多重因子檢驗分析平台
Luminex® 200™

儀器原理應用介紹
與保養操作教育訓練

何俊賢 資深經理
進階生物科技股份有限公司

level
Discovering new boundaries

進 階 生 物 科 技
Level Biotechnology Inc.

Outlines

- Luminex xMAP Technology
- Luminex 儀器介紹
- 應用介紹
  - ProcartaPlex/MilliPlex
  - QuantiGene Plex
- 保養操作步驟

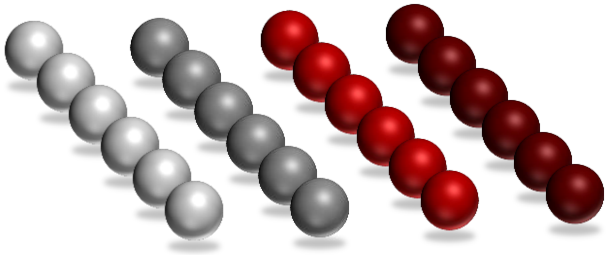
Luminex xMAP Technology原理

x= the unknown, **M**= Multi, **A**= Analyte, **P**= Profiling

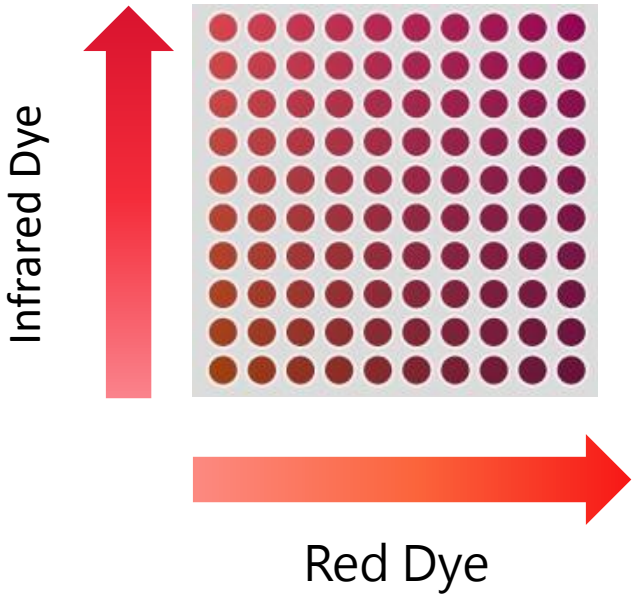
- 利用2種不同濃度染劑組合出100種顏色微珠
- 透過紅雷射激發微珠，再利用兩組濾鏡過濾判讀發散光中兩種顏色強度，還原微珠色碼編號
- 透過綠雷射激發微珠上抗體的PE螢光蛋白，再利用PMT收光後，得到螢光強度來判斷反應物的量。

