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ALA (RAPID PORPHYRIN TEST)

Principle

This test is used for rapidly detecting porphyrin as a means of speciating *Haemophilus* species. Enzymes which convert ALA (delta - aminolevulinic acid) to porphyrins in the biosynthesis of hemin (X factor) are produced by *Haemophilus parainfluenzae* but not by *H. influenzae*. The production of porphyrins is detected by examination with an ultra-violet (UV) light.

Reagents



BBL TAXO Differentiation Disks ALA. (Store refrigerated in the dark. Allow 10-15 minutes for the container to reach room temperature before opening).
Sterile distilled water

Other Materials

Petri dish
Inoculating loop
Gauze
Long-wave UV lamp
Forceps

Procedure

1. Place one ALA disk for each organism to be tested on the inside of a Petri dish using forceps.
2. Moisten each disk with one drop of sterile water.
3. Rub a loopful of the test organism onto the moistened disk holding it in place with sterile forceps.
4. Saturate gauze with water, squeeze out any excess and place it in the petri dish as far away from the disks as possible.
5. Incubate at 35°C.
6. Examine at hourly intervals for 6 hours by removing the top of the petri dish and exposing the disks to UV light in a darkened room. **NB:** Wear UV safety goggles when using the UV light.

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Interpretation

- A. Positive: Orange-red fluorescence
- B. Negative: No fluorescence observed

Precautions

- 1. Use for differentiating *Haemophilus* spp. only.
- 2. Best results are obtained when a heavy inoculum is used.
- 3. ALA is light sensitive. Disks must be protected from light.



Quality Control

Test the following positive and negative controls each time an unknown is tested:

Positive: *H. parainfluenza* (ATCC 7901)
Negative: *H. influenzae* (ATCC 35056)

Reference

Remel A.L.A. Disk package insert, 2010.

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Aminopeptidase test (Bactident)

Principle

L-alanine aminopeptidase is an enzyme which is localized in the cell envelope of bacteria and which is found in relevant activities almost exclusively in Gram-negative microorganisms.

This enzyme splits off the amino acid L-alanine from various substrates.

In the case of these test strips, the substrate L-alanine-4-nitroanilide is split into 4-nitroaniline and the amino acid L-alanine in the presence of alanine aminopeptidase. The presence of the L-alanine aminopeptidase is indicated by the yellow colouration of the 4-nitroaniline.

Reagents

Bactident® Aminopeptidase (for the detection of L-alanine aminopeptidase in microorganisms)

Sterile saline

Other Materials

Inoculating loop/sterile swab


Forceps

Isolated bacterial colony overnight incubation on blood agar, chocolate agar (or other non pigmented/chromogenic media)

Procedure

1. Using an inoculating loop, remove an individual, thickly-grown colony from the nutrient medium
2. In a small test tube, suspend the bacterial mass in 0.2 ml of distilled water.
3. Insert the aminopeptidase test strip into the test tube such that the reaction zone is completely immersed in the bacteria suspension
Do not touch the reaction zones of the test strips
4. Incubate the test tube in a water bath (or incubation cupboard) for 10 to a maximum of 30 minutes* at 37 °C.
5. Read off the reaction by comparison with the colour scale on the container.

* A clear yellow colouration of the bacteria suspension can be seen after only 10 minutes in the case of most aminopeptidase positive microorganisms; if no yellow colouration appears within this time, the incubation should be extended to a maximum of 30 minutes so that the weakly aminopeptidase positive strains can be recognized or the absence of Gram-negative microorganisms can be confirmed

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Interpretation

- A. Positive: Yellow
- B. Negative: Colorless after 30 minutes

Precautions and Limitations

1. Intended to aid in differentiation of gram negative bacilli from over-decolorized large gram positive bacilli (ex. *Bacillus* sp) for blood culture gram smears
2. Only bacterial colonies without strong intrinsic colourations should be used for the aminopeptidase test.
3. Do not use for suspect *Bacteroides vulgatus*, *Bacteroides fragilis*, *Campylobacter* species, *Veillonella parvula*



Quality Control

Test the following positive and negative controls each time an unknown is tested:

Positive: *E. coli* ATCC 25922
Negative: *S. aureus* ATCC 29213

Reference

Bactident aminopeptidase package insert, EMD Millipore, 2014.

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ANAEROBIC/CAMPYLOBACTER JAR SET UP

Anaerobic Jar

1. Anaerobic plates are kept in the nitrogen holding box until there is enough for a box or before every break and at end of shift.
2. Place the inoculated plates (max 12/box including control plate), biological indicators and anaerobic indicator strip into an anaerobic box.
3. Tear open an anaerobic foil sachet at the tear-nick indicated and remove the anaerobic paper sachet from within.
4. Immediately place the paper sachet in the box in appropriate slot.
5. Close and seal box (no catalyst required).
Note: The time between opening the foil sachet and sealing the box should not exceed one minute.
Note: Box and lid must be labelled with the same number.
6. Label box with date and place in walk in incubator.

Control Testing

An anaerobic indicator is added to each box as it is set up to visually check that anaerobic conditions have been achieved and maintained. Check the box after 2 hours incubation to make sure the indicator does not indicate oxygen present.

Biological Indicator


Inoculate a quarter anaerobic plate with the following test organisms:

Bacteroides fragilis ATCC 25285: growth

Clostridium sordellii ATCC 9714: growth

Pseudomonas aeruginosa ATCC 27853: no growth

Record results in the daily Anaerobic Jar QC worklist in LIS.

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Campylobacter Jar

1. Campylobacter plates are kept in the CO₂ incubator until there is enough for a box or until 4 p.m. (Any late cultures will be set up at the end of the shift).
2. Place a paper towel into the bottom of an anaerobic jar/container.
3. Place the inoculated plates (max 12/box including control plate) into the box. Include a plate freshly inoculated with the control organism.
4. Tear open a CampyGen foil sachet at the tear-nick indicated and remove the CampyGen paper sachet from within.
5. Immediately place the paper sachet in the box in appropriate slot.
6. Close and seal box (no catalyst required).
Note: The time between opening the foil sachet and sealing the box should not exceed one minute.
Note: Box and lid must be labelled with the same number.
7. Label box with date and place in 42°C incubator.

Biological Indicator



Inoculate a campylobacter agar plate with the following test organism:

Campylobacter jejuni ATCC 29428: growth

Record results in the daily Campylobacter Jar QC worklist in LIS.

Note: The technologist on the enteric bench is responsible for the daily subculturing of the control organism (4 new plates). The old control plate and the remaining 4 newly subcultured plates will be kept in the CO₂ incubator until the end of the day.

The 4 new subcultured plates are for setting up new boxes. If more are needed, the technicians will subculture new plates from the old control plate.

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API Test Strips

IDENTIFICATION OF ENTEROBACTERALES (API 20E)

Principle

The API 20E system facilitates the 24-hour identification of *Enterobacteriales* as well as 24 or 48-hour identification of other Gram negative bacteria.

The API 20E strip consists of microtubes containing dehydrated substrates for the demonstration of enzymatic activity and carbohydrate (CHO) fermentation. The substrates are reconstituted by adding a bacterial suspension. After incubation, the metabolic end products are detected by indicator systems or the addition of reagents. CHO fermentation is detected by colour change in the pH indicator.

Materials

API 20E strips - store at 2-8⁰C

0.85% sterile saline

Nitrate A - store at 2-8⁰C

Nitrate B - store at 2-8⁰C

Mineral oil

Zinc dust

Kovacs Reagent

Voges - Proskauer Reagents

Ferric Chloride

H₂O₂

Oxidase Reagent

OF Dextrose

Motility Medium

}

}

}

Store at 2-8⁰C

}

}

}

ID of non-

Enterobacteriales

Procedure

1. Preparation of Inoculum



- a) Add 5 ml. of 0.85% saline to a sterile test tube.
- b) Using a sterile inoculating loop, carefully touch the centre of a well isolated colony (2-3 mm. Diameter) and thoroughly emulsify in the saline.

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2. Preparation of the Strip

- a) An incubation tray and lid is supplied for each strip.
- b) Dispense 5 ml of water in to the tray.

3. Inoculation of the Strip

- a) Remove the cap from the tube containing the bacterial suspension and insert a 5 ml. Pasteur pipette.
- b) Tilt the API 20E incubation tray and fill the tube section of the microtubes by placing the pipette tip against the side of the cupule.

Note: The ADH, LDC, ODC, H₂S, AND URE reactions can be interpreted best if these microtubes are slightly underfilled.


- c) Fill both the TUBE and CUPULE section of [CIT], [VP] and [GEL] tubes.
- d) After inoculation, completely fill the cupule section of the ADH, LDC, ODC, H₂S and URE tubes with mineral oil.
- e) Using the excess bacterial suspension, inoculate an agar slant or plate (non-selective media such as nutrient agar, blood agar or tryptic (trypticase) soy agar is suggested) as a purity check and for oxidase testing, serology, and/or additional biochemical testing. Incubate the slant or plate for 18-24 hours at 35°C.

4. Incubation of the Strip

- a) After inoculation, place the plastic lid on the tray and incubate the strip for 18-24 hours at 35°C in a non-CO₂ incubator.
- b) Weekend incubation: The biochemical reactions of the API 20E should be read after 18-24 hours incubation. If the strips cannot be read after 24 hours incubation at 35°C, the strips should be removed from the incubator and stored at 2-8°C (refrigerator) until the reactions can be read.

5. Reading the Strip

- a) After 18 hours of incubation and before 24 hours incubation, record all reactions not requiring the addition of reagents.

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- b) If the GLU tube is negative (blue or green), do not add reagents. Reincubate a further 18-24 hours.
- c) If the GLU is positive (yellow):
 - i. Perform the oxidase test.



A portion of the growth from the agar slate or plate, inoculated from the 20E bacterial suspension, should be rubbed onto filter paper to which a drop of oxidase reagent (1% tetramethyl-p-phenylenediamine dihydrochloride) has been added. The area where the growth has been added will turn dark purple within 10 seconds if the reaction is positive and will be colourless or light purple if negative.

Note:

- (b) The oxidase test should NOT be performed using bacterial growth from selective media such as MacConkey, EMB, etc.

Note: (a) Before addition of reagents, observe GLU tube (positive or negative) for bubbles.

- (b) The nitrate reduction and indole tests must be performed last since these reactions release gaseous products which interfere with the interpretation of other tests on the strip. The plastic incubation lid should not be replaced after the addition of these reagents.
- ii. Add the reagents to TDA and VP tubes. If positive, the TDA reactions will be immediate, whereas the VP reaction may be delayed up to 10 minutes.
- iii. The Kovacs' reagent should then be added to the IND tube.
- iv. The Nitrate Reduction test should be performed on all oxidase positive organisms. The reagents should be added to the GLU tube after the Kovacs Reagent has been added to the IND tube.



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Interpretation

- a) Record results on a API 20E analytical profile sheet.
- b) The tests are separated into groups of three. The following numerical value is assigned to each reaction recorded:
 - 1- positive reaction in the first test of the group
 - 2- positive reaction in the second test of the group
 - 4- positive reaction in any test
 - 0- negative reaction in any test
- c) Refer to the [API WEBSITE for Identification Profile](#)

Reference

1. Murray P.A., et al. Manual of Clinical Microbiology, 7th ed., 1999.

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SUMMARY OF RESULTS - 18-24 HOUR PROCEDURE



TUBE	INCUBATION	POSITIVE	NEGATIVE	COMMENTS
ONPG		Yellow	Colourless	(1) Any shade of yellow is a positive reaction. (2) VP tube, before the addition of reagents, can be used a negative control.
ADH	18-24 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	Orange reactions occurring at 36-48 hours should be interpreted as negative.
LDC	18-24 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	Any shade of orange within 18-24 hours is a positive reaction. At 36-48 hours, orange decarboxylase reactions should be interpreted as negative.
ODC	18-34 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	Orange reactions occurring at 36-48 hours should be interpreted as negative.
CIT		Turquoise or Dark Blue	Light Green Or Yellow	(1) Both the tube and cupule should be filled. (2) Reaction is read in the aerobic (cupule) area.
H ₂ S		Black Deposit	No Black Deposit	(1) H ₂ S production may range from a heavy black deposit to a very thin black line around the tube bottom. Carefully examine the bottom of the tube before considering the reaction negative. (2) A "browning" of the medium is a negative reaction unless a black deposit is present. "Browning" occurs with TDA positive organisms.
URE	18-24 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	A method of lower sensitivity has been chosen. <i>Klebsiella</i> , <i>Proteus</i> and <i>Yersinia</i> routinely give positive reactions.
TDA	Add 1 drop 10% Ferric chloride.			(1) Immediate reaction. (2) Indole positive organisms may produce a golden orange colour due to indole production. This is a negative reaction.
		Brown-Red	Yellow	
IND	Add 1 drop Kovacs Reagent			(1) The reaction should read within 2 minutes after the addition of the Kovacs reagents and the results recorded. (2) After several minutes, the HCl present in Kovacs reagent may react with the plastic of the cupule resulting in a change from a negative (yellow) colour to a brownish-red. This is a negative reaction.
		Red Ring	Yellow	

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SUMMARY OF RESULTS - 18-24 HOUR PROCEDURE (cont'd)



TUBE	INCUBATION	POSITIVE	NEGATIVE	COMMENTS
VP	Add 1 drop of 40% Potassium Hydroxide, then 1 drop of alpha-naphthol.	Red	Colourless	(1) Wait 10 minutes before considering the reaction negative. (2) A pale pink colour which appears immediately after the addition of reagents but which turns dark pink or red after 10 minutes should be interpreted as positive. Motility may be observed by hanging drop or wet mount preparation.
GEL		Diffusion of the pigment	No diffusion	(1) The solid gelatin particles may spread throughout the tube after inoculation. Unless diffusion occurs, the reaction is negative. (2) Any degree of diffusion is a positive reaction.
GLU MAN INO SOR RHA SAC MEL AMY ARA		Yellow Or Gray Yellow	Blue or Blue-Green Blue or Blue-Green	COMMENTS FOR ALL CARBOHYDRATES Fermentation (<i>Enterobacteriales</i> , <i>Aeromonas</i> , <i>Vibrio</i>) (1) Fermentation of the carbohydrates begins in the most anaerobic portion (bottom) of the tube. Therefore, these reactions should be read from the bottom of the tube to the top. (2) A yellow colour at the bottom of the tube only indicates a weak or delayed positive reaction. Oxidation (Other Gram-negatives) (1) Oxidative utilization of the carbohydrates begins in the most aerobic portion (top) of the tube. Therefore, these reactions should be read from the top to the bottom of the tube. (2) A yellow colour in the upper portion of the tube and blue in the bottom of the tube indicate oxidative utilization of the sugar. This reaction should be considered positive only for non- <i>Enterobacteriales</i> gram negative rods. This is a negative reaction for fermentative organisms such as <i>Enterobacteriales</i> .

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SUMMARY OF RESULTS - 18- 24 HOUR PROCEDURE (cont'd)



TUBE	INCUBATION	POSITIVE	NEGATIVE	COMMENTS
GLU	After reading GLU reaction, add 2 drops 0.8% sulfanilic acid and 2 drops 0.5% N. N-dimethyl-alpha-naphthylamine NO ₂ N ₂ gas	Red Bubbles: Yellow after reagents and zinc	Yellow Orange after reagents and zinc	(1) Before addition of reagents, observe GLU tube (positive or negative) for bubbles. Bubbles are indicative of reduction of nitrate to the nitrogenous (N ₂) state. (2) A positive reaction may take 2-3 minutes for the red colour to appear. (3) Confirm a negative test by adding zinc dust or 20 mesh granular zinc. A pink-orange colour after 10 minutes confirms a negative reaction. A yellow colour indicates reduction of nitrates to the nitrogenous (N ₂) state.
MAN INO SOR Catalase	After reading carbohydrate reaction, add 1 drop 1.5% H ₂ O ₂	Bubbles	No bubbles	(1) Bubbles may take 1-2 minutes to appear. (2) Best results will be obtained if the test is run in tubes which have no gas from fermentation.

