The Wonderful World of Burkholderia

A Review

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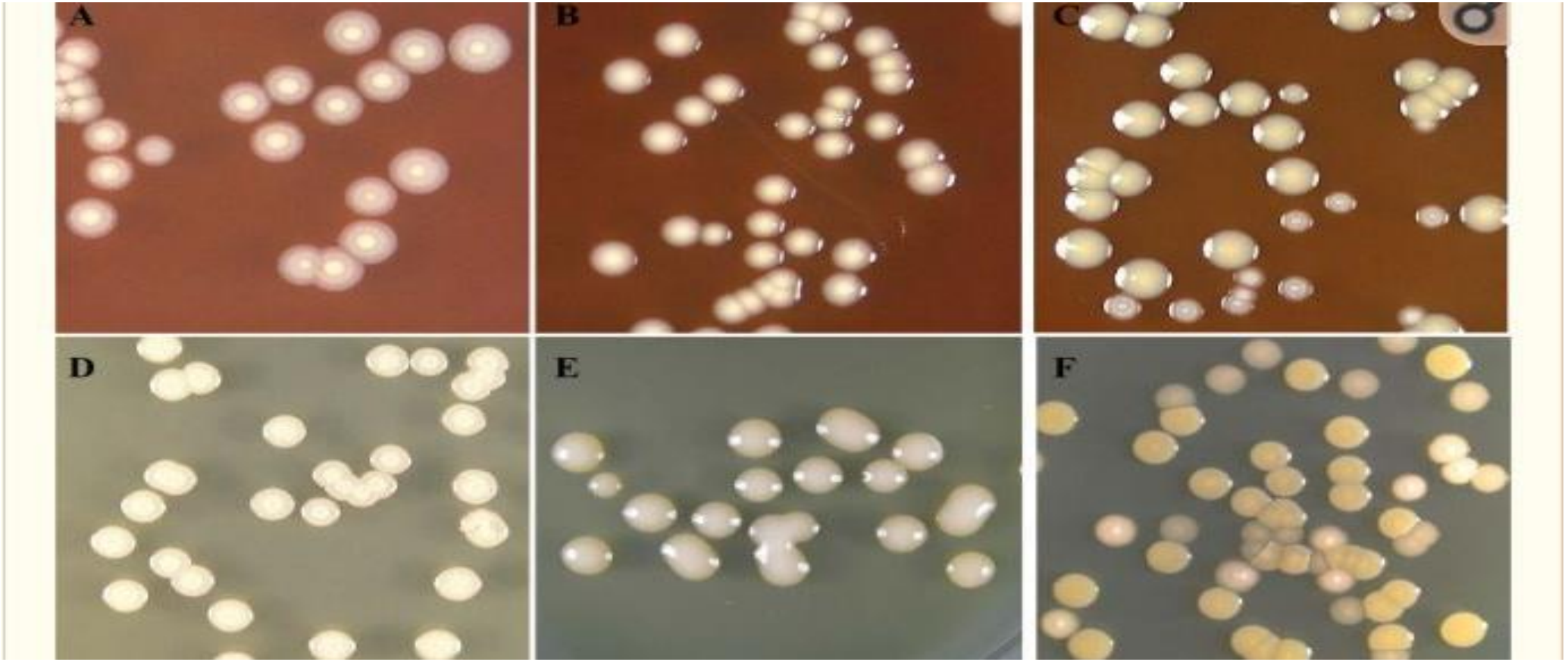
Colony Morphology Can Vary



