

臨床微生物學

痰液培養

細菌組 蘇柏安

UCL

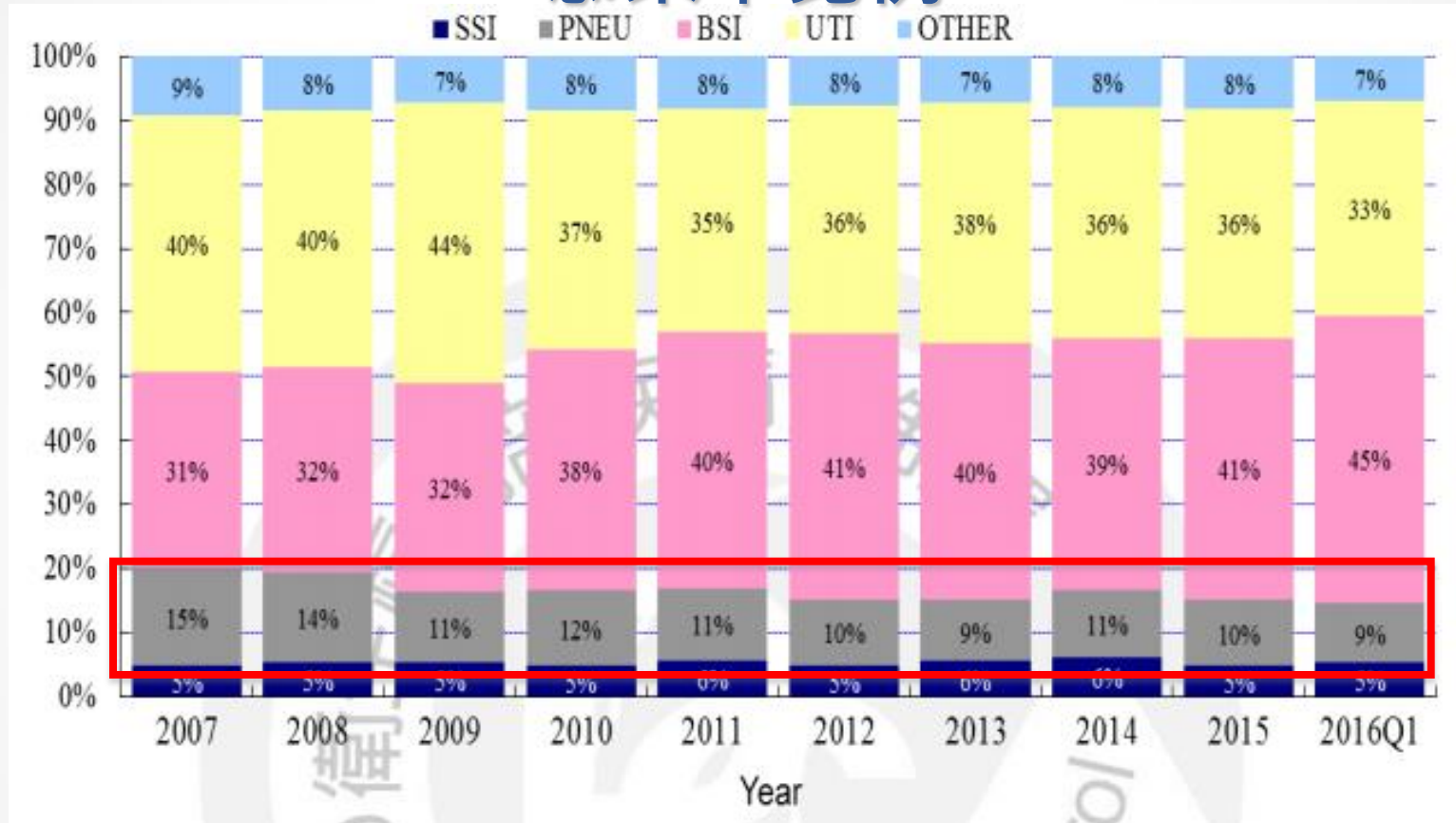


痰液培養目的

- 為了找出感染下呼吸道的細菌或黴菌, 症狀包含有肺炎及支氣管炎.
- ✓ 院內感染型肺炎
- ✓ 社區感染型肺炎

2007 – 2016第一季 醫學中心加護病房院內

感染率比例



院內感染率: 8-10%

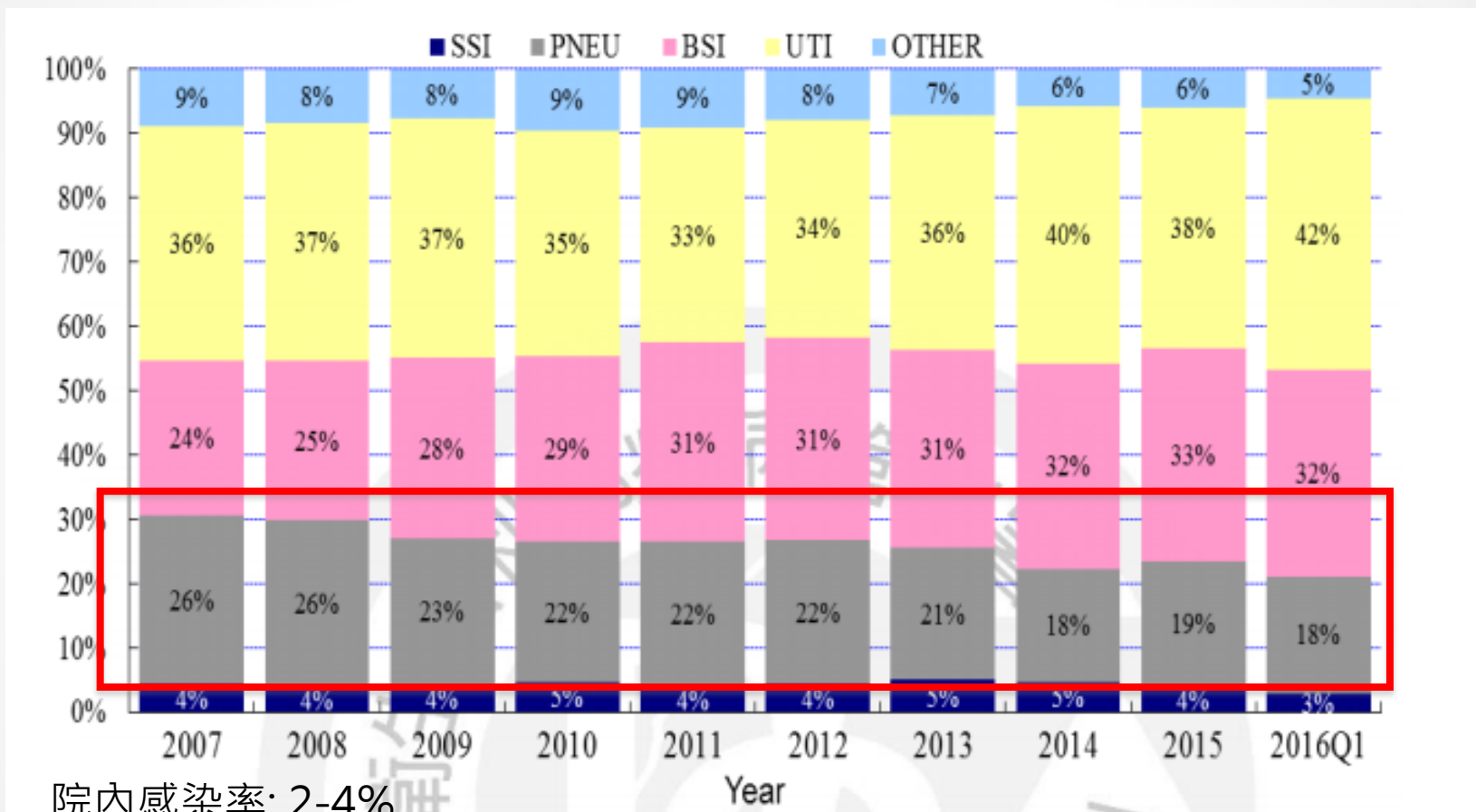
SSI: 手術感染
BSI: 血液感染

PNEU: 肺炎
UTI: 泌尿道感染

Taiwan CDC

2007 – 2016 第一季地區醫院加護病房院內

感染率比例



SSI: 手術感染
BSI: 血液感染

PNEU: 肺炎
UTI: 泌尿道感染

Taiwan CDC

院內感染型肺炎

- ✓ 住院後48小時發生之肺炎
- ✓ 住院病人發生機率為0.5-1.5%，通常為細菌感染
- ✓ 院內感染型肺炎死亡率高達20-71%，通常致死原因因為其造成之病發症而並非肺炎本身

痰液檢體收集和保存

- **檢體收集**
 - ✓ 自行咳出
 - ✓ 清晨第一口痰為最佳檢體
 - ✓ 以內插管及刷子刷取肺部
 - ✓ 支氣管沖出液
- **保存**
 - ✓ 無菌痰盒
 - ✓ 室溫 2小時, 或是 2-8 °c 24 小時.

可接受的痰液檢體標準

Murray, Washington 1975

格蘭氏染色

	Epithelial cells (LPF)	Neutrophils (LPF)
Group 1	> 25	< 10
Group 2	> 25	10-25
Group 3	> 25	> 25
Group 4	10-25	> 25
Group 5	< 10	> 25

可接受: Group 4, 5

Taiwan society of Laboratory medicine

L K Wong 1982

Geckler et al., Murray and Washington, and Van 等人認為最簡單之方法為定義一標準LPF下之上皮細胞數量, 超過即不合格.