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IDENTIFICATION OF NON-ENTERIC GRAM-NEGATIVE RODS (API 20NE)

Principle

The API 20NE system facilitates the identification of non-fastidious Gram-negative rods not belonging to the *Enterobacteriales* within 48 hours.

The API 20NE strip consists of microtubes containing dehydrated media and substrates. The media microtubes containing conventional tests are inoculated with a bacterial suspension which reconstitutes the media. After incubation, the metabolic end products are detected by indicator systems or the addition of reagents. The substrate microtubes contain assimilation tests and are inoculated with a minimal medium. If the bacteria are capable of utilizing the corresponding substrate, then they will grow.

Materials

API 20NE strips - store at 2-8 ⁰ C		
0.85% sterile saline		
Mineral oil		
Zinc dust		
AUX Medium	}	
James Reagent	}	
Nitrate 1 - store at 2-8 ⁰ C	}	Store at 2-8 ⁰ C
Nitrate 2 - store at 2-8 ⁰ C	}	
Oxidase Reagent		

Procedure

1. Preparation of Inoculum

- a) Add 2 ml. of 0.85% saline to a sterile test tube.
- b) Using a sterile inoculating loop, carefully touch the centre of a well isolated colony (2-3 mm. Diameter) and thoroughly emulsify in the saline. The suspension turbidity should be equal to a 0.5 McFarland standard.

2. Preparation of the Strip


- a) An incubation tray and lid are supplied for each strip.
- b) Dispense 5 ml of distilled water in to the tray.

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3. Inoculation of the Strip

- a) Remove the cap from the tube containing the bacterial suspension and insert a sterile pipette.
- b) Tilt the API 20NE incubation tray and fill the TUBE section of the NO₃ to PNPG microtubes by placing the pipette tip against the side of the cupule.
- c) Open an ampule of AUX Medium and add 200 uL of the bacterial suspension to the ampule. Mix well with a pipette while avoiding the formation of air bubbles.
- d) Using the AUX Medium bacterial suspension, fill both the TUBE and CUPULE section of [GLU] to [PAC]. Do not overfill the cupules. Fill to a flat or slightly convex meniscus.
- e) After inoculation, completely fill the CUPULE section of the 3 underlined tests, GLU, ADH and URE tubes with mineral oil.
- f) Using the excess bacterial suspension, inoculate an agar slant or plate (non-selective media such as nutrient agar, blood agar or tryptic (trypticase) soy agar is suggested) as a purity check and for oxidase testing, and/or additional biochemical testing. Incubate the slant or plate with the API 20NE strip.

4. Incubation of the Strip

- a) After inoculation, place the plastic lid on the tray and incubate the strip for 24 hours at 30⁰C in a non-CO₂ incubator.

5. Reading the Strip

- a) After 24 hours incubation, record all reactions not requiring the addition of reagents.
- b) Perform the oxidase test.

A portion of the growth from the agar slate or plate, inoculated from the 20NE bacterial suspension, should be rubbed onto filter paper to which a drop of oxidase reagent (1% tetramethyl-p-phenylenediamine dihydrochloride) has been added. The area where the growth has been added will turn dark purple within 10 seconds if the reaction is positive and will be colourless or light purple if negative.


- (b) The oxidase test should NOT be performed using bacterial growth from selective media such as MacConkey, EMB, etc.

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- c) Assimilation tests are observed for bacterial growth. An opaque cupule indicates a positive reaction.
- d) Protect the assimilation tests with the incubation tray lid during the reading of the Nitrate and TRP tests.
- e) Perform the Nitrate test.
 - i. Add one drop of Nitrate 1 and one drop of Nitrate 2 reagents to NO₃ cupule.
 - ii. After 5 minutes a red color indicates a positive reaction.
 - iii. A negative reaction may be due to the production of nitrogen. Add Zinc dust to the NO₃ cupule. After 5 minutes a colorless cupule indicates a positive reaction. A pink-red cupule indicates a negative reaction.
- f) Perform the TRP test.
 - i. Add one drop of JAMES Reagent.
 - ii. The reaction takes place immediately, producing a pink color in the entire cupule if the reaction is positive.

Interpretation

1. Use the API 20NE analytical profile index.
2. The tests are separated into groups of three. The following numerical value is assigned to each positive reaction recorded:
 - 1 - positive reaction in the first test of the group
 - 2 - positive reaction in the second test of the group
 - 4 - positive reaction in the third test of the group
3. Refer to the [API WEBSITE for Identification Profile](#)
4. The strip must be reincubated in the following cases:
 - i. If the profile cannot be found in the API web site.
 - ii. If the following note is indicated for the profile obtained:

**IDENTIFICATION NOT VALID
BEFORE 48-HR INCUBATION**

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- iii. If the strip is to be reincubated, remove the reagents from the NO₃ and TRP cupules and then cover these tests with mineral oil.
- iv. Reincubate the strip for another 24 hours at 30°C in a non-CO₂ incubator.
- v. Read all the tests again, except for NO₃, TRP and GLU.

READING TABLE



TESTS	SUBSTRATES	REACTIONS/ENZYMES	NEGATIVE RESULTS	POSITIVE RESULTS
NO ₃	Potassium nitrate	NITrate reduction to nitrites	NIT 1 + NIT 2 / 5 min colourless pink-red	
		NITrates to nitrogen	Zn / 5 min pink colourless	
TRP	tryptophane	indole production	JAMES / immediate colourless / pink pale green / yellow	
<u>GLU</u>	glucose	Acidification	blue to green	yellow
<u>ADH</u>	arginine	arginine dihydrolase	yellow	orange/pink/red
<u>URE</u>	urea	Urease	yellow	orange/pink/red
ESC	esculin	hydrolysis (β-glucosidase)	yellow	grey/brown/black
GEL	gelatine (with India ink)	hydrolysis (protease)	no pigment diffusion	diffusion of black pigment
PNPG	p-nitrophenyl-β-D-galactopyranoside	β-galactosidase	colourless	yellow
[<u>GLU</u>]	glucose	Assimilation	transparent	opaque
[<u>ARA</u>]	arabinose	Assimilation	transparent	opaque
[<u>MNE</u>]	mannose	Assimilation	transparent	opaque
[<u>MAN</u>]	mannitol	Assimilation	transparent	opaque
[<u>NAG</u>]	N-acetyl-glucosamine	Assimilation	transparent	opaque
[<u>MAL</u>]	maltose	Assimilation	transparent	opaque
[<u>GNT</u>]	gluconate	Assimilation	transparent	opaque
[<u>CAP</u>]	caprate	Assimilation	transparent	opaque
[<u>ADI</u>]	adipate	Assimilation	transparent	opaque
[<u>MLT</u>]	malate	Assimilation	transparent	opaque

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[CIT]	citrate	Assimilation	transparent	opaque
[PAC]	phenyl-acetate	Assimilation	transparent	opaque
OX	see oxidase test	cytochrome oxidase	colorless/ light purple	dark purple

Quality Control

To be performed on receipt of every new lot of strip by the QC bench technologist.

Reference

Reference Package Insert - api 20NE system for the identification, bioMerieux Inc., Missouri USA.

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SYSTEM FOR IDENTIFICATION OF NEISSERIA & HAEMOPHILUS (API NH)

Principle

The API NH strip consists of 10 microtubes containing dehydrated substrates, which enable the performance of 12 identification tests (enzymatic reactions or sugar fermentations), as well as the detection of a penicillinase (particular interest in *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Branhamella catarrhalis* (*Moraxella catarrhalis*) and *Neisseria gonorrhoeae*).

The reactions produced during incubation result in spontaneous color changes or are revealed by the addition of reagents.

After a 2-hour incubation period at a temperature of 35-37°C, the reading of the reactions is performed visually and identification is obtained by consulting the profile list.

Reagents

API NH strips	}	Store at 2-8°C
NaCl 0.85% Medium (2 ml)		
JAMES reagent		
ZYM B reagent		
Swab		
Incubation box		
Result sheet		
1 package insert		
McFarland Standard, point 4 on the scale		
Mineral oil		
Pipettes		
Ampule rack		
Ampule protector		

Procedure

1. Specimen Processing



The microorganisms to be identified must first be isolated as separate colonies by streaking the specimen onto Blood agar, Chocolate agar or Martin-Lewis agar according to standard microbial techniques.

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2. Preparation of Strip

Each strip is composed of 10 cupules. Each cupule has an open and closed area (cupule and tube). An incubation tray is supplied for each strip. It serves as a support and individual chamber while both protecting the strip from contaminants in the air and assuring the humid atmosphere necessary to avoid dehydration during incubation.

- Remove the strip from its individual packaging
- Place the strip in the incubation box
- Discard the desiccant sachet

Record the specimen number on the flat portion of the tray (do not record the number on the lid as it may be misplaced during handling).

3. Preparation of the Inoculum

- Open an ampule of NaCl 0.85% Medium (2 ml) with the ampule protector.
- Using a swab, pick up a few well-isolated colonies and prepare a suspension with a turbidity equivalent to **4 McFarland, ensuring it is well mixed.**
- The suspension should be used immediately after preparation.

4. Inoculation of the Strip



- Distribute the prepared bacterial suspension into the cupules, avoiding the formation of bubbles (tilt the strip slightly forwards and place the tip of the pipette or PSIpette against the side of the cupule):
 - Only fill the tube part of the first 7 microtubes (PEN to URE): about 50 µl.
 - Fill tube and cupule of the last 3 microtubes LIP/ProA, PAL/GGT, βGAL/IND: about 150 µl, avoiding the formation of a convex meniscus.
- Cover the first 7 tests (PEN to URE) with mineral oil (underlined tests).

NOTE: The quality of the filling is very important: tubes which are insufficiently or excessively full may cause false positive or false negative results.

- Close the incubation box.
- Incubate for 2 hours at 35-37°C **in aerobic conditions.**

5. Incubation

Incubate for 2 hours at 35-37°C in aerobic conditions.

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6. Reading the Strip

Refer to the Reactions Table for a description of how to read the reactions.

Note all spontaneous reactions (PEN to β GAL) and record them as + or -.

- Add 1 drop of ZYM B reagent to microtubes 8 and 9: LIP/ProA and PAL/GGT.
- Add 1 drop of JAMES reagent to microtube 10: β GAL/IND.
- **Wait 2 minutes** then read the reactions by referring to the Reading Table in this package insert and record them on the result sheet.
 - If the LIP reaction is positive (blue pigment), interpret the ProA reaction as **negative**, whether the ZYM B reagent has been added or not.
 - If, after a 2-hour incubation period, several reactions (fermentation, penicillinase) are doubtful, re-incubate the strip for another 2 hours and read the reactions again (the enzymatic tests should not be re-read in this case).

Reactions Table



TESTS	REACTIONS	SUBSTRATES	QTY (mg)	RESULTS	
				NEGATIVE	POSITIVE
1) <u>PEN</u>	PENicillinase	Penicillin G	1.36	Blue (penicillinase absent)	Yellow Yellow-green Yellow-blue (penicillinase present)
2) <u>GLU</u> 3) <u>FRU</u> 4) <u>MAL</u> 5) <u>SAC</u>	GLUcose (Acidification) FRUctose (Acidification) MALtose (Acidification) SACcharose/Sucrose (Acidification)	Glucose Fructose Maltose Sucrose	0.5 0.1 0.1 0.5	Red Red-orange	Yellow Orange
6) <u>ODC</u>	Ornithine DeCarboxylase	Ornithine	0.55	Yellow-green Grey-green	Blue
7) <u>URE</u>	UREase	Urea	0.41	Yellow	Pink-violet
8a) <u>LIP</u>	LIPase	5-bromo-3-indoxyl-caprate	0.033	Colorless Pale grey	Blue (+precipitate)
9a) <u>PAL</u>	Alkaline Phosphatase	Para-Nitrophenyl-phosphate 2CHA	0.038	Colorless Pale yellow	Yellow
10a) <u>βGAL</u>	Beta GALactosidaase	Para-Nitrophenyl-BD galactopyranoside	0.04	Colorless	Yellow

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Reactions Table (Cont'd)

TESTS	REACTIONS	SUBSTRATES	QTY (mg)	RESULTS	
				NEGATIVE	POSITIVE
8b) <u>ProA</u>	Proline Arylamidase If LIP is +, ProA is always -	Proline-4-methoxy- β naphthylamide	0.056	<u>ZYM B / 3 min</u> Yellow Pale orange (brown if LIP +)	
9b) <u>GGT</u>	Gamma Glutamyl Transferase	Gamma glutamyl 4-methoxy- β naphthylamide	0.049	<u>ZYM B / 3 min</u> Yellow Pale orange (yellow-orange if PAL +)	
10b) <u>IND</u>	INDole	Tryptophane	0.036	<u>JAMES / 3 min</u> Colorless	

Interpretation

- Record results on a API NH profile sheet.
- Refer to the [API WEBSITE for Identification Profile](#)

Limitation

- Not to be used for identification of gram negative bacilli
- May mis-identify Streptobacillus moniliformis as Aggregatibacter aphrophilus, do not report Aggregatibacter aphrophilus based on this test.

Quality Control

To be performed on receipt of every new lot of strip by the QC bench technologist.

QC organisms to be used:



<i>Neisseria gonorrhoea</i>	ATCC 31426
<i>Haemophilus influenzae</i>	ATCC 10211
<i>Branhamella catarrhalis</i>	ATCC 23246
<i>Haemophilus paraphrophilus</i>	ATCC 49917

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Reference

Reference Package Insert - api NH system for the identification of *Neisseria* and *Haemophilus*
 bioMerieux Inc., Missouri USA.

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API WEBSITE for Identification Profile

1. Read API biochemical strip as per reading procedure.

2. Go to API website:


<https://apiweb.biomerieux.com/servlet/Authenticate?action=prepareLogin>

Login: See Application Login Information in the internal manual

3. Enter reactions or profile number

4. Obtain identification

5. Copy the identification and percent probability to the LIS workcard.

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BACITRACIN DISK TEST

Principle

This is a screening test for the presumptive identification of Group A Streptococci which are susceptible to 0.04U bacitracin. Other beta-haemolytic streptococci are usually resistant to this concentration of bacitracin.

Reagents

Bacto Differentiation Disks Bacitracin (0.04U). Store refrigerated.
5% Sheep Blood Agar (BA)

Other Materials

Culture loop
Cotton swabs
Forceps

Procedure


1. Inoculate the surface of the BA with the suspect beta haemolytic *Streptococcus*. Streak for confluent growth.
2. Using aseptic technique, place a bacitracin disk onto the inoculated surface.
3. Incubate in O₂ at 35°C X 18-24 hr.

Interpretation

Susceptible: any zone of inhibition around the disk (Presumptive Group A Streptococcus).
Resistant: growth up to the edge of the disk

Precautions

1. Other beta-haemolytic streptococci may be susceptible to bacitracin. Therefore this test can be used only for the presumptive identification of Gp. A Strep.

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Quality Control


Test with known susceptible and resistant control strains weekly.

Susceptible: Gp.A Strep. (ATCC 19615)

Resistant: Gp.B Strep. (ATCC 13813)

Reference

1. Difco Differentiation Disks Bacitracin package insert.

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BILE ESCULIN TEST

Principle

This test determines the ability of an organism to grow in the presence of bile and to hydrolyze the glycoside esculin to esculetin and glucose. The test is used to presumptively identify Group D Streptococci.