BAP

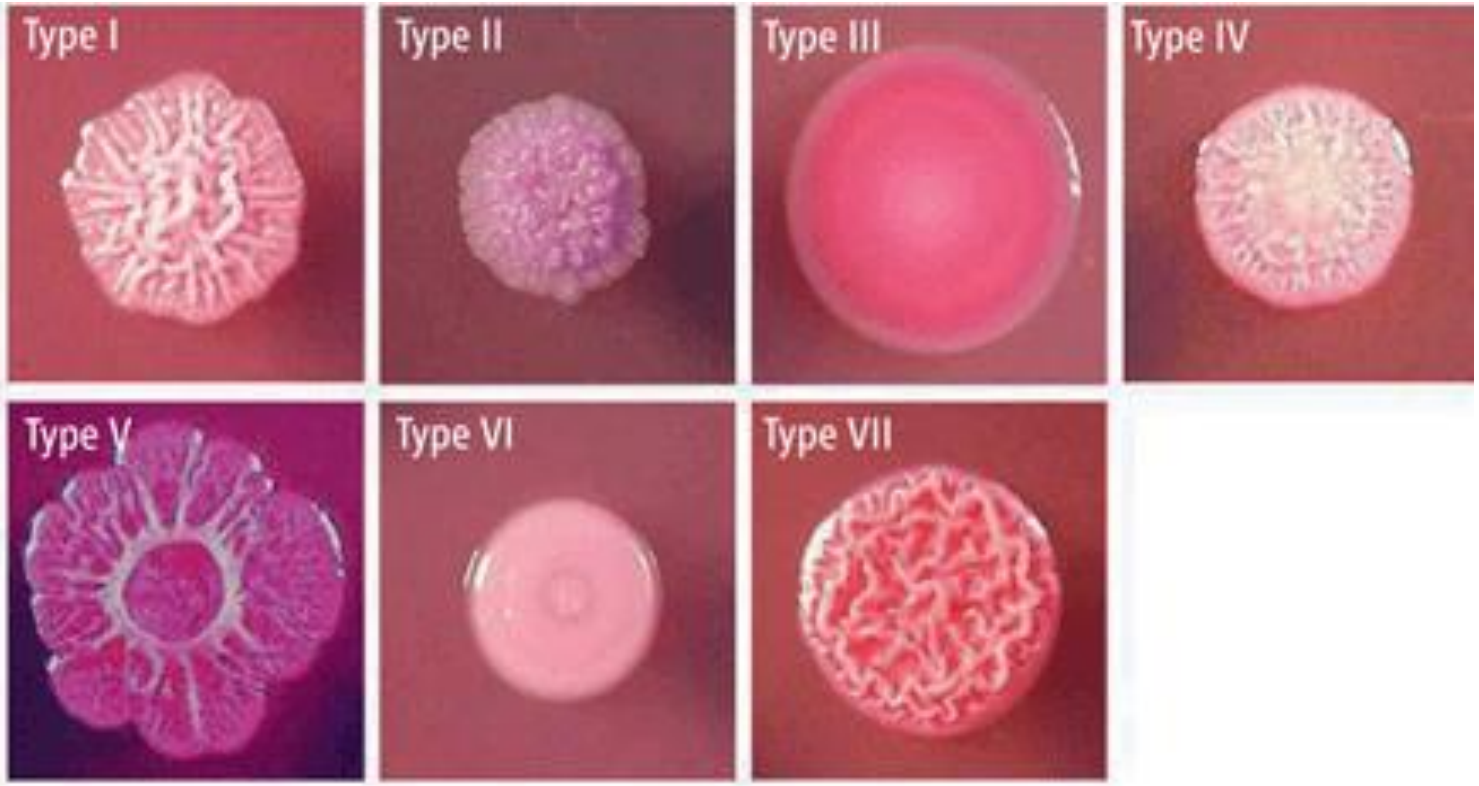
TSA

A & D = Non-mucoid colonies

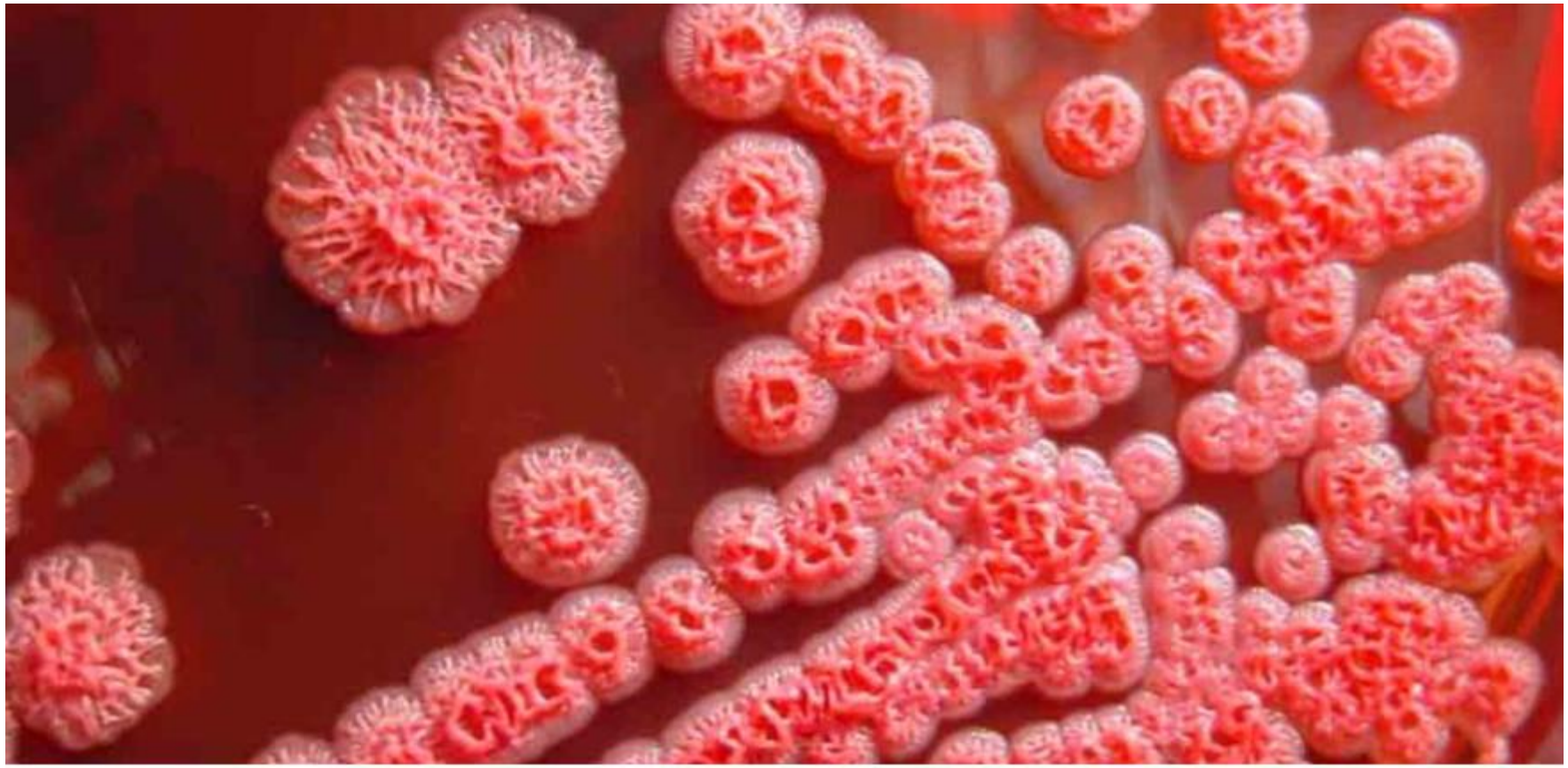
B & E = Mucoid colonies

C & F = Mixed Morphologies

Colony Morphology Can Vary



Colony Morphology Can Vary



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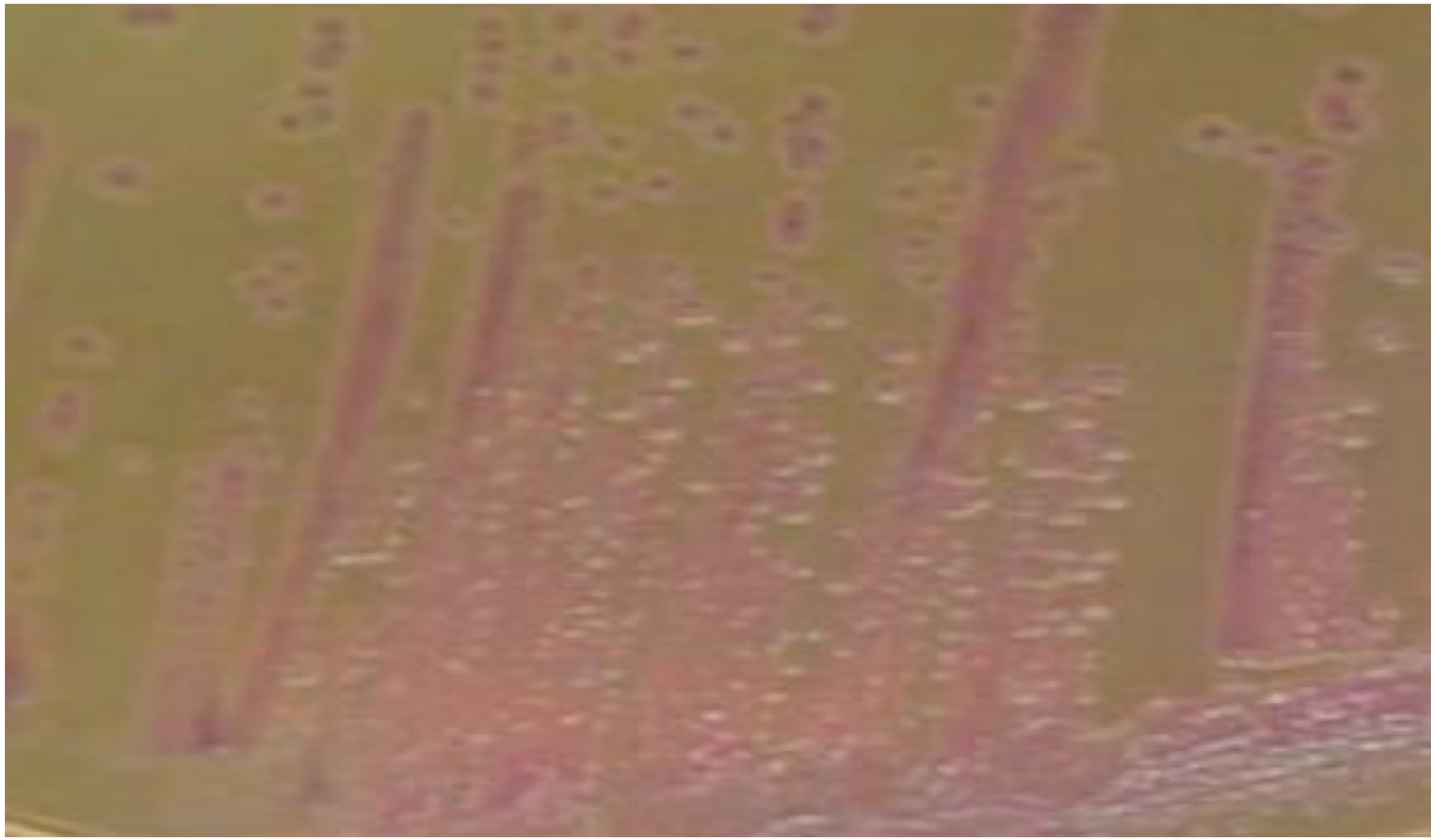
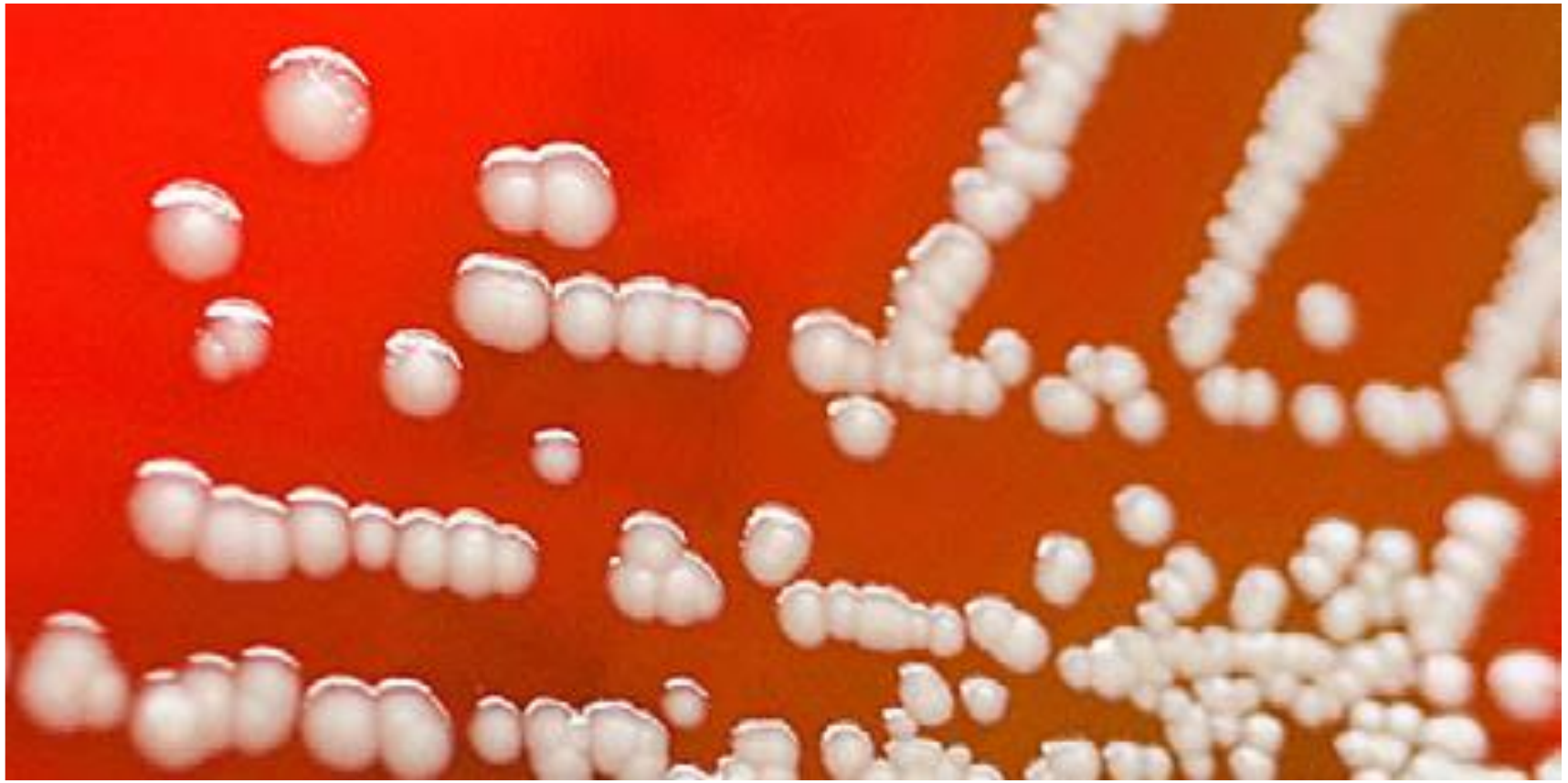
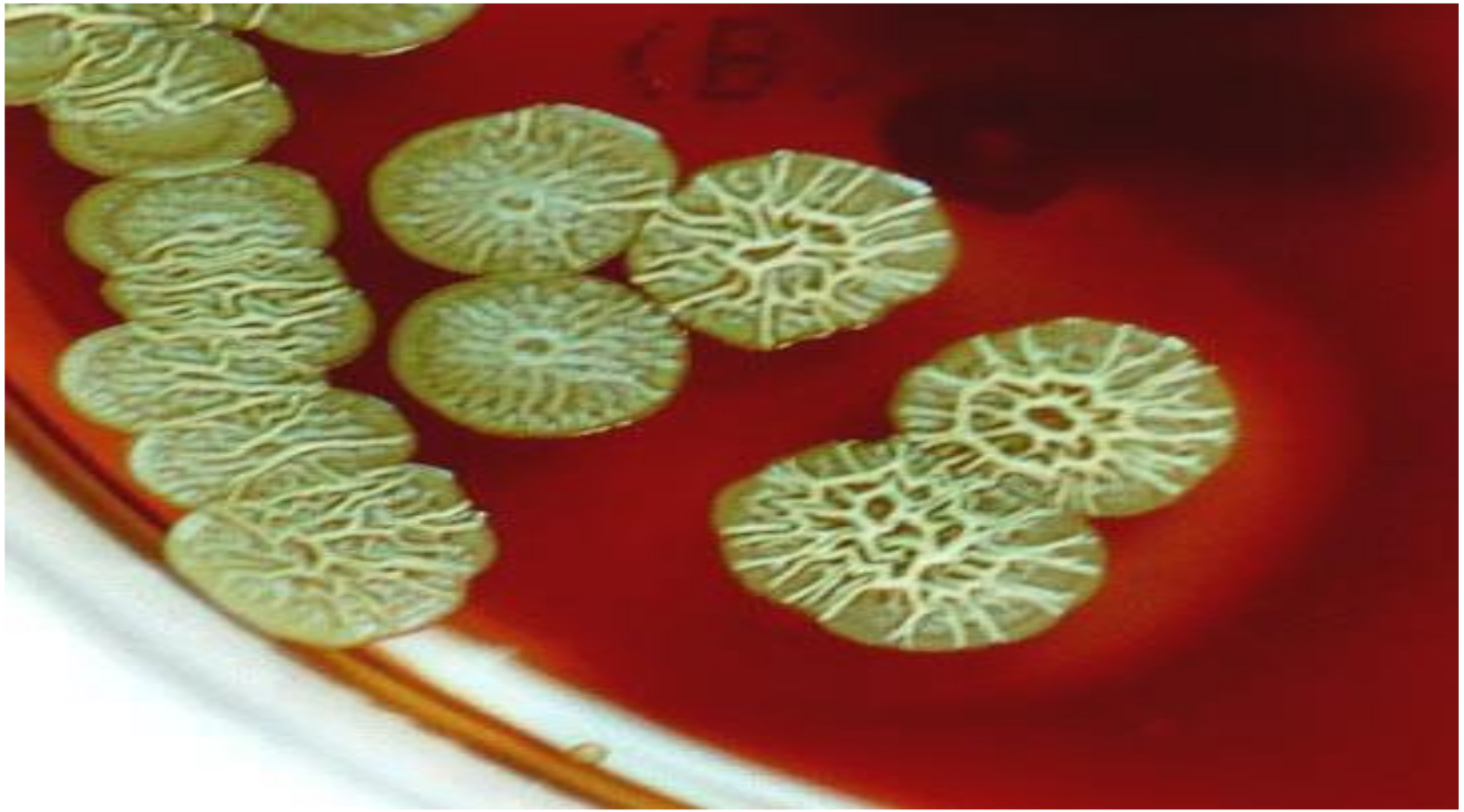


Colony Morphology Can Vary

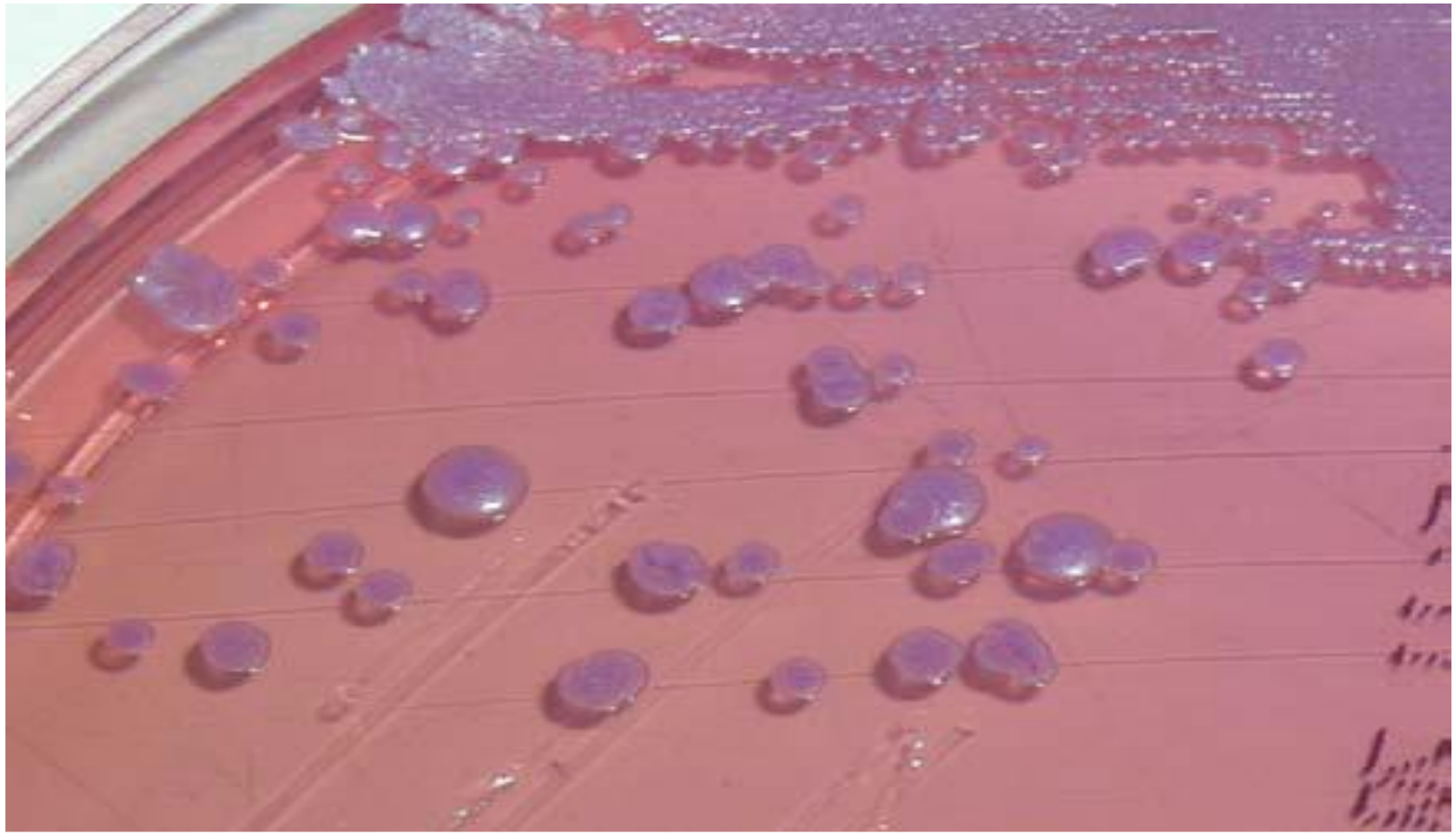


Colony Morphology Can Vary

Colony Morphology Can Vary



Colony Morphology Can Vary



The colonies are gorgeous, but…

Where do I begin with the identification?

