Identification of Anaerobes

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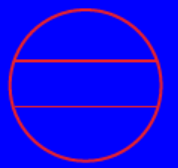


Damage to Anaerobes by Oxygen

- 短時間的暴露在有氧的情況
  - Oxidation of lipids in membranes
  - Inactivation of enzymes
  - Direct genetic damage

檢體的收集、傳送、保存、鑑定

- 檢體採集 ：
  - Needole & Syringe
  - Tissue & Biopsy
  - Swab
- 檢體傳送 ：
  - 無菌容器 (螺旋蓋)
  - Anaerobic Transport Medium
  - 儘速送檢
- 檢體保存
  - 室溫保存
- 培養基鑑定及判讀
  - 厭氧操作箱

不適合操作厭氧培養檢體

- Bronchial washing or brush
- Sputum
- Stool
- 腸道造口術引流液
- Nasopharyngeal / Throat swab
- Urethral swab
- Vaginal or cervical swab
- 皮膚表淺部位檢體
- 導尿或中段尿

Primary Media

- Primary specimens should be plated on non-selective, selective and differential media
- All media should be supplemented with vitamin K and hemin
- Media should be very fresh; old media slow growth, require prolonged incubation and more tech time for multiple examinations

培養基

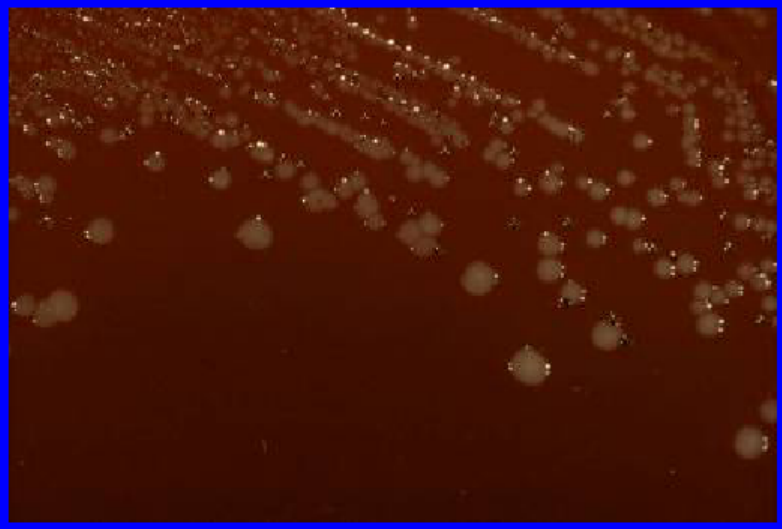
- Non-Selective medium
  - » Brucella CDC agar
- Selective & differential media
  - » Phenylethyl alcohol agar(PEA)
  - » Laked blood with kanamycin and vancomycin brucella agar (LKV)
  - » **Bacteroides bile-esculin-gentamicin agar (BBE)**
  - » **Cycloserine-Cefoxtin Fructose agar (CCFA)**
  - » **Egg yolk agar(EYA)**

Primary Setup for Anaerobes

Medium	Grows	Inhibits
Brucella CDC Agar	All	None
PEA	All Anaerobes	Enterics
Supplemented Thioglycolate or Chopped Meat Broth	All	None

Selective and differential media(1)

- Laked blood with kanamycin and vancomycin brucella agar (LKV)
  - Inhibits gram-positives and enterics
  - Grows Bacteroides, Prevotella, some fusobacteria
  - Enhances pigmentation of Prev. melaninogenica group



Selective and Differential Media(2)

- **Bacteroides bile-esculin-gentamicin agar (BBE)**
  - **20%Bile 下細菌生長是否受抑制**
  - **Esculin 水解**
  - **Rapid presumptive ID of B. fragilis group, and Bilophila.**
  - **Some strains of Fusobacterium mortiferum and F. varium may grow. (bile resistant)**
  - **Enterococci highly resistant to gentamicin will grow**

Selective and Differential Media(3)

- **Cycloserine-Cefoxtin Fructose agar (CCFA)**
  - 選擇性區分Clostridium difficile
  - *Clostridium difficile*：resistant to Cefoxtin
  - 黃色毛玻璃狀
  - 有臭味

Selective and Differential Media(4)

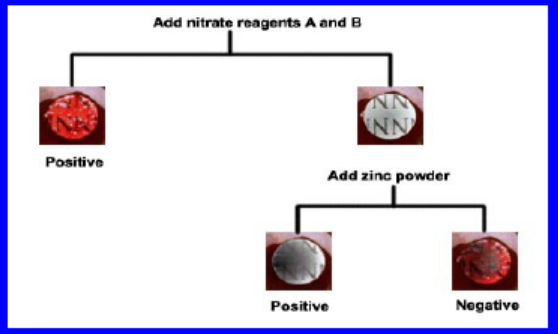
- **Egg yolk agar(EYA)**
  - 輔助性鑑別Clostridium SPP.
  - 是否含有Lecithinase及Lipase
    - » Lecithinase：培養基有一圈大的不透明環
    - » Lipase：虹彩多色光澤
    - » Proteolysis：細菌周圍會有Clear zone

Incidence of Anaerobes in Clinical infections

◆ B. fragilis group	35%	
– B. fragilis		(40-50%)
– B. thetaiotaomicron		(20%)
– Other Bf group		(20-40%)
◆ Prevotella-Porphyromonas etc.	17%	
◆ Fusobacterium	7%	
◆ Anaerobic cocci	28%	
◆ Clostridium	8%	
◆ Non-sporeforming GPB	5%	

Importance of basic tests : identify >75% clinical anaerobes

- Growth in 20% bile
- Catalase
- Indole
- Nitrate
- Susceptibility to kana、 vanco、Col
- Cell morphology
- Fluorescence



生化試驗

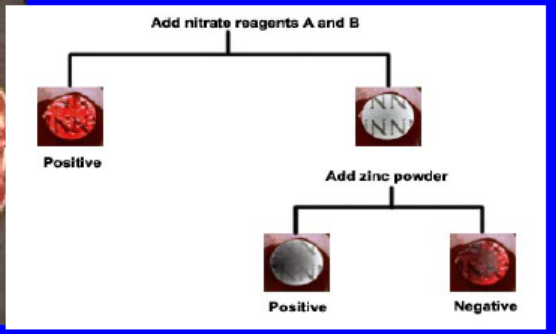
- 15%Catalase
  - 陽性:強氣泡產生
  - 陰性:無氣泡或20-30s少量氣泡

- **Spot Indole test:**
  - 陽性: 藍色
  - 陰性:不變色或粉紅色

- **Nitrate test:**
  - 陽性: 粉紅色
  - 陰性:無色

添加鋅粉→粉紅(陰性)

