	+	+	+	+	+	+	+	+	+	−	+	−	−	−	d	+	−	−	+	+	+	−	−	+	+	+	+	+	−	−	−	+	+	+	+	−

[a]Symbols and abbreviations (unless otherwise indicated): +, 90% or more strains positive; ±, 90% or more strains weakly positive; −, 90% or more strains negative; *d*, 11 to 89% of strains positive; ND, not determined. Parentheses indicate a delayed reaction.

[b]Positive is defined as a colony diameter of ≥6 mm after incubation on P agar at 34 to 35°C for 3 days and at room temperature (ca. 25°C) for an additional 2 days; exceptions are *S. succinus* (4 to 6 mm on tryptic soy agar) and *S. fleurettii* (8 to 12 mm on tryptic soy agar).

[c]Positive is defined by the visual detection of carotenoid pigments (e.g., yellow, yellow-orange, or orange) during colony development at normal incubation or room temperatures. Pigments may be enhanced by the addition of milk, fat, glycerol monoacetate, or soaps to P agar.

[d]Growth is in a semisolid thioglycolate medium. Symbols: +, moderate or heavy growth down the tube within 18 to 24 h; ±, heavier growth in the upper portion of the tube and weaker growth in the lower, anaerobic portion of tube; −, no visible growth within 48 h but very weak diffuse growth or a few scattered, small colonies may be observed in the lower portion of the tube by 72 to 96 h. Parentheses indicate delayed growth appearing within 24 to 72 h, sometimes noted as large, discrete colonies in the lower portion of the tube.

[e]Growth is on P agar or bovine, sheep, or human blood agar at 34 to 37°C. The subspecies of *S. equorum* and *S. succinus* grow slowly at 35 to 37°C; the optimum growth temperatures of *S. equorum* subsp. *equorum* and *S. equorum* subsp. *linens* are 30°C and 32°C, respectively, and those of *S. succinus* subsp. *succinus* and of *S. succinus* subsp. *casei* are 28°C and 32°C, respectively. Anaerobic species *S. saccharolyticus* and *S. aureus* subsp. *anaerobius* grow very slowly in the presence of air. *S. aureus* subsp. *anaerobius* requires the addition of blood, serum, or egg yolk for growth on primary isolation medium. *S. auricularis*, *S. lentus*, and *S. vitulinus* produce just-detectable colonies on P agar in 24 to 36 h, and these colonies remain very small (1 to 2 mm in diameter).

[f]The slide agglutination test using rabbit or human plasma detects the expression of clumping factor. Use human plasma for *S. lugdunensis* and *S. schleiferi*. Latex agglutination is less reliable for the detection of clumping factor in *S. lugdunensis*.

[g]Hemolysis on bovine blood agar. Symbols and abbreviations: +, wide zone of hemolysis within 24 to 36 h; (+), delayed moderate to wide zone of hemolysis within 48 to 72 h; (*d*), no or delayed hemolysis; −, no or only very narrow (1-mm) zone of hemolysis within 72 h. Some strains designated negative may produce a slight greening or browning of blood agar. Analysis of hemolysis for both *S. succinus* subspecies was performed on Wilkins-Chalgren anaerobe agar plates containing 5% sheep blood.

[h]Catalase and cytochrome synthesis cannot be induced in *S. aureus* subsp. *anaerobius* by the addition of H_2O_2 or hemin to the culture medium. Catalase activity can be induced in *S. saccharolyticus* by hemin supplementation. In this species, cytochromes *a* and *b* are present in small quantities.

[i]Determined by the modified oxidase test to detect the presence of cytochrome *c* (140).

[j]Determined primarily by commercial rapid identification tests (see the text).

[k]Positive (resistant) is defined by an MIC of ≥1.6 μg/ml or a growth inhibition zone diameter of ≥16 mm with a 5-μg novobiocin disk.

[l]Positive is defined by a growth inhibition zone diameter of <10 mm with a 300-U polymyxin B disk.