Key Tests

- Gram Stain
- Growth on MacConkey Agar
- Growth at 42°C Catalase
- Oxidase
- Indole
- Polymyxin B
- Motility (Use semi-solid agar only)
- Triple Sugar Iron Agar – *use only to determine glucose non-fermenter status*

Burkholderia species - TNTC

Are there really over 100 species of Burkholderia????

Burkholderia cepacia Complex

Tests	Burkholderia ambifaria	Burkholderia anthina	Burkholderia cenocepacia	Burkholderia cepacia	Burkholderia dolosa	Burkholderia multivorans	Burkholderia pyrrocinia	Burkholderia stabilis	Burkholderia vietnamiensis
Urea hydrolysis	−	−	V	V	+	+	−	−	V
Growth at 42 degrees C	V	V	V	V	+	+	V	−	−
Citrate	+	+	+	+	+	+	−	+	+
Lysine decarboxylase	+	+	+	+	−	V	+	+	+
Ornithine decarboxylase	−	−	V	V	−	V	V	+	−
Adonitol	V	V	V	V	+	+	+	V	−
ONPG (beta galactosidase)	+	V	V	V	+	V	+	−	V
Sucrose	+	V	V	V	−	−	V	−	V
Lactose	V	V	V	V	+	+	−	V	V
Trehalose			V		+	+	−	V	
Gelatin hydrolysis	+	−	V	V	V	V	V	V	V
D-Mannitol	+	+	+	+	+	+	V	+	+
D-Mannose	+	+	+	+	+	+	V	+	+
Growth on MacConkey agar	+	+	V	V	+	+	V	+	+
L-Arabinose	+	+	V	+	+	+	V	V	+
Nitrite to gas			−	−	−	−	V	−	−
Maltose	+	+	V	V	+	+	+	V	V
Oxidase	+	+	V	V	V	V	+	+	+
Yellow pigment	−	−	V	V	V	V	−	−	−
Motile			V	V	+	+	+	+	+
Beta hemolysis	V	V	V	V	−	−	V	−	V
D-Xylose	+	V	V	V	+	+	V	V	V
Esculin hydrolysis	V	−	V	V	V	V	−	V	−
Raffinose			V	V	−	−	−	V	V
Casein hydrolysis			V	V	−	−		V	−
Nitrate to nitrite	V	V	V	V	+	+	V	V	V
Cellobiose			V	V	+	+	V	V	V
Salicin			V	V	−	V	V	V	−
Dulcitol			V	V	+	+		V	V
D-Sorbitol			V	V	+	+		V	V
myo-Inositol			V	V	+	+		V	V
Glycerol			V		+	+		V	

\+ > 90%
V 10% - 90%
− < 10%

Other Important Burkholderia species

Tests	Burkholderia gladioli	Burkholderia mallei	Burkholderia pseudomallei	Burkholderia thailandensis
Nitrite to gas	−	−	+	+
Growth at 42 degrees C	−	−	+	+
Yellow pigment	+	−	+	+
Tartrate utilization	+	−	−	−
Motile	+	−	+	+
Starch hydrolysis	−	V	+	+
Maltose	−	V	+	+
Adonitol	+		−	+
Pyrrolidonyl-beta-naphthylamide	+		−	−
Dulcitol	+		+	−
Erthyritol	−		+	−
Arginine dihydrolase	−	+	V	V
Citrate	+	−	V	V
L-Arabinose			−	+
D-Mannitol	+	V	+	+
Beta hemolysis	V	−	−	−
Lipase	+	V	+	+
Glucose oxidizer	V	+	+	+
D-Xylose	+	V	+	+
Nitrate to nitrite	V	+	+	+
Growth on MacConkey agar	+	V	+	+
Sucrose	−	−	V	V
Oxidase	V	V	+	+
Lactose	V	V	+	+
ONPG (beta galactosidase)	V		−	−
Gelatin hydrolysis	V	−	V	V
Esculin hydrolysis	V	−	V	V
L-Rhamnose	−		V	V

\+ > 90%
V 10% - 90%
− < 10%

Biothreat Agents:

1. *Burkholderia mallei*
2. *Burkholderia pseudomallei*

Two Choices: RULE OUT *OR* SEND OUT

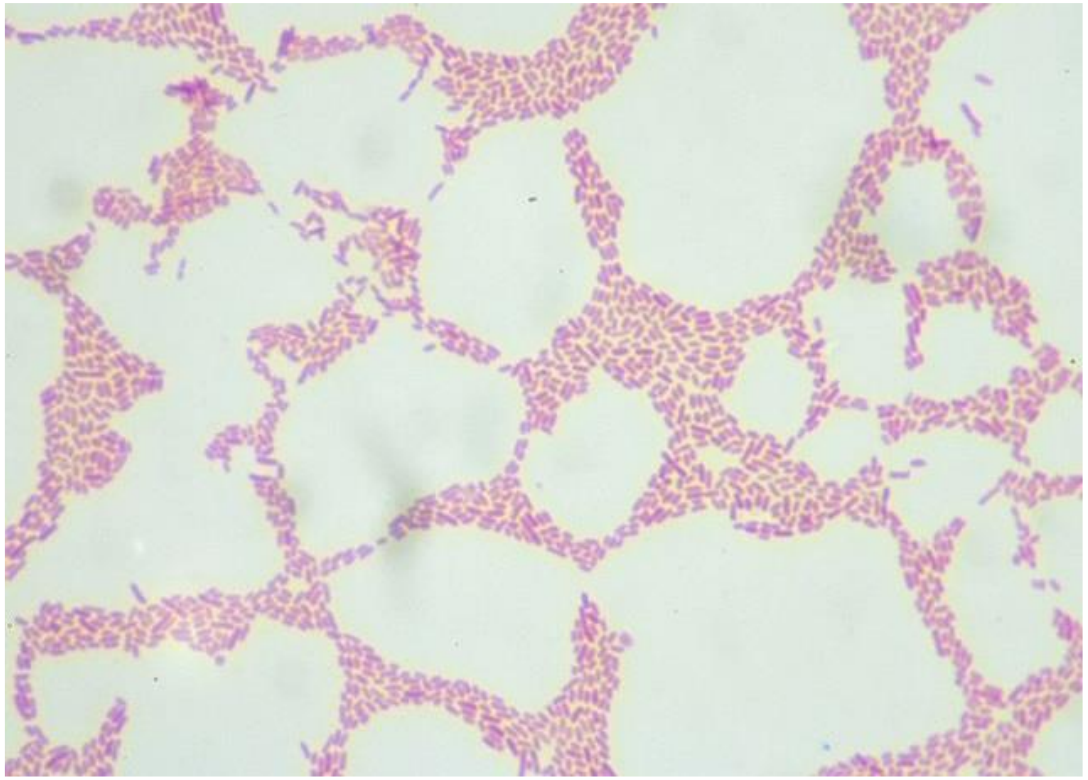
If unable to rule out, send the isolate to the Maryland State Public Health laboratory.

DO NOT SEND TO A COMMERCIAL REFERENCE LABORATORY.

Gram Stain

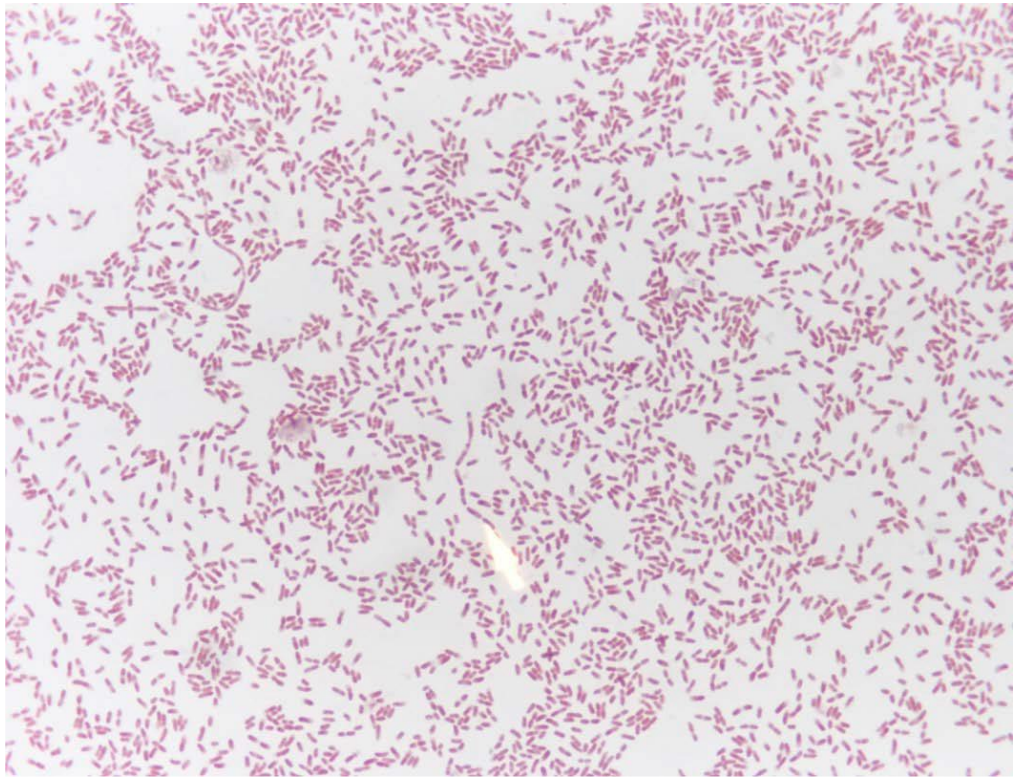
Burkholderia mallei

Gram negative coccobacilli with round ends, Chinese-letter forms in parallel or bundles



Burkholderia pseudomallei

May appear bipolar in direct smears



Key Characteristics

Burkholderia mallei

- Blood Agar = Nonhemolytic/No pigment
- MacConkey = Poor/No growth
- Growth at 42°C = No growth
- Catalase = Positive
- Oxidase = Variable/Mostly negative
- Spot Indole = Negative
- Polymyxin B = No zone
- Motility = Negative

Burkholderia pseudomallei

- Blood Agar = Nonhemolytic/Pigment
- MacConkey = Growth
- Growth at 42°C = Growth
- Catalase = Positive
- Oxidase = Positive (may be delayed)
- Spot Indole = Negative
- Polymyxin B = No zone
- Motility = Positive
- Diagnostic = Distinctive musty/earthy odor