Materials

Bile esculin agar plate – Store at 2-8°C
Culture loop

Procedure

1. Heavily inoculate a bile esculin plate with the suspect organism.
2. Incubate in O₂ at 35°C for 18-24 hr.

Interpretation

Positive: Presence of a dark brown to black colour on the slant.

Negative: No blackening of the medium. Growth may occur, but this does not indicate esculin splitting.


Quality Control

Each new lot of media should be tested with known control strains.

Positive:	<i>E. faecalis</i>	(ATCC 29212)
Negative:	Gp.B Strep.	(ATCC 13813)
No Growth:	Gp.A Strep.	(ATCC 19615)

References

1. MacFaddin JF, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore MD, 1980, p4-12.

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BILE SOLUBILITY TEST

Principle

Tests the ability of alpha haemolytic streptococci to lyse in the presence of bile salts. This test is used for the identification of *Streptococcus pneumoniae*.

Reagents

BBL Spot Test dropper (10% sodium desoxycholate). Store at room temperature.

Procedure

1. Hold the dropper upright and squeeze gently to crush the glass ampoule inside the dispenser.
2. Place 1 drop of the reagent directly on isolated colonies of suspected *S. pneumoniae*.
3. Keep the plates very level to prevent the reagent from running and washing a non-pneumococcal colony away, producing a false positive result.
4. Incubate at room temperature on the bench for 15-30 minutes until the reagent dries. Do not invert the plate; leave the lid ajar.
5. Examine the colonies for lysis.

Interpretation

Positive (bile soluble): Lysis of the colonies.

Negative (bile insoluble): No lysis of colonies.

Quality Control

Test with known positive and negative control strains weekly.

Positive: *S. pneumoniae* (ATCC 6303)
Negative: *S. sanguinis* (ATCC 10556)

References



1. Murray PA, et al. Manual of Clinical Microbiology, 7th ed., 1999; p. 1665.
2. BBL Desoxycholate Reagent Droppers package insert, June 2010.

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CATALASE TEST

Principle

Detects the presence of the enzyme catalase which hydrolyzes H_2O_2 to produce H_2O and O_2 . This test is used to differentiate Staphylococci (catalase positive) from Streptococci (catalase negative).

Reagents

Hydrogen peroxide (H_2O_2), 3%

Store in a dark bottle and avoid any undue exposure to light.

Keep refrigerated at all times when not in use.

Other Materials

Clean glass microscope slides

Plastic culture loop or wooden applicator stick



Procedure

1. Pick a colony from an 18-24 hr culture and place it on a clean glass slide. Avoid carry over of blood agar which can cause false positives.
2. Put one drop of 3% H_2O_2 over the organism on the slide. Do not reverse the order of the procedure as false positive results may occur. Do not mix.
3. Observe for immediate bubbling (gas liberation) and record the result.
4. Discard the slide into a discard container.

Interpretation

Positive test: Immediate bubbling, easily observed (O_2 formed)

Negative test: No bubbling or scant, delayed bubbling

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Precautions

1. Carry over of blood agar must be avoided.
2. Growth for testing must be from an 18-24 hr culture.
3. 3% H₂O₂ is caustic - avoid exposure to skin, wear gloves. If H₂O₂ does get on the skin, immediately flood the area with water.
4. Aerosols may be released by the bubbling of the O₂.
5. H₂O₂ is unstable and breaks down easily on exposure to light. The solution must be kept refrigerated in the dark.


Quality Control

H₂O₂ is very unstable and should be tested daily or immediately prior to its use.

Positive: *S. aureus* (ATCC 25923)
Negative: *E. faecalis* (ATCC 29212)

References

1. MacFaddin JF, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore MD., 1980, p51-58.
2. Murray PA, et al. Manual of Clinical Microbiology, 7th ed., 1999; pp 426-427.

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CRYPTOCOCCAL ANTIGEN

Principle

Latex particles coated with anti-cryptococcal globulin (ACGR) reacts with cryptococcal polysaccharide antigen (in CSF or serum) causing a visible agglutination.

Specimen Collection and Processing

5 mL of blood is collected in a serum separator tube and separated by centrifugation. The serum is removed to a vial and refrigerated until testing. Specimens are stored at -20°C after testing.

Spinal fluid is collected in clean, sterile, centrifuge tubes. Specimens are stored refrigerated after testing.

Note: Fungus culture should also be set up.


Reagents

Meridian CALAS (Cryptococcal Antigen Latex Agglutination System)

- GBDA - Glycine buffered diluent with albumin.
- AGC - Antiglobulin control. Rehydrate with 1.5 mL dH₂O.
- NC - Negative control. Rehydrate with 2.5 mL dH₂O
 - Note: **inactivate the negative at 56°C for 30 minutes** each day the test is used.
- CAC - Cryptococcal antigen control (Positive control).
- Pronase - Rehydrate with 2.5 mL dH₂O, can be stored at 2°C-8°C for approximately 1 month.
- Detect Latex (ACGR) - Anti-Cryptococcal globulin reagent.
- Control Latex (NGR) - Normal globulin reagent.

Note: Ensure that all reconstituted vials are thoroughly dissolved before use

All reagents are stored refrigerated. Do not interchange reagents with a kit having a different lot number. Allow reagents to warm to room temperature before use. Mix gently before use.

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Other Materials

- 100 °C heating block
- 56°C heating block
- x 0.1 mL pipettes
- Rotator
- Small serologic test tubes
- Test tube rack
- Marking pen
- Applicator sticks
- Eppendorf 5415R Centrifuge



The following are provided by Meridian:

- Capillary pipettes
- Slide cards

Procedure

Specimen preparation





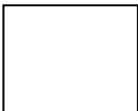
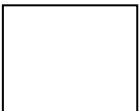
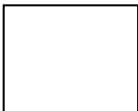
1. Store specimen refrigerated if testing is not done immediately.
2. Inactivate serum by mixing 200 µL of serum and 200 µL of pronase solution in a 12 x 75 mm tube and incubate at 56°C for 15 minutes. Further inactivate by heating at 100°C for 5 minutes. **This constitutes a 1:2 dilution.**
3. Each day, if using the test for the first time, heat inactivate the negative control at 56C for 30 minutes to avoid false QC reaction.
4. Centrifuge CSF at 1000g/3300 rpm in the Eppendorf 5415R centrifuge for 15 minutes. Inactivate the supernatant in a boiling water bath for 5 minutes.

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Performing the test

Note: Controls must be run each time a patient specimen is tested.

1. Set up and label the slide as follows:

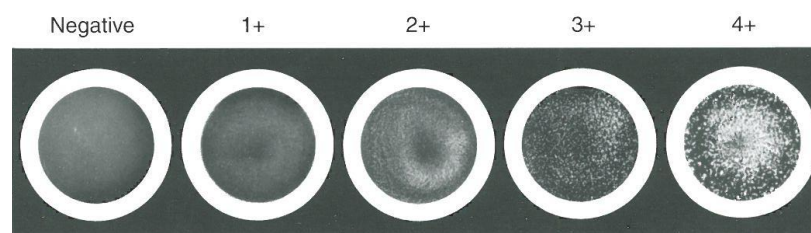
Detect Latex (NGR)		Not Used		
Control Latex (ACGR)				
CAC (POS Control)	AGC (Anti-globulin Control)	NC (Negative Control)	TEST	

2. Gently resuspend the latex particles in the **Detect Latex (ACGR)** and **Control Latex(NGR)** reagents. Rock each reagent just prior to use.
3. Place one drop of **Detect Latex (ACGR)** or **Control Latex (NGR)** into the designated spots.
4. Place 25 µL (one drop) of the cryptococcal antigen control (CAC) into the designated rings. Repeat with the negative control (NC) and anti-globulin control (AGC)
5. Place 25 µL of specimen in the designated rings.
6. Using a separate applicator stick, mix the contents of each ring thoroughly, spreading the contents over the entire surface area.
7. Place the slide on the rotator and rotate at 125 rpm for 5 minutes.
8. Read the reactions immediately.

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9. Rate the agglutination as follows: document agglutination results on worksheet.
A second technologist must double check/verify all results. Document verification on worksheet.

1+ = fine granulation against a milky background
 2+ = small but definite clumps against a slightly cloudy background
 3+ = large and small clumps against a clear background
 4+ = large clumps against a clear background



Positive Test: $\geq 2+$ agglutination (small but definite clumps or larger against a slightly cloudy or clear background)

Negative Test: $\leq 1+$ agglutination (fine granulation against a milky background) or a homogenous suspension of particles with no visible clumping)

Note: document 1+ agglutination in the system

Detect Latex (ACGR) + Positive Control = Positive
 Detect Latex (ACGR) + Negative Control = Negative
 Control Latex (AGC) + Anti-globulin Control = Positive
 Control Latex (AGC) + Positive Control = Negative
 Control Latex (AGC) + Negative Control = Negative

10. Patient specimens showing $\geq 2+$ agglutination in EITHER **Detect Latex** or **Control Latex** should be titrated against both **Detect Latex (ACGR)** and **Control Latex (AGC)** reagents.

- a) Prepare two-fold serial dilutions of the specimen using 200 μ L of GBDA in each of 8 test tubes labelled as follows:

Tube 1 2 3 4 5 6 7 8


Serum 1:4 1:8 1:16 1:32 1:64 1:128 1:256 1:512

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CSF 1:2 1:4 1:8 1:16 1:32 1:64 1:128 1:256

- b) Transfer one drop of each dilution into 2 rings.
- c) Add one drop of **Detect Latex** (ACGR) to one ring of each dilution.
- d) Add one drop of **Control Latex** (NGR) to each of the other rings.
- e) Mix using separate applicator sticks.
- f) Place the slide on the rotator and rotate at 125 rpm for 5 minutes.
- g) Rate the agglutination.
- h) If tube #8 gives an agglutination of 2+ or greater, the specimen must be further serially diluted until a titre may be obtained.

Interpretation of results

Negative: Negative result in initial screening tests against Detect Latex(ACGR).

Positive: The titre is reported as the highest dilution showing a 2+ or greater reaction with Detect Latex (ACGR) and negative with Control Latex (NGR).



Positive with Nonspecific Interference:

The titre with Detect Latex (ACGR) is at least 4 fold higher than the titre with Control Latex (NGR). (E.g. Detection Latex titer 1:32, Control Latex titer 1:8 or lower)

Uninterpretable (**Invalid**) test:

The titre with Detect Latex (ACGR) is less than 4-fold greater than the titre with Control Latex (NGR).
(E.g. Detection Latex 1:32, Control latex 1:16)

Note: If a Cryptococcal antigen result is uninterpretable, **retest** the sample at 1:10, 1:100, and 1:1000 dilutions for possible high antigen levels

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Reporting

Telephone all positive reports.

Negative Report: "Cryptococcal antigen not detected by latex agglutination. If the status of a patient suggests a cryptococcal infection, please submit follow-up specimens for additional testing."

Positive Report: "Cryptococcal antigen detected at a titre of ____ by latex agglutination."

Positives with titre $\leq 1:4$ add:

}CRLO "Titre of $\leq 1:4$ may be due to a very low level of antigen or non-specific cross-reactivity. Results should be interpreted within the context of the clinical signs, symptoms, and history of the patient."

Positive with Nonspecific Interference: "Cryptococcal antigen detected with nonspecific Interference."

Report result following serial dilutions performed after an initial uninterpretable result:



Initial Result	Repeat Result after Serial dilution	Final Report
Uninterpretable	Negative for all 1:10, 1:100, and 1:1000 dilution	Cryptococcal antigen not detected by latex agglutination. Sample was diluted up to 1:1000 and remained negative suggesting No evidence of a postzone phenomenon interfering with the test result. If the status of a patient suggests a cryptococcal infection, please submit follow-up specimens for additional testing."
	Positive at any dilution	Cryptococcal antigen detected at a titre of _____ by latex agglutination. (Sample was initially uninterpretable and became positive after a ____ dilution, suggesting the presence of a postzone phenomenon.)
	Uninterpretable at 1:1000 dilution	Cryptococcal antigen test invalid due to nonspecific interference) Result remained uninterpretable up to 1:1000 dilution. Unable to determine Cryptococcal antigen status. Recommend repeat testing with a new specimen if clinically indicated.

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Quality Control



The pattern of reactions for the controls must be as follows.

Detect Latex (ACGR)	+	N/A	-
Control Latex (NGR)	-	+	-
	CAC	AGC	NC

Failure to obtain this pattern indicates that the test must be repeated and the patient test results cannot be reported.

References

Product Insert, 1986 **Rev 7/01** Meridian Diagnostics Inc., 3471 River Hills Dr., Cincinnati, Ohio 45244. (513)-271-3700.

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E. coli O157 LATEX TEST (OXOID)

Principle

The Latex test will demonstrate by slide agglutination, *E. coli* strains possessing the O157 antigen. Sorbitol MacConkey Agar (SMAC) should be used as the primary screen. Non-sorbitol fermenting colonies (NSF) are tested with the latex reagents, to determine if the isolate belongs to the O157 serogroup, and is therefore a potential vero-cytotoxin (VT) producing strain.

Reagents

DR621M Test Latex -	consists of blue latex particles sensitized with specific rabbit antibody reactive with the O157 antigen.
DR622M Control Latex -	consists of blue latex particles sensitized with pre-immune rabbit globulins.
DR623M -	positive control suspension
DR624M -	negative control suspension

Storage

Do not freeze. Store at 2⁰C - 8⁰C. Do not use kit beyond the expiry date.



Procedure

NSF colonies may be taken from SMAC plates or alternatively NSF isolates may be inoculated onto non-selective agar media for testing.

For best results, sub NSF to a Blood Agar plate prior to testing.

It is necessary to test up to 10 separate NSF colonies to ensure a high probability of detection from mixed cultures.

- 1) Bring the latex reagents to room temperature. Make sure the latex suspensions are mixed by vigorous shaking.

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- 2) Dispense 1 drop of the Test latex onto a circle of the slide. Place it close to the edge of the circle.
- 3) Add some loopfuls or a pasteur pipette drop of saline to the circle. Ensure that the latex and saline do not mix at this stage.
- 4) Using a loop, pick off a portion of the colony to be tested and carefully emulsify in the saline drop.

Mix the Test latex and suspension together and spread to cover most of the reaction area using the loop.

- 5) Rock the slide in a circular motion, observing for agglutination. Do not rock the card for more than 1 minute and do not use a magnifying glass.
- 6) If no agglutination occurs, then proceed to test other NSF colonies if these are present.
- 7) If agglutination with the test reagent does occur, then it is necessary to test a further portion of the colony with the control reagent to ensure that the isolate is not an auto agglutinating strain.
- 8) When finished, dispose of the reaction slide into disinfectant.


Interpretation

- a) Positive result - Agglutination of the Test latex occurs within 1 minute.
No agglutination of the Control latex. *Perform biochemical tests to confirm that the organism is an *E. coli* strain.
- b) Negative result - no agglutination of the Test latex.
- c) Non-interpretable result - clumping of the Control latex.

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References

1. Borczyk A., Lior H., Crebin B. 1987. Int. J. Food. Microbiol. 4, 347-349.
2. Konowalchuk J., Speirs J. and Stavric S. 1977. Infect. Immune. 18, 775-779.
3. Scotland S., Day N. and Rowe B. 1980. FEMS Microbiol. Lett. 7, 15-17.
4. Centers for Disease Control. 1982. Morbid Mortal Wkly. 31, 580-585.
5. Karmali M., Steel B., Petric M. and Lim C. 1983. Lancet 8, 619-620.
6. Johnson W., Lior H. and Bezanson. 1983. Lancet 8, 76.
7. March S. and Ratnam. 1986. J. Clin. Microbiol. 23, 869-872.
8. Krishnan C., Fitzgerald V., Dakin S. and Behme R. 1987. J. Clin. Microbiol. 25, 1043-1047.

