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Prepared by QA Committee		
Issued by: Laboratory Manager	Revision Date: 4/20/2018	
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Vitek MS Guide to Bacteria and Yeast Identification:

1. Use Vitek MS for initial identification **along with** colonial morphology and/or gram stain.
 - a. Use [VTMS MALDI-TOF Manual](#) for acceptance rules
 - b. Use [VITEK-MS-V2-speciesList Created-Translated](#) list for translation
2. When Vitek MS fails, or manual tests warranted, set up tests according to reference tables.
 - a. For bone and joint fluids specimens, report organisms to the species level. If not identified in lab, send to PHOL.
3. If Vitek MS or manual tests do not provide a reliable identification,
 - a. For blood cultures and sterile sites, send to PHOL for identification
 - b. For non-sterile sites, consult charge/senior technologist.

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Reference Bacteria and Yeast Identification Tables and Flowcharts

AEROBIC BACTERIA

GRAM POSITIVE BACTERIA

GRAM POSITIVE COCCI - Catalase-Positive

Tests	<i>S. aureus</i>	CNST	<i>S. lugdunensis</i>	<i>S. saprophyticus</i>	<i>Micrococcus</i> species ⁵	<i>Stomatococcus</i> species ⁶
Pasteurex Slide Agglutination	+	-	-	-	-	-
Tube coagulase ¹	+	-	-	-	-	-
PYR ²	-	+/-	+	-	N/A	+
Ornithine decarboxylase ³	N/A	-	+	N/A	N/A	N/A
Novobiocin susceptibility ⁴	N/A	S	S	R	N/A	N/A
Oxidase	-	-	-	-	+	-
Bacitracin	N/A	N/A	N/A	N/A	S	N/A
LAP	N/A	N/A	N/A	N/A	N/A	+

1 compulsory test for Blood Cultures, Sterile Sites and Infection Control MRSA screens

2 compulsory test for Blood Cultures and Sterile Sites

3 compulsory test on PYR positive CNSTs from Blood Cultures and Sterile Sites.

4 test on urines from female of childbearing age (>12yrs, <60yrs)

5 obligate aerobe


6 catalase variable

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GRAM POSITIVE COCCI – Catalase-Negative

1. *Beta-hemolytic colonies on Blood Agar:*

Tests	Group A, B, C or G	<i>S.anginosus</i>
Bile Esculin	-	-/weak
Streptococcus Latex Agglutination	A, B, C or G	A, C, F or G or non-groupable
VP ¹	-	+

¹ compulsory test on all small beta-hemolytic colonies

2. *Non-hemolytic or α-hemolytic on Blood Agar:*



Test	<i>Streptococcus pneumoniae</i>	<i>viridans Streptococci</i>	<i>Aerococcus urinae</i>	<i>Leuconostoc/ Pediococcus</i>	<i>Streptococcus bovis</i> group	Others
Gram Stain Arrangement	g+dc	g+c	g+c clusters, tetrads	g+c	g+c	g+c
Bile Solubility	+	-	N/A	N/A	N/A	N/A
Optochin	S	R	N/A	N/A	N/A	N/A
PYR	N/A	-	-	-	-	N/A
LAP	N/A		+			
BE	-	-	-	-	+	V
Vancomycin	S	S	N/A	R	N/A	S
Other	Vitek GP-ID	Vitek GP-ID	N/A	API Strep Strip		Vitek GP-ID Will ID <i>Gemella</i> , <i>Strep. anginosus</i> group, non-haemolytic group B strep

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3. *Enterococcus* identification:

Test	<i>Enterococcus faecalis</i>	<i>Enterococcus faecium</i>	<i>Enterococcus gallinarum</i>	<i>Enterococcus casseliflavus</i>
BE	+	+	+	+
PYR	+	+	+	+
Yellow pigment	-	-	-	+
Vancomycin	S/R	S/R	I/R	I/R
Ampicillin	S	R	S	S
Other	Vitek GP-ID	API Strep strip	API Strep strip	API Strep strip

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GRAM POSITIVE BACILLI

Test	<i>Listeria</i> ¹	<i>C. jeikeium (JK)</i>	<i>C. urealyticum</i>	<i>C. pseudo-diphtheriticum</i>	<i>Lactobacilli</i>	<i>Rhodococcus equis</i>	Other
Gram Stain						Diptheroid-like	
Colony Morphology							
Catalase	+	+	+		-		
BE	+	-	-		V		
Motility	+	-	-		N/A		
Penicillin Resistance	N/A	Yes	N/A		N/A		
Urease			+	+		+	
Other	Set up Vitek id-gp card	Send to PHL	Send to PHL				Set up Vitek id-gp card

¹ from Blood and sterile site specimens: Send isolate to the Provincial Health Lab for Serogroup typing.