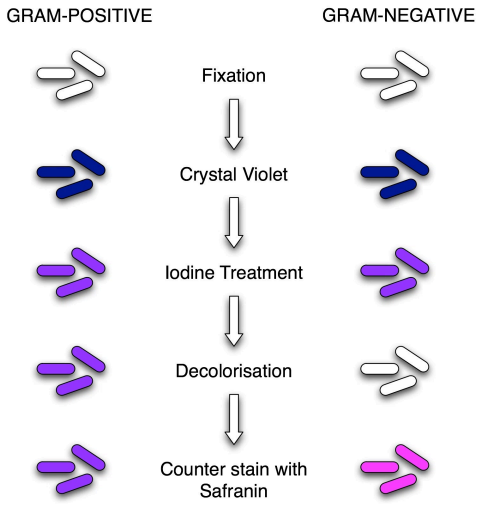
Importance of the Microbiology Laboratory

It is important for the microbiology laboratory to diagnose the presence of *S. aureus* since leaving the infection untreated may lead to other superficial infections (such as folliculitis, furuncles/carbuncles, hydradenitis suppurative and pyoderma), or acute life-threatening necrotizing fasciitis and myositis. Localized *S. aureus* infections may become invasive and cause bacteremia, and possibly lead to meningitis or endocarditis [1].

Laboratory tests

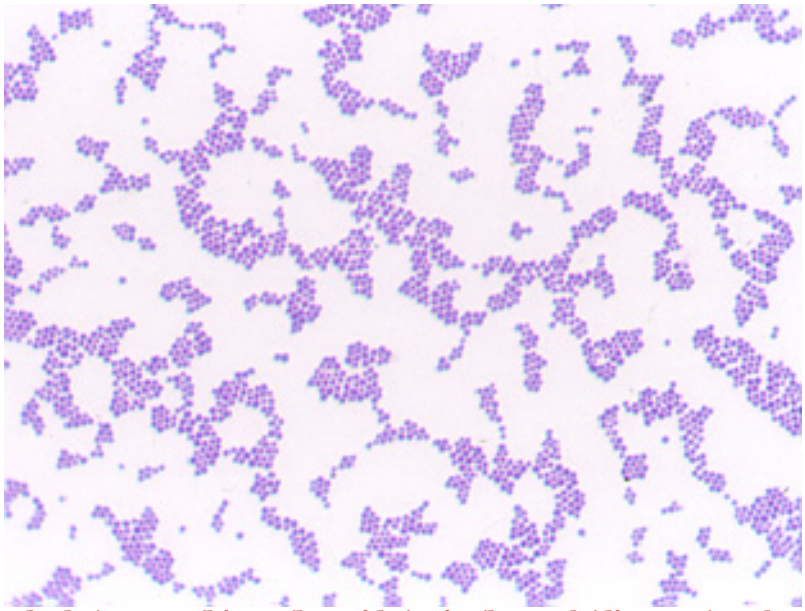
Gram stain

To complete a Gram stain, the microbe needs to be heat-fixed to a slide, and then crystal violet should be applied and allowed to react for 1 minute. The slide should be rinsed, and then flooded with iodine, allowing it to react for 1 minute. At this point, all bacteria should appear purple. Once again, the slide should be rinsed, and acetone should be applied for 5 seconds, and then rinsed with water again. At this point, Gram negative bacteria will be clear, while Gram positive bacteria will still appear purple. Safranin should then be applied for 30 seconds followed by the last rinsing step. Safranin will stain the Gram negative bacteria a light pink colour. The bacteria can be analyzed using a regular compound microscope.



http://i2.wp.com/microbeonline.com/wp-content/uploads/2013/04/gram-stain-mine.jpg

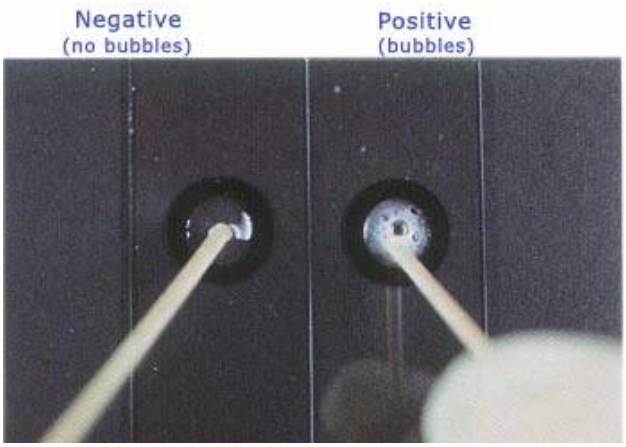
Using the compound microscope, *S. aureus* can be seen as gram positive (purple) cocci (spherical), arranged in pairs, tetrads or short chains.



