VITEK MS™

# 微生物鑑定質譜儀
# 之原理介紹與規範

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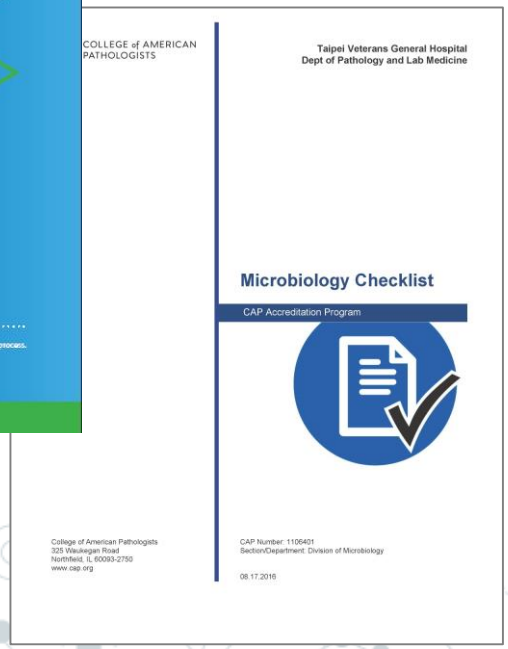
DITECH ENTERPRISE CO., LTD.

產品專員 林慧軒

# Outline

- 原理
- 優勢
- 相關國際規範
  - 菌株的選擇與製備
  - 結果判讀
  - 報告規範
  - 設備與安全
  - 人員訓練
  - 品質管理



# MALDI-TOF 原理

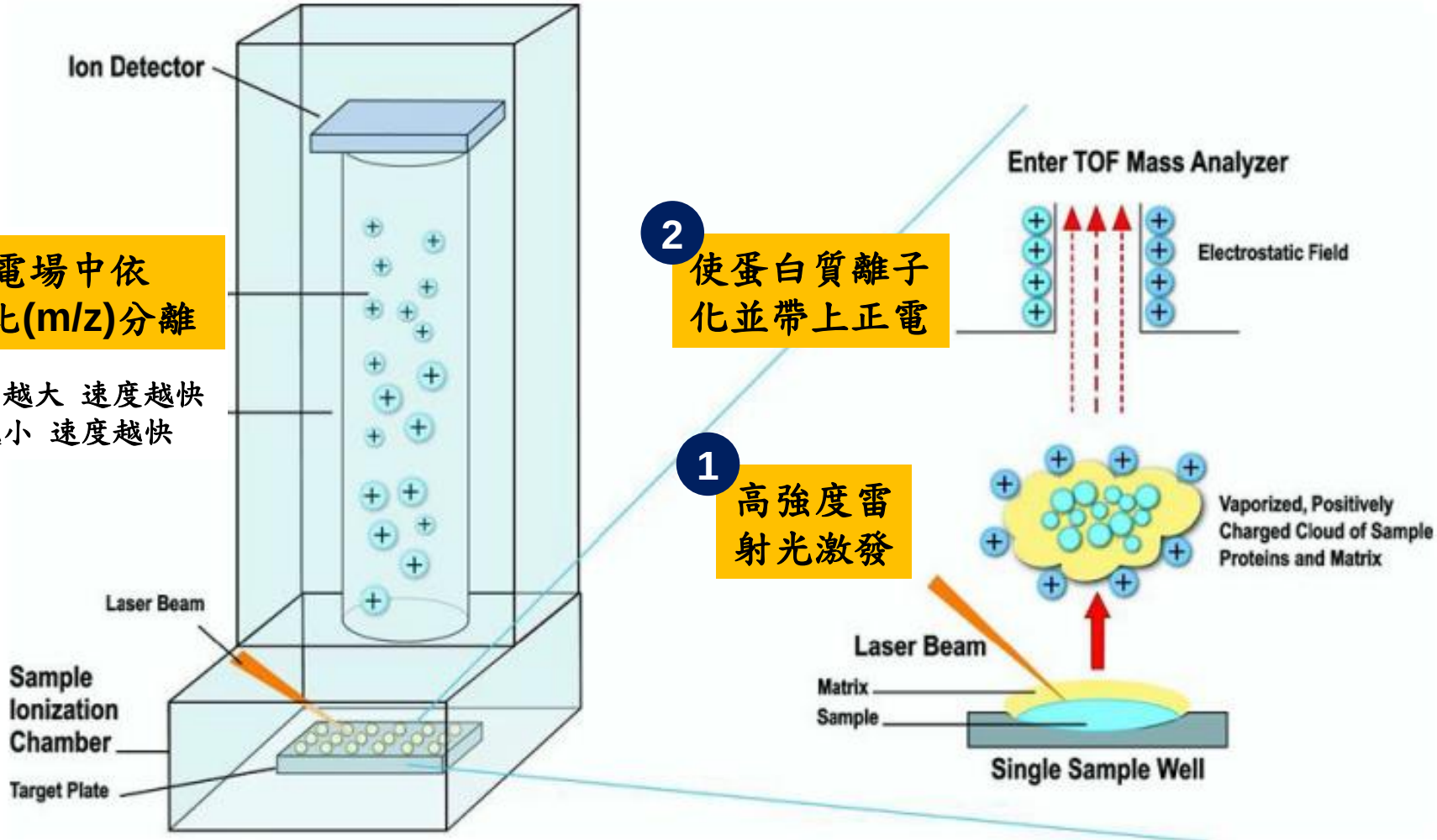
4 抵達偵測終點產生訊號(peak)並組成圖譜(spectrum)

