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## BACTERIA and YEAST WORK-UP

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## AEROBIC BACTERIA

### GRAM POSITIVE BACTERIA

#### GRAM POSITIVE COCCI - Catalase-Positive

Tests	<i>S. aureus</i>	CNST	<i>S. saprophyticus</i>	<i>Micrococcus</i> species <sup>6</sup>	<i>Stomatococcus</i> species <sup>7</sup>
T-DNase <sup>1</sup>	+	-	-	-	-
Staph-Slide Agglutination <sup>1,2,4</sup>	+	-	-	-	-
Tube coagulase <sup>1,4</sup>	+	-	-	-	-
Novobiocin susceptibility <sup>3</sup>	N/A	S	R	N/A	N/A
GenProbe <sup>5</sup>	≥1,500 PLU	≤1,200 PLU	N/A	N/A	N/A
Oxidase	-	-	-	+	-
Growth in 6.5% NaCl	+	+	+	+	-
Bacitracin	N/A	N/A	N/A	S	N/A
PYR	N/A	N/A	N/A	N/A	+
LAP	N/A	N/A	N/A	N/A	+

1 compulsory test for Blood Cultures

2 compulsory test for all specimens other than Blood Cultures

3 test on urines from female of childbearing age

4 compulsory test for Infection Control MRSA screens

5 confirmatory test if needed

6 obligately aerobic

7 catalase variable

## GRAM POSITIVE COCCI – Catalase-Negative

### 1. *beta-hemolytic large colonies on Blood Agar:*

Test	Group A, B, C or G streptococcus
Gram Stain	g+c
Catalase	-
BE	-
Streptococcus Latex Agglutination	Agglutination with Group A, B, C or G

### 2. *beta-hemolytic small colonies on Blood Agar:*

Test	<i>Streptococcus milleri</i> group
Gram Stain	g+c
Catalase	-
BE	-
Streptococcus Latex Agglutination	Agglutination with Group A, B, C, F, G or non-groupable
VP	+

### 3. *Non-hemolytic or α-hemolytic on Blood Agar:*

Test	<i>Streptococcus pneumoniae</i>	viridans Streptococci	Enterococci <sup>1,4</sup>	Leuconostoc/ Pediococcus	Others
Gram Stain	g+dc	g+c	g+c	g+c	g+c
Catalase	-	-	-	-	-
BE	-	-	+ <sup>1</sup>	-	V
Vancomycin	S	S	S / R <sup>3</sup>	R	S
Bile Solubility	+	-	N/A	N/A	N/A
Optochin	S	R	N/A	N/A	N/A
PYR	N/A	-	+	-	N/A
Vitek gpi card	ID	Some ID; report as viridans strep	ID <sup>2</sup>	Not in data base, set up API Strep strip	ID – will ID Gemella, Strep. milleri group <sup>5</sup> , non-haemolytic group B strep

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1. For urine, wounds and superficial specimens, report as *Enterococcus species* based on Bile Esculin result (do not need to further speciate).
2. If Vitek ID as *E. faecium* and Amp-S, or other *Enterococcus* other than *E. faecalis*, set up sugars: Xylose, Arabinose, MGP, PYR
3. *Enterococci species* that are I or R to Vancomycin must be checked for yellow pigment. For isolates negative for pigment, set up rapid xylose test. Positive xylose (within 2 hours) isolates are *E. gallinarum*. For non-yellow pigmented isolates and negative rapid xylose isolates, set up *Enterococcus* MIC panel. Unidentified isolates are to be sent to the Provincial Health Lab for identification.
4. *Enterococcus* identification:

Test	<i>Enterococcus faecalis</i>	<i>Enterococcus faecium</i>	<i>Enterococcus gallinarum</i>	<i>Enterococcus casseliflavus</i>
BE	+	+	+	+
Yellow pigment	-	-	-	+
Pyruvate	+	-	-	-
Rapid xylose (2hrs.)	-	-	+	+
Arabinose	-	+	+	+
MGP	-	-	+	+
Ampicillin	S	R	S	S

5. *Streptococcus milleri group* – VP+

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## GRAM POSITIVE BACILLI

Test	<i>Listeria</i>	<i>Arcanobacterium</i>	<i>Erysipelothrix</i>	<i>C. jeikeium (JK)</i>	<i>Lactobacilli</i>
Catalase	+	-	-	+	-
BE	+	-	-	-	V
Penicillin Resistance	N/A	N/A	N/A	Yes	N/A
Motility	+	-	-	-	N/A
Vancomycin Disc	N/A	N/A	N/A	NA	R/S
	Set up Vitek gpi card and rhamnose	Set up Vitek gpi card		Set up Vitek ani	Set up RapID ANA