http://faculty.ccbcmd.edu/courses/bio141/lecguide/unit3/bacpath/diseases/staphaureus/gpstaph.html

Catalase Test

The catalase test is a simple test to determine if a microbe has the catalase enzyme, which catalyzes a reaction converting hydrogen peroxide into water and oxygen molecules ($2\ H_2O_2 \rightarrow 2\ H_2O + O_2$) [4]. The test is done by applying a colony of bacteria to a sterile slide and adding a drop of hydrogen peroxide to the slide. No formation of bubbles would indicate a negative result (the bacteria does not have the catalase enzyme), while formations of bubbles would be a positive result (the bacteria has the catalase enzyme).



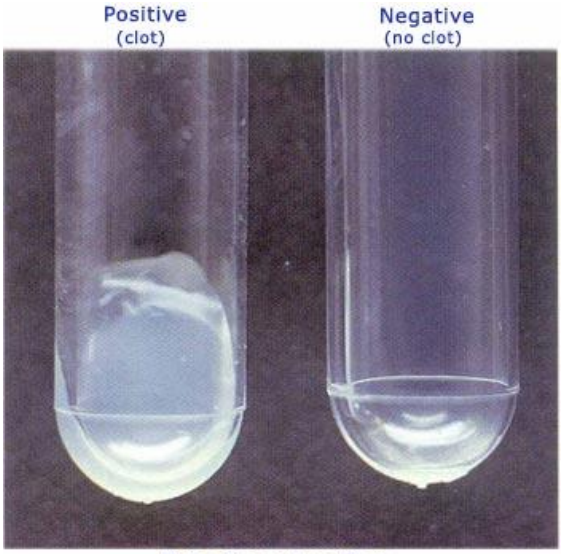
http://staff.um.edu.mt/amce1/Stains%20and%20Tests%20PAT2322/The%20Catalase%20test.htm

S. aureus is a catalase positive bacteria, therefore it is expected that bubbles would form when the catalase test is performed [1].

Tube Coagulase Test

The tube coagulase test is used to detect the extracellular free coagulase, which functions to convert fibrinogen (soluble) to fibrin (insoluble). To do this test, a well-isolated colony of bacteria is transferred onto 0.5ml of reconstituted rabbit plasma in a tube, and the tube is then incubated at 37°C for 4 hours. The tube is then slowly tilted 90° from the vertical, and is observed. Any amount of clotting represents a positive test [1].

The *S. aureus* should yield a positive tube coagulase test.

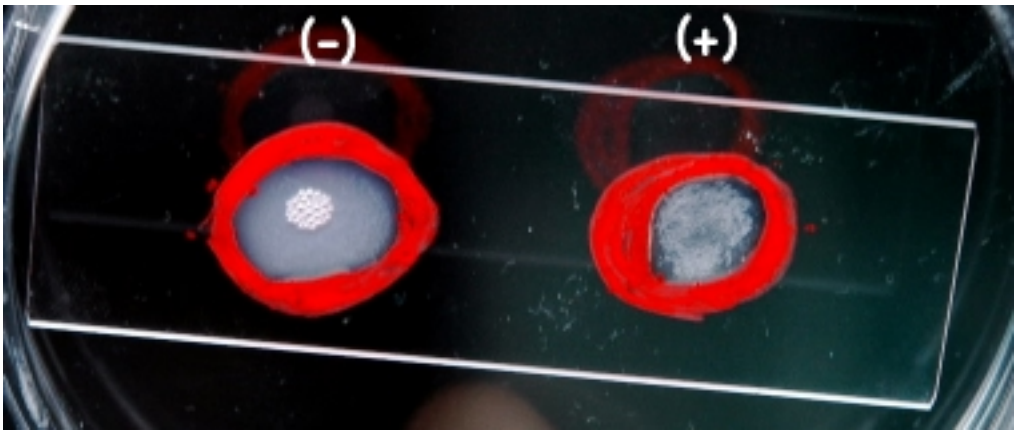


Tube Coagulase Test

http://staff.um.edu.mt/amce1/Stains%20and%20Tests%20PAT2322/The%20Coagulase%20Test.htm

Slide Agglutination Test

The slide agglutination test is used to determine if the bacteria contains a cell wall-bound coagulase (a clumping factor) [1]. For this test, a colony of bacteria is transferred to a slide, and an antibody that can bind to the cell wall-bound coagulase is added. A positive test would show microscopic appearance of clumps, while a negative test would not show any clumps.