Note:

- Catalase positive, Penicillin sensitive, Gram positive bacilli that are not *Listeria* or *Bacillus* species, report as “*Corynebacterium* species”; except in urine (rule out *C. urealyticum*).
- Catalase positive, Penicillin resistant, Gram positive bacilli that do not identify as *Corynebacterium jeikeium*, report as “*Corynebacterium* species”; except in urine (rule out *C. urealyticum*).
- For aerobic pigmented, Catalase positive, Gram positive bacilli, send isolate to PHOL for ID.
- Aerobic spore-forming bacilli:


Test	<i>Paenibacillus spp.</i>	<i>Bacillus cereus</i>	<i>Bacillus anthracis</i>	<i>Bacillus</i> species other than <i>B. cereus</i> or <i>B. anthracis</i>
Haemolysis on Blood Agar	-	+	-	+/-
Motility by Motility medium		+	-	+/-
PHOL for confirmation		No	Yes**	No

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**Send isolate to PHOL. Package the isolate in a SAFT PAK container, labeled “Bacillus species non-motile” and shipped as dangerous goods. Phone PHOL at 416-235-5706 to inform of bacillus species to rule out *B anthracis*.

- For faintly staining beaded gram positive bacilli, perform a modified Kinyoun (rule out Nocardia) and Kinyoun (rule out Mycobacterium)
- **All gram positive bacilli isolated from tissues and sterile sites**, perform a modified Kinyoun (rule out Nocardia) and Kinyoun (rule out Mycobacterium)

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GRAM NEGATIVE BACTERIA

GRAM NEGATIVE COCCI / DIPLOCOCCI

Test	<i>M. catarrhalis</i>	<i>N. gonorrhoeae</i>	<i>N. meningitidis</i> ¹	<i>N. species</i>
Oxidase	+	+	+	+
Catalase	+	+	+	+
Tributyryn	+	N/A	N/A	N/A
Other	N/A	API NH Phadebact	API NH	API NH

¹ from Blood and sterile site specimens: Send isolate to the Provincial Health Lab for Serogroup typing.

GRAM NEGATIVE BACILLI - Oxidase-Negative, Fermenter

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



* Use for urine isolates

Note:

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

Blood and Sterile sites*	
<i>Klebsiella pneumonia</i>	String Test

*Mandatory testing required

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GRAM NEGATIVE BACILLI – Oxidase-Negative, Non-Fermenter

Test	
Vitek-MS	Most ID
Vitek id-gn	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> ¹	<i>H. parainfluenzae</i>	<i>H. haemolyticus</i> ²
Gram	g-cb	g-cb	g-cb
Satellitism	+	+	+
ALA	-	+	-
Catalase	+	-	+

¹ identified from Blood and sterile site specimens: Send isolate to the Provincial Health Lab for Serogroup b typing.

² identified from Vitek-MS

If an isolate is identified from the Vitek-MS ID is *H. haemolyticus*, send the isolate to PHOL for confirmation and specify "rule out *H. influenzae*" on PHOL requisition.