上皮細胞數目 < 25/LPF

可接受的痰液檢體標準

格蘭氏染色

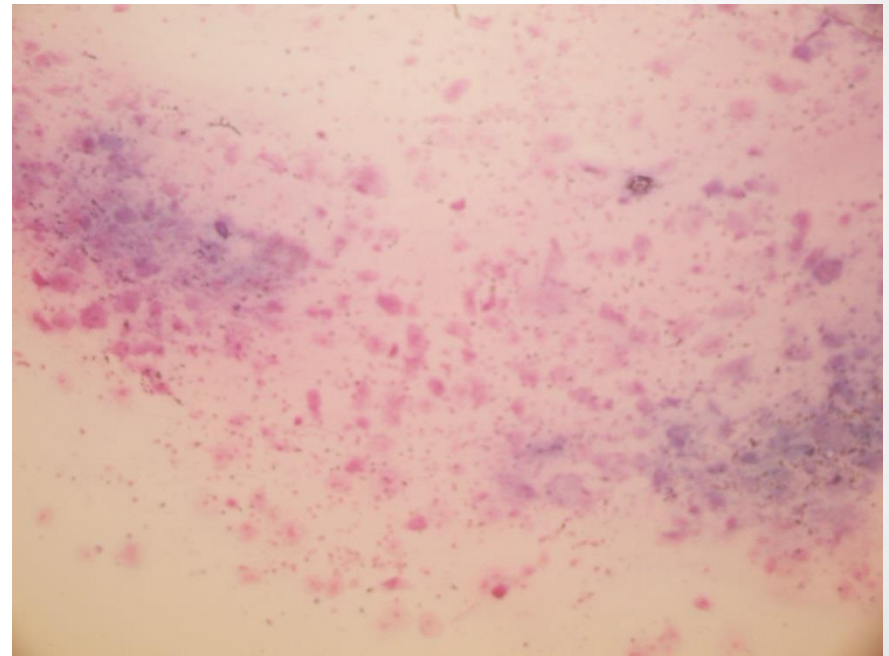
上皮細胞 < 10/LPF
白血球 > 25/LPF

上皮細胞 > 25/LPF
白血球 > 25/LPF



合格

100x



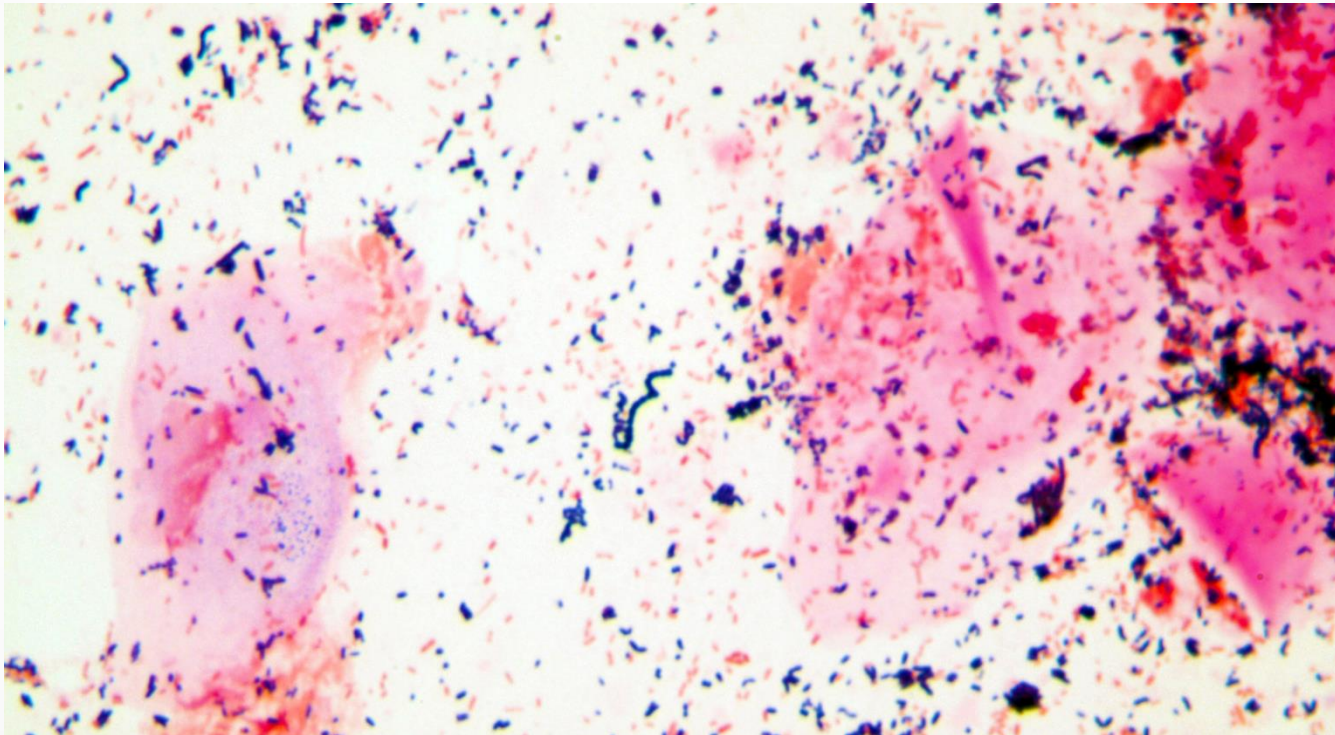
不合格

100x

可接受的痰液檢體標準

格蘭氏染色

上皮細胞 > 25/LPF
白血球 > 25/LPF



1000x

口腔常在菌叢污染

可能致病菌

一線絕對致病菌

Streptococcus pyogenes (Group A streptococcus)

Staphylococcus aureus

Pseudomonas aeruginosa

Stenotrophomonas maltophilia

Acinetobacter spp.

Burkholder cepacia

二線伺機性致病菌

> 2+, > *Streptococcus viridans*

Streptococcus pneumoniae

Group B, C, and G streptococcus

Moraxella catarrhalis

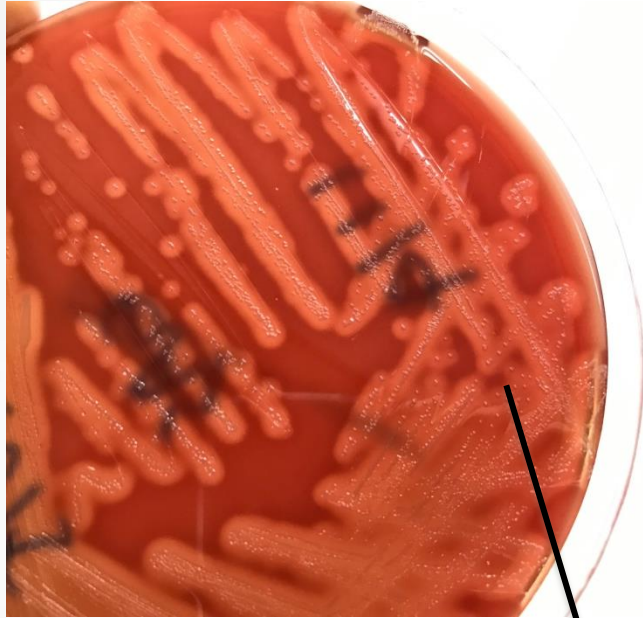
Neisseria meningitides

Enterobacteriaceae

Pasturella spp.