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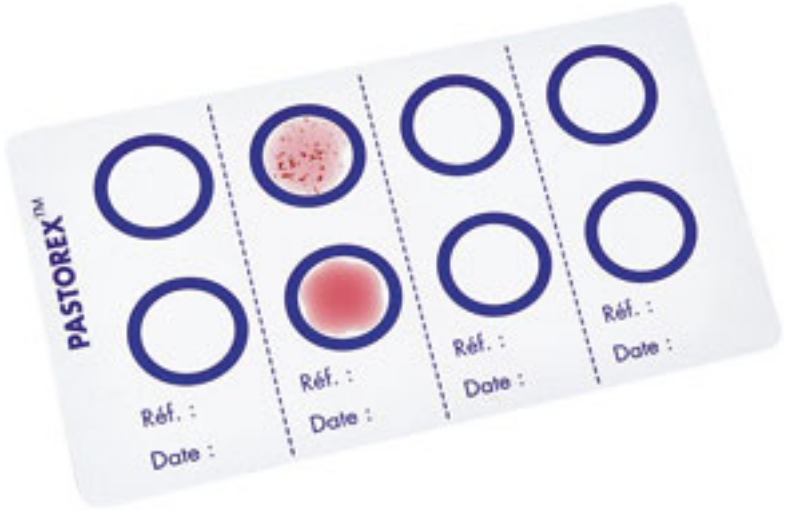
S. aureus should yield a positive result.

However, due to the low sensitivity, low specificity, and long incubation time of the classical slide agglutination tests, modern clinical microbiology laboratories use rapid latex and hemagglutination assays (RLHA) using antibodies. RLHA are designed to specifically detect (via antibodies) protein A, clumping factor A, and capsular polysaccharide serotypes 5 and 8 [1].



http://www.bio-rad.com/webroot/web/images/cdg/products/microbiology_us/product_overlay_content/global/cmd_staph_01e_overlay.jpg

Other tests

If there are concerns regarding the accuracy of the above tests in the establishing the presence of *S. aureus*, the image below lists other tests that may be used for identification of *S. aureus* from other clinically significant Staphylococcus species [1].

TABLE 3 Key tests for identification of the most clinically significant *Staphylococcus* species

Species	Result of test for[a]:																			
	Colony pigmentation[b]	Staphylocoagulase	Clumping factor[b]	Heat-stable nuclease	Alkaline phosphatase	Pyrrolidonyl arylamidase[b]	Ornithine decarboxylase	Urease[b]	β-Galactosidase[b]	Acetoin production	Novobiocin resistance[b]	Polymyxin B resistance[b]	Acid production (aerobically) from: D-Trehalose	D-Mannitol	D-Mannose	D-Turanose	D-Xylose	D-Cellobiose	Maltose	Sucrose
S. aureus subsp. *aureus*	+	+	+	+	+	−	−	*d*	−	+	−	+	+	+	+	+	−	−	+	+
S. epidermidis	−	−	−	−	+	−	(*d*)	+	−	+	−	+	−	−	(+)	(*d*)	−	−	+	+
S. haemolyticus	*d*	−	−	−	−	+	−	−	−	+	−	−	+	*d*	−	(*d*)	−	−	+	+
S. hyicus (veterinary)	−	*d*	−	+	+	−	−	*d*	−	−	−	+	+	−	+	−	−	−	−	+
S. intermedius (veterinary)	−	+	*d*	+	+	+	−	+	+	−	−	−	+	(*d*)	+	*d*	−	−	(±)	+
S. lugdunensis	*d*	−	(+)	−	−	+	+	*d*	−	+	−	*d*	+	−	+	(*d*)	−	−	+	+
S. pseudintermedius (veterinary)	−	+	−	ND	+[c]	+	ND	+	+	+	−	+	+	(±)	+	(±)	−	−	+	+
S. schleiferi subsp. *schleiferi*	−	−	+	+	+	+	−	−	(+)	+	−	−	*d*	−	+	−	−	−	−	−
S. saprophyticus subsp. *saprophyticus*	*d*	−	−	−	−	−	−	+	+	+	+	−	+	*d*	−	+	−	−	+	+

[a]Symbols and abbreviations (unless otherwise indicated): +, 90% or more strains positive; ±, 90% or more strains weakly positive; −, 90% or more strains negative; *d*, 11 to 89% of strains positive; ND, not determined. Parentheses indicate a delayed reaction.