→**無色(陽性)**



Identification disks

- Kana， Van、 Col
- 抑制環>=10mm(S)
- 抑制環<10mm(R)
- 依抗生素鑑定紙錠的感受性型式將厭氧菌分群

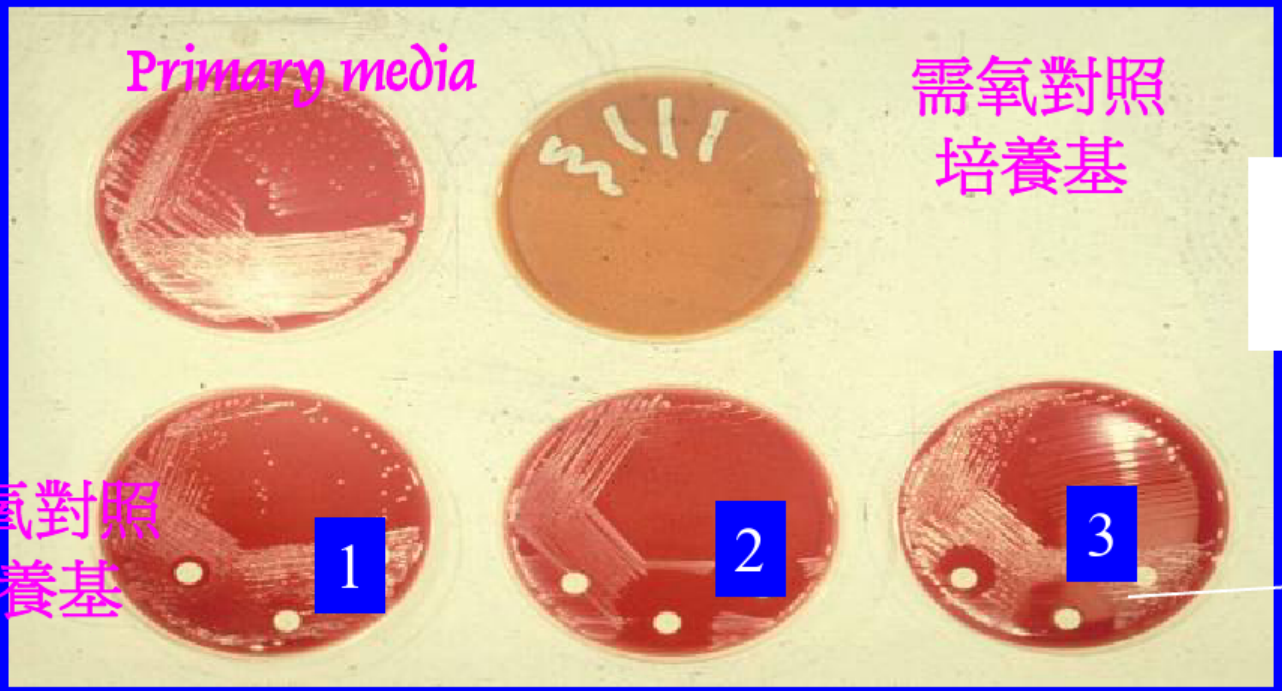
厭氧菌分群

Table 4.6.5–1 Identification by means of special-potency antimicrobial agent disks[a]

Organisms	Response[b] to:		
	Kanamycin, 1,000 µg	Vancomycin, 5 µg	Colistin, 10 µg
Gram positive	V	S[c]	R
Gram negative	V	R	V
Bacteroides fragilis group	R	R	R
Bacteroides ureolyticus group	S	R	S
Fusobacterium spp.	S	R	S
Porphyromonas spp.	R	S[d]	R
Prevotella spp.	R	R	V
Veillonella spp.	S	R	S

Aerotolerance test

厭氧環境	需氧環境	判讀
有長	有長	需氧菌
有長	無長	厭氧菌



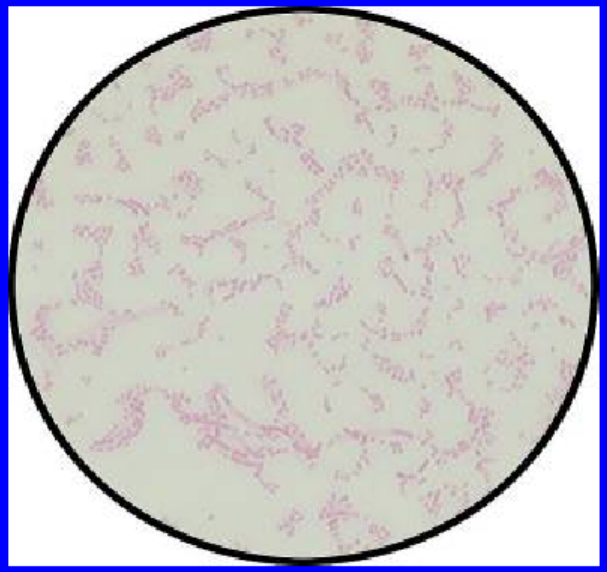
Gram stain抹片

1 2 3

貼Va/k/col

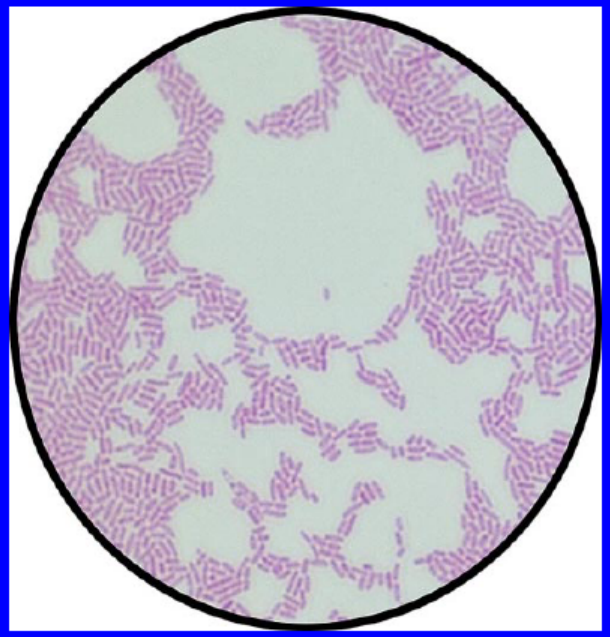
Gram stain-Cell morphology(GNB)

Fusobacterium

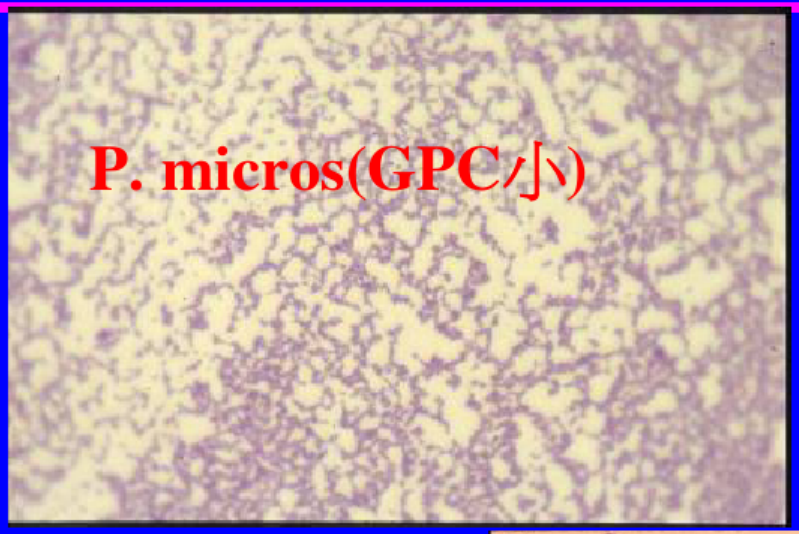


Prevotella/Porphyromonase

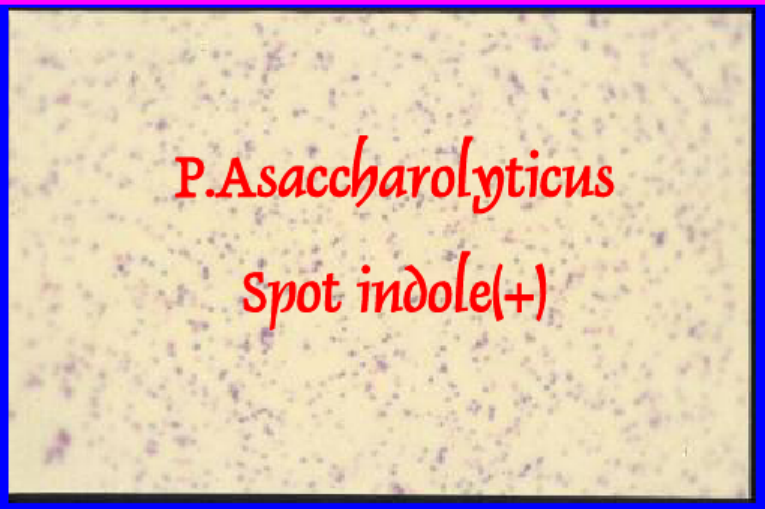
Bacteroides/Bilophila



Gram stain-Cell morphology(GPC)