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GRAM NEGATIVE BACILLI – Oxidase-Positive

Test	<i>Ps. aeruginosa</i>	Not <i>Ps. aeruginosa</i>
Cetrimide*	+	-
Vitek id-gn	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)

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Urine Pathogens Workup



Suspect Organism	Tests to be performed and expected result	Identification/Susceptibility
<i>E.coli</i> (Lactose fermenter)	Oxidase: Negative MUG: Positive + Indole: Positive	Vitek Susceptibility
Enterobacteriaceae	Oxidase: Negative	Vitek ID + Susceptibility
<i>Pseudomonas aeruginosa</i>	Oxidase: Positive Characteristic appearance + Cetrimide: Positive	Vitek Susceptibility
Non-fermenters	Oxidase: Negative/Positive	Vitek ID + Susceptibility or API NE + KB Sens if applicable
Yeast	Wet mount Germ tube-positive : Report as <i>C. albicans</i> -negative : Yeast, not <i>Candida albicans</i> ".	Refer to Mycology
Group B streptococcus	Strep. Latex Agglutination: Group B Positive Bile esculin: Negative	
<i>Staphylococcus</i> species: <i>aureus</i> <i>S. saprophyticus</i> CNST	Staph. Latex Agglutination: Positive Staph. Latex Agglutination: Negative Novobiocin: Resistant (set up on patients 12 - 60 yrs and females only) Novobiocin: Sensitive Staph. Latex Agglutination: Negative	Vitek Susceptibility Oxacillin screen Vancomycin screen
<i>Enterococcus</i> species	Bile esculin: Positive	Vitek Susceptibility Vancomycin screen
<i>Corynebacterium</i> species	Non-haemolytic colonies on Blood Agar Gram positive bacilli, small, palisade arrangement. Catalase: Positive Urea: Negative	

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Suspect Organism	Tests to be performed and expected result	Identification/Susceptibility
<i>Corynebacterium urealyticum</i>	Non-haemolytic colonies on Blood Agar Gram positive bacilli, small, palisade arrangement. Catalase: Positive Urea (rapid): Positive	API CORYNE for ID
viridans streptococcus	Alpha-haemolytic colonies on Blood Agar Gram positive cocci in chains Catalase: Negative	
<i>Aerococcus urinae</i>	Alpha-haemolytic colonies on Blood Agar Gram positive cocci in tetrads or clusters Catalase: Negative LAP: Positive + PYR: Negative	
<i>Lactobacillus</i> species	Alpha-haemolytic or non-haemolytic colonies on Blood Agar Gram positive thin bacilli Catalase: Negative	
<i>Bacillus</i> species	Gram positive large bacilli with square ends Motility: Positive	

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Enteric Pathogens Workup

Organism	TSI	ONPG	PPA	Motility	Indole	Urea
<i>S. typhi</i>	-/+ ¹	-	-	+	-	-
<i>S. arizonae</i>	d/+ H ₂ S	+	-	+	-	-
<i>S. paratyphi A</i>	-/+ ²	-	-	+	-	-
Other Salmonella	-/+ H ₂ S	-	-	+	-	-
<i>S. sonnei</i>	-/+	+	-	-	-	-
<i>S. dysenteriae</i>	-/+	d	-	-	d	-
<i>S. flexneri (1-5)</i>	-/+	-	-	-	d	-
<i>S. flexneri (type 6)</i>	-/+ ⁴	-	-	-	d	-
<i>S. boydii</i>	-/+	-	-	-	d	-
<i>Y. enterocolitica</i>	d/+	+	-	- ³	d	+ ⁵

¹ may produce small amounts of gas and /or H₂S

² occasionally produces H₂S weakly

³ non-motile at 35°C; motile at room temperature (read motility within 15 minutes after removal from incubator.

⁴ may produce a small amount of gas

⁵ after overnight incubation

“d” indicates variable results

E.coli O157



1. Oxidase test negative
2. MUG test negative
3. O157 latex agglutination test positive
4. Send the isolate to PHOL for confirmation and H typing.

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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 ₂	No growth	No growth	No growth	No growth*	No growth	No growth
Subculture CHOC CO ₂	No growth	No growth	No growth	No growth*	No growth	No growth
Subculture BRUC AnO ₂	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

* 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 BAL 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:

Germ tube: **Positive** - Report as "*Candida albicans*".

Germ tube: **Negative** - Report as "Yeast, not *Candida albicans*".