Gram negative rod

streptococcus)



G(+) 球菌
觸酶(-)
 β -溶血

致病因子

Streptolysin O

Streptolysin S

Strep. pyogenic 內毒素A

傳播方式

呼吸道 皮膚

臨床疾病

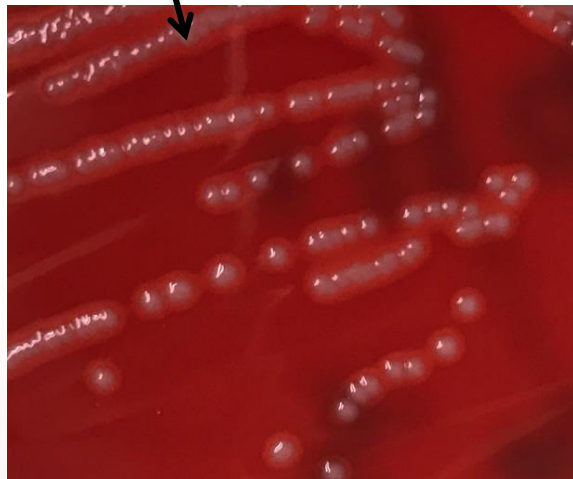
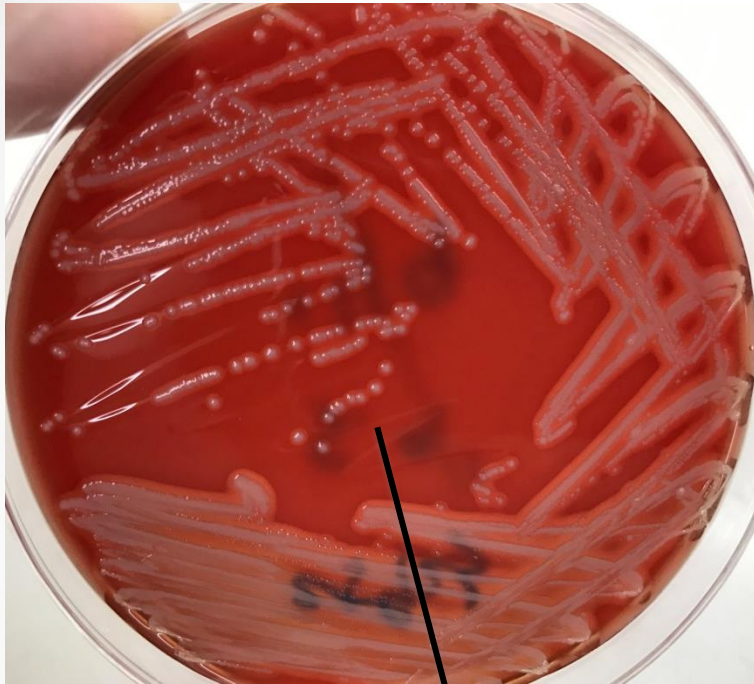
咽喉炎

猩紅熱

壞死性筋膜炎

毒休克

Staphylococcus aureus



G(+) 球菌

觸酶(+)

凝集酶(+)

β -溶血

致病因子

hemolysins

PTSAgs (superantigens)

Exfoliative toxins

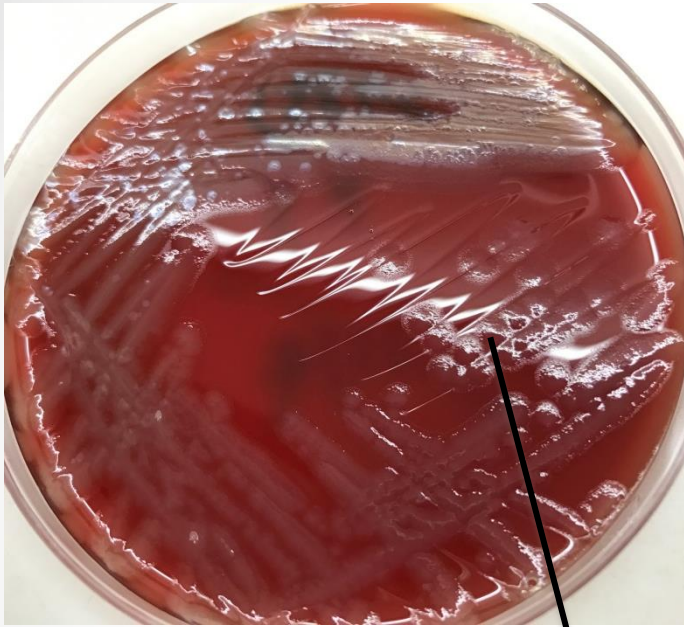
傳播

呼吸道 皮膚 食物

臨床疾病

膿瘍, 菌血症, 毒休克

Pseudomonas aeruginosa



G(-) 桿菌
Citrate(+)
觸酶(+)
oxidase(+)
可生長於42°C

致病因子

Exotoxins A

Phenazines (pyocanin, aeruginosin)