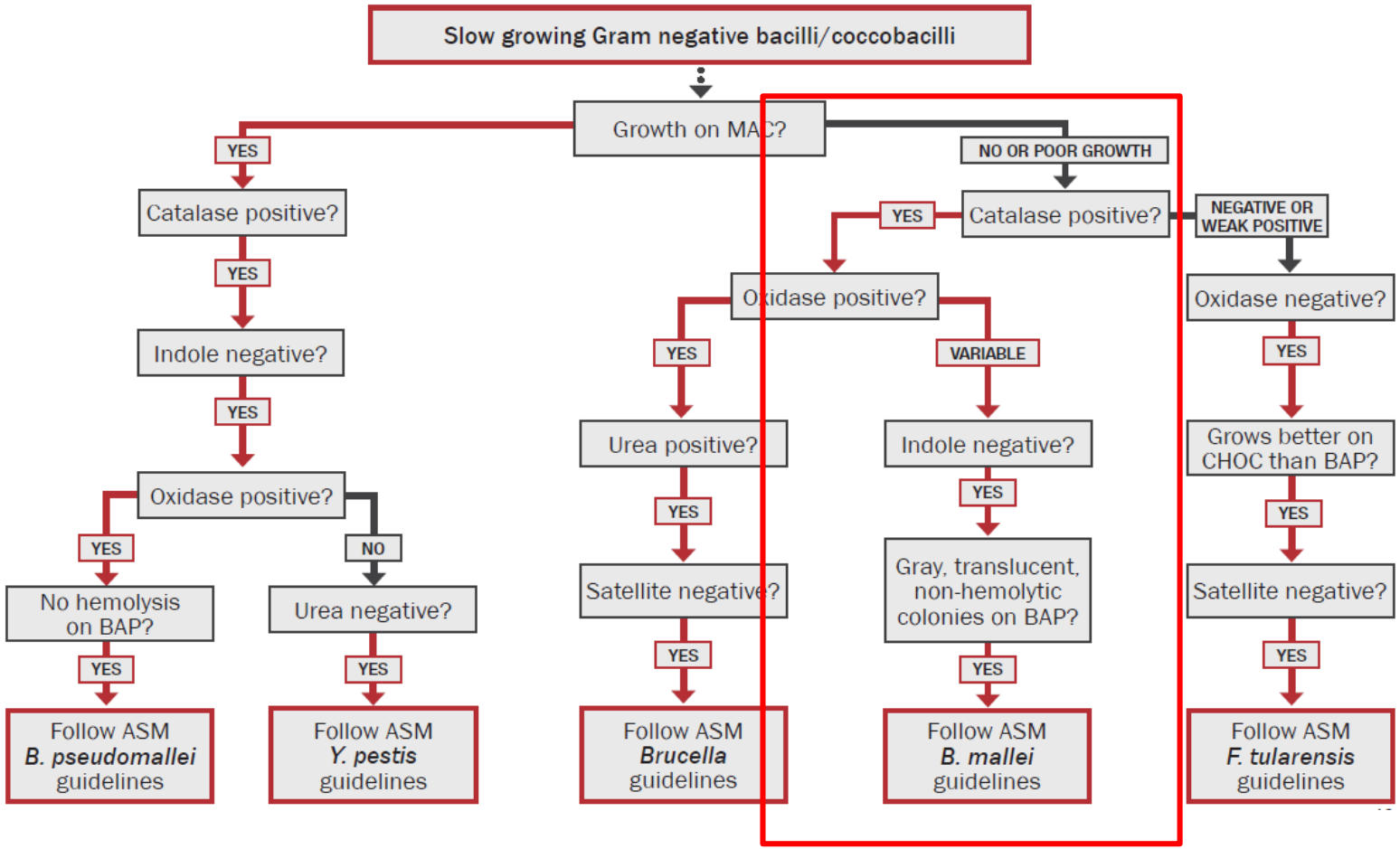
Flowcharts – Rule In/Rule Out

The gateway to tables and the Laboratory Response Network (LRN) Facilities

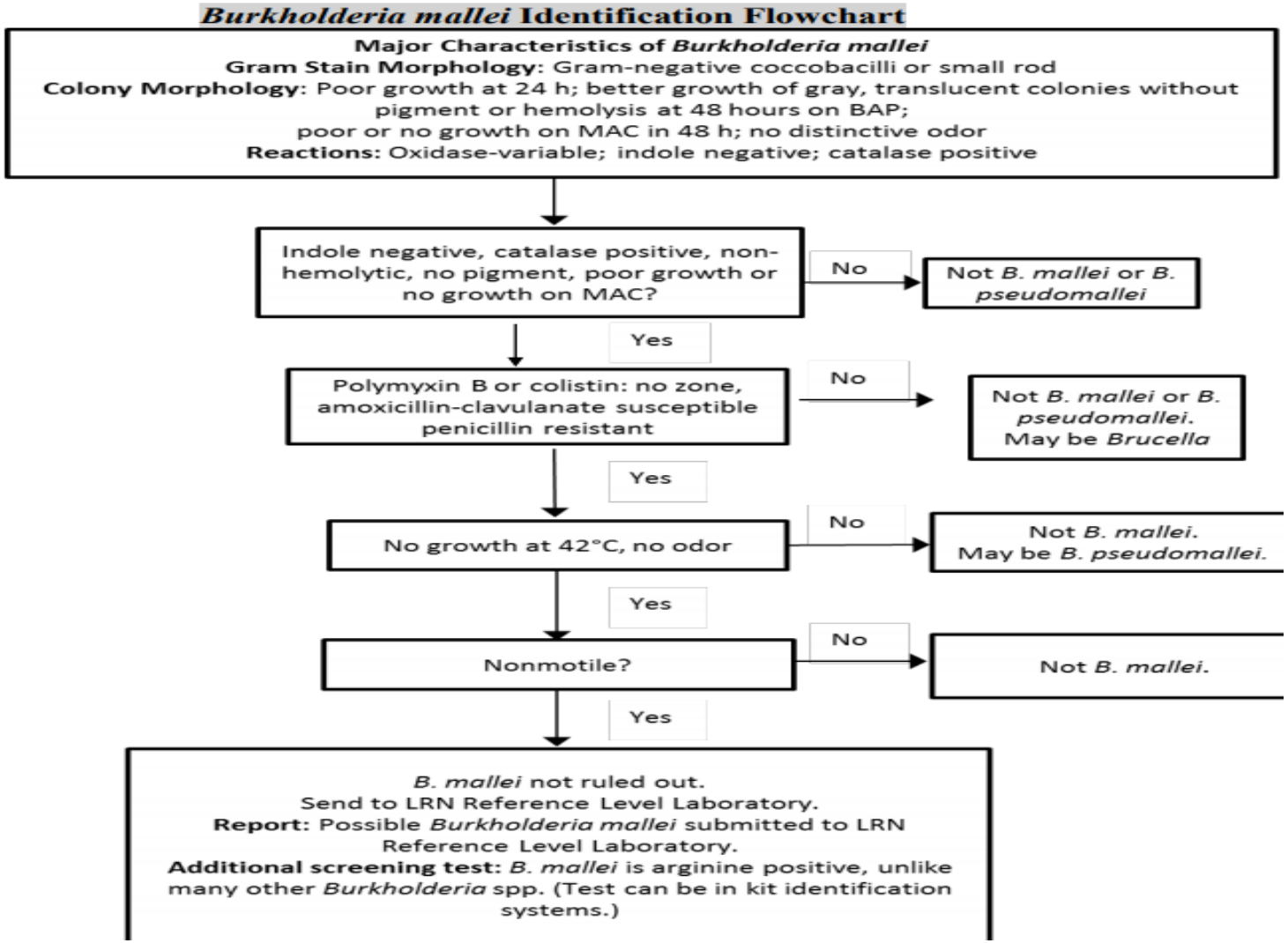
Burkholderia mallei

Oxidase variable; Indole negative; Catalase positive

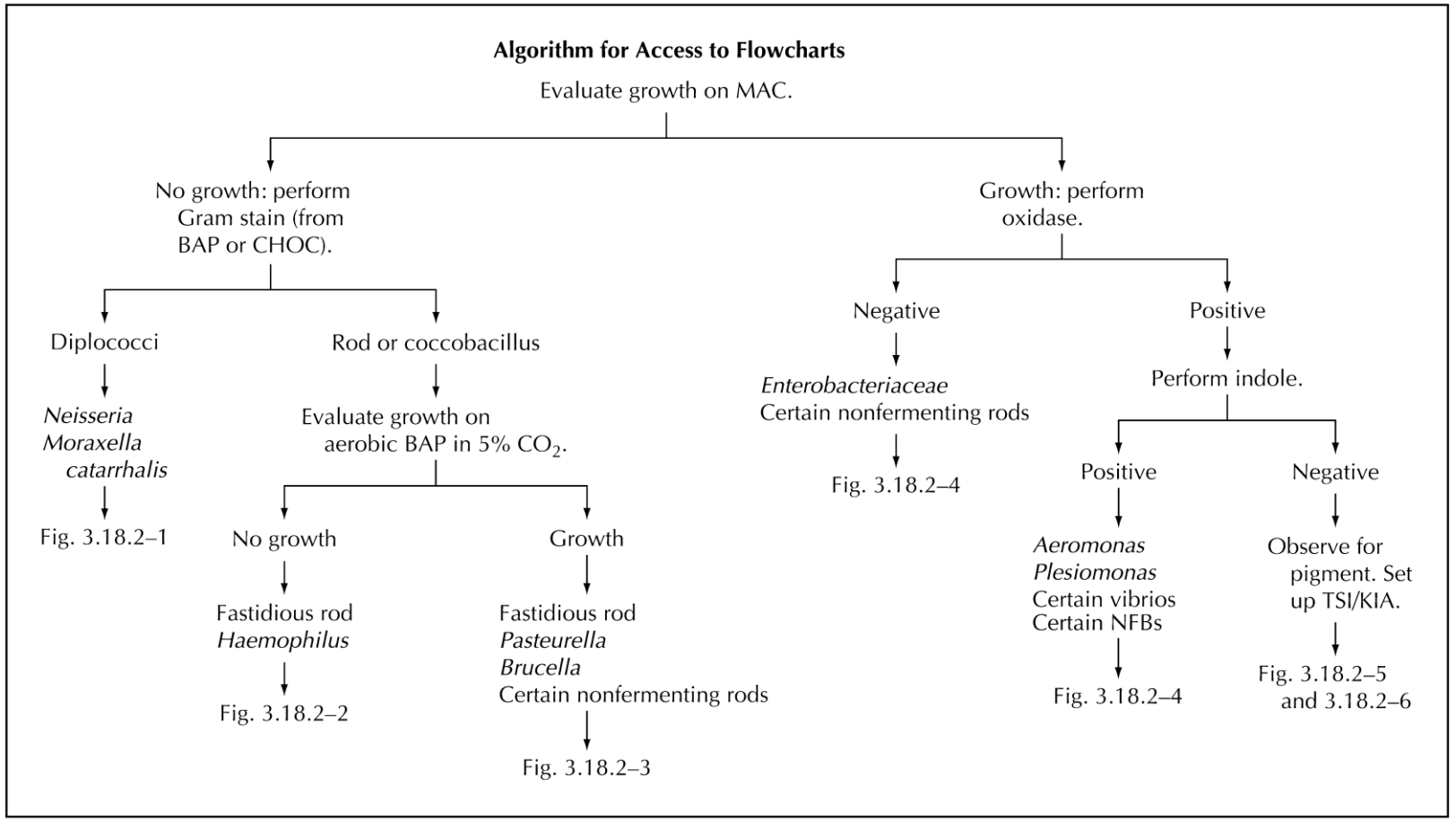
Go With The APHL Flow – *B. mallei*