3 在電場中依荷質比(m/z)分離

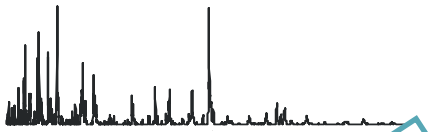
- 帶電量越大 速度越快
- 質量越小 速度越快

2 使蛋白質離子化並帶上正電

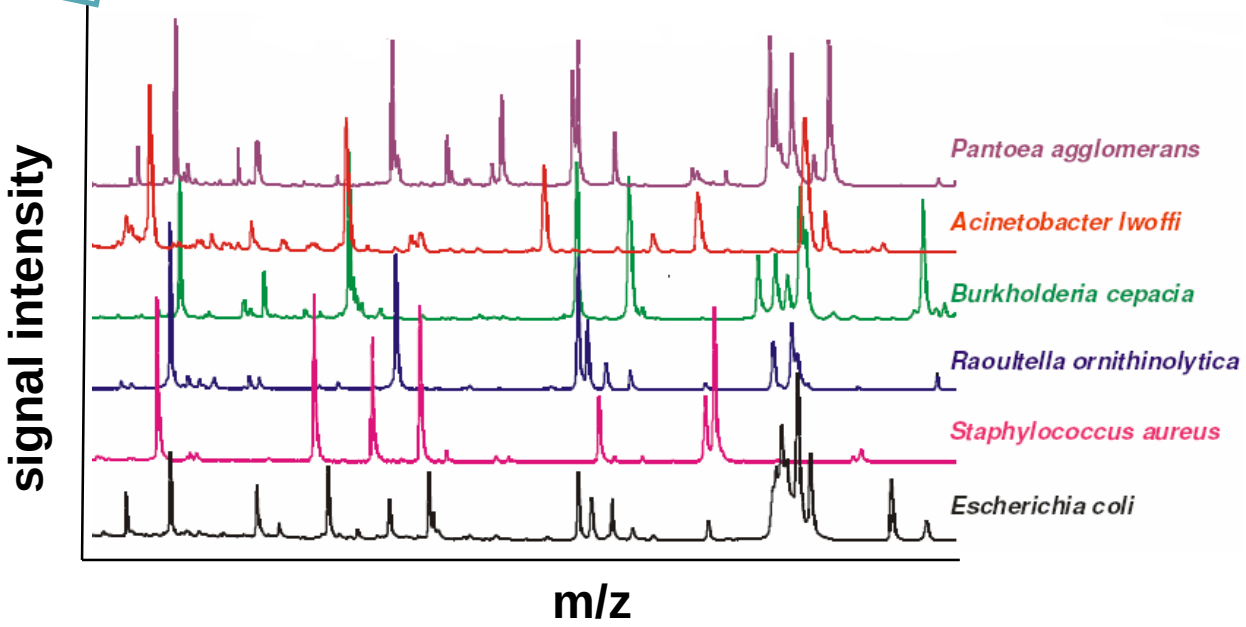
1 高強度雷射光激發



# MALDI-TOF 原理



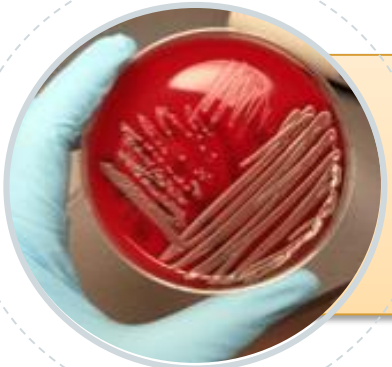
比對圖譜資料庫




**Microorganism Identification**

# 微生物鑑定質譜儀之優勢

# 傳統生化鑑定方法



隔夜
培養

+ - + + - ...

- ✓ 耗時
- ✓ 大量培養基使用
- ✓ 試劑需求複雜
- ✓ 需判別菌種以執行相對應試驗
- ✓ 需人眼判讀結果

# 微生物鑑定質譜儀之優勢

- ✓ 快速鑑定結果
- ✓ 資料庫涵蓋菌種廣泛
  GP, GN, yeasts, moulds, mycobacterium…
- ✓ 可同時大量上機
- ✓ 快速的製備即可得到高專一性結果
- ✓ 低試劑成本
- ✓ 操作簡單
- ✓ 菌量需求少
- ✓ 可鑑定出過往以生化方法難以鑑定之菌株

# Selecting Isolates for MALDI-TOF MS Analysis
# 菌株的選擇

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# 培養基

- **Pure culture**

- Solid vs Liquid Culture Media?
  - Solid media: purity can be visually assessed
  - Liquid media: generally need manipulation before analysis
  - 遵循製造商建議

- Selective vs Nonselective Culture Media?
  - Selective media may negatively affect the spectral quality, leading to ID failures or low-confidence ID.
  - 遵循製造商建議

# 培養條件

- Incubation Conditions
  - Microorganisms should be cultured under conditions that best support their growth, which vary by microorganism.
  - Refrigerated isolates should not be use.
  - 遵循製造商建議

- Incubation Duration
  - Freshly cultured isolates should be used
    Common bacteria and yeast: 18-24 hours
    Anaerobes: may need up to 72 hours
  - 遵循製造商建議
  - "Rapid incubation protocol"
    - Most frequently with blood culture (monomicrobial and urgent TAT)
    - Analyzing culture isolates after only a few hours of incubation.
    - Verify culture purity and concordant colony morphology at later time point.
    - Report findings as preliminary (pending later confirmation), unless the laboratory has validated the off-label procedure.

# 分析所需之生物質量(Biomass)

- 適當的菌量為**$1\times10^5$ - $1\times10^7$ CFU**

  For bacteria, approximately $1\times10^5$ to $1\times10^7$ CFU of microorganism are needed

- **過多的菌量並不必要**

  More biomass on the target will not necessarily improve spectral quality and can actually decrease the spectral quality for some microorganism types.