傳播

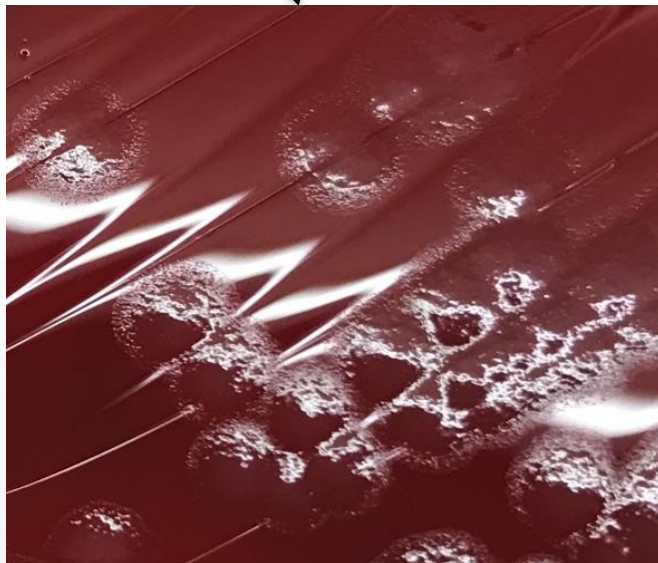
呼吸道 皮膚 泌尿道

疾病

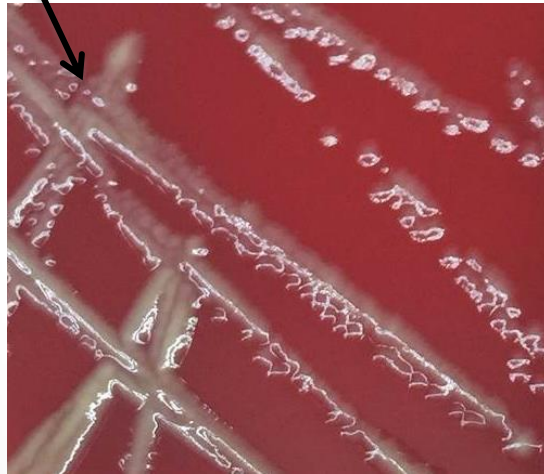
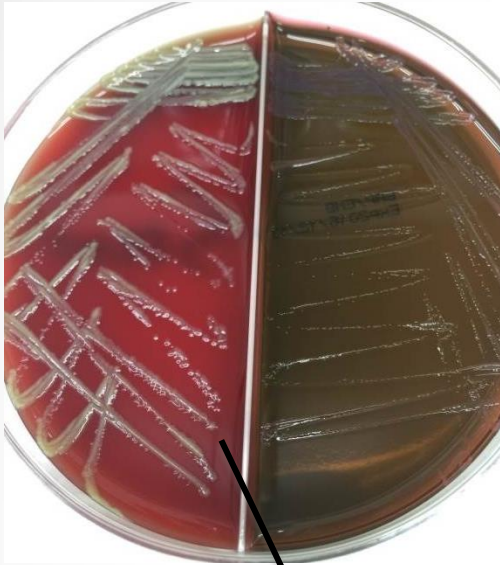
肺炎

壞死性腸炎

泌尿道感染



Stenotrophomonas maltophilia



G(-) 桿菌
觸酶(+)
Oxidase(-)

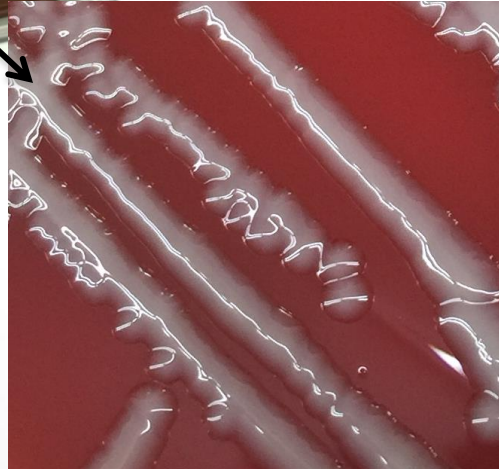
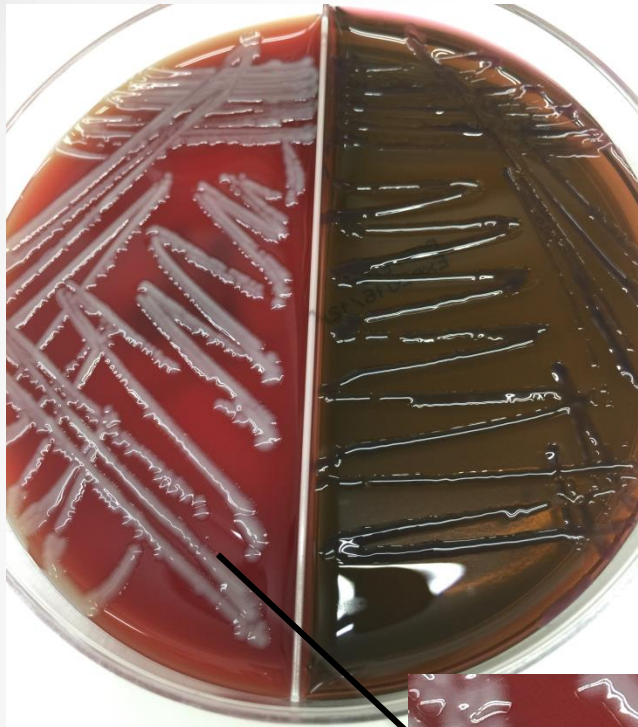
傳播

潮濕表面:
導尿管, 醫療器械, 內視鏡

臨床疾病

免疫不全之病人:
肺炎 泌尿道感染 血液感染

Acinetobacter baumannii



G(-) 桿菌
Oxidase(-)