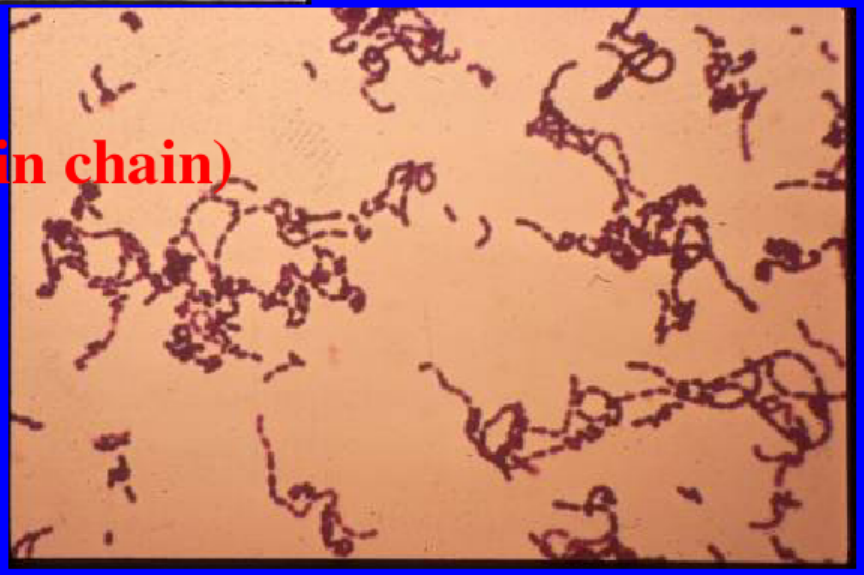
P. micros(GPC小)



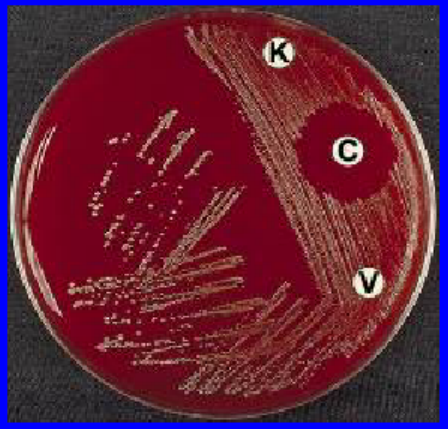
P.Asaccharolyticus

Spot indole(+)

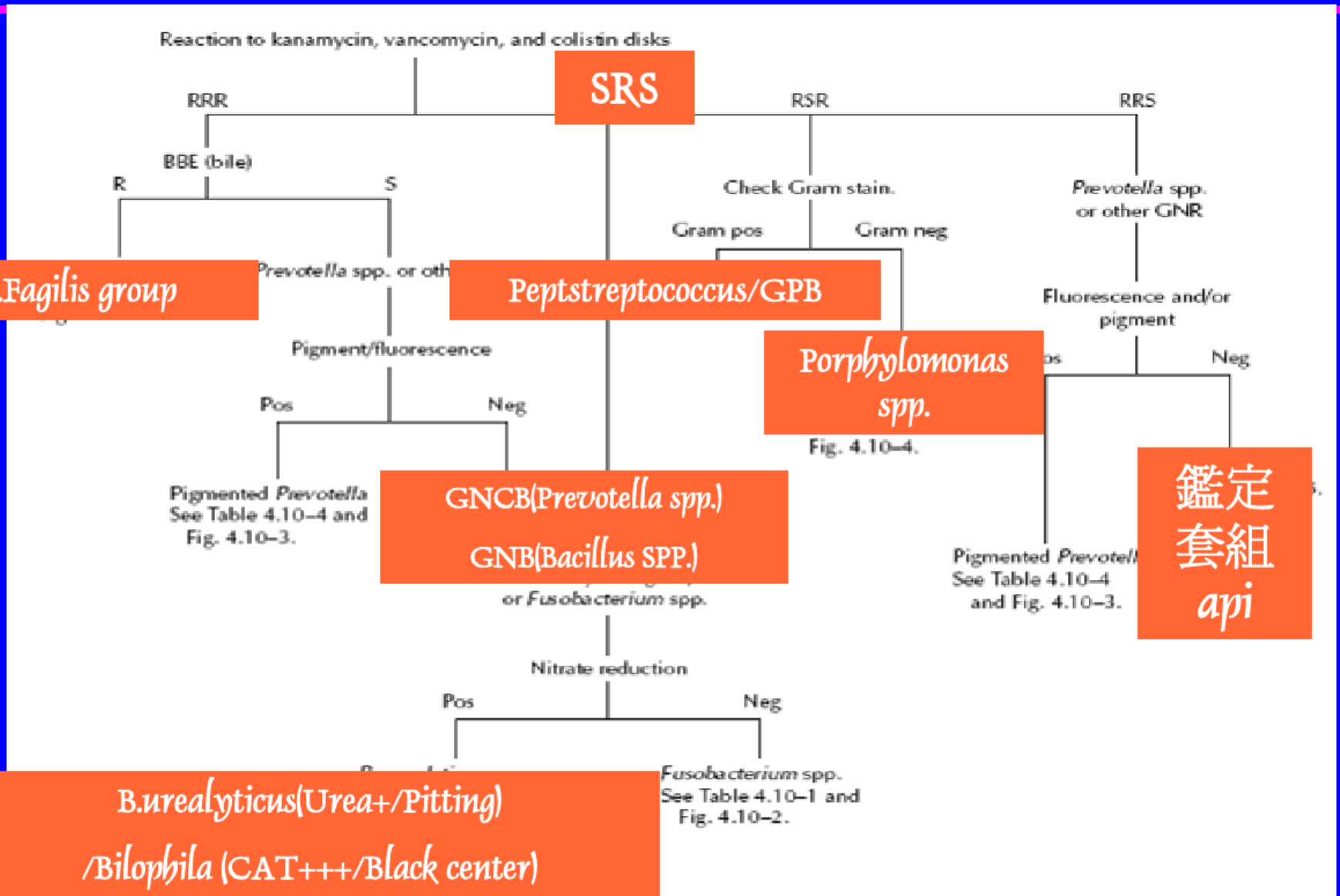
P. anaerobius(in chain)