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GERM TUBE TEST

Principle

This is a rapid test for the presumptive identification of *C. albicans*.

Reagents

Horse serum

A small volume to be used as a working solution may be stored at 2 to 8°C. Stock solution can be dispensed into small tubes and stored at -20°C.

Other Materials

Clean glass microscope slides
Glass coverslips
Vitek tubes (13 x 100 mm)
Pasteur pipettes

Procedure



1. Put 3 drops of serum into a small Vitek tube.
2. Using a Pasteur pipette, touch a colony of yeast and gently emulsify it in the serum. The pipette can be left in the tube.
3. Incubate at 37°C for 2-4 hours but no longer.
4. Transfer a drop of the serum to a slide for examination.
5. Coverslip and examine microscopically using x 40 objective.

Interpretation

Germ tubes are appendages half the width and 3 to 4 times the length of the yeast cell from which they arise. There is no constriction between the yeast cell and the germination tube.

Positive test: presence of short lateral filaments (germ tubes)

Negative test: yeast cells only

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Precaution

C. tropicalis may form pseudohyphae which may be falsely interpreted as germ tubes.



Quality Control

Set up known controls each time a test is run.

Positive: *C. albicans* (ATCC 10231)
Negative: *C. tropicalis* (ATCC 13803)

Reference

1. Murray PA, et al. Manual of Clinical Microbiology, 7th ed., 1999; pp. 1189-1191.

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KOEHLER ILLUMINATION



The microscope should be set up using Koehler illumination for all parasitology examinations. This ensures that all the light from the lamp is being focused onto the specimen and that the field to be examined is evenly illuminated.

Procedure

1. Turn the lamp on.
2. Bring the condenser up to the top position, with the top lens swung in.
3. Open the condenser diaphragm.
4. Place a specimen on the stage and focus with the 10x objective.
5. Close the field diaphragm.
6. Lower the condenser until the edge of the field diaphragm is in sharp focus.
7. Center the field diaphragm image with condenser centering screws.
8. Open the field diaphragm until the edge just disappears from view.
9. Remove one eyepiece.
10. Looking down the eyepiece tube, close the condenser diaphragm until the illumination is approximately 2/3 full.
11. Replace the eyepiece.
12. Repeat for each objective lens when changed.

Reference

1. Baron E., Tenenbaum S.M., Bailey & Scott's Diagnostic Microbiology, 8th ed., The C.V. Mosby Company, p64.

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LAP TEST

Principle

LAP (Leucine- β -naphthylamide) impregnated disks serve as a substrate for the detection of Leucine aminopeptidase. Following the hydrolysis of the substrate by the enzyme the resulting β -naphthylamine produces a red colour upon the addition of cinnamaldehyde reagent. This test is usually used, in conjunction with other tests, for the identification of streptococci and other catalase negative gram positive cocci.

Reagents

LAP discs
Cinnamaldehyde reagent (0.01% p-dimethylamino-cinnamaldehyde)
(Disks and reagents are both in LAP kit, Store at 2-8°C)
Glass slide
Inoculating loop
Forceps
Sterile distilled water

Procedure

1. Place a LAP disk onto a glass slide and moisten it with one drop of sterile distilled water.
2. Rub a loopful of the culture onto the moistened disk holding it in place with sterile forceps.
3. Leave at room temperature for 5 minutes.
4. After 5 minutes, add 1 drop of cinnamaldehyde reagent.

Interpretation

Positive: red colour within one minute

Negative: no colour change or slight yellow colour

Quality Control

Test known positive and negative controls each time an unknown is run.


Positive: *E. faecalis* (ATCC 29212)
Negative: *Leuconostoc* (ATCC 8923)

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Reference

Remel LAP test package insert July 2010.

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MOTILITY TEST MEDIUM

Principle

Motility Test Medium is a semi-solid agar designed to demonstrate motility by diffusion.

Motility Test Medium is a modification of the formula of Tittsler and Sandhoizer. The medium contains small amounts of agar and gelatin, as well as triphenyltetrazolium chloride (TTC). TTC is a soluble compound which is taken up by the bacterial cells. Once the substance has been absorbed by the bacteria, it is reduced, releasing the acid formazan, a highly pigmented red, insoluble compound.

Organisms are stabbed into the medium with an inoculating wire. If the organisms are motile, they will diffuse into the soft medium laterally from the line of inoculation, resulting in a diffuse, pink colour throughout the medium. Non-motile organisms grow along the line of inoculation only, producing a pinkish-red line with no diffusion.

Storage

Upon receipt store at 2 - 8°C, away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking or discoloration), or if the expiration date has passed.



Limitations

Motility tests often show a false-negative reaction. The organism may be weakly motile, or the flagella may be damaged due to heating, shaking, or other trauma. Hanging drop motility may be performed from an inoculated tryptone broth incubated for 2-4 hours to confirm motility results. Consult appropriate microbiological texts for procedure.

TTC may be inhibitory to some fastidious bacteria.

Most motility of bacteria should be interpreted at 35°C: however, certain bacteria such as *Yersinia enterocolitica* demonstrate the best motility at 25°C.

Organisms that require oxygen for growth, such as *Pseudomonas aeruginosa*, will produce a spreading film on the surface of the medium, and will not fan out from the inoculation line where oxygen is depleted.

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Procedure

Prior to inoculation, the medium should be brought to room temperature. Inoculate selected colonies of a pure 18 to 24 hour culture, or from a turbid broth culture 4-8 hours old. Using a straight needle, stab the centre of the medium about 1/4" from the top. Incubate the tubes with the caps loose at 35°C (see "Limitations") for 18 - 24 hours. Observe for motility.

Interpretation

Positive: A diffuse pink colour occurring throughout the medium.

Negative: A pinkish red line at the stab site with no diffusion.

Quality Control



Test the following positive and negative control organisms on receipt of each shipment:

Positive: *Escherichia coli* (ATCC 25922)

Negative: *Klebsiella pneumoniae* (ATCC 13883)

References

1. Finegold, S.M., and E. J. Baron, Bailey and Scott's Diagnostic Microbiology, 7th ed., C.V. Mosby, St. Louis, 1986. Koneman, E.W., et al., Color Atlas and Textbook of Diagnostic Microbiology, J.B. Lippincott, Philadelphia, 1979. Lennette, E.H., et al., Manual of Clinical Microbiology, 4th ed., American Society for Microbiology, Washington, D.C., 1985.
2. MacFaddin, J.F., Biochemical Tests for Identification of Medical Bacteria, Williams and Wilkins, Baltimore, 1980. Tittsler R.P., and L.A. Sandhoizer, J. Bacteriol., 31:575, 1936.

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OPTOCHIN SENSITIVITY TEST

Principle

This test is used to determine an organism's susceptibility to the chemical Optochin (ethylhydrocupreine hydrochloride) for the presumptive identification of *S. pneumoniae*.

Reagents

Bacto Optochin Disks (5 µg disk) Store refrigerated at 2-8°C
 5% Sheep Blood Agar (BA)

Other Materials


Culture loop
 Forceps
 Cotton swabs

Procedure

1. Inoculate the suspected alpha haemolytic colony onto a BA to obtain confluent growth.
2. Using aseptic technique place an Optochin disk onto the surface of the inoculated agar. Press down with forceps.
3. Incubate at 35°C in CO₂ for 18-24 hours.

Interpretation

Susceptible: Zone of inhibition of at least 14 mm
 Resistant: Zone of inhibition less than 14 mm

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Quality Control

Test with known susceptible and resistant control strains weekly:

Susceptible: *S. pneumoniae* (ATCC 6303)
Resistant: Viridans Strep. (LPTP 8610)

References

1. MacFaddin JF, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore MD., 1980, p245-249.
2. BD BBL package insert, July 2010.

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ORNITHINE DECARBOXYLASE

Principle

This test is used to determine an organism's ability to decarboxylate or hydrolyze the amino acid - ornithine, forming an amine - putrescine that produces an alkaline pH. It is primarily used for the differentiation of *S. lugdunensis* from other coagulase-negative staphylococci.

Reagents

Møller's ornithine Decarboxylase (1% ornithine) with bromcresol purple. OXOID. Store refrigerated 2-8°C.

Mineral Oil

Other Materials

Culture loop

Procedure

1. Inoculate one or two colonies of a 18 to 24 hour culture of staphylococci into the ornithine decarboxylase tube.
2. Overlay the inoculated tube with approximately 1 mL of mineral oil.
3. Incubate at 35°C in O₂ for 18-24 hours.
4. Observe for purple colour.

Interpretation



Positive: Turbid, purple colour (alkaline)

Negative: Bright yellow colour (acidic)

Quality Control


Test with known positive and negative control strains weekly:

Positive: *S. lugdunensis* (ATCC 700328)
Negative: *S. aureus* (ATCC 25923)

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References

1. MacFaddin JF, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore MD., 1980.
 2. Izenberg H.D. 2003. Decarboxylase-Dihydrolase Tests 3.17.15, in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.
- WWe are currently using Pro-Lab TestOxidase reagent on filter paper that is then kept for one month, this practice is not documented in the product insert

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OXIDASE

Principle

This test determines whether an isolate produces oxidase enzymes. This test is mainly used, in conjunction with other tests, for the identification of gram negative organisms and *Bacillus* species.

Reagents

API Oxidase Reagent

- 0.2% Aqueous ascorbic acid: Reconstitute ascorbic acid with 25 ml sterile distilled water. This solution may be refrigerated 2-8°C for up to 28 days. The expiry date must be written on the bottle.
- N,N,N,-Tetramethyl-p-phenylenediamine-dihydrochloride:
Reconstitute with 5 ml of the 0.2% aqueous ascorbic acid. It is recommended that this be re-constituted 4-5 hours before use. This solution may be refrigerated for up to 7 days at 2 - 8°C. The expiry date must be written on the bottle.

Other Materials



filter paper
oxidase tray
Wooden applicator stick

Procedure

- Place a filter paper in the oxidase tray and moisten entire paper with oxidase reagent. Allow to air dry. May be used for up to 1 week
- Transfer a portion of the colony to the filter paper using a wooden applicator stick.
- Observe for 30 seconds.

Interpretation

Positive: Development of a purple colour within 30 seconds
Negative: No colour change

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Quality Control



Test daily with known positive and negative controls.

Positive: *P. aeruginosa* (ATCC 27853)

Negative: *K. pneumoniae* (ATCC 13883)

References

1. API Oxidase package insert 3/80.
2. MacFaddin JF, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore MD, 1980, p249-260.

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PASTOREX STAPH PLUS TEST

Principle

A rapid slide latex agglutination test for the detection of clumping factor, capsular polysaccharide and protein A produced by most strains of *S. aureus*.

Reagents and Materials

Pastorex test latex suspension (store refrigerated 2-8°C)
Disposable reaction cards
Plastic stick

Procedure



1. Confirm the identification of a suspect *Staphylococcus* by Gram stain and catalase test.
2. Allow the latex reagent to warm to room temperature before use.
3. Shake the reagent so that all of the particles are re-suspended.
4. Dispense one drop of latex test reagent in one of the circles on the reaction card.
5. Dispense one drop of negative control reagent in another circle on the card.
6. Emulsify 1 to 3 colonies into the test latex with a loop for 10 seconds using the plastic sticks provided with the kit or a plastic loop, do not use wood toothpicks
7. Repeat step 6 for the negative control reagent.
8. Gently rock the card for 30 seconds and look for clumping.
9. Discard the card into a discard container.

Interpretation

Positive test: Clumping within 30 seconds with the test latex particles only.

Negative test: No clumping in either latex.

Uninterpretable test: Clumping in the negative control.

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Precautions

1. False positive results may occur after 40 seconds.
2. False positive agglutination can occur with organisms other than staphylococci.
3. Gloves recommended, may cause allergic skin reaction,

Quality Control

Test known positive and negative controls daily.

Positive: *S. aureus* (ATCC 25923)
Negative: *S. epidermidis* (ATCC 12228)

References

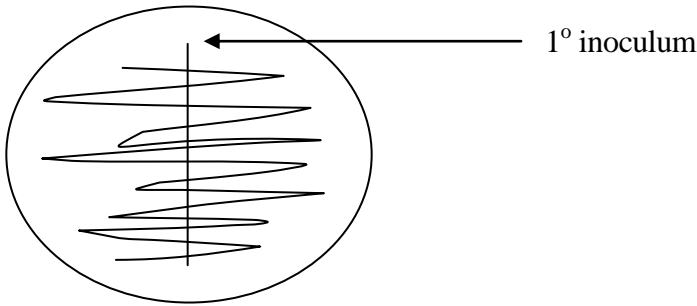
1. Pastorex Staph Plus package insert Jan 2015..

PLATE STREAKING METHODS

Blood Agar and MacConkey Agar for Urine Cultures

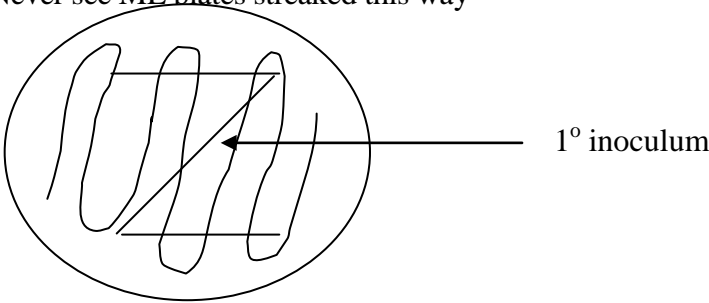
1 uL disposable loop

Inoculate in one continuous streak down the middle of the plate. With the same loop, streak out the entire plate at 90° to the initial inoculum. Streak a minimum of 15 lines.