### Note:

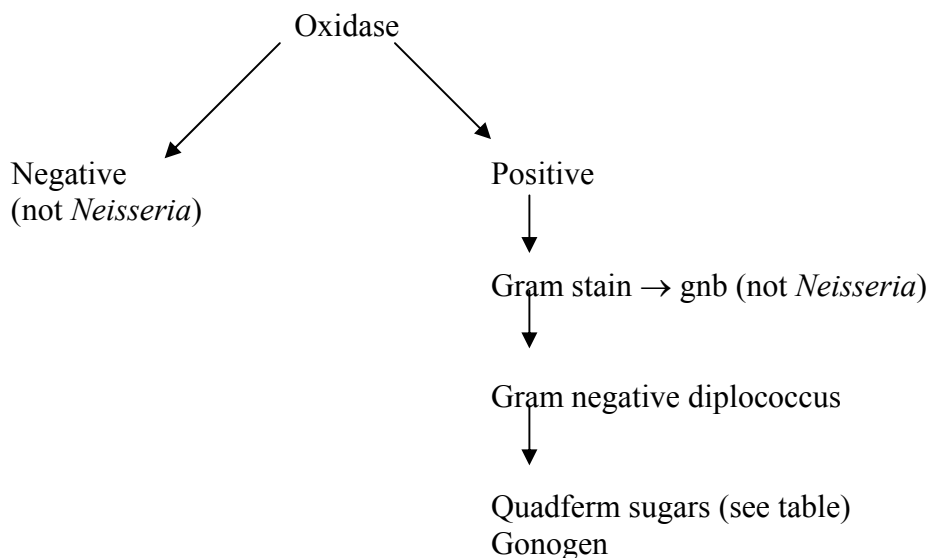
- Catalase positive, Penicillin sensitive, Gram positive bacilli that are not *Listeria* or *Bacillus* species, report as “*Corynebacterium* species”.
- Catalase positive, Penicillin resistant, Gram positive bacilli that do not identify as *Corynebacterium jk*, report as “*Corynebacterium* species”.
- For aerobic pigmented, Catalase positive, Gram positive bacilli, send isolate to PHL for ID.
- Aerobic spore-forming bacilli - observe for haemolysis and set up motility test (use motility medium). If motile, report as *Bacillus* sp. If non-motile, non-haemolytic and large bacilli send isolate to PHL.

## GRAM NEGATIVE BACTERIA

### GRAM NEGATIVE COCCI / DIPLOCOCCI

Test	<i>M. catarrhalis</i>	<i>N. gonorrhoeae</i>	<i>N. meningitidis</i>	<i>N. species</i>
Oxidase	+	+	+	+
Catalase	+	+	+	+
Tributyryn	+	N/A	N/A	N/A
apiNH	N/A	ID	ID	Some ID
Vitek nhi	N/A	N/A	N/A	ID
Gonogen	N/A	ID	ID	Some ID

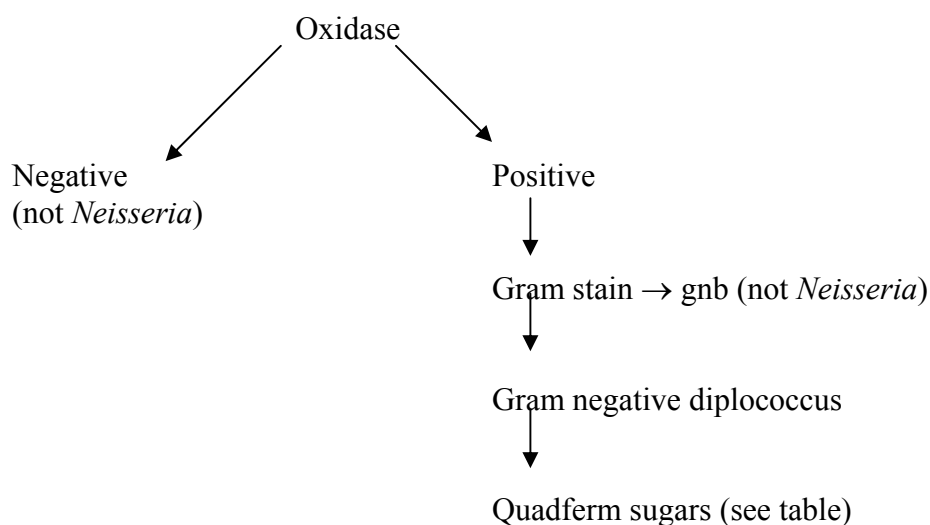
#### Identification of *Neisseria gonorrhoeae*



#### Quadferm Sugars

Well#	Test	Result Colour	Interpretation
3	Carbohydrate control (CRTL)	Red	Negative
4	Glucose (GLU)	Yellow	POSITIVE
5	Maltose (MAL)	Red	Negative
6	Lactose (LAC)	Red	Negative
7	Sucrose (SUC)	Red	Negative
9	Dnase (DNA)	Red	Negative
10	Penicillin (β-LAC)	Red Yellow	Negative POSITIVE

### Identification of *Neisseria meningitidis*



<b>Quadferm Sugars</b>			
<b>Well#</b>	<b>Test</b>	<b>Result Colour</b>	<b>Interpretation</b>
3	Carbohydrate control (CRTL)	Red	Negative
4	Glucose (GLU)	Yellow	POSITIVE
5	Maltose (MAL)	Yellow	POSITIVE
6	Lactose (LAC)	Red	Negative
7	Sucrose (SUC)	Red	Negative
9	Dnase (DNA)	Red	Negative
10	Penicillin (β-LAC)	Red	Negative
		Yellow	POSITIVE

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### GRAM NEGATIVE BACILLI - Oxidase-Negative, Fermenter

Test	<i>E. coli</i>	Not <i>E. coli</i>
MUG*	+	-
INDOLE*	+	+/-
Vitek gni+	ID	ID
api20E	Use when not ID by gni+	Use when not ID by gni+

\* Use for urine isolates

**Note:**

Unidentified isolates are to be sent to the Provincial Health Lab for identification.