- 遵循製造商建議

(Example from VITEK® MS Workflow User Manual)

# 菌株的選擇

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| Organism | Acceptable Media for MALDI-TOF MS Analysis | Acceptable Incubation Conditions Before Analysis | Acceptable Score for Reporting |
|---|---|---|---|
| Yeasts | • SAB<br>• SABCL<br>• IMA<br>• BAP<br>• BHI<br>• *Candida* chromogenic agar | 30°C or 35°C air or $CO_2$ for up to 72 hours | ≥2.0 for species-level reporting, unless otherwise noted on the trusted list<br><br>1.99 to 1.7 for genus-level reporting |
| Enteric gram-negative bacilli | • BAP<br>• MAC | 35°C air or $CO_2$ for up to 5 days | |
| Nonfermenting or fastidious gram-negative bacilli | • BAP<br>• MAC<br>• CHOC | 35°C air or $CO_2$ for up 5 days | |
| | • CBP | 42°C; microaerophilic or $CO_2$ | |
| Gram-positive bacteria | • CNA<br>• BAP<br>• CHOC<br>• MRSA agar<br>• MSA<br>• VRE chromogenic agar<br>• Group B *Streptococcus* chromogenic agar | 35°C, 4°C, or room temperature up to 5 days | |
| Anaerobic organisms | • Brucella agar | 35°C in anaerobic atmosphere for up to 5 days | |
| | • BAP<br>• PEA<br>• LKV<br>• BBE | Ambient air and/or room temperature for up to 5 days | |

- 實驗室應制定針對不同菌種所適用的培養基與培養環境對照表

Laboratories may wish to develop a brief reference guide to summarize appropriate media and incubation conditions for various organism groups.

- 遵循製造商建議

Refer to manufacturers recommendations, the use of other media must be validated.

Appendix A. Example Quick Reference Guide for MALDI-TOF MS Identification Testing of Microorganisms

# Preparing Isolates for MALDI-TOF MS Analysis
# 菌株製備

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# 菌株製備方式

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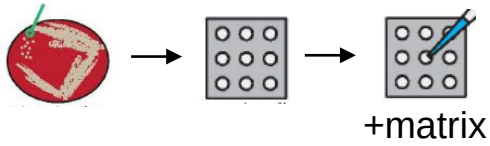
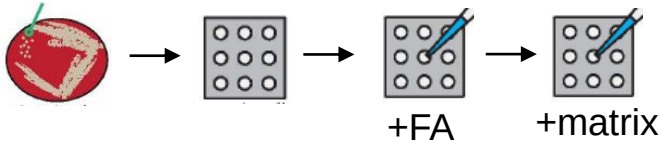
**CLSI M58**

## Direct Colony Transfer



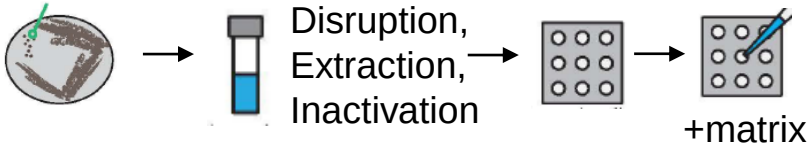

GN, GP, Anaerobes

## On-Target Extraction




Yeasts

## Tube Extraction

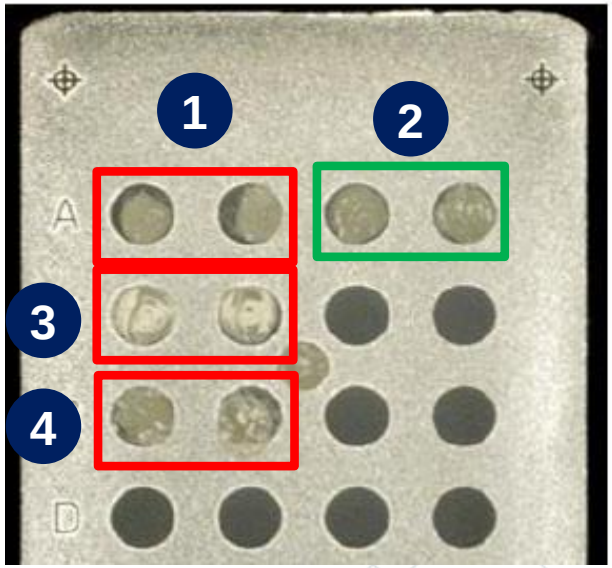



Moulds, Mycobacteria and Nocardia

*FA: formic acid

# 菌株製備注意事項

- 不建議使用swab沾取菌落 (會有fiber殘留疑慮)
- 不要刮取slide
- 不要取到media
- 盡量完整覆蓋spot
- 讓spot在室溫中自然乾燥不要加熱