V/K/CL 鑑定紙錠結果對照

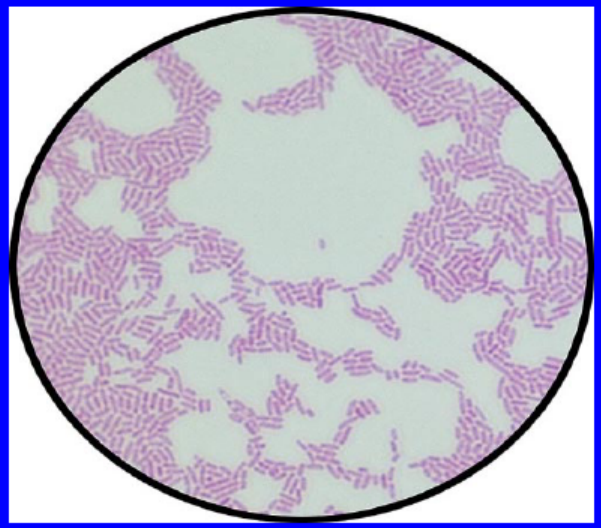


厭氧菌鑑定流程

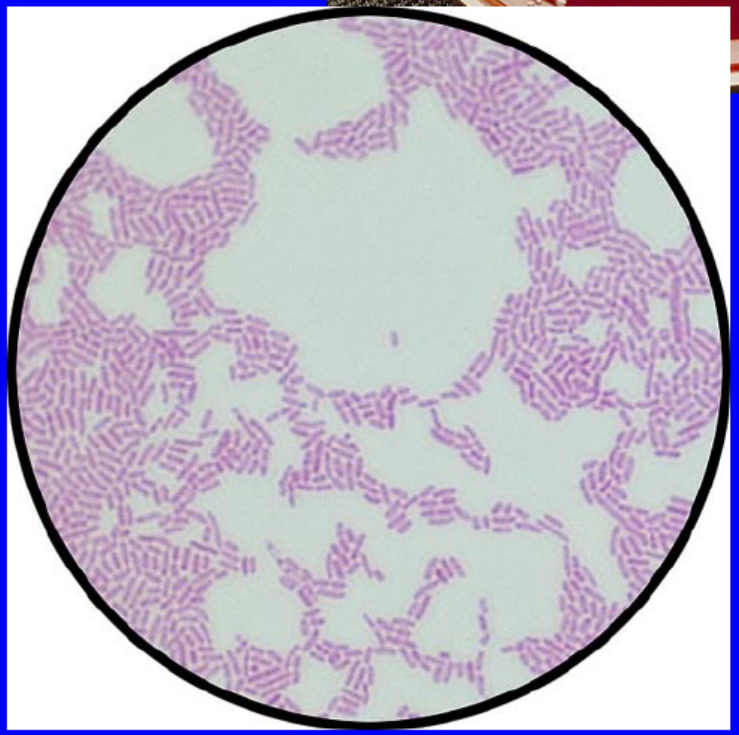

Reaction to kanamycin, vancomycin, and colistin disks
SRS
RRR
RSR
RRS
BBE (bile)
R
S
Check Gram stain.
Gram pos
Gram neg
Prevotella spp. or other GNR
B.Fagilis group
Peptstreptococcus/GPB
Porphylomonas spp.
Fig. 4.10–4.
Fluorescence and/or pigment
Pos
Neg
Pigment/fluorescence
Pos
Neg
Pigmented Prevotella See Table 4.10–4 and Fig. 4.10–3.
GNCB(Prevotella spp.)
GNB(Bacillus SPP.)
or Fusobacterium spp.
Pigmented Prevotell See Table 4.10–4 and Fig. 4.10–3.
鑑定套組 api
Nitrate reduction
Pos
Neg
Fusobacterium spp. See Table 4.10–1 and Fig. 4.10–2.
B.urealyticus(Urea+/Pitting)
/Bilophila (CAT+++/Black center)

Bacteroides fragilis group

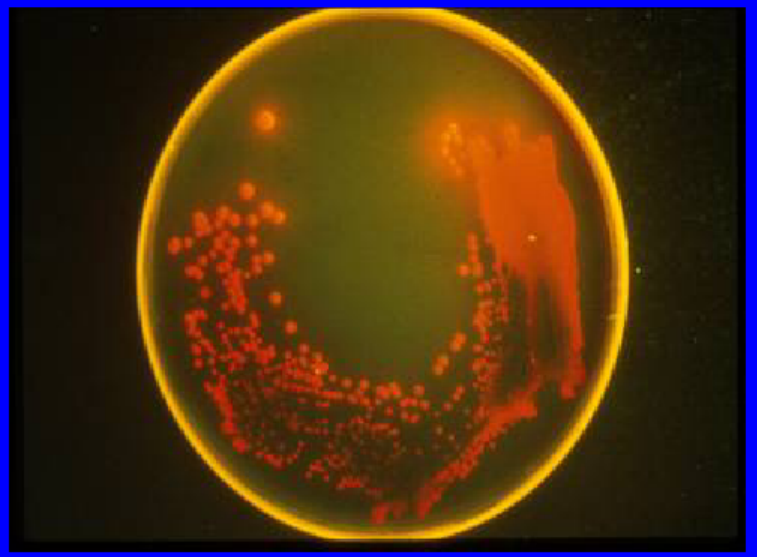
- BBE(生長)bile resistant
- kana®, vanco®, colistin® disks
- Gram stain: 淺色、GNB(多型性)
- Black colony on BBE >1mm



B. fragilis group



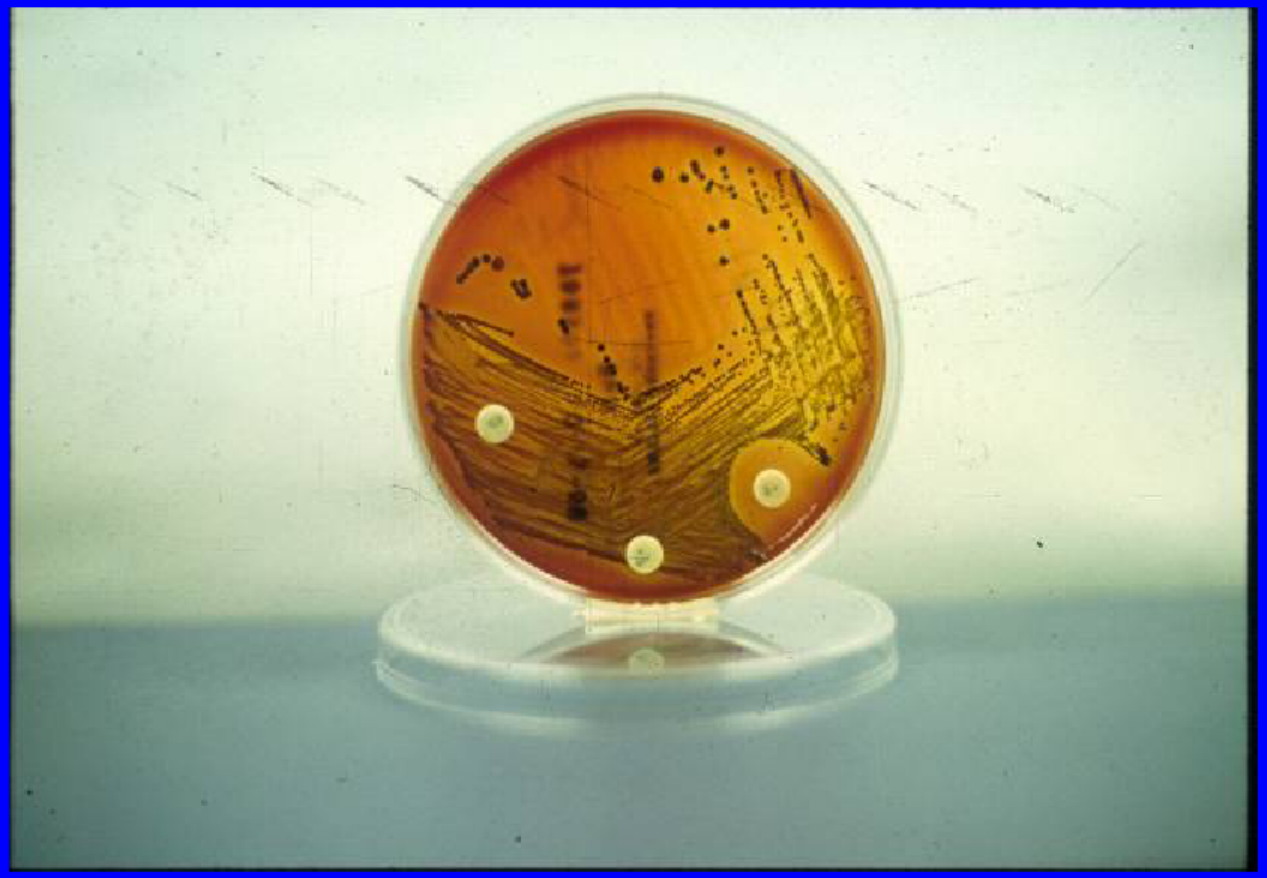
Prevotella species



Prevotella species

- Isolated from oral and pelvic infections, occ abdominal and soft tissue
- Growth inhibited on BBE (but may turn agar black from hydrolysis of esculin if colonies plated)
- kana®, vanco®, colistin(S/R)
- Catalase usually negative