Go With The ASM Sentinel Lab Flow – *B. mallei*

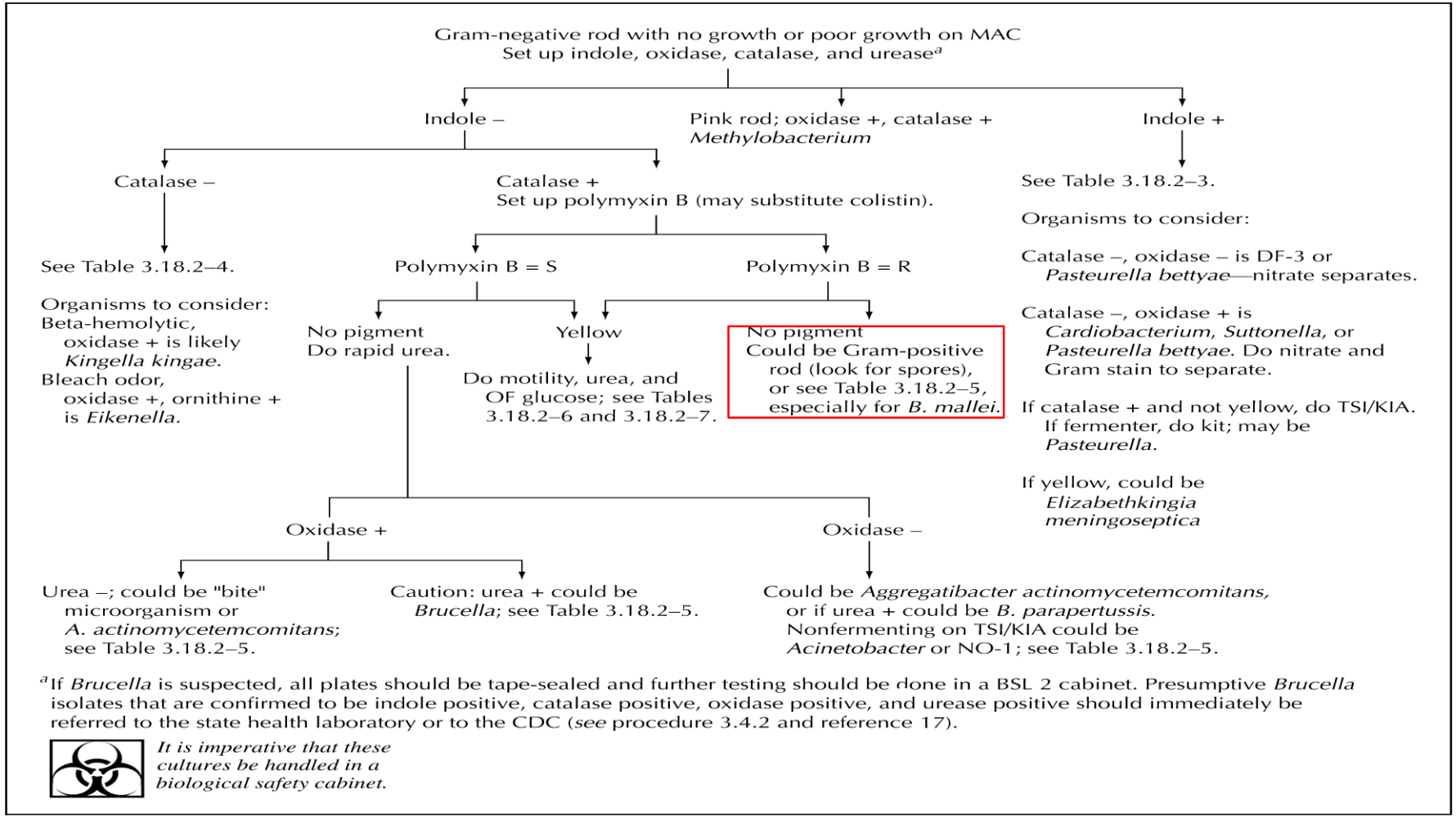


Go With The ASM CMPH Flow – *B. mallei*



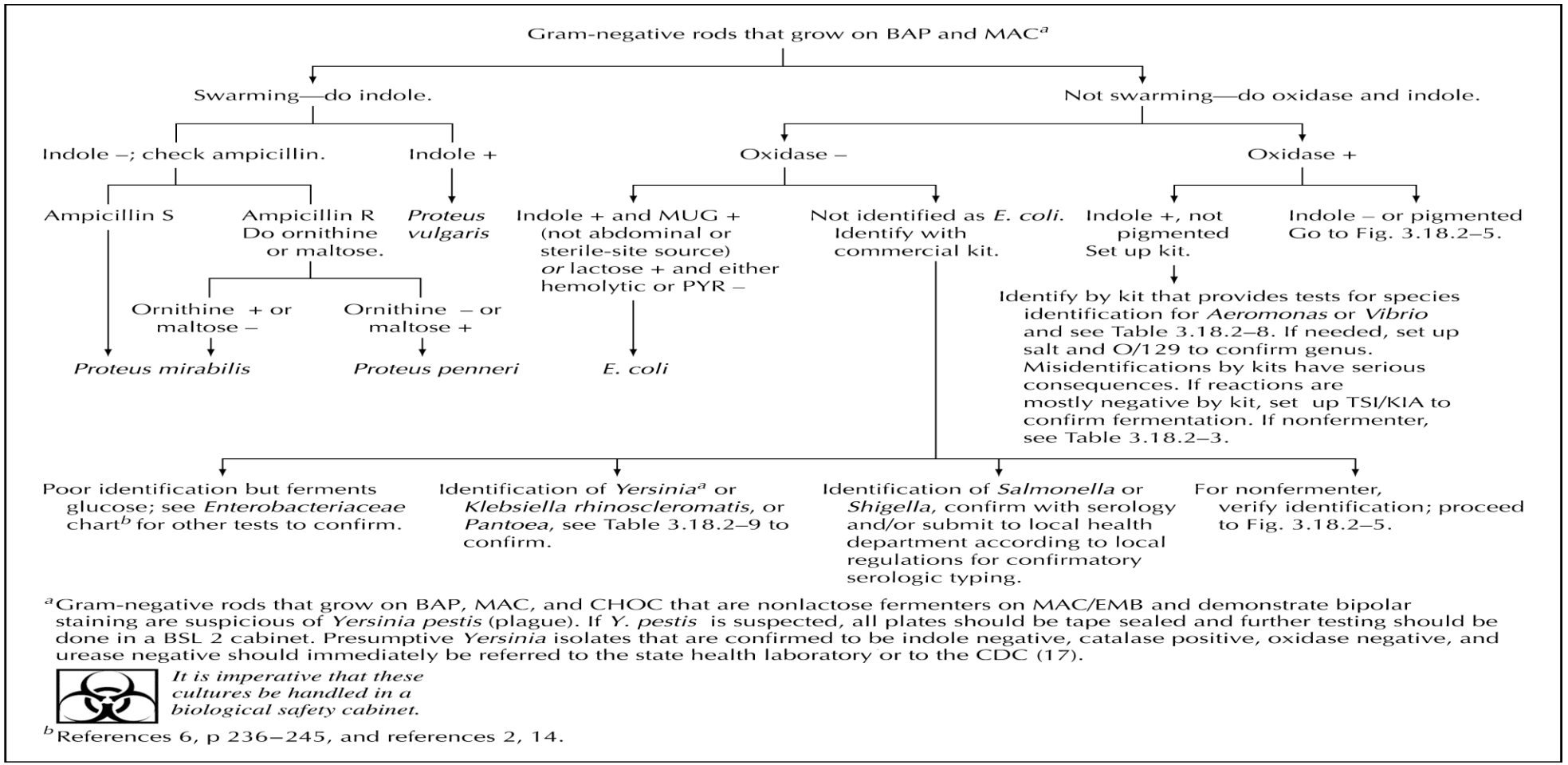
Go With The ASM CMPH Flow – *B. mallei*

Figure 2-3



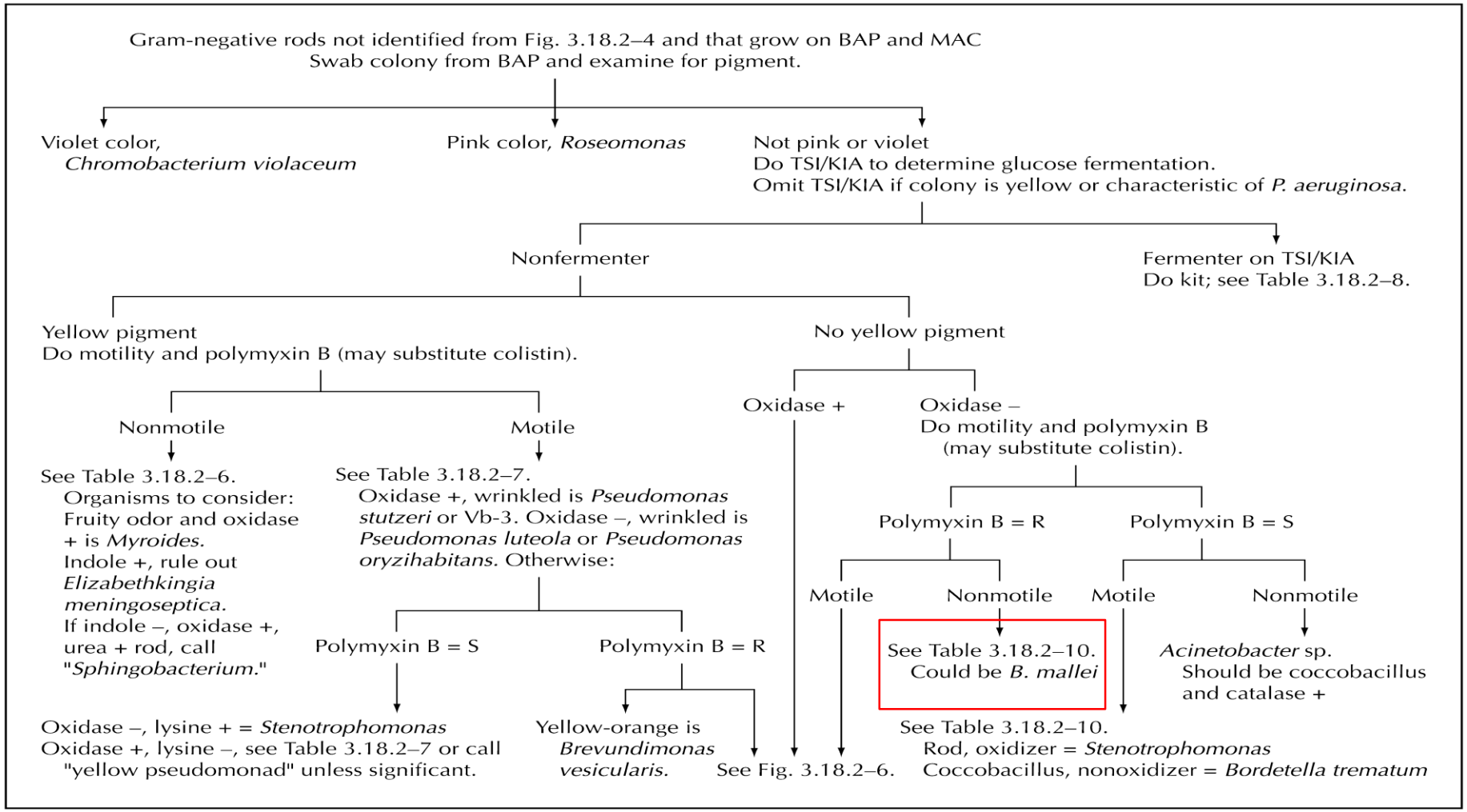
Go With The ASM CMPH Flow – *B. mallei*

Figure 2-4



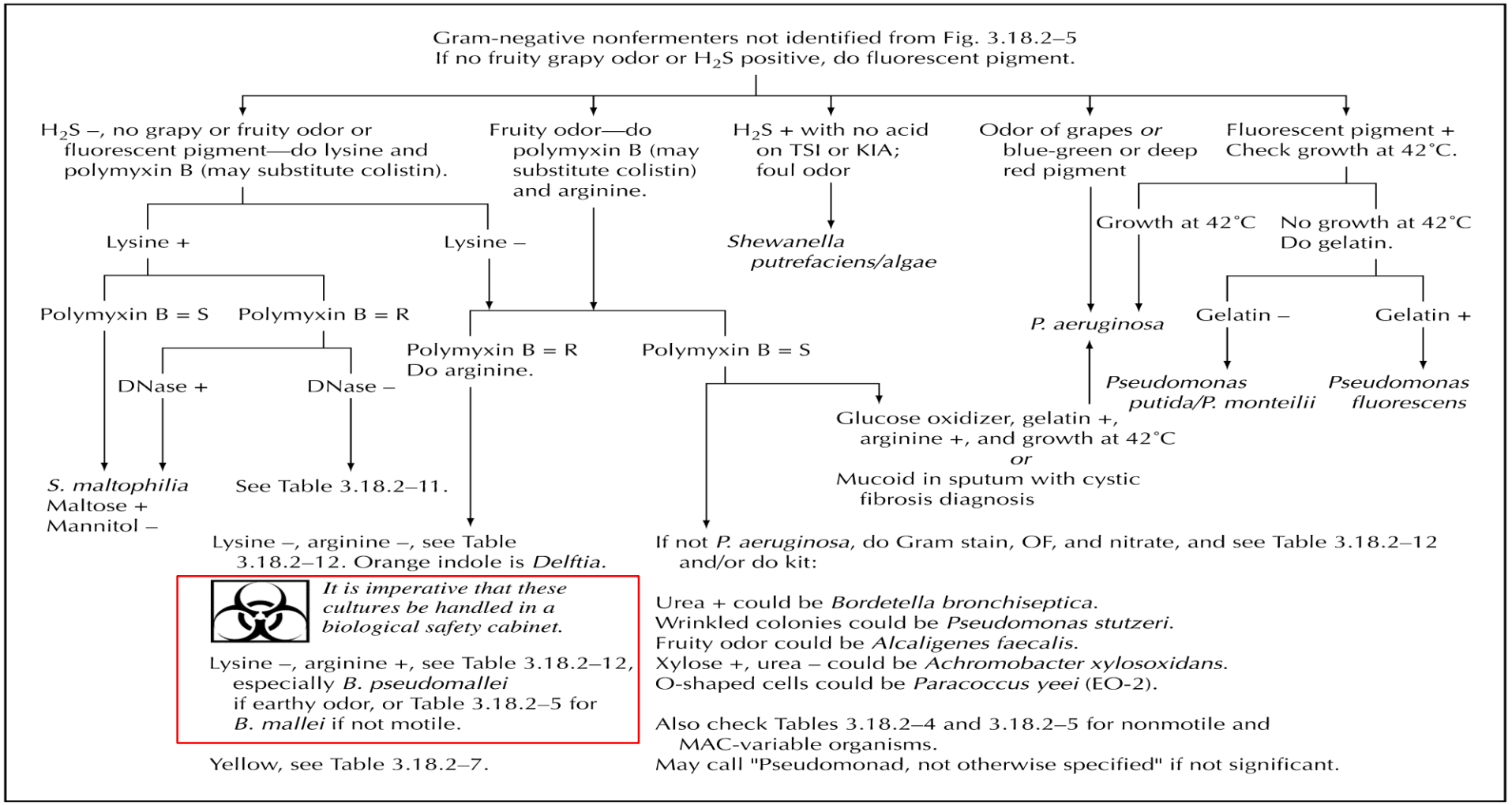