1：菌量過少，且無完整覆蓋孔位
2：菌量適當，均勻且完整覆蓋孔位
3：菌量過多
4：超出孔位範圍，可能造成汙染

**Figure 5. Examples of Spotting.**

CLSI M58 p.15, 17

# Interpreting Results, Troubleshooting, and Performing Supplemental Testing

# 結果判讀、疑難排解及補充試驗

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# Comparing MALDI-TOF MS Results With Microscopic and Plate Morphology

為避免分析前、中、後有任何錯誤影響鑑定結果，所有鑑定菌種應對照其特性是否符合：

- Gram stain characteristics
- Colony morphology
- Rate of growth
- Culture conditions (eg, aerobic vs anaerobic)
- Results of any biochemical testing performed
- AST results

*…also by reliance on "logical progression" programmed into the LIS*

**而在某些檢體出現不尋常的鑑定結果時也應特別注意其特性或加做試驗確認**

[*manually reviewing phenotypic features, and in some cases, performing supplemental tests, should be considered for some specimen types, for unfamiliar, unclaimed, or highly significant microorganisms, and when split identifications are obtained using MALDI-TOF MS.*]

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# 多筆可信度相似的鑑定結果是否執行加做試驗？

**若這些結果的屬名不同，必須執行加做試驗區分**

When multiple species from different genera are listed, supplemental testing should be performed to determine the correct genus designation.

**若只差別在種名不同，可視臨床需求決定是否執行**

When multiple species from a single genus are listed, it is important to first consider the clinical need for a species-level identification.

(EX)

- 痰液檢體中的*Enterococcus* spp. 可能不需要進一步區分
- **無菌部位**檢體則需要進一步區分

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# Table 2. 沒有結果或出現非預期鑑定結果 –可能原因及圖譜品質問題

| Poor-Quality or No Mass Spectrum Generated – No ID Obtained |
|---|
| • Matrix was not added before analysis. Matrix沒加 |
| • Too much or too little biomass was used in analysis. 菌量太多/太少 |
| • Biomass and/or matrix do not cover the entire target spot. 沒有塗滿 |
| • Isolate is mucoid; too much capsular material was transferred to target. 菌太黏 |
| • Organism type is not responsive to the direct colony transfer technique; extraction is needed. 需要萃取 |
| • Organism biomass is contaminated with agar or primary specimen. 取到agar或原檢體培養基質 |
| • Culture is mixed (organism not properly isolated). 有雜菌 |
| • Reagents have expired/evaporated/crystallized or were incorrectly prepared. 試劑不當保存/使用 |
| • Organism is too old or too young. 菌太老/年輕 |
| • Organism was refrigerated before analysis. 菌有被冷藏過 |
| • Scratches or dents are on the target slide or plate. 樣本版刮傷 |
| • Laser intensity is inadequate. 雷射強度不適當 |
| • Detector needs maintenance or replacement. 偵測器需保養或更換 |
| • Uneven target spot application. 塗不均勻 |

**VITEK MS 試劑**

IVD開封即用設計，無須配製及額外品管
僅需依製造商規範保存與使用

→ 試劑使用方便

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# Table 2. 沒有結果或出現非預期鑑定結果 –可能原因及圖譜品質問題

| Good-Quality Mass Spectrum Generated – Unexpected ID, Split ID, or No ID Obtained |
|---|
| • The sample was transferred to the wrong spot on the target slide or plate (inaccurate sample tracking). 塗錯地方 |
| • Sample cross-contamination is present (this may occur from sloppy spotting, using a single pipette to apply the matrix to multiple spots, or from inadequate cleaning of reusable target slides or plates). 交叉汙染 |
| • The culture is mixed (the microorganism not properly isolated before analysis).有雜菌 |
| • Reagents are contaminated with microorganisms. 試劑汙染 |
| • Organism is not represented in the database. 資料庫無此菌種 |