[b]Descriptions are the same as those in Table 2.

[c]Alkaline phosphatase reactions tested positive in the STAPH-ZYM gallery but negative in the API STAPH gallery.

Streptococcus pyogenes

Streptococci species are characterized by being catalase-negative, Gram-positive cocci. The cocci are less than 2 μm in diameter, and usually grow in chains. They are also facultative anaerobes and are incapable of respiratory metabolism due to a lack of heme compounds [5].

Their classification is based on their ability to undergo a hemolytic reaction, their colony size and the presence of Lancefield antigens [5].

Primarily, the hemolytic reaction is what sets streptococci apart. Such bacteria produce exotoxins, called hemolysins, which lyse red blood cells and hemoglobin [6]. Beta-hemolysis is the complete destruction of red blood cells and hemoglobin around and underneath the colony of bacteria. Streptococci produce streptolysins (type of hemolysin), and can be categorized as type O or type S [6]. Streptolysin O is oxygen-liable and displays maximum reaction under aerobic conditions. On the other hand, streptolysin S is oxygen-stable, but displays maximum reaction under anaerobic conditions [6]. Beta-hemolytic streptococci are also known as pyogenic streptococci, and include the most commonly known human pathogen *Streptococcus pyogenes*.

The second distinguishing factor of the pyogenic streptococci are their colonies, which are usually larger than 0.5mm.

Finally, they are further characterized by the presence of Lancefield antigens. These are defined as "serologically distinguishable groups into which most streptococci can be divided and which are based on the polysaccharide antigens present in the streptococcal cell wall" [7]. This grouping was developed by Rebecca Lancefield in 1933 when she observed the formation of antibodies which caused a precipitation of streptococci from solution [7].

Streptococci are commensal bacteria, therefore they do not generally harm the host. Accordingly, they are usually found on mucous membranes or as transient skin microbiota. Transmission can occur by direct contact or via droplets.