Go With The ASM CMPH Flow – *B. mallei*

Figure 2-5



Go With The ASM CMPH Flow – *B. mallei*

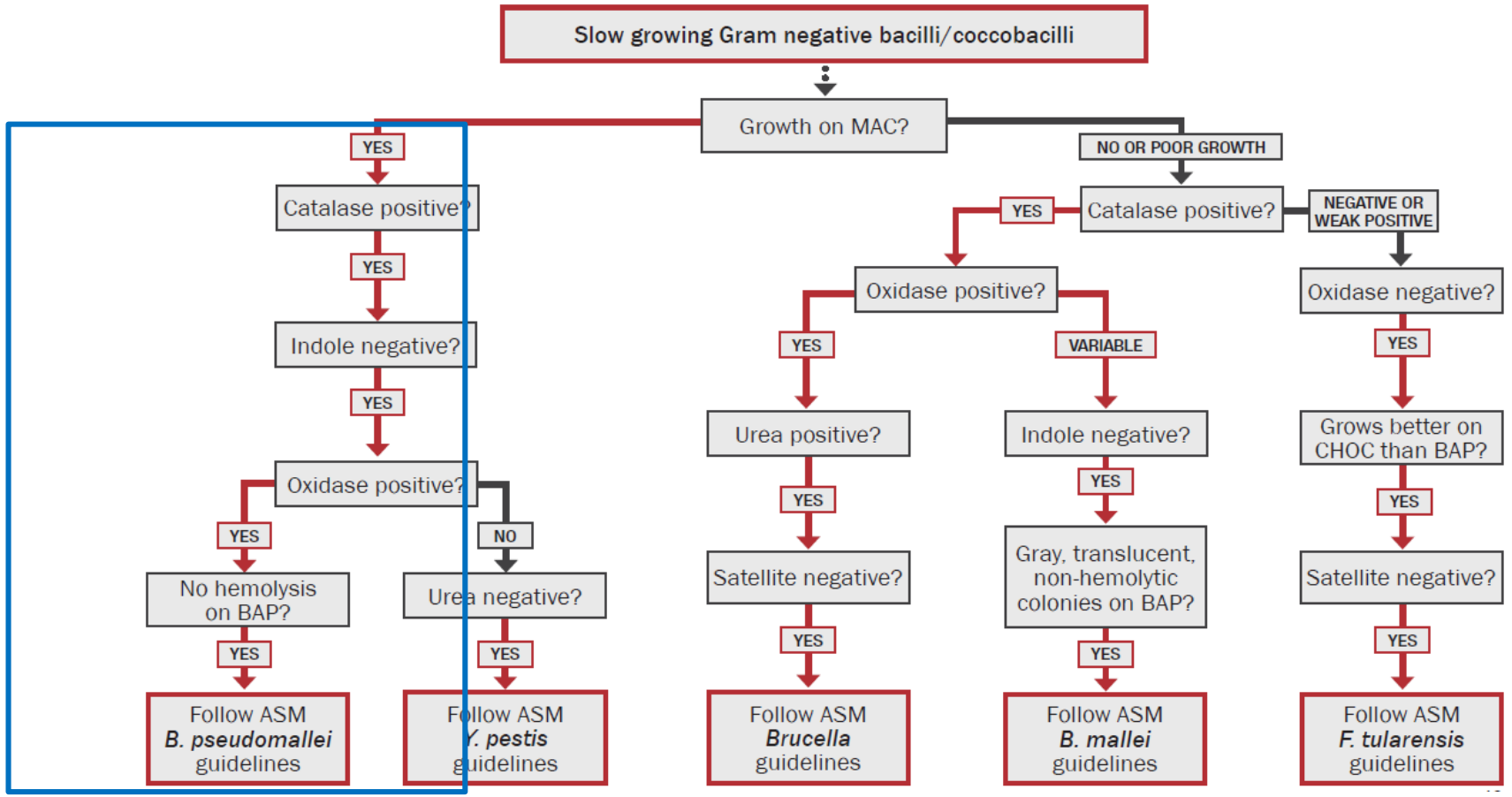
Figure 2-6



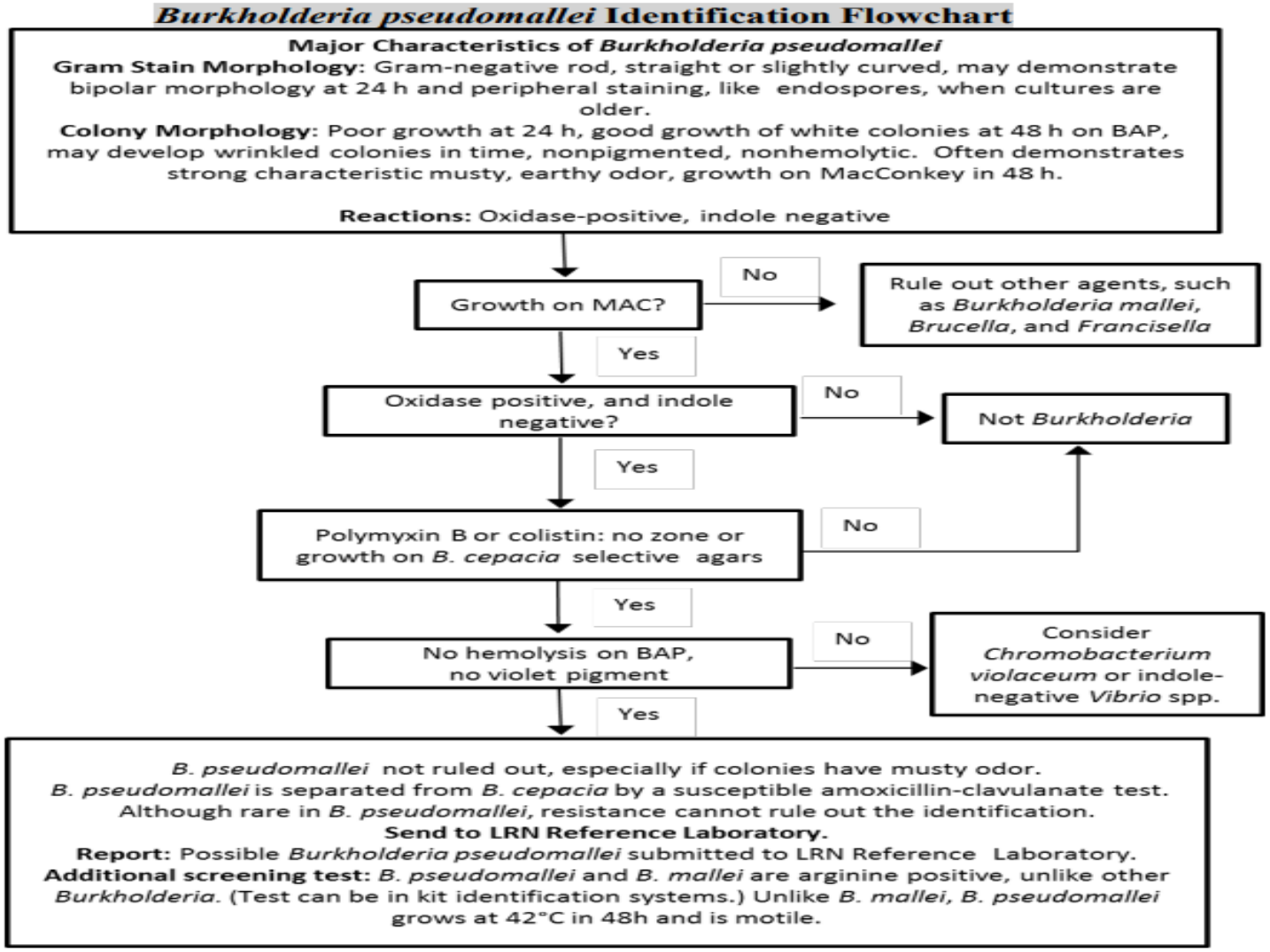
Burkholderia pseudomallei

Oxidase positive; Indole negative; Definitely down to earth

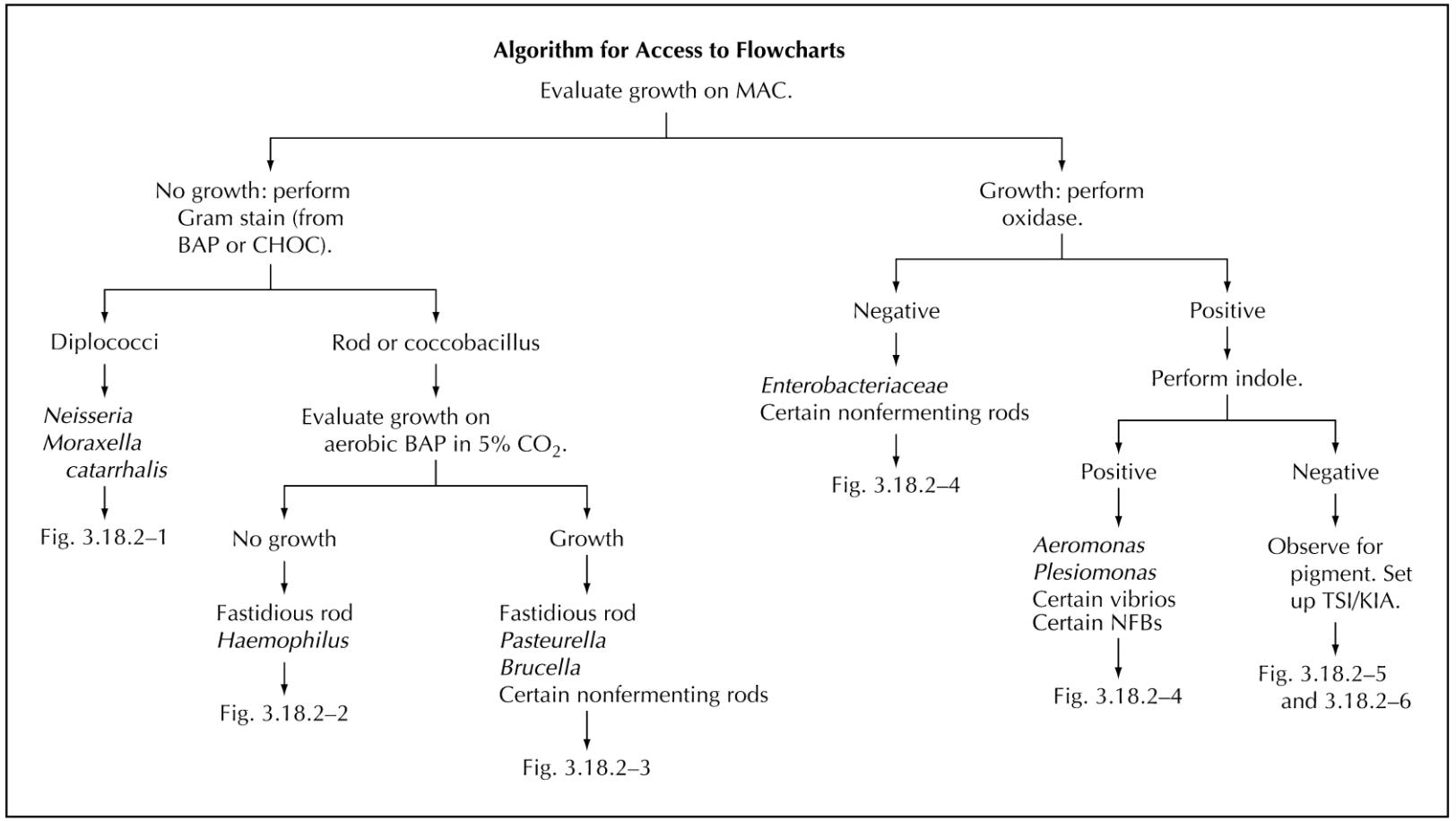
Go With The APHL Flow – *B. pseudomallei*