**VITEK MS 樣本片**

- 拋棄式設計，無交叉汙染風險
- 每片均有獨立條碼辨識，具良好溯源性

→ 降低沒有結果或非預期結果的發生率

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# Reporting Guidelines

# 報告規範

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# 報告的一般注意事項

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**實驗室應自行建立規範，決定鑑定結果應發至哪個菌名層級**

[ It is important for laboratories to establish internal reporting guidelines to aid technologists in deciding when to report recovered microorganisms using the precise taxonomy provided by MALDI-TOF MS-MIS, and when to report using more general terms (eg, “normal regional flora”). ]

可參考以下幾點：

- The **body site** from which the specimen is isolated (normally sterile or normally colonized with resident microbiota)
- The manner in which the specimen was obtained (**invasive** vs **noninvasive** procedures)
- Specimen quality
- Host factors (immune status, patient history, the presence of foreign material at the site of infection)

# 菌名分類

**實驗室應定期更新菌名，以避免不熟悉某些質譜鑑定結果**

**Table 4. Examples of Clinically Important Microorganisms With Recent Taxonomic Revisions**

| Former Name | New Name |
|---|---|
| *Haemophilus aphrophilus* | *Aggregatibacter aphrophilus* |
| *Micromonas micros* | *Parvimonas micra* |
| *Peptostreptococcus asaccharolyticus* | *Peptoniphilus asaccharolyticus* |
| *Arcanobacterium bernardiae* | *Trueperella bernardiae* |
| *Stomatococcus mucilaginosus* | *Rothia mucilaginosa* |
| *Aspergillus fumigatus** | *Aspergillus lentulus* |
| *Geotrichum capitatum* | *Saprochaete capitata* |
| *Pichia farinosa* | *Millerozyma farinosa* |
| *Pichia ohmeri* | *Kodamaea ohmeri* |
| *Rhodotorula rubra* | *Rhodotorula mucilaginosa* |

*建議：可建立“translation table”*

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# Reporting to the Group or Genus Level

某些菌種因太相近而無法區分，可直接發至屬名

[ within certain genera, MALDI-TOF MS-MIS cannot reliably distinguish among closely related species or subspecies. ]

(EX) *Achromobacter xylosoxidans*/*Achromobacter denitrificans*”

加做試驗

若無特別需求，*Achromobacter* sp.

---

由於加做試驗可能受到限制，可藉由染色結果發出「可能」結果，例如“Probable *Actinomyces* sp.”

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# Facilities and Safety

# 設備與安全

# 化學危害

VS

可在實驗桌上操作小量的
matrix及FA solutions

管式萃取、去活化及**清潔重複使用的target slides**需有防護設備及用具

# 試劑安全注意事項

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CLSI M58共對五種質譜鑑定可能使用試劑說明其危害，
只有TFA被點出必須於生物安全櫃操作並配戴眼睛及臉部防護

**Table 7. Safety Considerations for Commonly Used Reagents**

| Chemical | Safety Considerations |
|---|---|
| TFA三氟乙酸<br>(Trifluoroacetic acid) | • TFA is harmful if inhaled.<br>• TFA is corrosive and causes burns.<br>• Protective clothing and gloves should be worn when handling.<br>• TFA should only be handled in a chemical fume hood using face and eye protection.<br>• TFA should be stored in appropriate containers (may corrode glass), separately from flammable liquids. |

