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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 Manualfor 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 culturs and sterile sites, send to PHOL for identification
    b. For non-sterile sites, consult charge/senior technologist.

Reference Bacteria and Yeast Identification Tables and Flowcharts

AEROBIC BACTERIA

GRAM POSITIVE BACTERIA

GRAM POSITIVE COCCI - Catalase-Positive

Tests	***S. aureus***	**CNST**	***S. lugdunensis***	***S. saprophyticus***	***Micrococcus* species**[5]	***Stomatococcus* 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	N/A	N/A	**N/A**	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 used to rule out S. saprophyticus from urines from female of childbearing age (>12yrs, <60yrs)
5 obligate aerobe
6 catalase variable

GRAM POSITIVE COCCI – Catalase-Negative

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

Tests	Group A, B, C or G	S.anginosus
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	***Streptococcus pneumoniae***	**viridans *Streptococci***	***Aerococcus urinae***	***Leuconostoc/ Pediococcus***	***Streptococcus bovis* 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 *Gemella*, *Strep. anginosus* group, non-haemolytic group B strep

3. *Enterococcus* identification:

Test	***Enterococcus faecalis***	***Enterococcus faecium***	***Enterococcus gallinarum***	***Enterococcus casseliflavus***
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

GRAM POSITIVE BACILLI

Test	***Listeria*[1]**	***C. jeikeium (JK)***	***C. urealyticum***	***C. pseudo-diptheriticum***	***Lactobacilli***	***Rhodococcus equis***	**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] **For *L. monocytogenes* from Blood and sterile site specimens:** Send isolate to the Provincial Health Lab for whole genome sequencing.

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

******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)

GRAM NEGATIVE BACTERIA

GRAM NEGATIVE COCCI / DIPLOCOCCI

Test	*M. catarrhalis*	*N. gonorrhoeae*	*N. meningitidis*[1]	*N. species*
Oxidase	+	+	+	+
Catalase	+	+	+	+
Tributyrin	+	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	*E. coli*	Not *E. coli*
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*	
Klebsiella pneumonia	String Test

*Mandatory testing required

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	***H. influenzae*[1]**	***H. parainfluenzae***	***H. haemolyticus*[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.

GRAM NEGATIVE BACILLI – Oxidase-Positive

Test	***Ps. aeruginosa***	**Not *Ps. aeruginosa***
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	***H. pylori***
Gram stain	Small, gram negative gull-shaped or spiral
Catalase	+
Oxidase	+
Urea slant (rapid)	+

Urine Pathogens Workup

Suspect Organism	Tests to be performed and expected result	Identification/Susceptibility
E.coli (Lactose fermenter)	Oxidase: Negative MUG: Positive + Indole: Positive	Vitek Susceptibility
Enterobacterales	Oxidase: Negative	Vitek ID + Susceptibility
Pseudomonas aeruginosa	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 *C. albicans* -negative : Yeast, not *Candida albicans*".	Refer to Mycology
Group B streptococcus	Strep. Latex Agglutination: Group B Positive Bile esculin: Negative	
Staphylococcus species: *aureus* CNST	Staph. Latex Agglutination: Positive Staph. Latex Agglutination: Negative (if no ID from MALDI, send to PHOL for patients 12 - 60 yrs and females only to rule out S. saprophyticus)	Vitek Susceptibility Oxacillin screen Vancomycin screen
Enterococcus species	Bile esculin: Positive	Vitek Susceptibility Vancomycin screen
Corynebacterium species	Non-haemolytic colonies on Blood Agar Gram positive bacilli, small, palisade arrangement. Catalase: Positive Urea: Negative	

Suspect Organism	Tests to be performed and expected result	Identification/Susceptibility
Corynebacterium urealyticum	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	
Aerococcus urinae	Alpha-haemolytic colonies on Blood Agar Gram positive cocci in tetrads or clusters Catalase: Negative LAP: Positive + PYR: Negative	
Lactobacillus species	Alpha-haemolytic or non-haemolytic colonies on Blood Agar Gram positive thin bacilli Catalase: Negative	
Bacillus species	Gram positive large bacilli with square ends Motility: Positive	