致病因子

AbaR resistance island
 β - lactamase
Efflux pumps
生物膜形成
莢膜

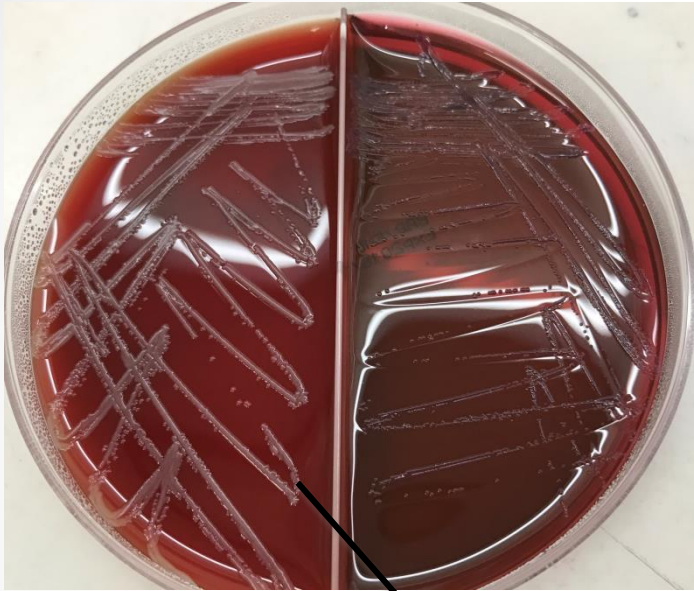
傳播

醫療器械表面 (抗乾燥)

臨床疾病

院內感染：
肺炎 泌尿道感染 血液感染

Burkholderia cepacia



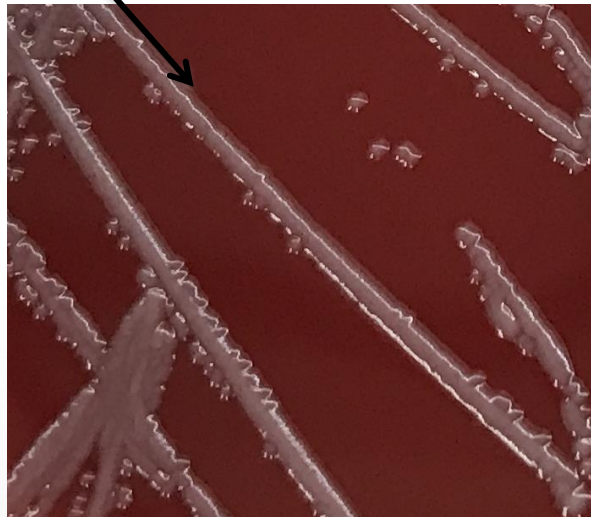
G(-) bacilli
觸酶(+)

觸酶

附著於醫療器械表面

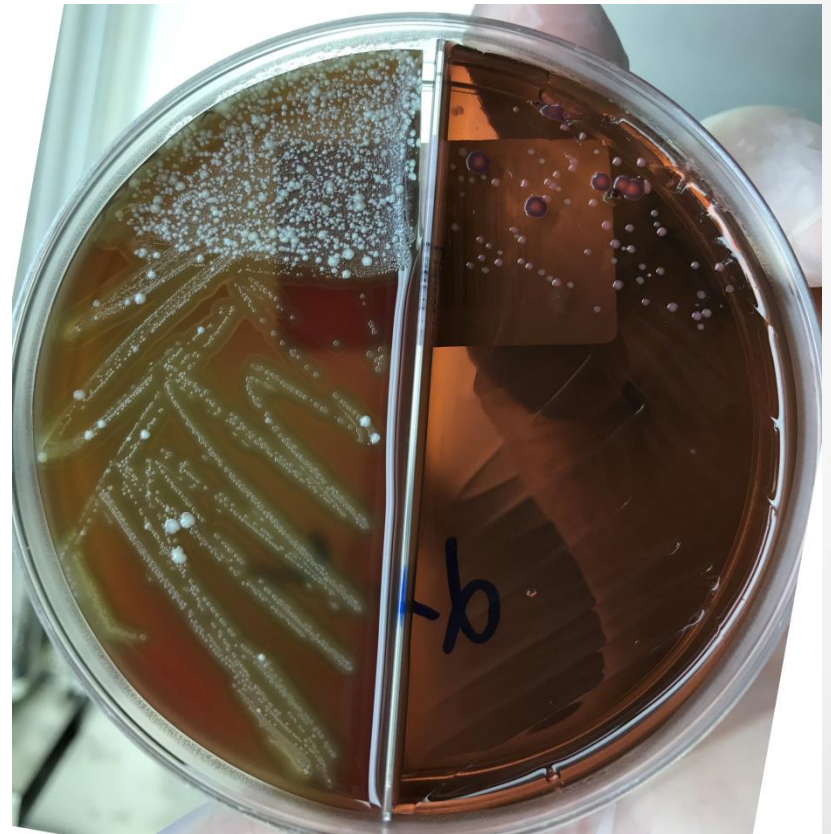
臨床疾病
肺炎

免疫不全之病人
(肺纖維囊腫)



痰液培養(次培養前)