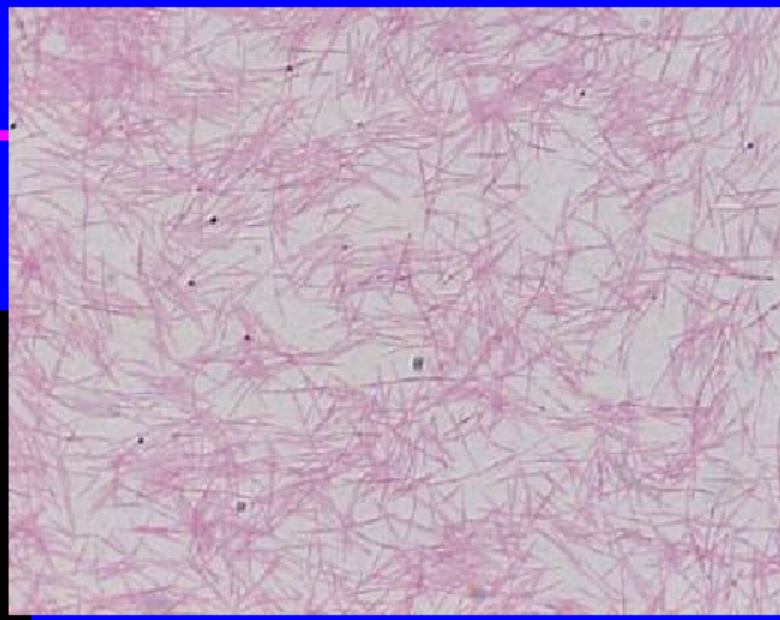
Porphyromonas (vanco sens)

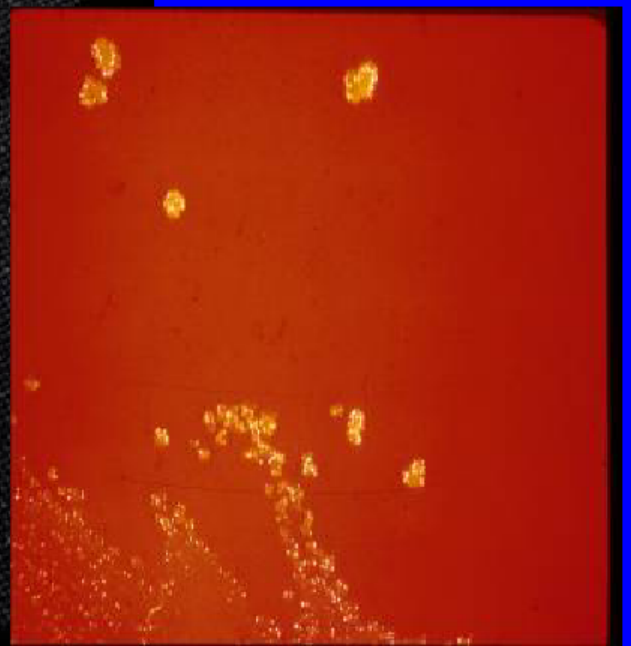


Fusobacterium spp

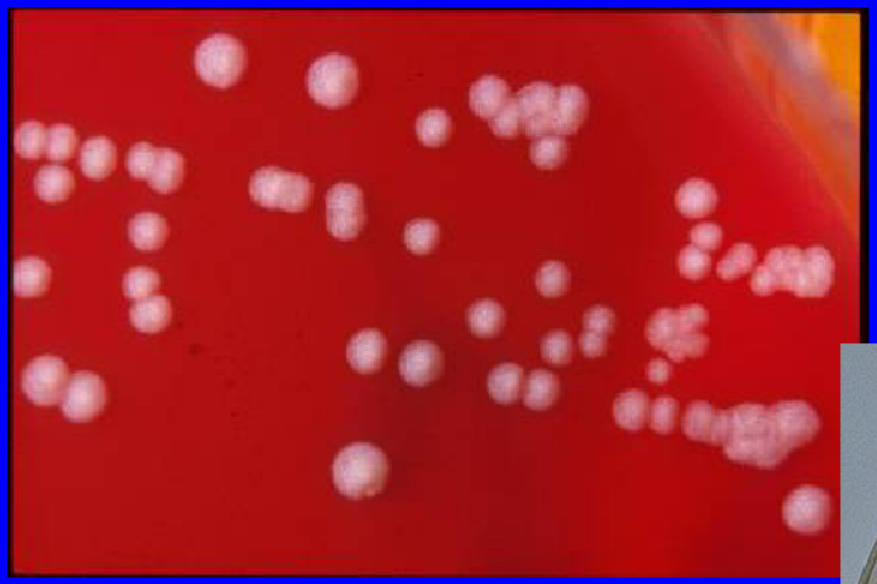
species	bile	NO_3	ind	lipase	esc	cells
nucleatum	S	-	+	-	-	s,pt
necroph.	S^R	-	+	+	-	r,pleo
naviforme	S	-	+	-	-	boat
gonidiaform.	S	-	+	-	-	r,gon
russii	S	-	-	-	-	r
canifelinum	S	-	+	-	-	pt
mortiferum	R	-	-	-	+	pleo
varium	R	-	$+^-$	$-^+$	-	r,pleo

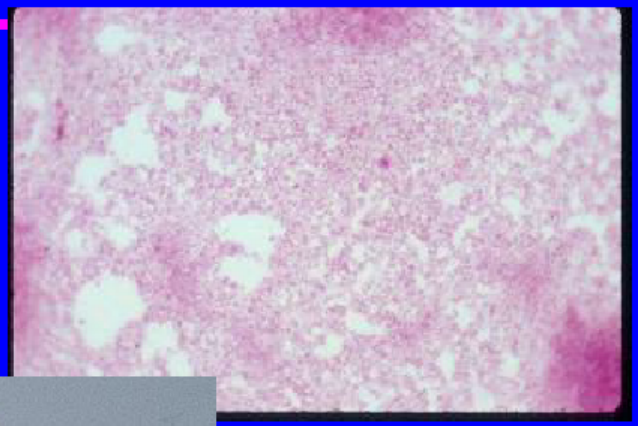
F. nucleatum





Fusobacterium necrophorum





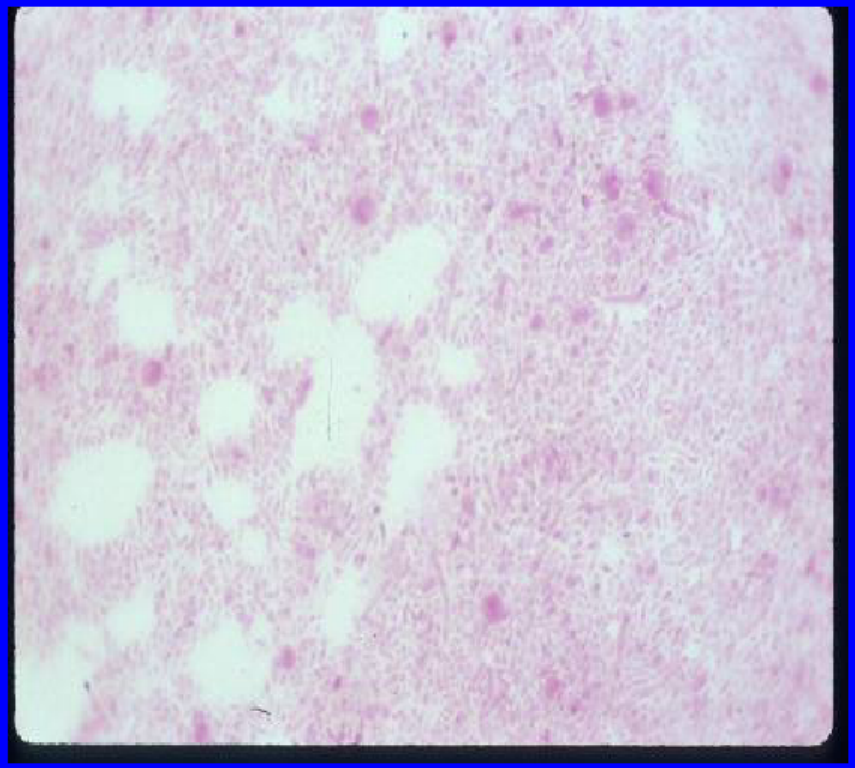
Fusobacterium necrophorum

- kana(S) and colistin(S) 、vanco®
- indole and lipase positive; beta-hemolytic
- cells have rounded ends or may be very pleomorphic