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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Annual Review	May 12, 2003	Dr. T. Mazzulli
Annual Review	May 26, 2004	Dr. T. Mazzulli
Annual Review	May 12, 2005	Dr. T. Mazzulli
Yeast ID moved from Respiratory and Wounds Manuals	April 6, 2005	Dr. T. Mazzulli
Identification of <i>Neisseria gonorrhoeae</i> added	April 6, 2005	Dr. T. Mazzulli
Identification of <i>Neisseria meningitidis</i> added	April 6, 2005	Dr. T. Mazzulli
Identification of <i>H. pylori</i> moved from Respiratory Manual	April 6, 2005	Dr. T. Mazzulli
Identification for <i>S. lugduensis</i> in blood and sterile sites	Feb 14, 2006	Dr. T. Mazzulli
Annual Review	July 12, 2006	Dr. T. Mazzulli
Annual Review	August 13, 2007	Dr. T. Mazzulli
Annual Review	October 9, 2008	Dr. T. Mazzulli
Change reporting - If motile, report as " <i>Bacillus</i> sp. not <i>B. anthracis</i> ."	March 04, 2009	Dr. T. Mazzulli
Added Kinyoun and Modified Kinyoun for gram positive bacilli workup to rule out <i>Nocardia</i> and <i>Mycobacterium</i>	June 3, 2009	Dr. T. Mazzulli
Annual Review	October 10, 2009	Dr. T. Mazzulli
Annual Review	October 10, 2010	Dr. T. Mazzulli
Added shipping information for " <i>Bacillus</i> " to PHOL	January 11, 2011	Dr. T. Mazzulli
Annual review	May 31, 2011	Dr. T. Mazzulli
Changed <i>S. anginosus</i> BE reaction from – to -/weak	July 10, 2012	Dr. T. Mazzulli
Added <i>Bacillus</i> ID table	July 10, 2012	Dr. T. Mazzulli
Annual review	July 10, 2012	Dr. T. Mazzulli
Added Bacteria and Yeast ID Bench workflow to accommodate MS ID	November 04, 2013	Dr. T. Mazzulli
Annual Review	November 04, 2013	Dr. T. Mazzulli
Updated workflow for <i>Neisseria gonorrhoeae</i>	April 17, 2014	Dr. T. Mazzulli
Updated workflow for Enterococcus	April 24, 2014	Dr. T. Mazzulli
Annual Review	June 12, 2014	Dr. T. Mazzulli
<i>H. haemolyticus</i> ID by Vitek-MS workup	January 25, 2015	Dr. T. Mazzulli
Urine ID by MS and <i>E.coli</i> ID by pink colonies	January 25, 2015	Dr. T. Mazzulli

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Enteric Pathogen Workup moved from Enteric Manual	January 25, 2015	Dr. T. Mazzulli
Vitek MS Guide to Bacte/Yeast Identification reformatted. Updated tables: mandatory minimum tests in red for gpc catalase + and catalase – strep tables Added notes for <i>Rhodococcus equi</i> : Urea + & <i>Corynebacterium pseudodiphtheriticum</i> : urease + Added String test as mandatory test for sterile sties K.pneumo	September 30, 2015	Dr. T. Mazzulli
Added To gnc <i>Neisseria meningitides</i> flow chart: *Neisseria meningitidis identified from Blood and sterile site specimens: Send isolate to the Provincial Health Lab for Serogroup typing.	October 15, 2015	Dr. T. Mazzulli
p.2 Under: Vitek MS Guide to Bacteria and Yeast Identification per Bench, Added: For bone and joint fluids specimens, report organisms to the species level. If not identified in lab, send to PHOL	January 7, 2016	Dr. T. Mazzulli
Annual Review Updated MSH logo in header Updated Enterococcus , GNC and GPB table for biochemical ID	June 8, 2016	Dr. T. Mazzulli
Vitek MS Guide for ID (Page 2.) Updated instructions if MS or biochemicals do not provide an id: a. For blood cultur and sterile sites, send to PHOL for identification b. For non-sterile sites, consult charge/senior technologist. Yeast section and Urine ID section, updated yeast from non-sterile urine sites, <u>when Vitek MS fails</u> , ID at mimimum by germ tube.	February 26 th , 2018	Dr. T. Mazzulli
Annual Review	April 16, 2018	Dr. T. Mazzulli

UNIVERSITY HEALTH NETWORK/MOUNT SINAI HOSPITAL, DEPARTMENT OF MICROBIOLOGY

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