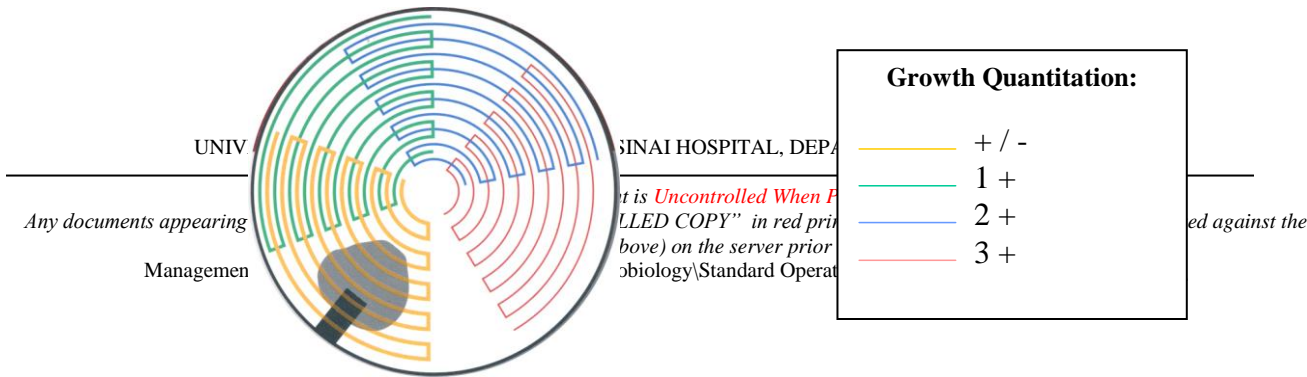



Martin-Lewis Agar

Inoculate plate with specimen swab in a "Z" pattern across the plate (with continuous rotation of the swab while inoculating). Streak the entire plate with a sterile loop at 90° to the initial inoculum. Streak a minimum of 15 lines ???. Never see ML plates streaked this way



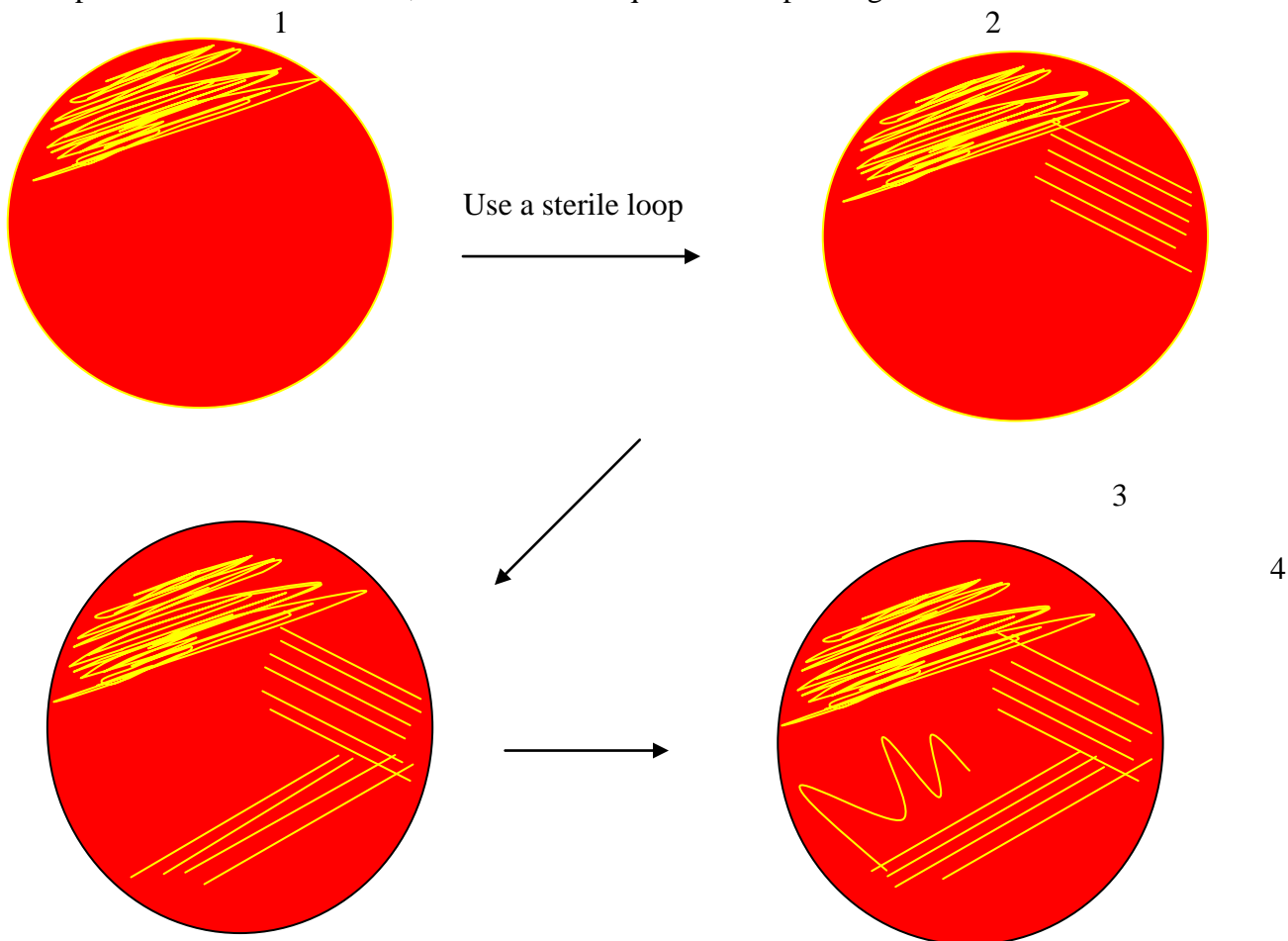
Isoplater Streaking





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Manual Streaking

Inoculate specimen with swab or loop onto the entire first quadrant of the agar plate. Use a sterile loop and streak out the second, third and fourth quadrants as per diagram:



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PYR TEST

Principle

PYR (L-pyrrolidonyl- β -naphthylamide) impregnated disks serve as a substrate for the detection of pyrrolidonyl peptidase. Following the hydrolysis of the substrate by the enzyme the resulting β -naphthylamine produces a red colour upon the addition of cinnamaldehyde reagent. This test is used, in conjunction with others, for the identification of catalase negative, gram positive cocci including Enterococci and Group A Streptococci.

Reagents

Dry slide PYR

Cinnamaldehyde reagent (0.01% p-dimethylamino-cinnamaldehyde)

(Slide and reagents are both in PYR kit, store at room temperature.)

Glass slide

Inoculating loop

Forceps

Sterile distilled water


Procedure

1. Aseptically cut a piece of dry slide and place on a glass slide. Do not touch the reaction area.
2. Moisten it with one drop of sterile distilled water.
3. Rub a loopful of the culture onto the moistened slide holding it in place with sterile forceps.
4. Leave at room temperature for 2 minutes.
5. After 2 minutes, add 1 drop of cinnamaldehyde reagent.

Interpretation

Positive: Pink or cherry red colour within one minute

Negative: No colour change or slight yellow colour

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Quality Control



Test knows positive and negative controls each time an unknown is run.

Positive: Group A streptococcus (ATCC 19615)

Negative: Group B streptococcus (ATCC 13813)

Reference

BD BBL Dryslide PYR package insert June 2010.

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QUANTITATION OF ORGANISMS & CELLS ON SMEARS & CULTURE

Microscopic:

Gram Smear:

Report as:

± <1 per oil immersion field	Few
+ 1 - 5 per oil immersion field	1+
++ 5 - 10 per oil immersion field	2+
+++ >10 per oil immersion field	3+

Culture:

Report as:



± few colonies in primary inoculum	Scant growth
+ confluent growth in primary inoculum	Light growth
++ growth up to 2nd quadrant	Moderate growth
+++ growth in or >3rd quadrant	Heavy growth

Size of colonies:

lg	- large
med	- medium
sm	- small
tiny	- tiny
ppt	- pinpoint

References

- H.D. Izenberg. 2003. in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C
- Acid-fast Bacilli (AFB): Change in Reporting the Enumeration of AFB Smears, Labstract, Ontario Public Health Laboratories, January 2007

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RapID ANA II SYSTEM

Principle

The RapID ANA II System is a qualitative micromethod employing conventional and chromogenic substrate for the identification of medically important anaerobic bacteria of human origin.



The tests used in it are based upon the microbial degradation of specific substrate detected by various indicator systems. The reactions are a combination of conventional tests and single-substrate chromogenic tests.

Materials

1. RapID ANA II panels – Store at 2 - 8°C
2. Suspension fluid – Store at 2 - 8°C
3. Kovacs spot indole reagent – Store at 2 - 8°C
4. RapID ANA II reagent – Store at 2 - 8°C
5. RapID ANA ID forms

Procedure

Make an equivalent McFarland #3 turbidity suspension of 18-24 hours AnO₂ culture (not more than 72 hours) in the supplied suspension fluid. Mix it thoroughly - can be used up to 15 minutes. Inoculate an agar (BA FAA) plate for purity and incubate for 24 hours anaerobically. Peel the lid off the panel marked "peel to inoculate". Using the Pasteur pipette, transfer the entire contents into the right upper corner of the panel. Seal the panel. Level the contents in the panel and slowly tilt the panel so that every chamber receives an equal amount of suspension. Incubate the panel at least four hours (not more than six hours) in non-CO₂ incubator at 35-37°C. After the incubation period, read the panel prior to adding the reagents and write results on the ID form. Add the reagents as per instructions. Allow 30 seconds but not more than two minutes. Read it and score on the form.


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Interpretation and Identification

Please follow the guidelines from the manufacturer and see <http://remel.com/eric/> for identification code.

See internal manual for login information.

See RapID ANA II System Insert #iii08-1/94.

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RapID VP TEST

Purpose

To aid in the identification of *S. anginosus group*

Media

MR - VP broth – Store at 2-8°C

Procedure



1. Transfer approximately 0.2 ml of VP broth into a sterile 13 x 100 test tube.
2. Using a sterile inoculating wire inoculate the test organism heavily into the broth.
3. Incubate the tube at 35°C for 5 hours.
4. After incubation add 1 drop of alpha-naphthol and 1 drop of 40% KOH.
5. Shake the tube gently for one minute to expose the medium to air. Allow 10-15 minutes for reaction to develop.

Interpretation

- Positive - Red colour
 Negative - No colour change within 10-15 minutes

Precautions

The order of adding reagents are important; alpha-naphthol followed by 40% KOH.

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Quality Control


Quality control must be performed on each new lot of Rapid VP reagent before being put into general use and once weekly with the following organisms:

S. pyogenes (ATCC 19615): Negative

Streptococcus species Group F (ATCC 12392): Positive

Reference

Ruoff, K.L., Ferraro, M.J. 1986. Presumptive identification of *S. milleri* in 5h J Clin Microbiol 24:495-497.

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SALMONELLA SEROLOGICAL AGGLUTINATION TEST

I. Introduction:

Remel Salmonella serological agglutination is a slide agglutination test using somatic (O), flagella (H) or capsular (Vi) antisera to aid in the identification of *Salmonella* species. Antibodies in the serum will agglutinate with homologous bacterial antigens from culture colonies.

II. Reagent:

1. Somatic (O) antisera
2. Flagella (H) antisera
3. Capsular (Vi) antisera

Sera should be stored at 2 to 8°C and used until the expiration date shown on the bottle label.

On storage, some sera become slightly turbid and does not necessarily indicate deterioration and normally will not interfere with results. Gross turbidity indicates contamination and should be discarded.

III. Materials:

1. Glass slide
2. Bacteriology loop
3. Sterile saline
4. China marker

IV. Procedure:

Testing should be performed on suspect colonies from a **fresh** culture (18-24hours) sub cultured on a **non-selective agar** e.g. BA, TSI. **Do not** use MAC or HEK plates to perform agglutination test.


1. With a China marker, divide two slides into two sections or make 2 circles.

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- On the first slide, mark the first section as “O”, the second as “H”. On the second slide, mark the first section as “Vi” and the second as a negative saline control.
- Put one drop of each reagent in their respective section. Add a drop of saline to the negative control section.
- Using a clean loop for each section, emulsify the organism into each section to give a homogenous, fairly dense suspension.
- Rock the slide gently and observe for agglutination using indirect lighting over a dark background. Agglutination should be strong and clearly visible within one minute.
- If there is agglutination in the saline control, the test is invalid.

V. Interpretation of results

Somatic (O) antisera	Flagella (H) antisera	Capsular (Vi) antisera	Saline	Interpretation
+	+	+	-	Possible <i>Salmonella typhi</i>
+	-	+	-	Possible <i>Salmonella typhi</i>
+	+	-	-	Probable <i>Salmonella</i> spp, not <i>typhi</i>
+	-	-	-	Probable <i>Salmonella</i> spp, not <i>typhi</i>
-	+	+	-	Possible <i>Salmonella typhi</i>
-	-	+	-	Possible <i>Salmonella typhi</i>
-	-	-	-	Not <i>Salmonella</i> spp
+/-	+/-	+/-	+	INVALID

Note: Cross-reactions may occur with Enterobacteriales.

See Vitek-MS-V2-speciesList Created-Translatedlist for reporting isolate species

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VII. Quality Control:

Quality control testing should be run with each shipment and on new kit lot number received and performs QC weekly.

QC Organisms	Somatic (O) antisera	Flagella (H) antisera	Capsular (Vi) antisera
<i>Salmonella typhimurium</i> ATCC#14028	+	+	-
<i>Salmonella paratyphi</i> ATCC#9150	+	+	-
<i>Salmonella typhi</i> ATCC#19430	-	+	+
<i>E.coli</i> ATCC #25922	-	-	-

VIII. Reference:

Remel Salmonella Polyvalent Agglutination Sera insert, June 2013.

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SATELITE TEST (STAPH STREAK) FOR *H.INFLUENZAE*

I. Introduction

Haemophilus Influenzae is a bacterium that requires hemin (X) and NAD (V) factors to thrive in a blood agar medium. While X factor is readily available in blood Agar, V factor is not. *Staphylococcus aureus* is cross inoculated to hemolyze red blood cells (RBC) in the Blood Agar, thus releasing the required V factor. As a result, *H. Influenzae* will grow in proximity to the "staph steak". This phenomenon is known as satellitism


A *Staph* streak is performed to support recovery of presumptive *Haemophilus influenzae* on blood agar, and can be useful in terms of biosafety to aid in differentiating organisms associated with suspected RG3 cultures and small gram-negative bacilli/coccobacilli seen in Gram stain such as *Brucella species*.

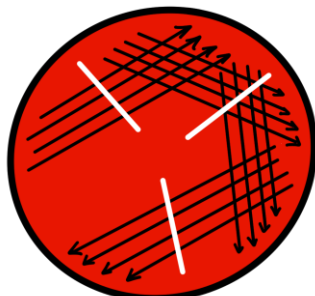
II. Materials

- Blood Agar
- *S. aureus* (ATCC 25923) colonies
- Sterile loop
- Clinical sample (primary sample or organism colony)

III. Procedure

1. Inoculate blood agar sample and streak for isolated colonies.
2. Using a plastic loop, take generous loop full of *S. aureus* (ATCC 25923) and cross inoculate a straight line through quadrant 1, 2, 4 of the streaked blood agar.

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3. If RG3, seal the plate with parafilm / follow RG3 protocols.
4. Incubate accordingly to BA and safety protocols.

IV. Results

Positive:

Satellitism: Growth of colonies in the vicinity of *S. aureus* colonies larger than those further from it.


Negative:

Absence of growth in the vicinity of *S. aureus* colonies.

Uniform growth around agar independent of *S.aureus* colonies

V. Reference

Clinical microbiology procedures handbook / editor in chief, Amy L. Leber,
Department of Laboratory Medicine, Nationwide Children's Hospital, Columbus, Ohio.
4th edition. | Washington, DC : ASM Press, [2016]

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SHIGELLA SEROLOGICAL TESTING

I. Introduction:

Serological testing is performed to identify *Shigella species*. Testing is performed by a slide agglutination test using (O) antisera.

II. Reagent:

Remel *Shigella* Polyvalent Agglutination Sera:

1. *Shigella dysenteriae* polyvalent (1-10)
2. *Shigella flexneri* polyvalent (1-6, x, y)
3. *Shigella sonnei* phase 1 & 2
4. *Shigella boydii* polyvalent 1 (1-6)
5. *Shigella boydii* polyvalent 2 (7-11)
6. *Shigella boydii* polyvalent 3 (12-15)

Sera should be stored at 2-8°C and used until the expiration date shown on the bottle label.

III. Materials:

1. Glass slide
2. Bacteriology loop
3. Sterile saline
4. China marker

IV. Procedure:

Testing should be performed on suspect colonies from a **fresh** culture (18-24hours) sub cultured on a **non-selective agar** e.g. BA, TSI. **Do not** use MAC or HEK plates to perform agglutination test.



1. With a China-marker, divide 6 slides into two. Make a circle in each section.
2. Put a drop of saline in each circle.
3. Using a loop, emulsify the organism in each drop of saline to give a homogenous, fairly dense suspension.
4. Add one drop of undiluted antiserum of each sera to one of the suspensions and mix. The last suspension serves as a control.