F. mortiferum / varium

- kana(s), colistin(s)
- BBE(生長)bile-resistant
- F. mort is indole neg; esculin:pos
- F. varium is indole pos^{neg}; esc:neg
- Gram stain:多型性、

Fusobacterium mortiferum / varium

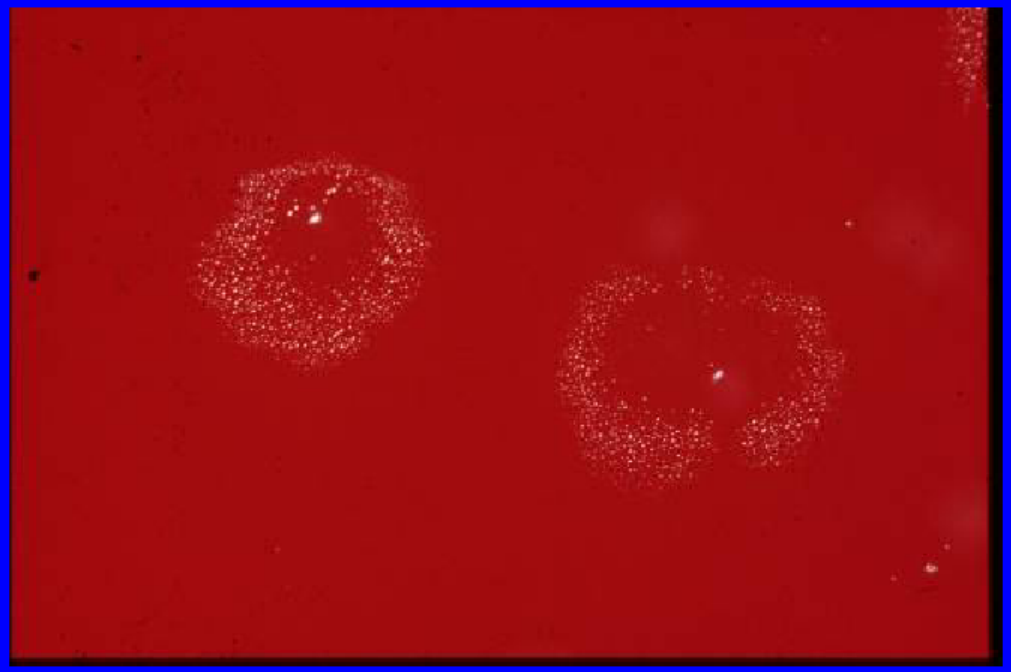


Bacteroides ureolyticus-like group

- kana(s), colistin(s), vanco®
- Small transparent/translucent colonies
- Pitting of agar is variable
- Most reduce NO3

Bacteroides ureolyticus

Transparent colonies, pitting of agar



Bilophila wadsworthia

- Original isolates from appendicitis
- Later from many other sources (chronic sinusitis, cholesteatoma, pleural fluid, liver abscess, blood)
- Grows on BBE, black centers in colonies (H_2S +)
- Catalase very positive !!!!!
- B-lactamase pos, resistant to many b-lactam agents

Bilophila wadsworthia

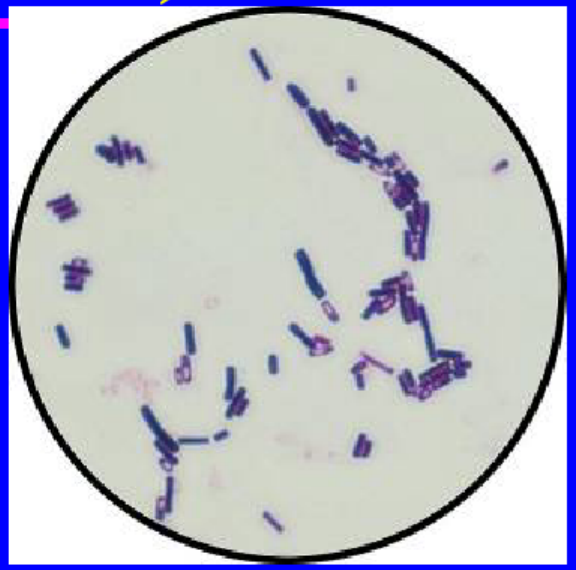
Gram-negative cocci

Genus	NO_3	catalase	glucose
Veillonella	+	V	-
Acidaminococcus	-	-	-
Megasphaera	-	-	+

Gram-positive cocci

	SPS	Cat	Ind	Ure	a-glu
P. anaerobius(鏈)	S	-	-	-	+
P. asaccharolyticus	R	-$^{+}$	+	-	-
M. micros(GPC小)	R/S	-	-	-	-
F. magna(GPC大)	R	-$^{+}$	-	-	-
A. prevotii	R	-	-	+/-	+
A. tetradius	R	-	-	+	+

Most frequent clinical isolates of Clostridium spp. (IUMC)

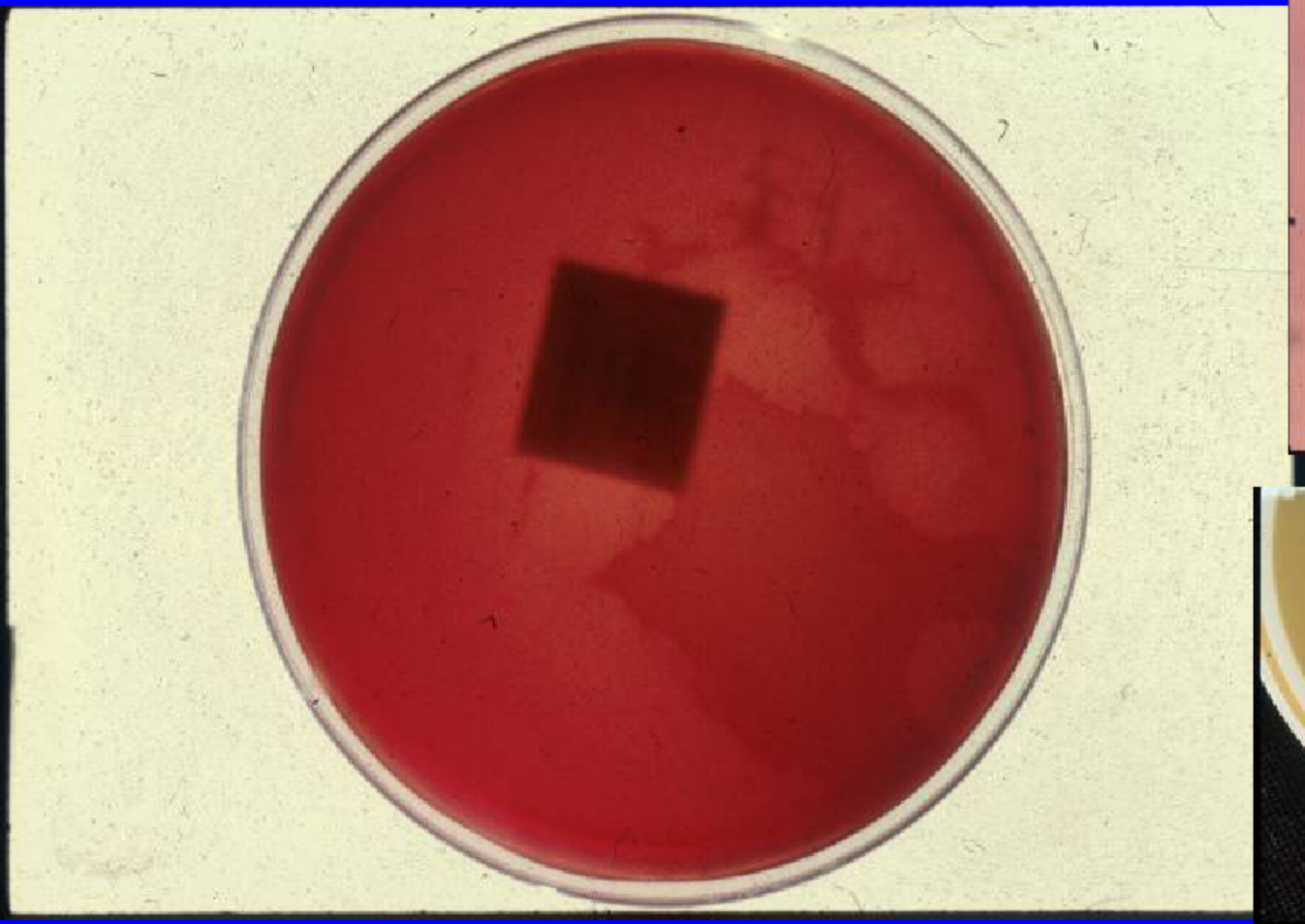


- C. perfringens 20%
- C. clostridioforme grp 16%
- C. innocuum 15%
- C. ramosum 14%
- C. difficile 11%
- <4% C. butyricum, C. cadaveris, <1% C. sporogenes, C. bifermentans, C. glycolyticum, C. tertium, C. septicum, C. sordellii, C. paraputrificum, C. symbiosum, C. subterminale, C. barati