Enteric Pathogens Workup

Organism	TSI	ONPG	PPA	Motility	Indole	Urea
S. typhi	-/+[1]	-	-	+	-	-
S. arizonae	d/+ H_2S	+	-	+	-	-
S. paratyphi A	-/+[2]	-	-	+	-	-
Other Salmonella	-/+ H_2S	-	-	+	-	-
S. sonnei	-/+	+	-	-	-	-
S. dysenteriae	-/+	d	-	-	d	-
S. flexneri (1-5)	-/+	-	-	-	d	-
S. flexneri (type 6)	-/+[4]	-	-	-	d	-
S. boydii	-/+	-	-	-	d	-
Y. enterocolitica	d/+	+	-	-[3]	d	+[5]

[1]may produce small amounts of gas and /or H_2S
[2]occasionally produces H_2S 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. 0157 latex agglutination test positive
4. Send the isolate to PHOL for confirmation and H typing.

ANAEROBIC BACTERIA

Test	***Peptostrepto-coccus* species**	***Cuti-bacterium* species**	**Anaerobic non-spore forming Gram positive bacilli**	***Clostridium* species**	***B. fragilis***	**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 "*Cutibacterium* species".

- Anaerobic, small Gram positive bacilli that are catalase negative should be reported as "Anaerobic non-spore forming Gram positive bacilli".

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 (≥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*".

REFERENCES

H.D. Izenberg. 2003. Guildlines for Identification of Aerobic Bacteria, 3.16.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

H.D. Izenberg. 2003. Schemes for Identification of aerobic Bacteria, 3.18.1.1 – 3.18.2.1 in Clinical Microbiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

Record of Edited Revisions

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Annual Review	May 12, 2003	Dr. T. Mazzulli
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Yeast ID moved from Respiratory and Wounds Manuals	April 6, 2005	Dr. T. Mazzulli
Identification of *Neiserria gonorrhoeae* added	April 6, 2005	Dr. T. Mazzulli
Identification of *Neiserria meningitidis* added	April 6, 2005	Dr. T. Mazzulli
Identification of H. pylori moved from Respiratory Manual	April 6, 2005	Dr. T. Mazzulli
Identification for *S. lugduensis* 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 "*Bacillus* sp. not *B. antracis*."	March 04, 2009	Dr. T. Mazzulli
Added Kinyoun and Modified Kinyoun for gram positive bacilli workup to rule out *Norcardia* and *Mycobacterium*	June 3, 2009	Dr. T. Mazzulli
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Added shipping information for "Bacillus " to PHOL	January 11, 2011	Dr. T. Mazzulli
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Changed *S. anginosis* BE reaction from – to -/weak	July 10, 2012	Dr. T. Mazzulli
Added Bacillus ID table	July 10, 2012	Dr. T. Mazzulli
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Added Bacteria and Yeast ID Bench workflow to accommodate MS ID	November 04, 2013	Dr. T. Mazzulli
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Updated workflow for *Neiserria gonorrhoeae*	April 17, 2014	Dr. T. Mazzulli
Updated workflow for Enterococcus	April 24, 2014	Dr. T. Mazzulli
Annual Review	June 12, 2014	Dr. T. Mazzulli
H. haemolyticus ID by Vitek-MS workup	January 25, 2015	Dr. T. Mazzulli
Urine ID by MS and E.coli 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 Rhodococcus equi: Urea + & *Corynebacterium pseudodiptheriticum*: urease + Added String test as mandatory test for sterile sties K.pneumo	September 30, 2015	Dr. T. Mazzulli
Added To gnc *Neisseria meningitides* 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
Annual Review	July 26, 2017	Dr. T. Mazzulli
Vitek MS Guide for ID (Page 2.) Updated instructions if MS or biochemicals do not provide an id: a. For blood culturs 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, when Vitek MS fails, ID at mimimum by germ tube.	February 26, 2018	Dr. T. Mazzulli
Annual Review	April 16, 2018	Dr. T. Mazzulli
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Full document review included in all updates. Bi-annual review conducted when no revision had been made within 2 years.

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Minor formatting change	April 11, 2021	Jessica Bourke
Nomenclature update – cutibacterium, enterobacterales	April 20, 2021	Wayne Chiu
Updated listeria sendout, only monocytogenes for wgs	June 23, 2021	Wayne Chiu
Removed novobiocin. for CNST if no ID from MALDI, send out to PHOL to rule out sapro on female of childbearing age	Nov 2, 2021	Wayne Chiu
Removed cephalothin and nalidixic acid for H. pylori identification	July 12, 2022	Wayne Chiu