Importance of the Microbiology Laboratory

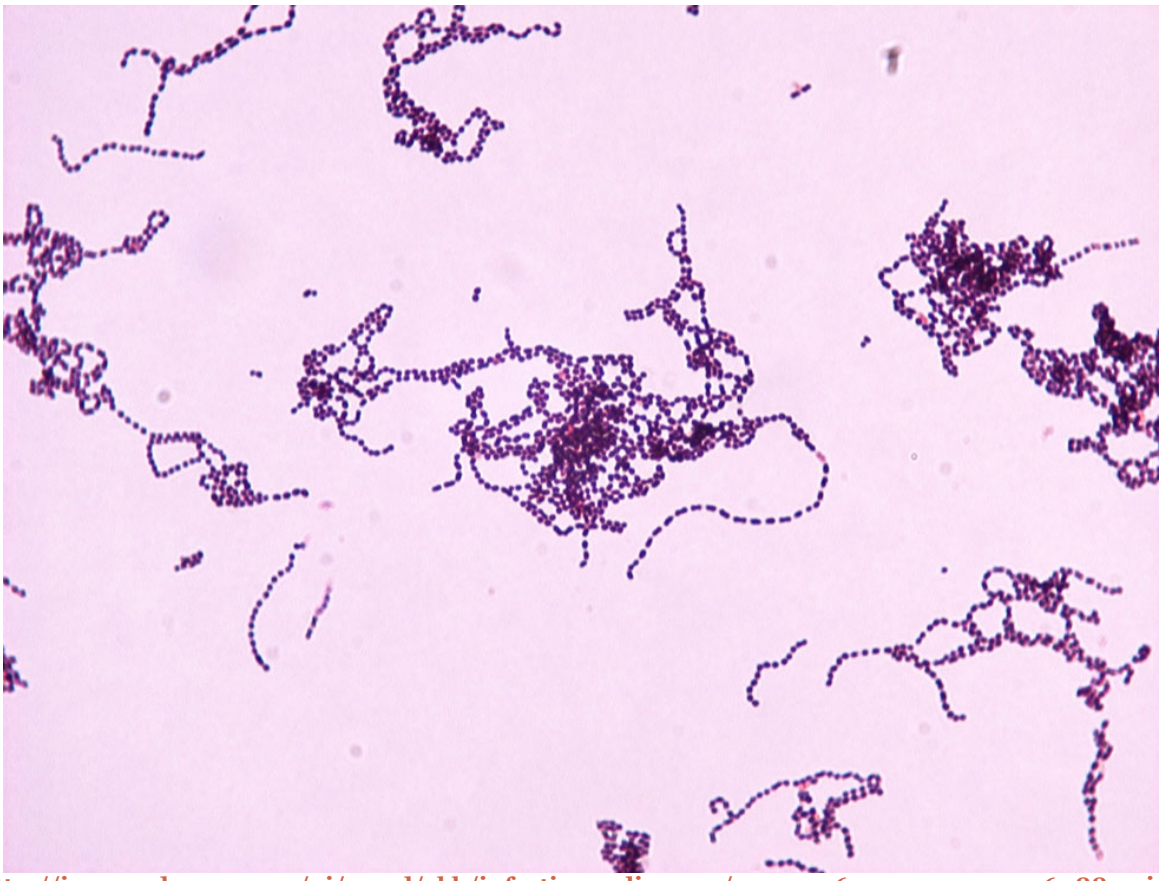
As a commensal bacteria, *S. pyogenes* may cause colonization, and subsequently lead to infection. Although skin colonization may not be dangerous, the risk of infections caused by S. pygoenes in the upper respiratory tract exists. Furthermore, it is a common cause of bacterial pharyngitis, impetigo, childbed fever and puerperal sepsis [5]. Therefore, accurate identification of this bacteria is important in prevention of further infections.

Laboratory tests

Gram Stain

The procedure for Gram staining is as outlined above.

S. pyogenes are Gram positive, and therefore should appear purple under a compound microscope. The bacteria should be spherical (cocci), less than 2 μm in diameter, and form chains or pairs.



http://img.medscape.com/pi/emed/ckb/infectious_diseases/211212-1641790-225243-1608843.jpg

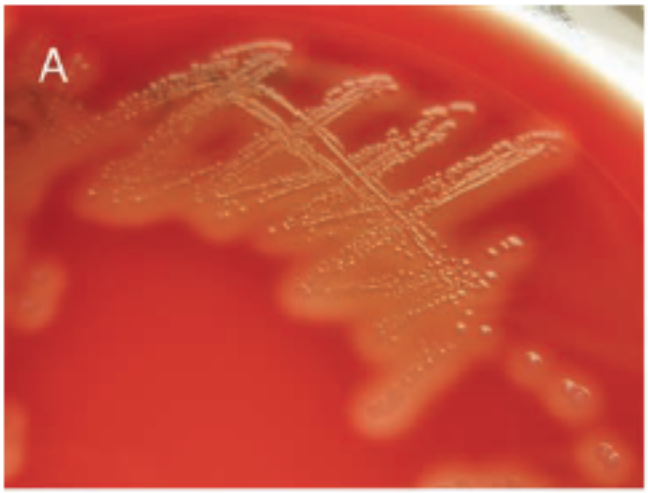
Catalase Test

The procedure for catalase test is as outlined above.

The catalase test for *S. pyogenes* should be negative, as determined by the lack of formation of oxygen bubbles.

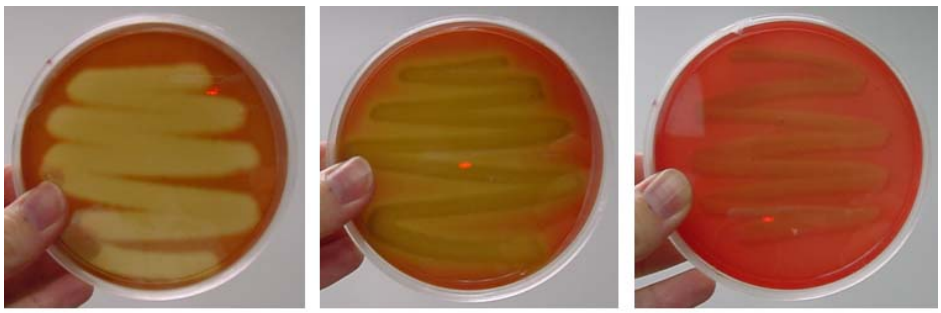
Colony description

Beta-hemolytic, pyogenic streptococci form colonies that are larger than 0.5mm after a 24 hour incubation period, and appear gray or almost white, moist and glistening [5]. This can be seen in the figure below [5].