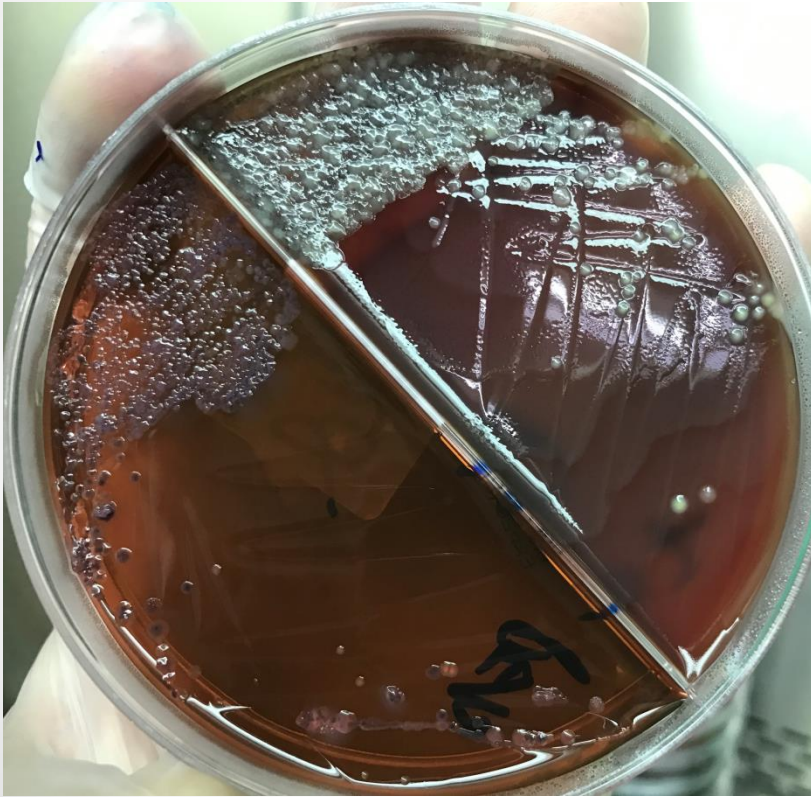
BAP/EMB, CAP, PEA



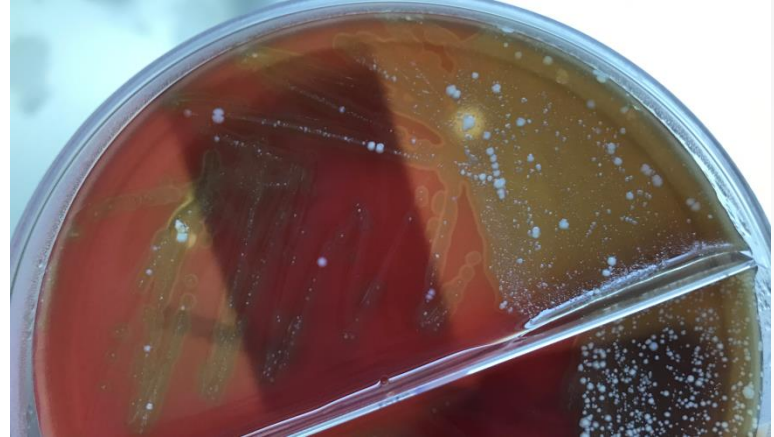
BAP/EMB

痰液培養(次培養前)

BAP/EMB, CAP, PEA



BAP/EMB



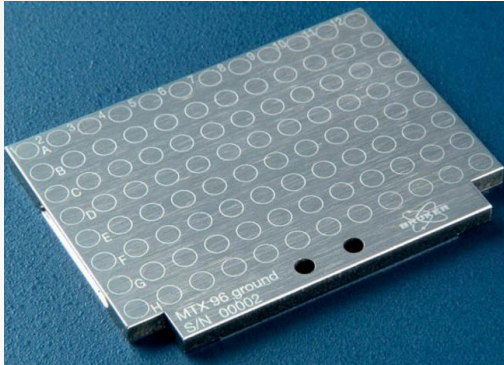
PEA



CAP

菌種鑑定

質譜儀



利用細菌菌種獨特蛋白質組成進行鑑定

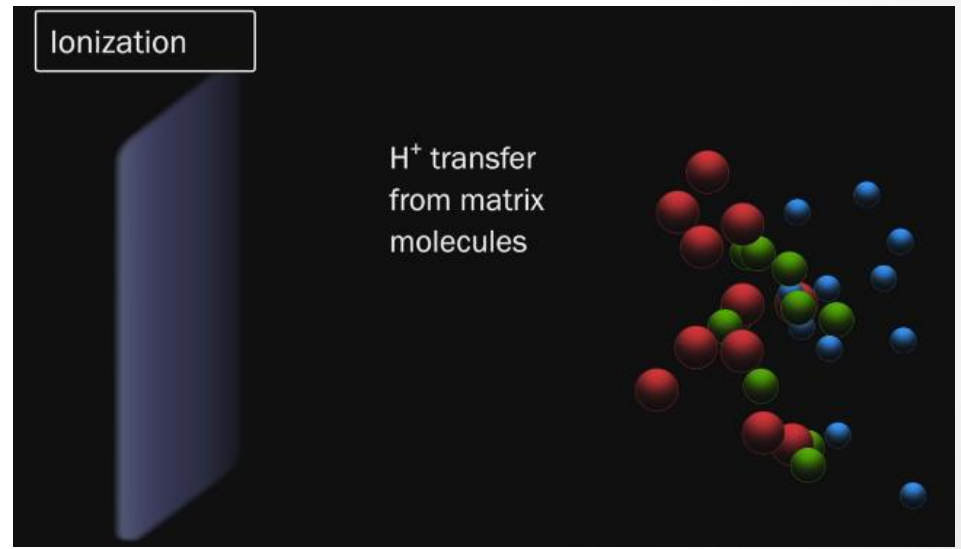
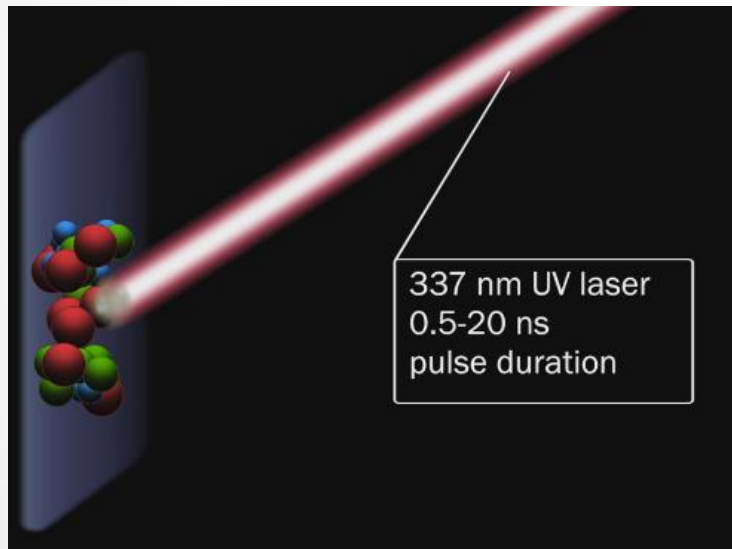
- ✓ 離子化分析物
- ✓ 將離子進行分類
- ✓ 偵測離子