**VITEK MS 樣本片**

拋棄式設計，無須重複清洗

→ 降低接觸毒化物機率

# 試劑耗材使用注意事項

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| | | |
|---|---|---|
| **Matrix**<br>• Spotting (Subchapter 2.2.1)<br>• Chemical Hygiene (Subchapters 3.1.1., 3.1.2, and Table 7) | Applied to every sample on target and required for analysis.<br><br>Multiple functions:<br>• Assists with protein extraction<br>• Absorbs energy from laser<br>• Transfers ions to proteins | • Manufacturer specific; instructions for use and storage should be followed exactly as written.<br>• Reagent log for all shipments and/or new lot numbers should be maintained.<br>• Lid should be kept tightly closed when not in use to prevent evaporation. Appearance of crystals on the bottom of the container necessitates user action (action dependent on manufacturer).<br>• Optimal ratio of matrix to organism smear (spot) and a homogeneous preparation should be observed for each "spot" to ensure the generation of ions and quality spectra. |
| **Target Slide or Plate**<br> | A flat slide or plate where sample preparation takes place and on which the isolated organisms is smeared onto the target.<br><br>It is traceable by a unique barcode and each spot represents one isolate. The target slide or plate is placed directly into MALDI-TOF MS for analysis. | • Manufacturer specific; instructions for use and storage should be followed exactly as written.<br>• Options include disposable (one-time use) and reusable polished steel targets.<br>• Should not use for testing if the target appears scratched, dirty, bent, "bleeds" into surrounding spots, or chipped.<br>• Touching the surface of the target with fingers should be avoided.<br>• Writing or applying stickers on the targets should be avoided. |

**VITEK MS 樣本片**

每片均有獨立條碼辨識，具良好溯源性 → 符合M58規範

# 生物危害

- 第一級及第二級危險群的微生物等常規性的細菌或酵母菌皆可在BSL-2等級實驗室的實驗桌上，直接轉移至MALDI-TOF MS target slides或plates上

- 第三級及第四級危險群的微生物 (如：*Burkholderia pseudomallei, Bacillus anthracis, Brucella spp., Francisella spp., Yersinia pestis, Mycobacterium tuberculosis*)

  - 須於生物安全櫃中進行去活化後，才可拿出操作

  - 只能使用一次性target slides

**VITEK MS 樣本片為拋棄式設計**

**→ 符合M58規範**

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# Personnel:
# Guidelines for Training and Competence Assessment

# 人員訓練規範及能力評估

# 人員訓練規範及能力評估

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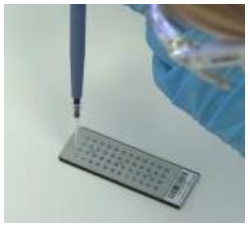
**QA program**

- Initial training
- Periodic competence assessment

可參考CLSI QMS03

- Appropriate isolate selection 適宜的菌株選擇
  - correct media, age of colony, avoid mixed cultures…
- Safe handling and processing 安全操作方式
- Correlating MALDI-TOF MS results with isolate characteristics 將MS結果與菌株特徵比對
  - gram stain, morphology, susceptibility results…



**Target slide preparation**

- Good spotting technique 好的塗片技巧
- Accurate pipetting of matrix to ensure coverage of the entire spot 將基質完整的覆蓋spot
- Reproducible accurate results 可重複做出準確的結果

# 人員訓練規範及能力評估

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**Appropriate instrument system operation**

- Instrument operation (software and hardware) and safety
- Maintenance procedures
- Troubleshooting
- Waste disposal procedures
- Reagent preparation
- Cleaning instructions for reusable target slides or plates

**Results reporting**

- Data analysis
- Test result interpretation
- Supplemental testing
- Troubleshooting

可參考Appendix C and D.

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# Process Management:
# Ensuring Quality in MALDI-TOF MS Testing

# MS鑑定品質之確認

# Quality Control

- **Positive control**
  以製造商建議之菌株驗證整體鑑定品質

- **Negative control** (reagents only)
  - Detect reagent contamination
  - Control for proper slide cleaning

# MIC. 16605 Mass Spectrometer Controls

Phase II

- For FDA-approved platforms, the organisms or calibrator(s) required by the manufacturer must be used.

  需使用製造商建議之菌株

- Appropriate controls would include at least one bacterium, with a representative yeast and mycobacterium also being run if these organisms are being tested for that day/routinely.