Characteristics of C. perfringens

- Box car shaped GPB
- Double zone of beta-hemolysis on BA
- Lecithinase positive (egg yolk agar)
  (alpha toxin)
- Reverse camp test positive
- Produces abundant gas in liquid media

C. perfringens

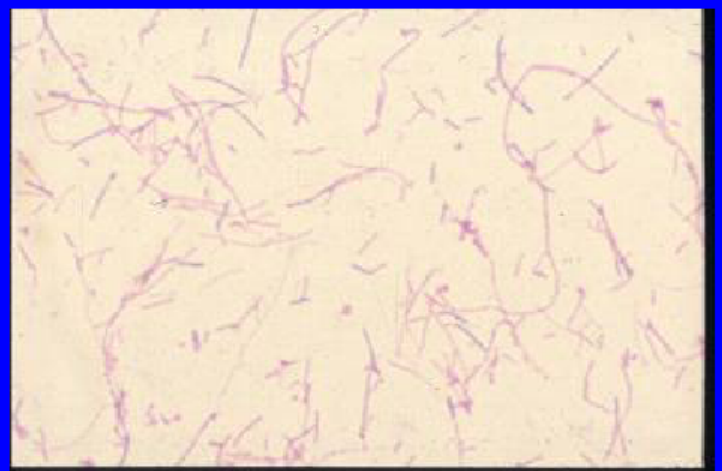


Clostridium innocuum

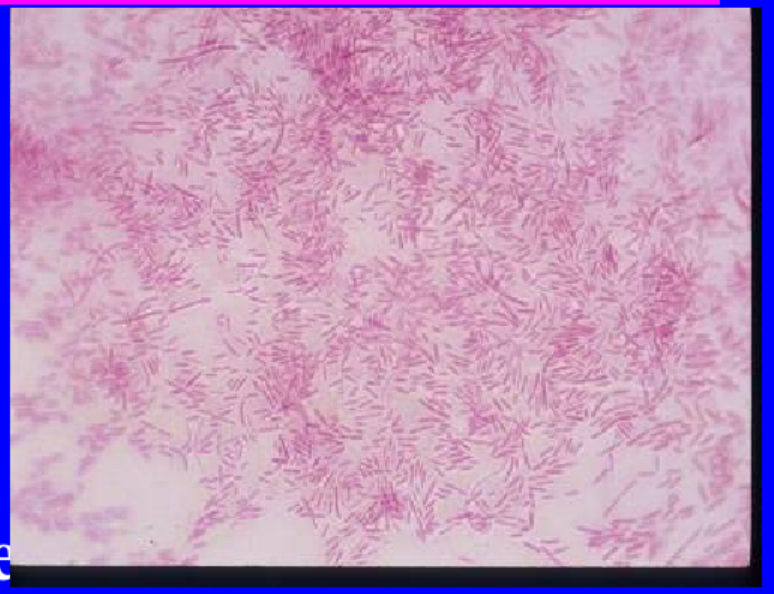
- Resistant to cefoxitin, other cephs, vancomycin (MIC= 8-16), some strains to clindamycin, quinolones
- Grows on CCFA medium – resembles C. difficile
- Disks- resistant to Ka, Va, Cl
- Misidentified in preformed enzyme kits (often as C. subterminale)
- Gram variable rod, occ large terminal spores
- Isolated from IA infections, blood

Clostridium ramosum

- Gram-variable long slender rod
- Chains in broth media
- Spores are rarely visible – terminal
- May be resistant to antimicrobials
- Identify using enzyme kits, biochemicals



Clostridium clostridioforme group

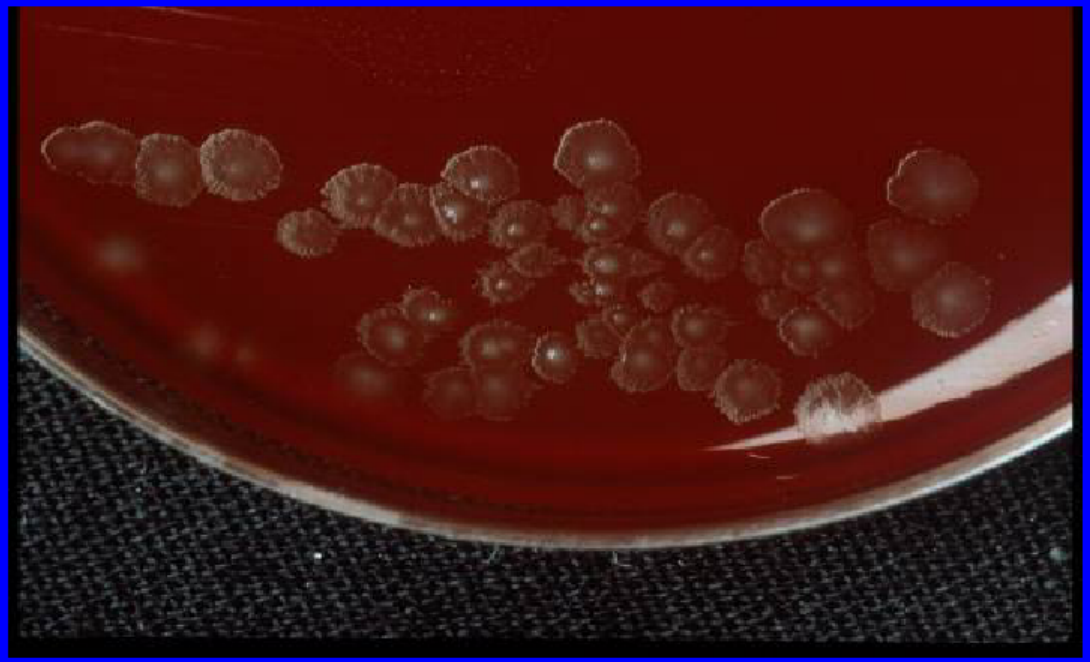


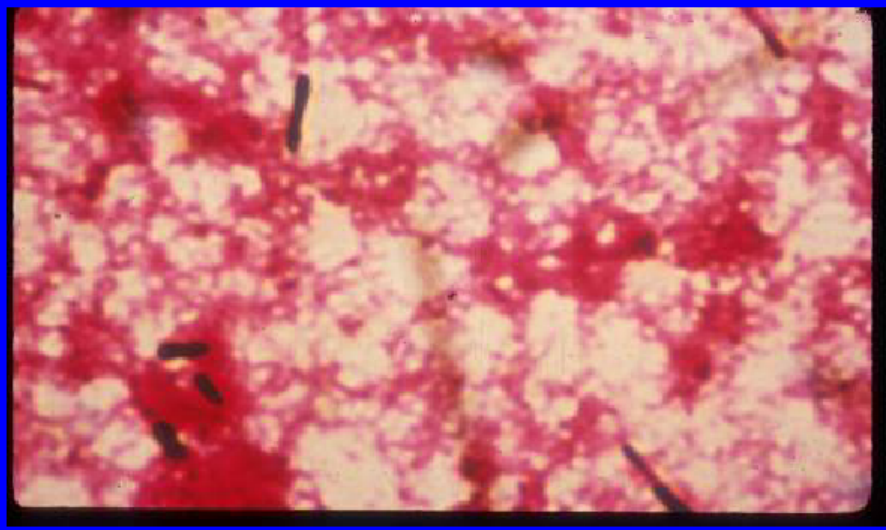
- Typically stains Gram-negative
- Spores are rarely seen
- vanco(s) and kana(s)
- One of most frequently encounte
- ~10% produce beta-lactamase; most generally more resistant to antimicrobials
- Identify with enzyme kits or biochems
- 染成GN