MALDI-TOF 質譜儀

MALDI (Matrix Assisted Laser Desorption Ionization)

✓ 離子化分析物. (基質, 337nm UV laser)

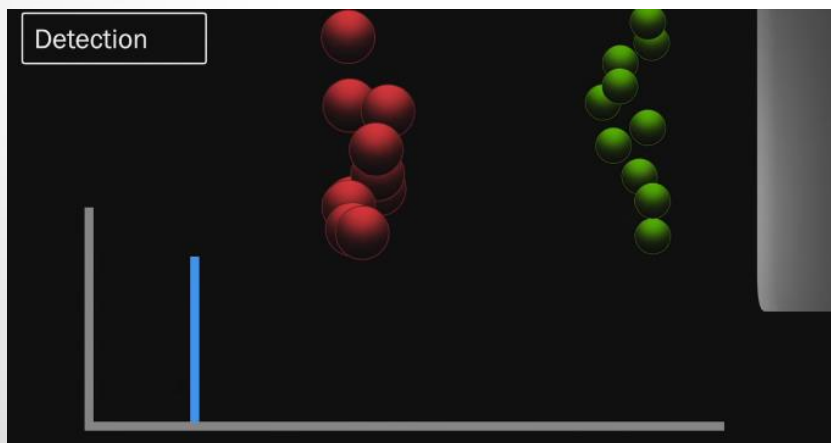
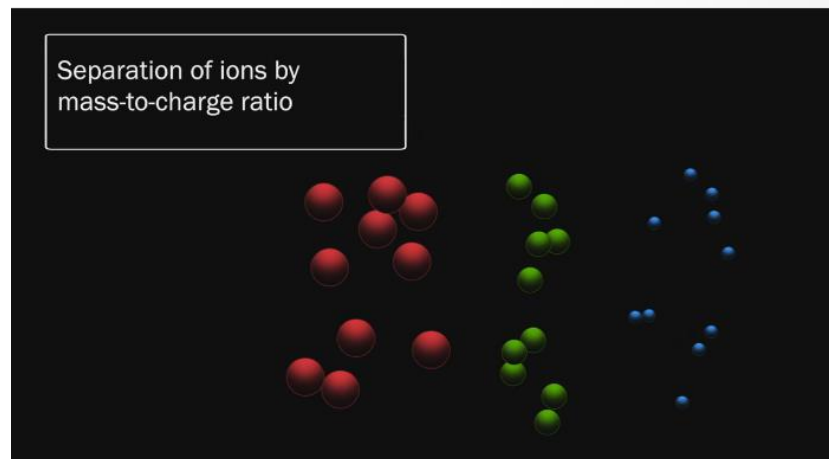


MALDI-TOF 質譜儀

TOF (Time of Flight)

✓ 分類並偵測離子

- 於電場進行離子加速.
- 持續提供動能.
- 低離子質電比者加速度較快.



菌種鑑定 & 藥物敏感性測試

BD Phoenix™ ID/AST Manual Panel Inoculation

McFarland 0.5 ~0.6

- 1** Open panel package.
INOCULATE WITHIN
2 HOURS.



- 4** After panel inoculation, place closures
securely on the panel to seal.

Prepare Purity Plate.
Using a sterile loop,
recover a small drop from
the inoculum fluid either
before or after inoculating
the panel. Inoculate an agar
plate (any appropriate medium)
for purity check. Incubate plates
for 18 - 24 hours or 18 - 48 hours
for yeast, at 35°C under appropriate
conditions.



- 2** Prepare Phoenix™ ID Broth.
Inoculate panel with prepared
ID Broth within (≤) 60 MINUTES.

PREPARE ID BROTH.

- Add organism to ID Broth.
- Vortex.
- Make .50 - .60 McFarland (Standard inoculum)*.
- OR
- Make .20 - .30 McFarland (Low inoculum)**.
- OR
- Make 2.00 - 2.40 McFarland (Yeast inoculum).



- 5** Load panel(s) into BD Phoenix instrument
within (≤) 30 MINUTES after inoculation.



- 3** Prepare Phoenix™ AST or AST-S Broth.
(ID/AST, AST Panels only)
Inoculate panel with prepared broth within (≤) 30 MINUTES.

PREPARE AST or AST-S BROTH***

- Add one free falling drop of AST or AST-S Indicator**** into AST or AST-S Broth. Tube can be used for up to 2 hours if exposed to light or up to 8 hours in the dark. Mix by inverting.
- Add 25 µL prepared ID Broth (Standard inoculum).
- OR
- Add 50 µL prepared ID Broth (Low inoculum).



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SHP-228



BD Phoenix™ ID/AST

* Not applicable for yeast ID panels.
** Not applicable for strep or yeast ID panels.
*** For gram-negative or gram-positive panels
use Phoenix AST Indicator and AST Broth.
For strep panels use Phoenix AST-S Indicator and AST-S Broth.
**** AST or AST-S indicator is good for 14 days after opening (refrigerated).