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5. Rock the slide gently and observe for agglutination using indirect lighting over a dark background. Agglutination should be strong and clearly visible within one minute.
6. If there is agglutination in the saline control, the test is invalid.
7. If the isolate is biochemically a *Shigella* species, but fails to agglutinate with the grouping sera, send isolate to PHOL for confirmation.

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V. Interpretation of Results:


<i>Shigella dysenteriae</i> polyvalent (1-10)	<i>Shigella flexneri</i> polyvalent (1-6, x, y)	<i>Shigella sonnei</i> phase 1 & 2	<i>Shigella boydii</i> polyvalent 1 (1-6)	<i>Shigella boydii</i> polyvalent 2 (7-11)	<i>Shigella boydii</i> polyvalent 3 (12-15)	Saline	Interpretation
+	-	-	-	-	-	-	<i>Shigella dysenteriae</i> (1-10)
-	+	-	-	-	-	-	<i>Shigella flexneri</i> (1-6, x, y)
-	-	+	-	-	-	-	<i>Shigella sonnei</i> phase 1 & 2
-	-	-	+	-	-	-	<i>Shigella boydii</i> (1-6)
-	-	-	-	+	-	-	<i>Shigella boydii</i> (7-11)
-	-	-	-	-	+	-	<i>Shigella boydii</i> (12-15)
-	-	-	-	-	-	-	Negative
+/-	+/-	+/-	+/-	+/-	+/-	+	INVALID

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VI. Quality Control:

Quality control testing should be run with each shipment and on new kit lot number received and performs QC monthly .

QC Organisms	<i>Shigella dysenteriae</i> polyvalent (1-10)	<i>Shigella flexneri</i> polyvalent (1-6, x, y)	<i>Shigella sonnei</i> phase 1 & 2	<i>Shigella boydii</i> polyvalent 1 (1-6)	<i>Shigella boydii</i> polyvalent 2 (7-11)	<i>Shigella boydii</i> polyvalent 3 (12-15)	Saline
<i>Shigella dysenteriae</i> ATCC 13313	+	-	-	-	-	-	-
<i>Shigella flexneri</i> ATCC 12022	-	+	-	-	-	-	-
<i>Shigella sonnei</i> ATCC 25931	-	-	+	-	-	-	-
<i>Shigella boydii</i> ATCC 9207	-	-	-	+	-	-	-
Saline Control	-	-	-	-	-	-	-

VII. Reference:

Oxoid Remel *Shigella* Polyvalent Agglutination Sera Package Insert.

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SHIGELLA WELLCOLEX LATEX AGGLUTINATION

I. Introduction:

Wellcolex Colour Shigella provides a simple, rapid qualitative procedure for detection and species identification of shigella present on solid culture media.

II. Reagent:

1. Latex reagent 1
2. Latex reagent 2
3. Red Positive Control
4. Blue Positive Control

Reagent kit to be stored at 2 to 8°C and used until the expiration date shown on the box.

III. Materials:

Materials provided:

1. Disposable sampling sticks
2. Disposable reaction cards
3. Disposable sample dispensers
4. Disposable suspension tubes

Material required but not provided:

1. 0.45% sterile saline
2. The test should be performed on a flatbed rotator operating at approximately 80 rpm.

IV. Procedure:



1. Use disposable sample dispensers dispense 0.5 ml saline in the disposable suspension tubes.
2. From an overnight culture pick few colonies average-sized (1 to 2 mm) suspected Shigella colonies from the culture plate using the flat end of a sampling stick and emulsify the bacteria in the saline.
3. Resuspend latex reagent 1 and 2 by shaking vigorously for a few seconds.

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Dispense one drop of each latex reagent into a separate circle on a reaction card without bubbles.

- Transfer one drop of bacterial suspension to each of the two circles.
- Using a sampling stick, mix the contents of each circle and spread to cover the whole area of the circle.
- Place the card on the flat bed rotator and run at 150 ± 5 rpm for 2 minutes.

V. Interpretation of results

Colour of clumps	Reagent 1	Reagent 2
Red	Shigella sonnei	Shigella dysenteriae
Blue	Shigella flexneri	Shigella boydii
None	Negative	
Purple	Not interpretable (further isolation procedures required)	

Positive reaction report:

Shigella species,

“~presumptive identification, confirmation to follow.”



“~NOTE: Occasionally, some E. coli may identify as presumptive Shigella species using our current in-lab methodology; confirmation by Public Health Lab reference methodology to follow.”

VII. Quality Control:

Quality control testing should be run with each shipment and on new kit lot number received and performs QC weekly.

Positive Control Procedure:

- Into 4 separate reaction circles, dispense 2 drops of latex reagent 1 and 2 (one drop per circle).
- Add one drop of **each** positive control to 2 of the reaction circle (one containing latex reagent 1 and one containing latex reagent 2).
- Mix the reagents using a separate disposable sampling stick for each positive control.
- Rotate the card at 150 ± 5 rpm for 2 minutes on a rotator.

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The color of the agglutinated latex in reagent 1 and reagent 2 should correspond to the color of the positive control (blue or red).

Negative Control Procedure:

Repeat the test procedure using saline instead of the test sample. There should be no significant agglutination.

VIII. Reference:

1. Oxoid Wellcolex Colour Shigella insert

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SPOT INDOLE

Principle

Spot indole reagent is for determination of the ability of micororganisms to spit indole from the tryptophan molecule by the spot test method.

Reagents and Materials

Inoculating loop
 Spot Indole Reagent – store product in at 2-30°C
 Filter paper

Procedure

1. Dispense 1 of 2 drops of reagent onto a piece of filter paper.
2. Smear growth from culture onto saturated filter paper
3. Observe for blue colour within 1-3 minutes

Interpretation

Positive Test – Blue colour within 3 minutes

Negative Test – Pink colour

Precautions



- Product should not be used if reagent colour has changed
- Test colonies from media without glucose
- Do not test from MAC or EBM agar. May produce false positives.
- Certain strains of *Proteus vulgaris*, *Providencia spp.*, and *Aeromonas spp.* Will give false negative results.

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Quality Control

Positive: *E. coli* ATCC 25922

Negative: *Proteus mirabilis* ATCC 12453

Reference

Remel Thermo Scientific package insert July 2012.

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STAINING METHODS

ROUTINE STAINS

GRAM STAIN

Principle

Bacteria can be recognized as gram positive (blue-black/purple) if they retain the primary dye complex of crystal violet and iodine in the face of attempted decolourization, or as gram negative (pink) if decolourization occurs as shown by the cell accepting the counterstain safranin.

Generally the mechanism of the Gram stain is: The fixed bacteria are stained with the triphenylmethane dye, crystal violet. Next the smear is flooded with Grams solution which oxidatively forms an insoluble complex with the crystal violet. The smear is then flooded with the organic solvent, acetone-alcohol. Depending on cell permeability the crystal violet-iodine complex will be washed from Gram negative bacteria in solvent but not from Gram positive bacteria. Upon counterstaining with safranin, organisms which had been discolorized by the ethanol (Gram negative) will stain pink. Gram positive organisms which retained the crystal violet will appear blue-black/purple microscopically.

Materials

Methanol
 Crystal violet solution
 Grams Iodine solution
 Acetone alcohol
 Safranin solution

Store solutions at room temperature. Refer to MSDS for storage requirements of alcohols.

Procedure



1. Prepare the film on the slide and allow to air dry.
DO NOT HEAT TO DRY FILM.
2. When film is dry, flood slide with methanol to fix smear. Let smear dry completely before staining.
3. Flood slide with crystal violet - leave 1 minute.

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4. Wash gently with water.
5. Flood slide with Grams Iodine - leave 1 minute.
6. Wash iodine from slide with acetone-alcohol mixture. Add a few more drops of acetone-alcohol until no more colour comes from film - usually 30 seconds.
7. Wash gently with water.
8. Flood slide with safranin - leave 1 minute.
9. Wash gently with water. Clean back of slide with tissue and place slide in tray.



Precaution

1. At no time should the film (smear) be exposed to too much heat. When the specimen is still wet, heat causes coagulation of the protein resulting in heavy overstaining which cannot be removed by the decolourizer. A thick smear will also show more tendency to "lift off" during staining.
2. Rinsing the Grams Iodine off with the decolorizer gives more stability to the CV-GI complex and false over decolorizing will not take place.
3. Flooding a hot slide with crystal violet will cause the stain to precipitate and make decolourizing much more difficult.

Note: Gram positive bacilli which are beaded or branching should be stained using the [Modified Kinyoun](#) and non-modified [KINYOUN](#)

Quality Control

Run control slides concurrently with unknowns at least once daily using known smears containing Gram positive and Gram negative bacteria.

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GRAM STAINING MACHINE-MIDAS III OPERATION

Principle

Adapting the principle of Gram Stain, the **MIDAS III** will carry fixed smears through different staining solutions for a pre-set time to produced a Gram stained smear.

Materials

Staining Rack
Methanol
Crystal violet solution
Gram's Iodine solution
Acetone alcohol (50%/50%)
Safranin solution

Store solutions at room temperature. Refer to MSDS for storage requirements of alcohols.

Instrument Programming Procedure

1. Press "Program"
2. Follow the instructions on the screen and program the duration of each staining step as follow:

For **Program 1** (thin smears)



Step	Station	Reagent	Time Set
1	1	Crystal Violet	1 minute
2	5	Water	1 minute; flow rate 1500
3	2	Gram's Iodine	1 minute
4	5	Water	1 minute; flow rate 1500
5	3	Acetone Alcohol	20 seconds
6	5	Water	1 minute; flow rate 1500
7	4	Safranin	1 minute
8	5	Water	1 minute; flow rate 1500
9	6	Dry bath	2 minutes

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For **Program 2** (thick smears):

Step	Station	Reagent	Time Set
1	1	Crystal Violet	1 minute
2	5	Water	1 minute; flow rate 1500
3	2	Gram's Iodine	1 minute
4	5	Water	1 minute; flow rate 1500
5	3	Acetone Alcohol	45 seconds
6	5	Water	1 minute; flow rate 1500
7	4	Safranin	1.5 minutes
8	5	Water	1 minute; flow rate 1500
9	6	Dry bath	2 minutes

3. Press “End” to save program.



Procedure

Daily Set-up:

1. Remove staining station covers.
2. Discard old stains.
3. Wash containers with tap water.
4. Fill containers with fresh stains/decolourizer.
5. Record stain change in LIS micqc.

Staining Procedure:

1. Fix dry smears with methanol and air dry completely.
2. Load fixed smears on MIDAS III slide holder
3. Load holder onto the prongs of MIDAS III.
4. Press “RUN”, then “1” for regular thin smears OR “2” for thicker sputum smears.
5. Press “Enter”
6. Remove smear holder when staining is complete.



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Quality Control

Run control slides concurrently with unknowns once per shift using known smears containing Gram positive and Gram negative bacteria.

Reference

MIDAS III operation manual.

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ACRIDINE ORANGE STAIN

Principle

Acridine orange is a fluorescent dye which will bind to the nucleic acid of bacteria and other cells. It is recommended for use for the detection of microorganisms in direct smears. It is useful for the rapid screening of specimens from normally sterile sites (eg. CSF) and blood smears, or smears containing proteinaceous material where differentiation of organisms from background material may be difficult.

Reagents



Acridine Orange spot test dropper. Stored at room temperature
 Absolute Methanol

Procedure

1. Prepare a smear of the specimen to be stained.
2. Allow to air dry.
3. Fix with methanol for 1 to 2 minutes.
4. Hold the dropper upright and squeeze gently to crush the glass ampoule inside the dispenser.
5. Flood the slide with the acridine orange and stain for 2 minutes.
6. Rinse thoroughly with tap water and allow to air dry.
7. Examine with a fluorescent microscope using low and oil immersion objectives.

Interpretation

Bacteria and fungus stain bright orange. The background appears black to yellow green. Leukocytes will stain yellow, orange and red.


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Quality Control

Run a control slide concurrently with unknowns each time using known smears containing Gram positive and Gram negative bacteria.

References

1. BD Acridine Orange Stain package insert. June 2010.

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STAINS FOR GRAM POSITIVE BRANCHING / BEADED BACILLI

ACID FAST STAIN FOR MYCOBACTERIA (KINYOUN)

Principle

To stain Mycobacteria present in specimens and cultures.

Mycobacteria are different to stain with common aniline dyes. However, they will stain with basic fuchsin. Once stained, they retain the dye despite treatment with mineral acids i.e. HCl H₂SO₄. This property of acid fastness may be due to a lipid fraction called mycolic acid. Mycobacteria also exhibit degrees of resistance to decolourization with alcohol.

Materials

Kinyoun Carbol fuchsin
 3% HCl in 95% ethanol
 Brilliant green

Store reagents at room temperature. Store alcohols as per MSDS.

Procedure



1. Prepare smear over an area of 2-3 sq. cm.
2. Heat fix smear with methanol.
3. Place slide on stain rack and allow to cool. Flood with Kinyoun Carbol fuchsin for 5 min.
4. Rinse off stain with water.
5. Decolourize with 3% acid alcohol for 3 mins.
6. Rinse with water.
7. Repeat decolourization for 1-2 mins. or until no red appears.
8. Rinse with water.

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9. Flood slide 3-4 mins. with Brilliant green.
10. Rinse with water.
11. Air dry. DO NOT BLOT.

Microscopy

Place a drop of oil between the specimen and coverslip and oil again on top. Smears are examined with oil immersion lens. The coverslip prevents cross contamination.

Interpretation

The filaments of *Mycobacterium* species appear red-stained against a green background.

Quality Control

Mycobacterium Control Slide (Remel R40144) set up simultaneously with the processing of clinical specimens.

Control Results:



Positive Control: Red-stained bacilli

Negative Control: Blue-green-stained bacilli

References

Baker, F.J., Breach, M.R. 1980. Medical Microbiological Technique, p. 15

Murray, PA. et al. Manual of Clinical Microbiology, 8th edition, 2003 ASM, Washington, D.C.

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ACID FAST STAIN FOR NOCARDIA (MODIFIED KINYOUN)

Principle

Nocardia species possess the unique characteristic of resisting decolorization with 1% sulfuric acid.

Materials

1. Carbol-fuchsin

Basic fuchsin solution	(3 g basic fuchsin in 100 mL 95% ethyl alcohol)
	10 mL
Phenol 5% aqueous	90 mL
2. Decolourizer (1% sulfuric acid)

H ₂ SO ₄ (concentrated)	1 mL
Distilled water	99 mL
3. Methylene blue

Methylene blue	0.3 g
Distilled water	100 mL

Store reagents at room temperature.

Procedure

Prepare 2 smears: one for the **Modified Kinyoun** (procedure as follows), the other slide to be stained in parallel with **KINYOUN** method. * *Do not make the smears too thick.*

Retrieve a Modified Kinyoun control smear for parallel staining.


1. Shake stains before use
2. Fix the smear by methanol, allow to air dry.
3. Flood the slide with Carbol fuchsin and allow to stand for 5 minutes
4. Rinse Carbol Fuchsin off the slide with tap water for 5 seconds
5. Wash the slide with 50% ethanol using the squirt bottle. This should be done for 5-7 seconds and all the film from the dye should be washed off.
6. Rinse slide with tap water for 5 seconds
7. Decolorize the slide by adding 1% sulfuric acid for 2 minutes. Place the slide on the rack over the sink and add fresh 1% sulfuric acid every 30 seconds - at 0s, 30s, 60s, 90s)
8. Rinse with tap water for 5 seconds

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9. Flood the slide with methylene blue for 1 minute to counter stain
10. Rinse off methylene blue dye with tap water and then let slide air dry

Microscopy

Place a drop of oil between the specimen and coverslip and oil again on top. Smears are examined with oil immersion lens. The coverslip prevents cross contamination.