Go With The ASM Sentinel Lab Flow – *B. pseudomallei*

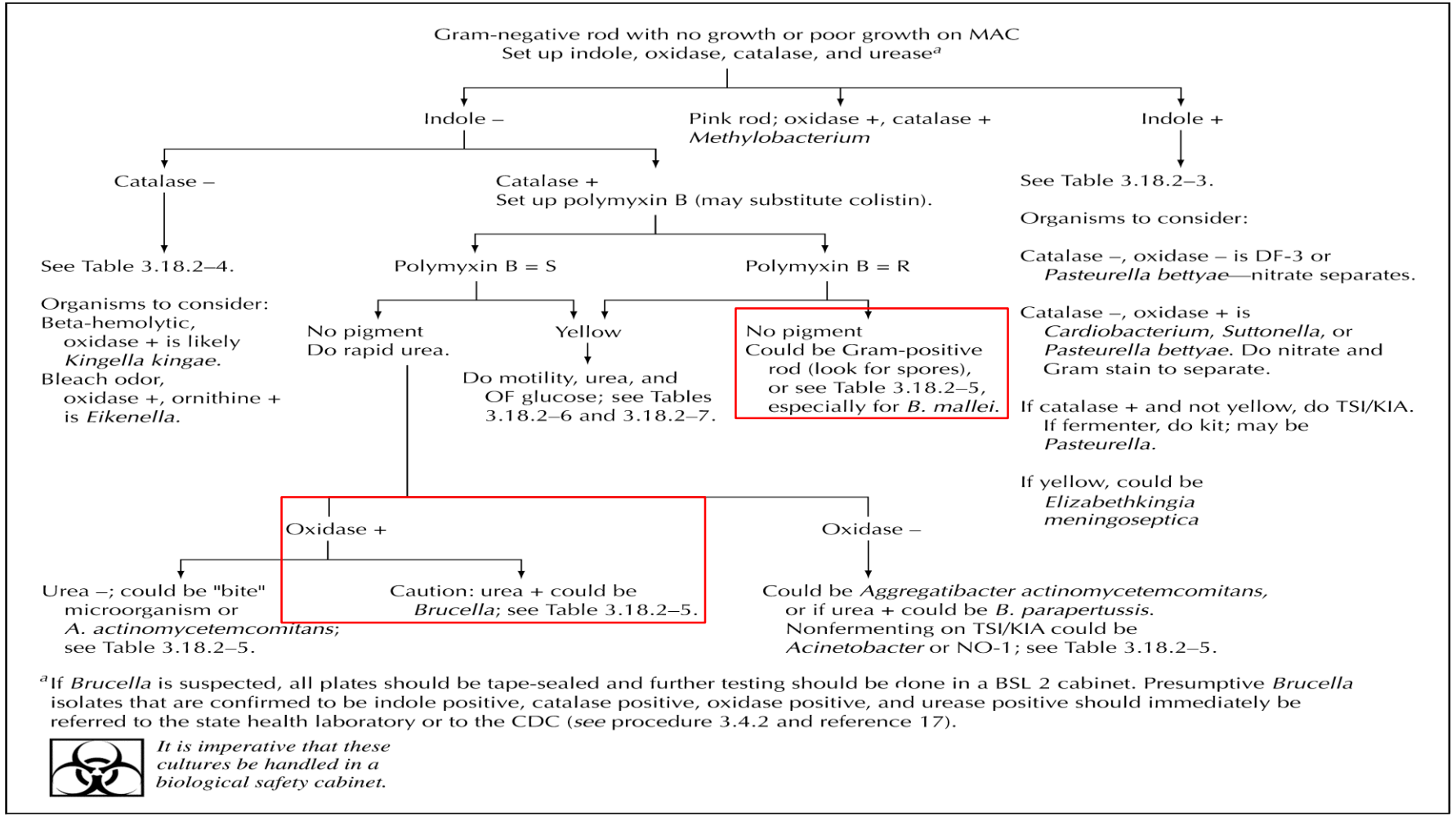


Go With The ASM CMPH Flow – *B. pseudomallei*



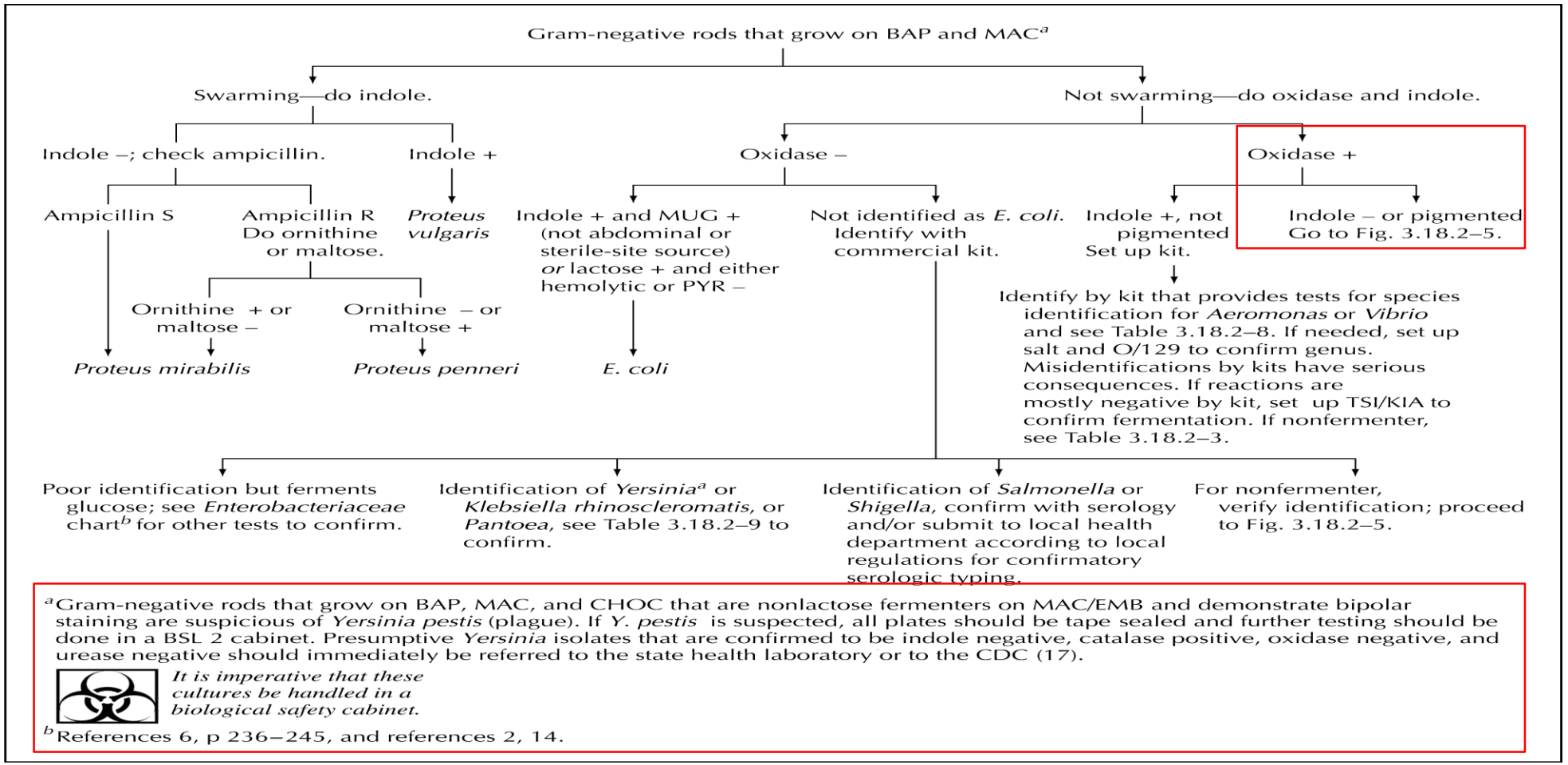
Go With The ASM CMPH Flow – *B. pseudomallei*

Figure 2-3



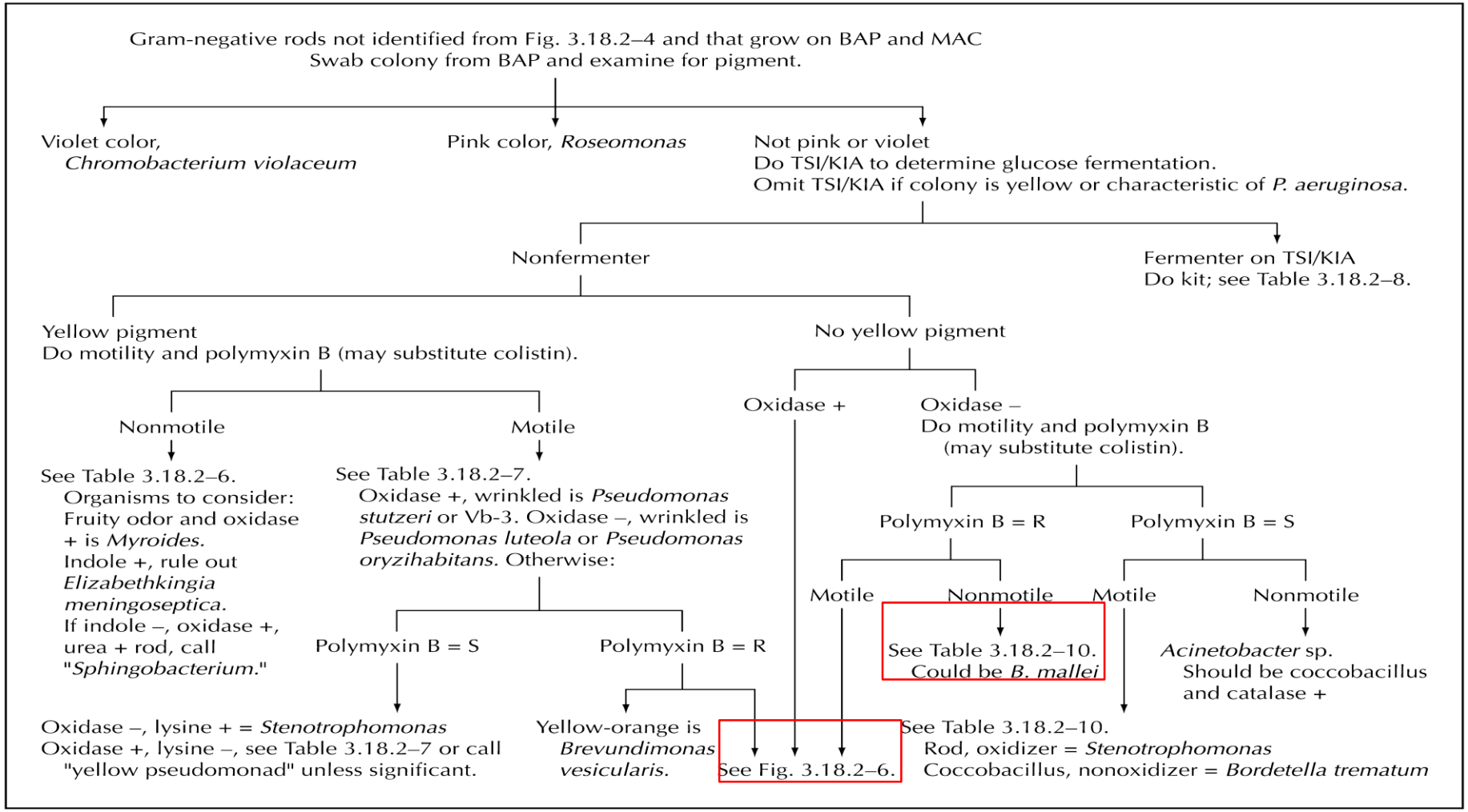
Go With The ASM CMPH Flow – *B. pseudomallei*

Figure 2-4



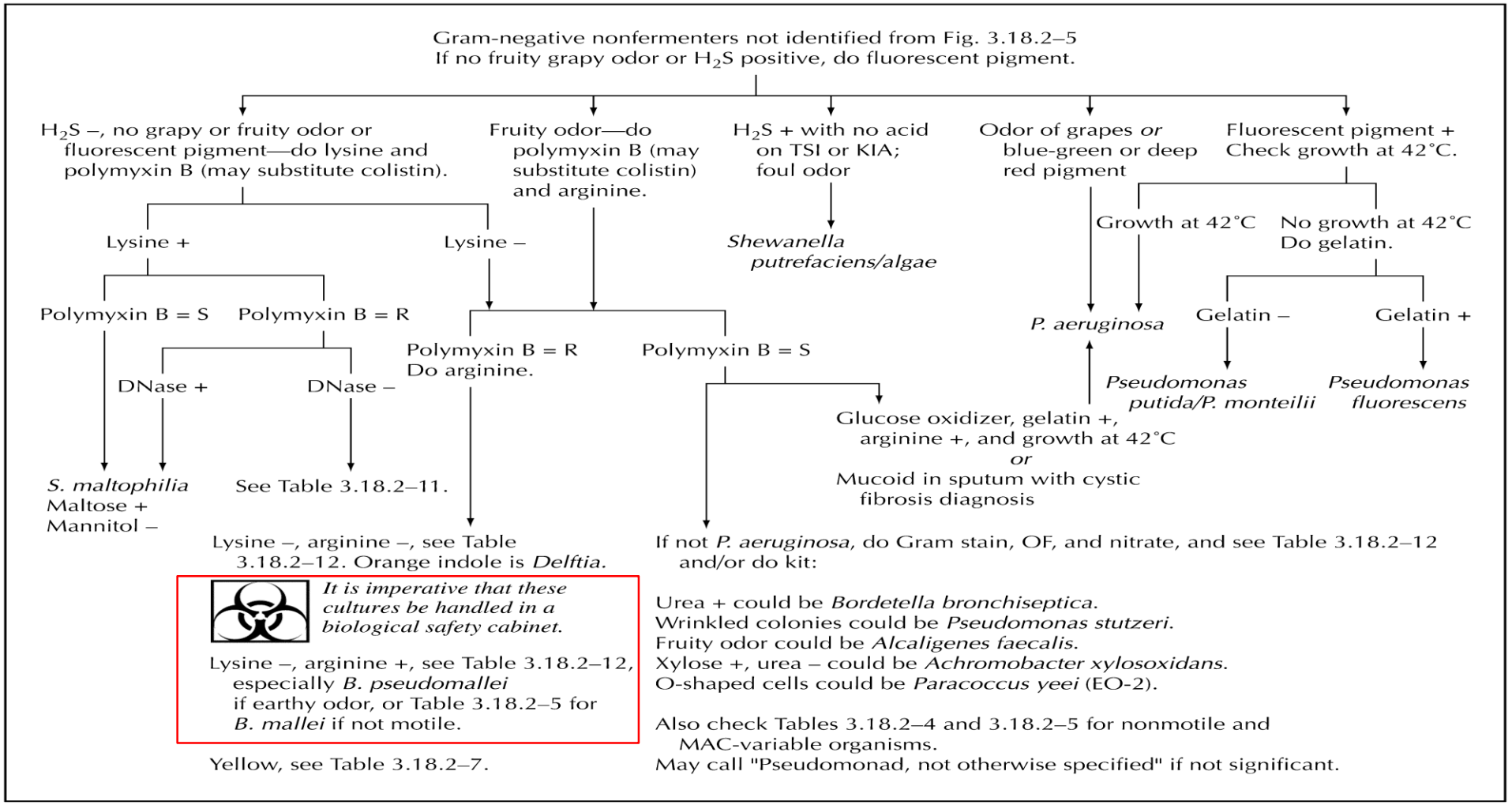
Go With The ASM CMPH Flow – *B. pseudomallei*

Figure 2-5



Go With The ASM CMPH Flow – *B. pseudomallei*

Figure 2-6



Tables

Troubleshooting Organism Mimicry

Table 2-5

Table 3.18.2–5 Biochemical differentiation of non-yellow-pigmented, Gram-negative rods that are catalase positive and indole negative but do not grow well on MAC[a]