Clostridium septicum

- Swarmer; subterminal spores
- Bacteremia associated with malignancies (leukemia, lymphoma, cancer of large bowel, diabetes, gas gangrene)
- Portal of entry – ileocecum
- High mortality
- Susceptible to usual antimicrobials

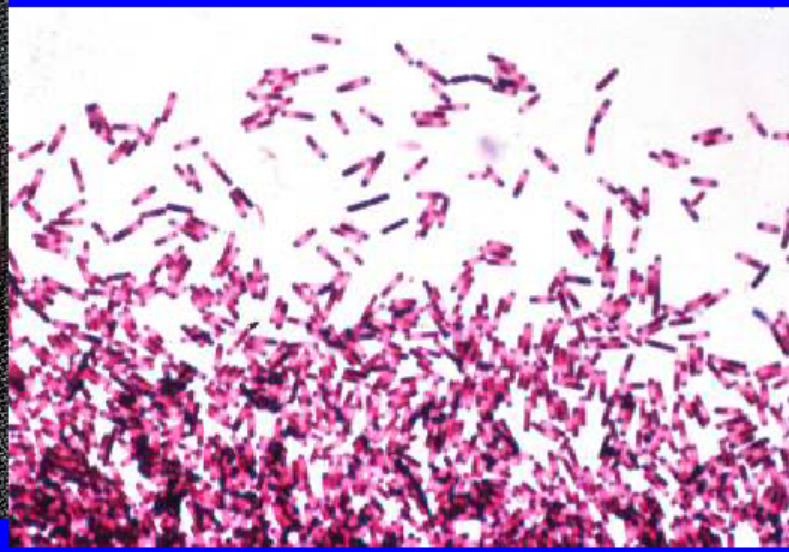
Clostridium septicum (swarmer)





Clostridium difficile

- PMC and antibiotic associated colitis
- Also isolated from clinical specimens
- Resistant to cefoxitin –grows on CCFA



Clostridium tertium

- Aerotolerant
- Aerobic growth shows no spores – resembles lactobacillus colonies and cell morphology
- Anaerobic growth – irregular edged colonies, terminal spores
- Associated with hem. malignancy
- Occasionally other infections
- Catalase(-)

Gram-positive bacilli – nonspore-forming

	catalase	nitrate	indole
Propionibacteria	+	+	+-
Actinomyces	-+	+	-
Eggerthella lenta	+	+	-
"Eubact. grp."	-	-+	-
Lactobacilli	-	-	-
Bifidobacteria	-	-	-

Propionibacterium species

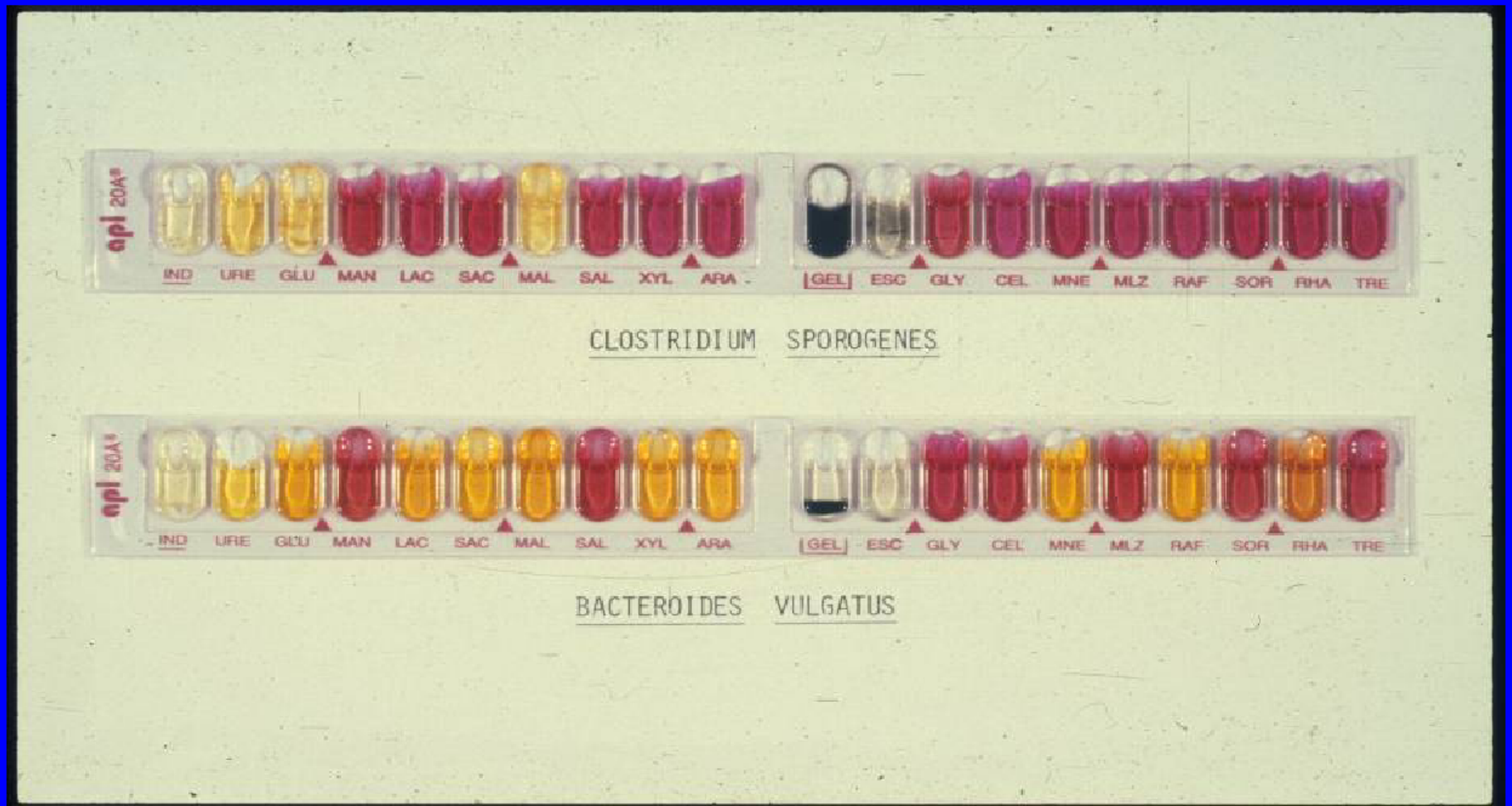
- Normal skin flora
- Acne vulgaris
- Infections: surgical procedures, foreign bodies
- Endophthalmitis
- Infections of bone, joints, CNS
- P. propionicum – lacrimal canaliculitis

Propionibacterium acnes

- Frequent skin contaminant in blood, csf cultures
- Occasionally pathogenic (shunts, implants, post-op cultures from eye)

- Metronidazole®

API 20A


api 20A
IND URE GLU MAN LAC SAC MAL SAL XYL ARA
GEL ESC GLY CEL MNE MLZ RAF SOR RHA TRE
CLOSTRIDIUM SPOROGENES
api 20A
IND URE GLU MAN LAC SAC MAL SAL XYL ARA
GEL ESC GLY CEL MNE MLZ RAF SOR RHA TRE
BACTEROIDES VULGATUS