### GRAM NEGATIVE BACILLI – Oxidase-Negative, Non-Fermenter

Test	
Vitek gni+	Some ID
api20E	Some ID
api20NE	Most ID

**Note:**

Unidentified isolates are to be sent to the Provincial Health Lab for identification.

### GRAM NEGATIVE BACILLI – Oxidase-Negative or Weak Positive ?*Haemophilus* species

Test	<i>H. influenzae</i> <sup>1</sup>	<i>H. parainfluenzae</i>
Gram	g-cb	g-cb
Satellitism	+	+
ALA	-	+
Catalase	+	-

**Note:**

Identify by Vitek nhi card if required or refer to the Provincial Health Lab.

<sup>1</sup> If isolated from blood or sterile sites, test for Serogroup b by latex agglutination.



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### GRAM NEGATIVE BACILLI – Oxidase-Positive

Test	<i>Ps. aeruginosa</i>	Not <i>Ps. aeruginosa</i>
Cetrimide*	+	-
Vitek gni+	ID	Some ID
api20E	ID	Some ID
api20NE	ID including mucoid strains	Most ID
Growth at 42°C	+	+/-

\* Growth **AND** green pigment

**Note:**

Unidentified isolates are to be sent to the Provincial Health Lab for identification.

### Identification of *H. pylori*:

Test	<i>H. pylori</i>
Gram stain	Small, gram negative gull-shaped or spiral
Catalase	+
Oxidase	+
Urea slant (rapid)	+
Cephalothin 30µg	S (inhibition)
Nalidixic acid 30µg	R (no zone)

### ANAEROBIC BACTERIA

Test	<i>Peptostreptococcus</i> species	<i>Propionibacterium</i> species	Anaerobic non-spore forming Gram positive bacilli	<i>Clostridium</i> species	<i>B. fragilis</i>	Anaerobic Gram negative bacilli
Gram	g+c	g+b small, branching	g+b small	g+b, large	g-b	g-b
Catalase	N/A	+	-	N/A	N/A	N/A
Subculture BA CO <sub>2</sub>	No growth	No growth	No growth	No growth*	No growth	No growth
Subculture CHOC CO <sub>2</sub>	No growth	No growth	No growth	No growth*	No growth	No growth
Subculture BRUC AnO <sub>2</sub>	Growth	Growth	Growth	Growth	Growth	Growth
BBE	N/A	N/A	N/A	N/A	+	-
RapID ANA**	N/A	N/A		Some ID	N/A	ID
Vitek ani**	N/A	N/A				N/A

\* Some *Clostridium* species can grow aerobically

\*\* Usually not done – report as anaerobic gram positive or gram negative bacilli

- Anaerobic, small Gram positive bacilli resembling diphtheroids that are catalase positive should be reported as “*Propionibacterium* species”.
- Anaerobic, small Gram positive bacilli that are catalase negative should be reported as “Anaerobic non-spore forming Gram positive bacilli”.

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## YEAST IDENTIFICATION

### Identify yeast as per site of isolation:

1) Sterile sites and biopsy specimens:

- a) Germ tube: **Positive** - Report as "*Candida albicans*" "isolated".
- b) Germ tube: **Negative** - Report as "Yeast" "isolated" "identification to follow" and send the isolate to Mycology for identification.

2) Respiratory sites isolates:

**Significant growth** – For sputum ( $\geq 2+$  growth and predominant **OR** 1+ growth and predominant and if pus cells are seen on gram stain) OR for bronchoscopy specimen (amount greater than that of commensal flora):

- a) Germ tube: **Positive** - Report as "*Candida albicans*"
- b) Germ tube: **Negative** - Rule out Cryptococcus using Urease test. If Urease is negative, report as "Yeast, not *Candida albicans* or Cryptococcus". If Urease is positive, confirm purity, subculture isolate onto a SAB plate and send the SAB and original plate to Mycology for further identification ASAP.

**Insignificant growth** – i.e. any amount of yeast other than what has defined as significant growth.

Rule out Cryptococcus using Urease test. If Urease is negative, report as part of Commensal flora **without** specifically mentioning the presence of yeast. If Urease is positive, confirm purity, subculture isolate onto a SAB plate and send the SAB and original plate to Mycology for further identification ASAP.

3) Voided urines, superficial sites, wounds and drainage fluids:

No Germ tube performed. Report as "Yeast" with quantitation.

4) Isolates from all other sites:

- a) Germ tube: **Positive** - Report as "*Candida albicans*".
- b) Germ tube: **Negative** - Report as "Yeast, not *Candida albicans*".

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