Interpretation

MODIFIED Kinyoun stain: the filaments of *Nocardia* species and *Rhodococcus* appear red-stained against a blue background.

Kinyoun stain: the filaments of *Nocardia* species and *Rhodococcus* should **NOT** show red-stained bacilli.



Quality Control

Positive control: *Nocardia* species.

Negative control: E coli

References

Murray, PA. et al. Manual of Clinical Microbiology, 8th edition, 2003 ASM, Washington, D.C.

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REPORTING FOR GRAM POSITIVE BRACHING / BEADED BACILLI

If initial Gram smear shows:

- 1+ pus cells
- 3+ gram positive bacilli, branching
- or
- 3+ gram positive bacilli , beaded

Stain new smear with [Modified Kinyoun](#) and [KINYOUN](#) for further characterization

Reporting:

Report as GRAM Test Comment:

Both modified and non-modified Kinyoun stains = negative:

Both Modified acid-fast and acid-fast stains: negative.



This suggests possible *Actinomyces* species. Culture confirmation to follow.

Both modified and non-modified Kinyoun stains = positive:

Modified acid-fast stain: positive. Acid-fast stain: positive. This suggests possible *Mycobacterium* species. Culture confirmation to follow.

Modified Kinyoun stain = positive; non-modified Kinyoun stain = negative:

Modified acid-fast stain: positive. Acid-fast stain: negative. This suggests possible *Nocardia* species. Culture confirmation to follow.

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FUNGAL STAINS

FUNGI-FLUOR™ STAIN

Purpose

The Fungi-Fluor™ stain is used for the rapid identification of various fungal elements in fresh or frozen clinical specimens.

Principle

The active, colourless, fluorescing dye in the staining solution is Cellufluor which is the disodium salt of 4,4'-bis[4-anilino-6-bis-(2-hydroxyethyl) amino-s-triazin-2-ylamino]-2,2'-stilbenedisulfonic acid. Fungi-Fluor™ staining solution is a 0.05% solution of this dye in deionized water with potassium hydroxide added as a clearing agent. The Fungi-Fluor™ counter staining solution B is an aqueous solution of Evans Blue dye used to reduce background fluorescence. Cellufluor binds nonspecifically to beta-linked polysaccharides found in chitin and cellulose which are present in fungal cell walls.

When exposed to long wave UV light, fungal cell walls will fluoresce.

NB: Collagen, elastin, cotton fibres, plant material, some cells, cell inclusions and parasite cyst forms (e.g. Acanthamoeba) may fluoresce.

Materials

Staining Solution A

Counterstaining Solution B


Absolute alcohol

Water

Fluorescent Microscope (Leitz Ortholux with G filter module exciting filter BP 350-460, suppression filter LP515 or equivalent)

Precautions

1. Store in a dark or opaque bottle, tightly sealed, at room temperature.

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- Avoid eye or skin contact: use gloves and protective glasses.

Procedure

- Prepare smear of specimen and allow to air dry.
- Fix on the rack with absolute methanol for 5 minutes until dry. Fixed smears can be held indefinitely until ready to stain and examine.
- Add one drop of Fungi-Fluor solution A (Cellufluor) for 1 minute.
- Rinse gently with tap water.
- Apply coverslip to wetted slide and examine with the fluorescent microscope using the designated filter. If there is a delay, add fresh distilled water to the coverslip just prior to examination.
- Optional for thicker smears. Add few drops of the counterstain Fungi-Fluor solution B. Rinse gently with tap water and then proceed as in step 5 above.

NB: Gram stained smears can be overstained with Fungi-Fluor after removing immersion oil with alcohol. Similarly, Fung-Fluor stained slides may be overstained with other stains such as GMS, PAS, Geimsa, etc.


Quality Control

Stain a smear of *Candida albicans* daily.

Interpretation

Use 25x or 40x objective.

Fungal cell walls will fluoresce apple-green. Observe for characteristic morphology to differentiate from artifacts and background. When the counterstain is used, fungi will appear yellow-green against a red-orange background.


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Appearance of other structures / organisms:

- i) Fungal elements - intense peripheral staining with characteristic morphology.
- ii) *Pneumocystis carinii* - fainter staining cyst wall (5-7 µm diameter) and intensely staining internal "been-shaped" or "double-parenthesis-like" structures with apposed sides flattened.
- iii) *Acanthamoeba* sp. cysts - intensely staining double wall with wrinkled outer wall (10-25 µm diameter)

References

1. Manufacturers' Instructions (Data Sheet #316). Fungi-Fluor™ kit - Polysciences, Inc., Sept 2012.
2. V.S. Baselski et al. "Rapid Detection of *Pneumocystis carinii* in Bronchoalveolar Lavage Samples by Using Cellufluor Staining". J. Clin. Micro. 28:393-394, Feb. 1990.
3. D. H. Larone. Medically Important Fungi. A Guide to Identification. 3rd ed. 1995, ASM Press.

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INDIA INK

Purpose

The procedure is applicable only to suspected positive Cryptococcal cultures.

Procedure



1. For suspected Cryptococcal cultures, make a wet preparation using saline on a clean glass slide, then add a small drop of India Ink and mix.
2. Apply a large coverslip ((22 x 40 mm) over the mixture and press it gently to obtain a thin mount.
3. If India Ink is too thick (dark), dilute it by 50% with saline.
4. Allow the preparation to stand for few minutes to settle.
5. Scan under low power in reduced light. Switch to high power if necessary.

Interpretation

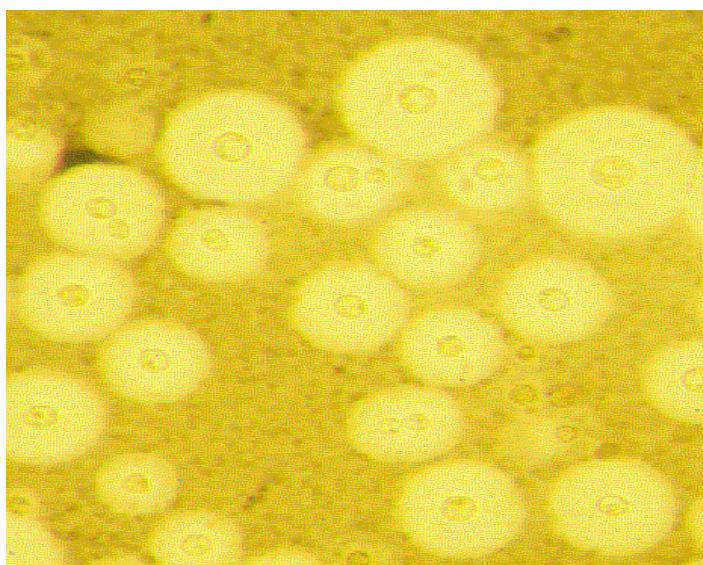
The mucoid capsule appears as a clear halo that surrounds the yeast cell or lies between the cell wall and the surrounding black mass of India Ink particles. Capsules may be broad or narrow. The yeast cells may be round, oval or elongate. Buds may be absent, single or rarely multiple and may be detached from the mother cell but enclosed in a common capsule attached.

Reference

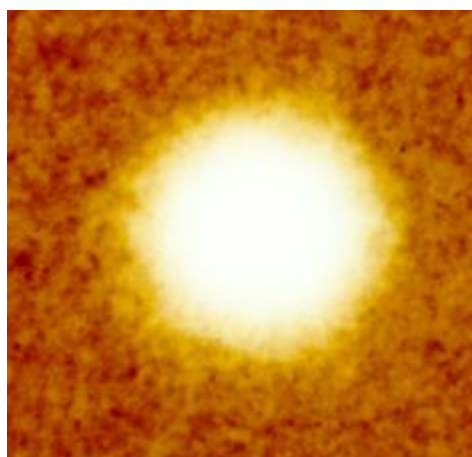
Murray PA, et al. Manual of Clinical Microbiology 7th ed., 1999, ASM Press.


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India Ink positive for Cryptococcus



Artefacts produced by reaction of India Ink (False Positive)



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LACTOPHENOL ANILINE BLUE (LPCB)

Purpose

To determine the morphology of the conidiogenous cells and the conidia that they give rise to in order to identify a filamentous fungus.

Principle

LPCB contains lactic acid as a clearing agent, phenol as a disinfectant, glycerol to prevent drying and Aniline Blue which is the dye that stains fungi. LPCB is a wet preparation.

Procedure

1) TEASE PREP

The test must be performed in the Laminar Airflow Biosafety Cabinet. First, observe the gross morphology of the colony carefully to determine whether or not the culture is mouldy, granular or a mixture of both. It is important to prepare the LPCB preparation by "teasing" the fungus not "chopping" it.

Materials required for LPCB staining:

1. LPCB reagent
2. Probe to get the specimen
3. Teasing needles
4. Glass slides
5. Coverslips
6. Lead or wax pencils
7. Disinfectant bucket
8. Electric incinerator
9. Clear nail polish
10. Slide tray

Tease Prep Procedure:


1. Sterilize the loop and the needles in the incinerator and allow them to cool.
2. Label slide, place 2 drops of LPCB reagent on the slide.

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3. Cut a small piece of the fungus from a granular or colored part of the colony, somewhere away from the central part towards the periphery and place the piece of the fungus in the LPCB in the upside down position.
4. Hold the thallus with the needle and gently tease the inverted side of the specimen into the staining (LPCB) fluid.
5. After enough teasing, remove all the solid particles and the agar from the mixture and discard in the disinfectant container.
6. Put a coverslip gently onto the LPCB preparation and hold the slide preparation briefly over the incinerator opening. Heating the slide will help to stain the cell wall of the fungi and kill the spores on the surface of the slide.
7. Seal the preparation with nail polish if necessary for a permanent mount.
8. Examine under the light microscope using the low power objective.


2) SCOTCH TAPE PREP FROM PLATE CULTURE (Not done on suspected dimorphic fungi)

1. Place a drop of LPCB onto a clean glass slide, make a circle with Vaseline.
2. Take a small piece of clear scotch tape and loop back on itself with sticky sides out.
3. Hold the tip of the loop securely with forceps.
4. Press the sticky side firmly to the surface of the fungal colony.
5. Pull the tape gently away from the colony.
6. Open up the tape strip and place it on the drop of LPCB on the glass slide, making sure that the entire sticky side adheres to the slide. Add cover slip..
7. Examine under the light microscope.

Reference

Davise H. Larone. Medically Important Fungi. A Guide to Identification. 3rd ed. 1995, ASM Press.

Martha E. Kern. Medical Mycology. 1985.

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STREPTOCOCCAL GROUPING

Principle

This test is used to determine the Lancefield group of an isolate. Latex particles labelled with specific group antisera will agglutinate in the presence of the corresponding antigen after extraction with nitrous acid.

Reagents

Pro-lab Streptococcal grouping Latex kit. Store at 2-8°C.

Other Materials

Droppers
 Disposable slides
 Wooden stirring sticks
 13x100 mm test tubes

Safety Precaution

Wear protective gloves.
 Wear eye and face protection.

Procedure


1. Label one test tube for each isolate.
2. Add 2 drops of Extraction Reagent 1 to each tube.
3. Suspend 4 beta-haemolytic colonies in the Extraction Reagent 1.
4. Shake tube to mix.
5. Add 2 drops of Extraction Reagent 2 to each tube.
6. Shake tube to mix.
7. Add 2 drops of Extraction Reagent 3 to each tube. Mix
8. Dispense one drop of each latex suspension to be tested onto separate circles on the test card.
9. Using a pasteur pipette, add one drop of extract to the latex suspension.
10. Mix the latex and extract with the wooden stick using the complete area of the circle.
11. Gently rock the card for 2 minutes and look for agglutination.

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Interpretation

Positive: Strong visible agglutination within 2 minutes.

Negative: Milky appearance without visible agglutination.

Precautions

- a) False positive reactions can occur with *Streptococcus porcinus* and *Streptococcus pseudoporcinus* with latex group B. Confirm with another method (eg. MALDI) before reporting as *S. agalactiae*
- b) False positive reactions have been known to occur with organisms from unrelated genera e.g. *E. coli*, *Klebsiella* sp., *Pseudomonas* sp



Quality Control

Test reagents are checked weekly.

Each test should be tested with at least one extra grouping latex suspension as a negative control.

Reference

1. Pro-lab Streptococcal Grouping package insert.

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STRING TEST

Principle


Hypermucoviscous *K. pneumoniae* (also known as hypervirulent *K. pneumoniae*) are associated with severe disease. In the mid-1980s and 1990s, reports were first published from Taiwan of a hypervirulent *K. pneumoniae* strain associated with community-onset pyogenic liver abscesses with a propensity for metastatic spread to distant sites including the eye and central nervous system causing endophthalmitis and meningitis in otherwise healthy individuals. Reports from other Asian Pacific Rim countries (Korea, Vietnam, and Japan) followed. Since then, reports in North America, South America, the Caribbean, Europe, and elsewhere have occurred. The syndrome is now recognized worldwide. Association with abscess formation at sites other than the liver is now recognized. Anecdotal reports suggest *K. pneumoniae* may undergo relapse months to years after their original infection. (Virulence 2013)

The string test is used to detect hypermucoviscous phenotype associated with hypervirulent strains of *Klebsiella pneumoniae* phenotype. However, it is not clear if all hypervirulent strains are string test positive. Whether non-virulent strains may possess a positive string test has also not been well studied. Of note, having a hypermucoviscous phenotype does not necessarily equate to having a mucoid phenotype. (BMJ 2013) (Virulence 2013)

Detection of rmpA: (regulator of mucoid phenotype A), a gene known as an extracapsular polysaccharide synthesis regulator, has been shown to be present in most but not all hypermucoviscous/hypervirulent *K. pneumoniae*. It is responsible for the overproduction of extracellular polysaccharide, distinct from capsule production. Other regulator genes able to switch on and off the hypermucoviscosity phenotype may also play a role. (CID 2006)

K1 and K2 capsular serotypes are common in hypermucoviscous *K. pneumoniae* strains but these are not specific as non-hypermucoviscous strains may also possess these serotypes nor are they sensitive as hypermucoviscous strains may have non-K1/K2 serotypes. (AAC 2014)

Experts advise clinicians dealing with *K. pneumoniae* bacteremia from an unknown focus make an effort to identify the hypermucoviscosity status of the strain. If isolates are positive for hypermucoviscosity by means of the string test or rmpA (regulator of mucoid phenotype A), it is advised that a detailed search for the underlying infectious origin, particularly abscess formation at metastatic sites be conducted, involving a new thorough physical examination and appropriate imaging tests. (CID 2006) (Virulence 2013)

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Materials

10ul standard bacteriologic loop

Precautions

Isolate subcultured from the freezer must be subculture twice before testing to avoid false-negative string test results (CID 2006))

Procedure

String test is performed on all *K. pneumonia* isolates from blood cultures and sterile sites.

1. Obtain a fresh culture on 5% sheep blood agar incubated at 37°C overnight
 - if isolate is being subcultured from the freezer, subculture twice before testing to avoid false-negative string test results (CID 2006))
2. Using a standard 1ul bacteriology loop, touch a colony from the culture and hold for 1 second, then lift upwards and stretch a mucoviscous string from a colony.
3. Measure string formation in mm
4. Send the isolate to NML for “*K. pneumoniae* hypermucoviscosity PCR” testing (includes rmpA detection and serotyping) for:
 - An isolate testing positive for string test
 - An isolate testing negative for string test but specifically requesting testing for rmpA and serotyping
 - An isolate upon microbiologists approval

Interpretation

Positive: Formation of a viscous string ≥ 5 mm in length.