Organism(s)[b]	MAC	Oxidase	Urea[c]	Nitrate	Glucose	Arginine	Other differential trait(s)
Aggregatibacter actinomycetemcomitans	—	V	—	+	F, gas V	—	Tiny rod; esculin —; acid slant and butt in TSI; sucrose fermentation —.
Avibacterium gallinarum	V	+	—	+	F	—	Rod, acid slant and butt in TSI; sucrose +
Bordetella parapertussis	—	—	+	—	n-o	NA	Coccobacilli, brown pigment on MH agar, beta-hemolytic
Brucella[c]	V	+	+	+	O	NA	Coccoid tiny cells; work in safety cabinet
Burkholderia mallei	V	V	V	+	O	+	Coccobacilli, hazardous; resistant to polymyxin B; citrate —
Capnocytophaga canimorsus/cynodegmi (DF-2)	—	+	—	—	F[b]	+	Rod; gliding motility, ONPG +; from dog and cat bites
Neisseria zoodegmatis (EF-4b)	V	+	—	+	O	—	Reduces nitrite with no gas; no reaction in TSI; from dog and cat bites
Neisseria animaloris (EF-4a)	V	+	—	+, gas	F	V	May reduce nitrite with no gas; 73% acid in butt of TSI; from dog and cat bites
CDC group EO-5	—	—	W	—	O	—	Coccobacilli; 20% are yellow
CDC group NO-1	V	—	—, W	+	n-o	—	Rod; from dog and cat bites. *Acinetobacter* organisms that fail to grow on MAC are coccoid and nitrate —.
Methylobacterium spp.	V	+	V	V	n-o, O	NA	Vacuolated rod pink in 72 h; grows faster on CHOC; motile
Moraxella spp.	V	+	—	V	n-o	NA	Coccoid, thick cells
Neisseria weaveri	V	+	—	—	n-o	—	Rod; PDA V, nitrite +; Gilardi rod group 1 similar, except nitrite — and PDA +
Oligella ureolytica	V	+	+	+, gas V	n-o	NA	Coccoid, PDA +, motile
Oligella urethralis	V	+	—	—	n-o	NA	Coccoid, PDA +, nitrite +
Paracoccus yeei (EO-2)	V	+	V	+	O	NA	Coccoid but large cells with vacuoles, appear as "o-shaped" cells; mucoid colony
Pasteurella haemolytica	V	+	—	+	F	—	Acid slant and butt in TSI; beta-hemolytic
Psychrobacter immobilis	V	+	V	V	n-o, O	NA	Coccoid; may have rose-like odor
Psychrobacter phenylpyruvicus	V	+	+	V	n-o	NA	Coccoid, PDA +, nitrite —, 6.5% salt +

[a] For indole-positive strains, see Table 3.18.2–3. All strains nonmotile, except as noted, but even with those, motility is difficult to demonstrate. See Table 3.18.2–4 for catalase-variable rods. Data are from references 5, 22, 26, and 28. W, weak reaction; NA, not applicable or available. See footnote *c* to Table 3.18.2–1 and footnote *a* to Table 3.18.2–3 for other abbreviations and symbols.

[b] See reference 8 for guidelines on when DNA target sequencing may be useful for identification.

It is imperative that these cultures be handled in a biological safety cabinet.

[c] If *Brucella* is suspected, all plates should be tape sealed and further testing should be done in a BSL 2 cabinet. Presumptive *Brucella* isolates that are confirmed to be indole positive, catalase positive, oxidase positive, and urease positive should immediately be referred to the state health laboratory or to the CDC (*see* procedure 3.4.2 and reference 17).

Table 2-10

Table 3.18.2–10 Biochemical reactions of non-glucose-fermenting, Gram-negative rods that are catalase positive, oxidase negative or delayed, and grow well on MAC within 48 h[a]

Organism(s)[b]	Motility	Pigment	Polymyxin B	Lysine	Glucose	Urea	Nitrate	Arginine	Mannitol	Maltose	Other differential trait(s)
Acinetobacter spp. Saccharolytic	–	–	S	–	O	–	–	V	–	NA	Coccobacilli; most are *A. baumannii,* the only one that grows at 42°C
Asaccharolytic	–	–	S	–	n-o	–	–	V	–	–	Coccobacilli; not all grow on MAC; nitrate negativity and rod shape separates them from NO-1
Bordetella trematum	+	–	NA	–	n-o	–	V	–	–	–	Coccobacilli
Burkholderia gladioli/ Pandoraea	+	Variable yellow	R	–	O (O)	V	V	–	V	V	Rod; see Table 3.18.2–11 for species identification; DNase –
Burkholderia mallei	–	–	R	–	O	V	+	+	V	V	Coccobacilli, hazardous; MAC and oxidase V, citrate –; no growth at 42°C
Pseudomonas oryzihabitans/luteola	+	Yellow	S	–	O	V	V	V	+	+	Wrinkled colonies; PYR +
Roseomonas spp.	V	Pink	R	NA	n-o	+	V	NA	–	–	Coccoid, mucoid, delayed positive oxidase; does not absorb long-wave UV light
Stenotrophomonas maltophilia	+	Variable yellow	V	+	O	–	V	–	–	+	Rod; DNase +, PYR –

[a] Reactions from references 5, 22, and 28. All strains are catalase positive. For oxidase-negative, yellow-pigmented organisms, see Table 3.18.2–7. See footnote *c* to Table 3.18.2–1 and footnote *a* to Tables 3.18.2–3 and 3.18.2–5 for abbreviations and symbols.

[b] See reference 8 for guidelines on when DNA target sequencing may be useful for identification.

Table 2-12

Table 3.18.2–12 Biochemical reactions of nonyellow Gram-negative rods that are oxidase positive and grow well on MAC within 48 h[a]

Organism(s)[d]	Polymyxin B	Glucose	Nitrate	Arginine	Urea	PYR	Xylose	Mannitol	Sucrose	Maltose	Other differential trait(s)
Achromobacter xylosoxidans subsp. *denitrificans*	S	n-o	+, gas	−	−	+	−	−	−	−	*Achromobacter piechaudii* is similar but nitrite − and no gas from nitrate.
Achromobacter xylosoxidans subsp. *xylosoxidans*	V	O	+, gas V	V	−	+	+	−	−	−	Oxidizes xylose better than glucose
Alcaligenes faecalis[c]	S	n-o	−	−	−	−	−	−	−	−	Fruity odor; nitrite +, acetamide +
Bordetella avium/hinzii	S	n-o	−	V	−+	+	−	−	−	−	*B. avium* is beta-hemolytic, nitrite −
Bordetella bronchiseptica	S	n-o	+	−	+	−	−	−	−	−	Grows on SS agar
Brevundimonas diminuta	R	n-o/O	−	−	V	−	−	−	−	−	Brown on MH agar
Burkholderia cepacia complex, *Ralstonia*, *Pandoraea*	R	O (O)	V	−	V	V	V	V	V	V	See Table 3.18.2–11 for species identification.
Burkholderia pseudomallei[b]	R	O	+, gas	+	V	−	+	+	V	+	White opaque colonies with sheen, then wrinkled, not beta-hemolytic, earthy odor; lactose +
CDC group Ic	NA	O	+	+	V	NA	−	−	−	+	
Comamonas spp.	S	n-o	+	−	−	+−	−	−	−	−	
Delftia acidovorans	R	n-o	+	−	−	+	−	+	−	−	Orange color of colony with Kovács' indole
Ochrobactrum anthropi and unnamed *Achromobacter* groups B, E, and F	V	O	V, gas V	V	+	+	+	V	V	V	PDA +
OFBA-1	S	NA	+, gas	+	−	NA	NA	NA	NA	NA	Beta-hemolytic; turns OF base +
Oligella ureolytica	NA	n-o	+, gas V	NA	+	NA	−	−	−	−	PDA +
Paracoccus yeei (EO-2)	S	O	+	−	V	NA	+	−	−	−	Coccoid but large cells with vacuoles; mucoid colonies
Pseudomonas alcaligenes, *Pseudomonas* CDC group 1, *Pseudomonas pseudoalcaligenes*	S	n-o	V	V	−	−	W	−	−	−	*P. pseudoalcaligenes* is nitrate + but no gas and 42°C +; *P. alcaligenes* is nitrate V and 42°C −; *Pseudomonas* CDC group 1 is nitrate + with gas.
											Pseudomonas fluorescent group
	S	O	V	+	V	V	+	V	V	−	See Fig. 3.18.2–6 for species separation.
Pseudomonas stutzeri	S	O	+, gas	−	V	−	+	V	−	+	Wrinkled colonies
Cupriavidus pauculus (IVc-2)	V	n-o	−	NA	+	+	−	−	−	−	No growth on SS agar
Rhizobium radiobacter	V	O	V	−	+	+	+	+	+	+	PDA +
Shewanella putrefaciens/algae	S	O	+	−	V	+	−	−	+	+	H_2S +, ornithine +, brown, foul smelling

[a] All strains are motile and indole negative. Also see Table 3.18.2–5 for nonmotile, Gram-negative rods that are MAC variable. Verify that strains are nonfermenting rods using TSI or KIA. For fermenting rods, see Table 3.18.2–8. Data are from references 5, 22, 28, 35, and 36. SS, salmonella-shigella. See footnote *c* to Table 3.18.2–1 and footnote *a* to Tables 3.18.2–3, 3.18.2–5, and 3.18.2–7 for other abbreviations and symbols for reaction key.

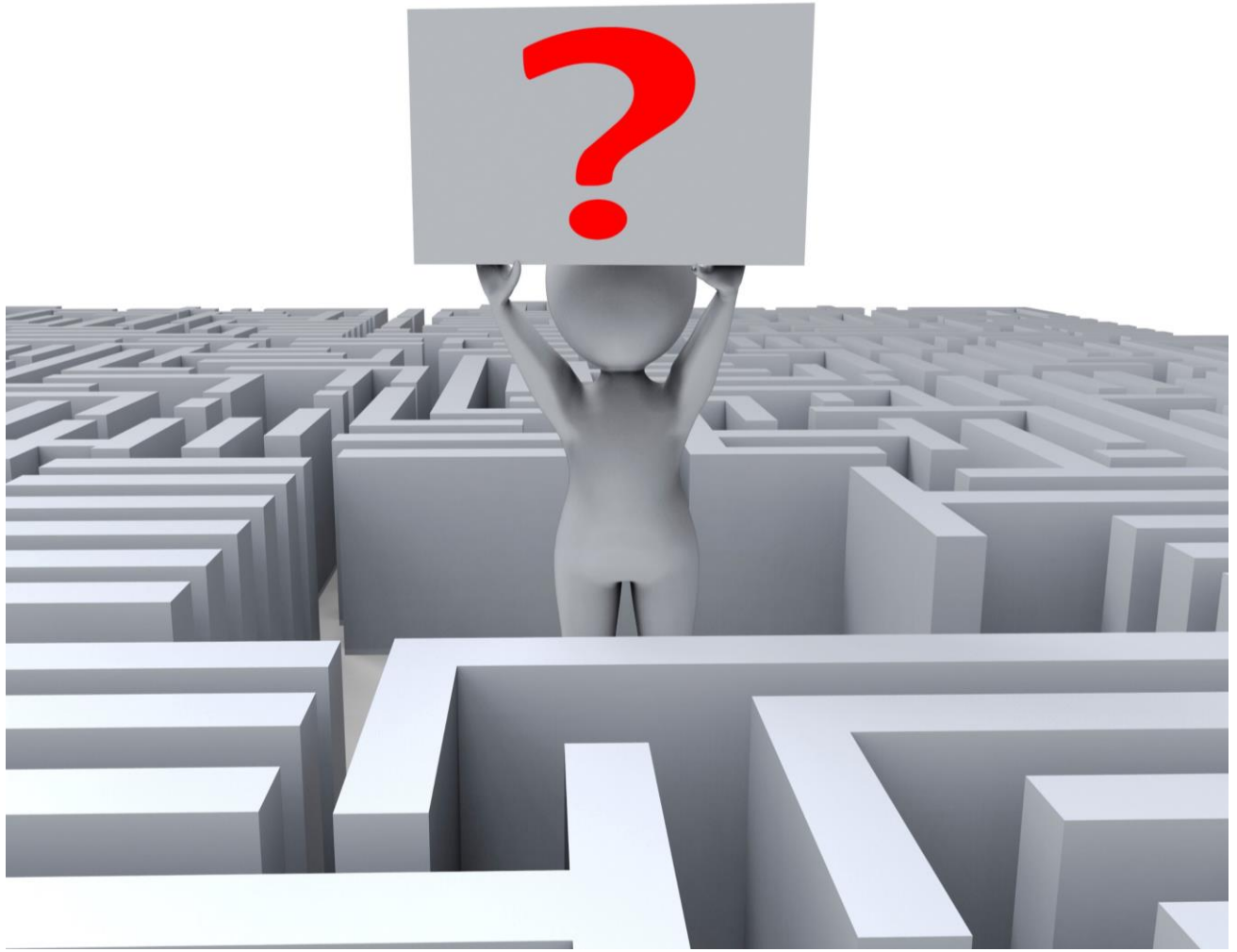
[b] *B. mallei* can have similar reactions but is nonmotile, has no odor, and does not produce gas from nitrate.

[c] To separate *Alcaligenes faecalis* from other related nonoxidizers: *Ralstonia gilardii* is nitrite negative; nonyellow *Myroides* is urea and PYR positive but nonmotile and polymyxin B resistant; and Gilardi rod group 1 is nonmotile and PDA positive.

[d] See reference 8 for guidelines on when DNA target sequencing may be useful for identification.

Now…

Are you ready for the real thing?



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