  品管菌株需包含至少一株細菌、酵母菌與分枝桿菌

# MIC. 16605 Mass Spectrometer Controls

Phase II

- In formats of testing where a target is reused, a blank control needs to be run after each cleansing to assess the cleanliness of the target.
  若重複使用樣本片，需在每一次清洗後執行空白品管以確保清潔度。
- Appropriate control organisms or calibrators are tested on each day of patient testing.“

  *Evidence of Compliance: records at defined frequency

  每日執行品管並留下紀錄

“

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# VITEK MS符合各項品管規範

- 各類品管菌株明確
- 提供拋棄式樣本片，無須重複清洗
- 自動留存品管結果

# Calibration

- Calibration automatically begins when a new sample run is initiated.

  當開始分析新一批次檢體時，系統需可自動進行校正

- The calibration must result in a correct organism identification with a high level of confidence.

  校正必須產出高可信度的正確鑑定菌名

- The run will not continue nor will the results be reported if the calibration does not meet required specifications.

  當校正結果不符允收時，系統不應給予鑑定報告。

# MIC. 16595 Mass Spectrometer Calibration

Phase II

- **A calibration control is run each day of patient testing, with each change in target plate, or according to manufacturer's recommendations and these records are maintained.**

*Evidence of Compliance: Records of calibration

每日、每次更換樣本盤時，需執行校正並留下記錄

瑞博企業有限公司
DITECH ENTERPRISE CO., LTD.

# VITEK MS符合各項校正規範

- 自動執行校正
- 校正結果不符允收時，不給予鑑定結果
- 自動留存校正結果

瑞博企業有限公司
DITECH ENTERPRISE CO., LTD.

# VITEK MS™

- 效能
- 試劑
- 耗材
- 實驗室安全
- 校正品管

CLSI

cap

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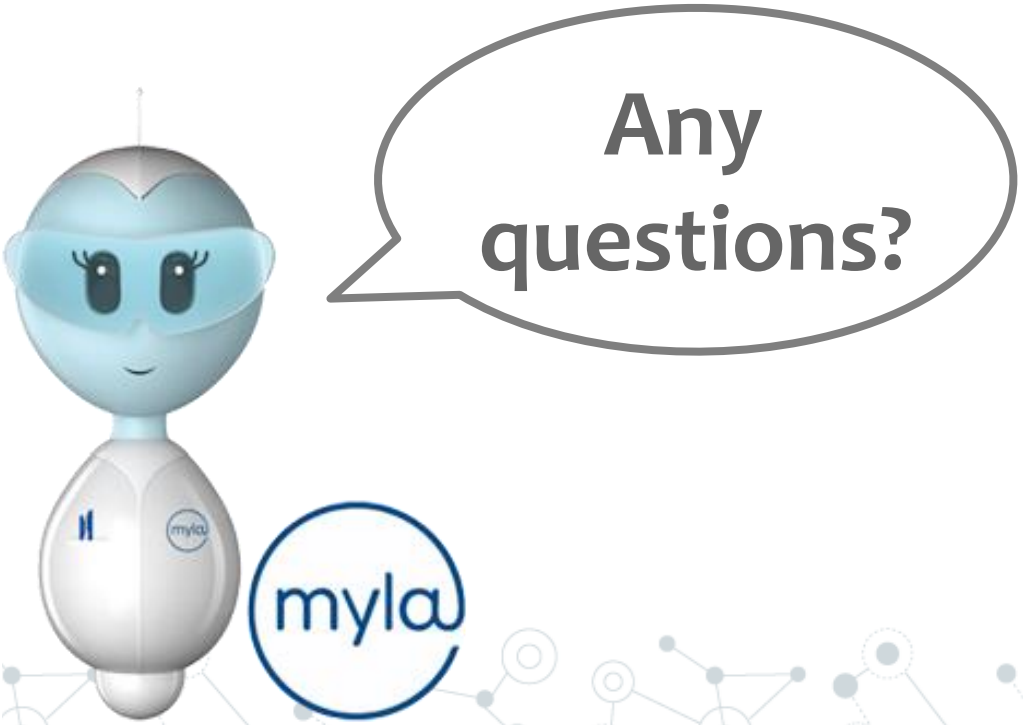
# THANKS FOR YOUR ATTENTION!