Negative: Formation of a viscous string < 5 mm in length or No String Formation

Reporting

Negative – Testing for rmpA and serotyping was not specifically requested:



Report *Klebsiella pneumoniae* isolated.

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Negative - Testing for *rpmA* and serotyping was specifically requested:

Report: *Klebsiella pneumoniae* isolated with comment:

“This isolate has a NEGATIVE String Test not consistent with a hypermucoviscous *K. pneumoniae* phenotype but the negative likelihood ratio of this test has not been established.
Further characterization from the National Microbiology Laboratory to follow as requested.”

“*K. pneumoniae* hypermucoviscosity PCR resulting from NML:

Report: *Klebsiella pneumoniae* isolated

“This isolate has a NEGATIVE String Test not consistent with a hypermucoviscous *K. pneumoniae* phenotype but the negative likelihood ratio of this test has not been established.

Further characterization from the National Microbiology Laboratory is as follows:

K1 serotype {insert results from NML}

K2 serotype

K5 serotype

iroB (salmocheilin)

iucA (aerobactin)

ybtA (yersinaibactin)

cibA (colibactin)

peg-344 (transporter)

rpm (regulator mucoid phenotype)



HBV-Kp PCR Genotype:

as reported by the National Microbiology Laboratory (NML),

1015 Arlington St. Winnipeg, MB. Canada, R3E 3R2

NML Specimen No. {insert number on NML report}

Consultation with medical microbiology or infectious diseases is advised.”

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Positive: Report *Klebsiella pneumoniae* isolated with comment:

“This isolate has a POSITIVE String Test consistent with a hypermucoviscous *K. pneumoniae* phenotype.
Further characterization from the National Microbiology Laboratory to follow.
Consultation with medical microbiology or infectious diseases is advised.”

“*K. pneumoniae* hypermucoviscosity PCR resulting from NML:

Report: *Klebsiella pneumoniae* isolated with comment:

“This isolate has a POSITIVE String Test which is consistent with a hypermucoviscous *K. pneumoniae* phenotype.
Further characterization from the National Microbiology Laboratory is as follows:

K1 serotype {insert results from NML}

K2 serotype

K5 serotype

iroB (salmochelin)

iucA (aerobactin)

ybtA (yersiniaibactin)

cibA (colibactin)

peg-344 (transporter)

rmp (regulator mucoid phenotype)



HBV-Kp PCR Genotype:

as reported by the National Microbiology Laboratory (NML),

1015 Arlington St. Winnipeg, MB. Canada, R3E 3R2

NML Specimen No. {insert number on NML report}

Consultation with medical microbiology or infectious diseases is advised.”

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Quality Control

Test the following positive and negative controls each time an unknown is tested:

Positive: *K. pneumoniae* (ATCC 13383)

Negative: *K. pneumoniae* (CAP98D)

Reference



Hadano Y. String Test, BMJ Case Rep 2013. doi:10.1136/bcr-2012-008328

Liu, Ying Mei et al. Clinical and Molecular Characteristics of Emerging Hypervirulent *Klebsiella pneumoniae* Bloodstream Infections in Mainland China, Antimicrobial Agents and Chemotherapy, September 2014, Vol. 58:9, p. 5379–5385.

Shon, Alyssa S. Bajwal, Rajinder P S. Russo, Thomas A. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: A new and dangerous breed, Virulence, Vol. 4:2, p. 107-118.

Yu, Wen-Liang et al. Association between *rmpA* and *magA* Genes and Clinical Syndromes Caused by *Klebsiella pneumoniae* in Taiwan, Clinical Infectious Diseases, May 2006; Vol. 42, p. 1351–1358.

Fang, Chi-Tai. Chuang, Yi-Ping. Shun, Chia-Tung. Chang, Shan-Chwen. Wang, Jin-Town. A Novel Virulence Gene in *Klebsiella pneumoniae* Strains Causing Primary Liver Abscess and Septic Metastatic Complications, Journal of Experimental Medicine, March 2004, Vol. 199:5 p. 697-705.

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TUBE COAGULASE TEST

Principle

This test is used to speciate staphylococci by determining the ability of an isolate to clot plasma by producing the enzyme coagulase.

Reagents

Rabbit plasma (pre dispensed) – Stored at -20°C. Thaw before use.

Other Materials

Culture loop or wooden applicator stick

Procedure

1. Emulsify a large loopful of a pure colony of *Staphylococcus* into the plasma.
2. Incubate at 35°C for 4 hr, observing every 30 minutes for clot formation.
3. If there is no visible clot at the end of 4 hours, leave at room temperature overnight and observe for clot formation.


Interpretation

Positive: Clot formation

Negative: No clot formation

Precautions

- 1) When observing the tube, do not shake or agitate the tube.

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Quality Control

Each time a coagulase test is performed; known positive and negative cultures must be tested.

Positive: *S. aureus* (ATCC 25923)
Negative: *S. epidermidis* (ATCC 12228)

References

1. MacFaddin, J.F., Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore MD, 1980, pgs. 64-77

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UREA SLANT

Principle

To determine the ability of an organism to split urea by the action of the enzyme urease forming two molecules of ammonia with resulting alkalinity

Materials

Urea Slant – Store at 2-8°C
Bacteriology loop

Procedure

1. From one isolated colony, heavily inoculate the urea slant.
2. Incubate O₂, 35°C.
3. Read at 3 hours and again at 18-24 hours.



Interpretation

Positive: Intense pink-red colour.
Rapidly positive: 1 to 6 hours (*Proteus* spp.)
Delayed positive: ≥ 18 hours

Negative: No colour change

Precautions

Urea test media rely on the demonstration of alkalinity, thus are not specific for urease. The utilization of peptones or other proteins may cause an increase in pH.

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Quality Control

Controls should be set up weekly.

<i>P. mirabilis</i> (ATCC 12453):	Positive - 4 hours
<i>K. pneumoniae</i> (ATCC 13883):	Weak positive - 18 hours
<i>E. coli</i> (ATCC 25922):	Negative

References

1. MacFaddin JF, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore MD, 1980, p298-308.

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Record of Edited Revisions

Manual Section Name: Technical Manual



Page Number / Item	Date of Revision	Signature of Approval
Annual Review	August 3, 2003	Dr. T. Mazzulli
Annual Review	May 16, 2004	Dr. T. Mazzulli
Annual Review	April 20, 2005	Dr. T. Mazzulli
Annual Review	April 10, 2006	Dr. T. Mazzulli
API website login change	March 14, 2007	Dr. T. Mazzulli
Annual Review	April 11, 2007	Dr. T. Mazzulli
Gen-Probe for S. aureus ID added	April 11, 2007	Dr. T. Mazzulli
Gram Staining machine MIDAS III added	April 11, 2007	Dr. T. Mazzulli
Fluorochrome stain for Mycobacterium – name of stains added to the procedure steps.	April 11, 2007	Dr. T. Mazzulli
Page number changes	April 11, 2007	Dr. T. Mazzulli
Added quantitation of AFB smear to Quantitation of Smear and Culture	April 11, 2007	Dr. T. Mazzulli
Annual Review	April 11, 2007	Dr. T. Mazzulli
Operation of Gen-Probe Luminometer Instructions changed	July 30, 2007	Dr. T. Mazzulli
Optochin Test – change from MHB to BA	July 30, 2007	Dr. T. Mazzulli
ACCUPROBE S. aureus Operation of GEN-PROBE luminometer change	Dec 4, 2007	Dr. T. Mazzulli
Change Gram quantitation for \pm to report as “Few”	April 1, 2008	Dr. T. Mazzulli
New procedure change for Streptococcus grouping	April 29, 2008	Dr. T. Mazzulli
Anaerobic jar organisms – wrong QC organism listed – changed	April 29, 2008	Dr. T. Mazzulli
Annual Review	April 29, 2008	Dr. T. Mazzulli
Added API strep strip	April 29, 2008	Dr. T. Mazzulli
Removed ACCUPROBE for S. aureus ID	April 29, 2008	Dr. T. Mazzulli
Removed CRYSTAL for MRSA ID	April 29, 2008	Dr. T. Mazzulli
Cryptococcal Antigen revised	April 29, 2008	Dr. T. Mazzulli
Gram Stain revised	July 27, 2010	Dr. T. Mazzulli
Annual Review	April 29, 2009	Dr. T. Mazzulli
Annual Review	April 29, 2010	Dr. T. Mazzulli

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Annual Review	April 29, 2011	Dr. T. Mazzulli
Added Phadebact Monoclonal	December 12, 2011	Dr. T. Mazzulli
Annual Review	April 20, 2012	Dr. T. Mazzulli
Annual Review	May 31, 2013	Dr. T. Mazzulli
Shigella Latex Agglutination added	December 16, 2013	Dr. T. Mazzulli
For N. gonorrhoeae, confirmed ID by API NH before reporting	December 16, 2013	Dr. T. Mazzulli
Added reporting for characterization of beaded or branching gpb	December 16, 2013	Dr. T. Mazzulli
Do Modified Kinyoun and non Modified Kinyoun stain for all beaded or branching Gram positive bacilli	June 17, 2014	Dr. T. Mazzulli
Removed Kinyoun and added a second fluorochrome stained smear as confirmation stain for positive fluorochrome stained smear	June 17, 2014	Dr. T. Mazzulli
Reformat headers/footers Removed sections: 1) Anaerobic Id Potency Disk 2) SPS disk 3) Gene Probe 4) Gonogen 5) Hippurate Test 6) Neisseria Id sugars 7) Rapid Yeast Plus Test 8) SIM 9) Tetrazolium Reduction Test 10) Xylose Fermentation Changes: -Anaerobic jars –using C. sordellii not C. novyii -Campy 42C removed “walk in” from type of incubator -Strep strip API changed copule to cupule -rapid VP changed milleri to Strep. Anginosus group. -Kinyoun: changed 1 hour fixing smear to fix on heating block. Crypto Ag: -change from water bath to heating block -corrected centrifugation speed; details on how to rate positive and negative test, Added nonspecific interpretation and reporting comment. Annual Review	July 25, 2014	Dr. T. Mazzulli
Salmonella serology agglutination test added	March 10, 2015	Dr. T. Mazzulli

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Revised decolourization step for Modified Kinyoun Addition of mycology staining methods Removed High level link and replaced with Blacta link in TOC Annual Review	March 11, 2015	Dr. T. Mazzulli
Added String test	September 29, 2015	Dr. T. Mazzulli
Update of wording of stains for GP branching bacilli -updated QC slide for kinyoun stain from to Oxoid from microbiologics -kinyoun background from blue to green -Removed reporting phrase for gpb beaded/branching “Further characterization of gram positive bacilli, branching / Further characterization of gram positive bacilli”	October 20, 2015	Dr. T. Mazzulli
String Test p.123 Procedure - changed look from 10ul loop to 1ul loop. -Added to hold loop on colony for 1 second then lift upward	December 3, 2015	Dr. T. Mazzulli
Added Remel Shigella Serology testing	March 1, 2016	Dr. T. Mazzulli
Annual Review Updated MSH logo in header Updated references Included containers with jars to anaerobic and campy plate set up. Bile solubility, updated negative control to <i>S. sanguinis</i> ATCC 10556 Catalase updated negative control from GAS to <i>E.faecalis</i> ATCC 29212 0157 serology: added for best results to sub NSF to BA plate prior to testing Pastorex Staph Plus test: clumping within 30sec Archive: PRO-AMP-GLU-AMP tests for Neisseria PYR: change from disk to BD BBL dry slide Kinyoun QC slide changed from oxoid to remel Tube coagulase reagent updated to pre-dispensed (non reconstituted). Added spot indole procedure. Updated references.	March 6, 2016	Dr. T. Mazzulli

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Updated all staining procedures to methanol fixation and added methanol to staining materials. “When film is dry, flood slide with methanol to fix smear. <u>Let smear dry completely</u> before staining.” Removed heat fixation for all staining methods.	April 12, 2016	Dr. T. Mazzulli
Updated Shigella serological procedure to include “When to test” statement under procedure: Testing should be performed on suspect colonies from a fresh culture (18-24hours) sub cultured on a non-selective agar e.g. BA, TSI. Do not use MAC or HEK plates to perform agglutination test.	April 25, 2016	Dr. T. Mazzulli
Welcollex test: <ul style="list-style-type: none"> Saline modified from 0.85% to 0.45%. 150RPM changed to 80RPM 	September 7, 2016	Dr. T. Mazzulli
Annual Review Modified Kinyoun staining procedure updated, including ethanol step.	April 5, 2017	Dr. T. Mazzulli
Modified Cryptococcus Antigen: Addition of documenting agglutination on worksheet, addition of having second verifier with documentation and agglutinating visual photo added showing degrees of grading agglutination.	May 17, 2017	Dr. T. Mazzulli
Updated Rapid ANA Eric website/login information Corrected Crypto antigen volumes from 500ul serum/pronase to 200ul as per package instructions.	August 15, 2017	Dr. T. Mazzulli
Annual Review Updated Cryptococcus positive comment for titres $\leq 1:4$	April 03, 2018	Dr. T. Mazzulli
Removed API ID and password. Refer to seniors	May 09, 2018	Dr. T. Mazzulli
Storage temperature completed for reagents.	May 28, 2018	Dr. T. Mazzulli
Annual Review	April 10, 2019	Dr. T. Mazzulli
Safety Precaution added to Streptococcal Grouping and Indole test	Feb 21, 2020	Dr. T. Mazzulli
Annual Review	May 25, 2020	Dr. T. Mazzulli

Full document review included in all updates. Biennial review conducted when no revision had been made within 2 years.


Page Number / Item	Date of Revision	Edited by:
Specified freq of neg control heat inactivation	Dec 31, 2020	Dorna Zareianjahromi
Minor formatting change	April 11, 2021	Jessica Bourke

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Nomenclature update – Clostridioides, Enterobacterales	April 19, 2021	Wayne Chiu
Added limitation to API NH	May 7, 2021	Wayne Chiu
Fixed rotator rpm for wellcolex	July 22, 2021	Wayne Chiu
Updated limitation for strep grouping – porcinus/pseudoporcinus false group B pos	Dec 23, 2021	Wayne Chiu
Updated Cryptococcus Antigen SOP: Document 1+ agglutination in the workcard Added extra comment to the negative report: <i>If the status of a patient suggests a cryptococcal infection, please submit follow-up specimens for additional testing.</i>	May 12, 2022	Qin Lliu
Added aminopeptidase (bactident) test	July 13, 2022	Wayne Chiu
Added glove recommendation for pastorex staph test	Oct 31, 2022	Wayne Chiu
Changed pastorex pos qc S. aureus strain to 25923 to match bench plate strain	Nov 30, 2022	Wayne Chiu
Addition of Satellite test	May 6, 2024	Jessica Bourke
Biennial Review- Updated Cryptococcal Antigen SOP: <ul style="list-style-type: none"> Added 1:10, 1:100, and 1:1000 serial dilutions to address potential high antigen levels and updated the corresponding reporting procedures. Deleted the following SOPs: <ul style="list-style-type: none"> Identification of Corynebacterium (API CORYNE) Identification of Streptococcaceae (API 20 Strep) Cetrimide Pseudomonas selective agar Indole test MUG test (PGUA) ONPG-Phenylalanine-Motility Medium (ONPG-PAM) Phadebact Monoclonal GC PRO-AMP GLU-AMP tests RapID MGP test BACTO 3-STEP gram stain procedure Fluorochrome stain for mycobacterium 	May 6, 2025	Qin Liu

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Page Number / Item	Date of Revision	Edited by:
<ul style="list-style-type: none"> • Thermonuclease test • Tributyrin test • TSI (Triple Sugar Iron) 		

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