Blood agar test

The blood agar test is an agar medium that isolates and detects hemolytic activity of streptococci or other fastidious microorganisms. The medium contains beef extract and peptone, and blood is added (sheep, rabbit or horse) [8]. For this test, the bacteria is aseptically spread across the medium. The three possible results are alpha-, beta- or gamma-hemolysis. Alpha-hemolysis is only partial lysis of red blood cells and hemoglobin, beta-hemolysis is the complete lysis of red blood cells and hemoglobin around and underneath the colony of bacteria, and gamma-hemolysis is no lysis.



http://iws2.collin.edu/dcain/CCCCD%20Micro/Hemolysis3.jpg

S. pyogenes should display beta-hemolysis, characterized by a clear area (complete lysis) around and underneath the colony.

Lancefield Antigen Immunoassays

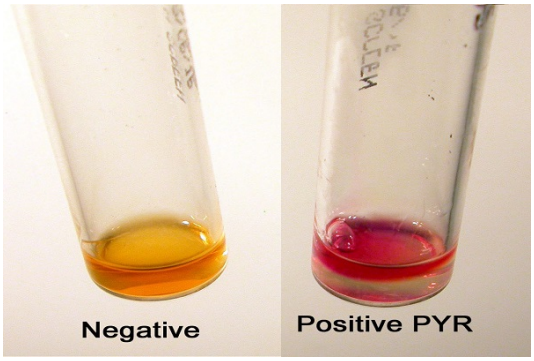
Many modern clinical microbiology laboratories stock commercially available Lancefield antigen immunoassays. These assays have rapid antigen extraction and cause agglutination. Generally, the bacteria reacts with polyclonal anti-Strep A antibodies conjugated to coloured particles on the test strip. The liquid then moves along the strip by capillary action, and reacts with various reagents [9]. If a high enough concentration of *S. pyogenes* antigen is detected, a coloured line forms at the test region of the strip, indicating a positive test result. If no coloured line forms, the test is interpreted as negative.

http://www.ultimed.org/produkte/strep-dipstick/

As long as an adequate sample was loaded onto the immunoassay, the *S. pyogenes* should yield a positive test result as indicated by a coloured line on the Strep A test strip.

PYR Test

S. pyogenes is the only beta-hemolytic streptococci that contains the pyrrolidonyl aminopeptidase (enzyme), and is therefore a useful test for distinguishing it from other bacteria in that group [5]. In this test, the L-pyttolidonyl-beta-maphthylamide is hydrolyzed by the enzyme to beta-naphthylamide, and therefore produces a red colour when cinnamaldehyde reagent is added. Care should be taken to complete this test on a pure culture, in conjunction with the previously mentioned tests in order to eliminate other genera of bacteria (ie. Enterococcus, Lactococcus, etc.) [5].



http://www.microbiologynotes.com/wp-content/uploads/2015/07/Broth-PYR-Test.jpg

S. pyogenes should yield a positive PYR test, as indicated by the red pigment of the broth.

Bacitracin Susceptibility Test

S. pyogenes is the only human beta-hemolytic streptococci that has a susceptibility to bacitracin [5]. Bacitracin is a type of antibiotic, usually applied topically for skin and eye infections. The test is completed by isolating 3 or 4 colonies of pure culture, inoculating them onto a sheep blood agar plate and applying a bacitracin disk (0.04 U). The plate is then incubated overnight at 35°C in 5% CO_2. Any zone of inhibition around the antibiotic indicates a positive test.

https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/refphoto/z7021_bacitracin_spyogenes_19615_hugo.jpg

S. pyogenes should yield a positive result, as indicated by a zone of inhibition around the antibiotic disc. However, there have been several instances of bacitracin-resistant *S. pygenes* samples, therefore, this test should be completed in conjunction with other identifying tests [5].

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