100種顏色組合- Bead Barcode

- 每個獨特顏色set的微磁珠，具備各自獨特的Barcode

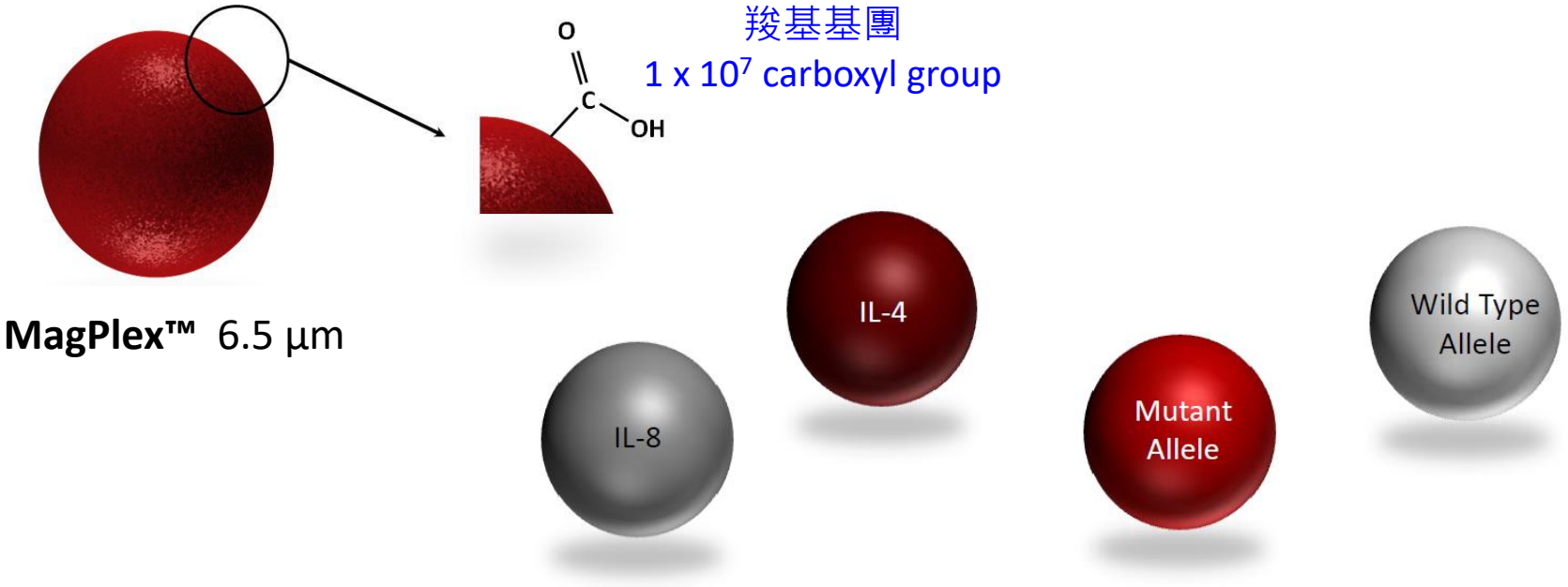


同beads set具相同顏色



Luminex xMAP Technology應用

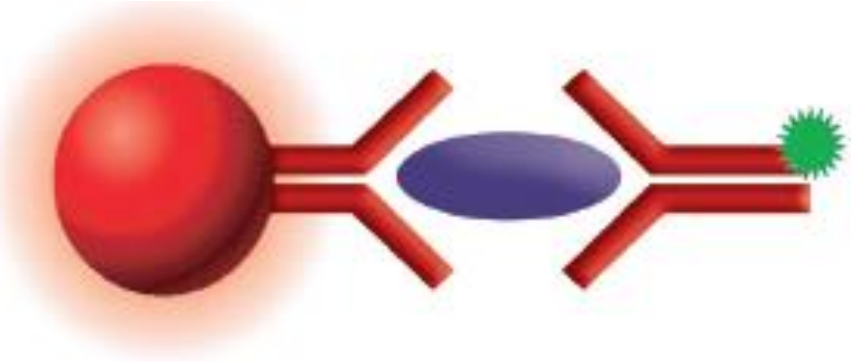
- 每個獨特顏色set的微磁珠，具備各自獨特的Barcode
- 藉由微磁珠表面上羧基基團來鍵結不同抗體、蛋白peptide或oligo nucleotide
- 因此每個獨特Barcode的微磁珠，便可代表一種獨特Target偵測微磁珠



xMAP® technology application

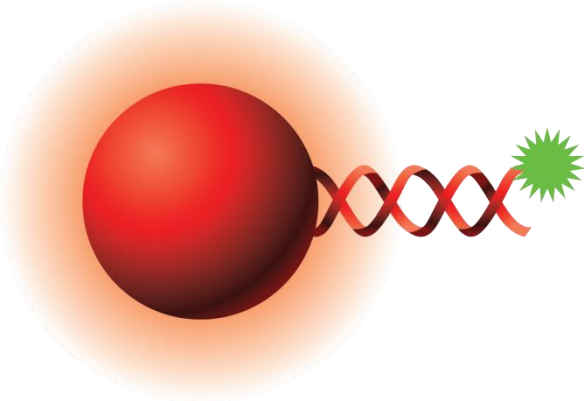
Bead 可以根據實驗需求偶聯特定的生物檢測目標

- **Immunoassays**



- 三明治法 (sandwich capture)
- 多重ELISA
- 受體(receptor) - 配體(ligand)作用分析
- 蛋白質相互作用分析

- **Nucleic Acid Assays**

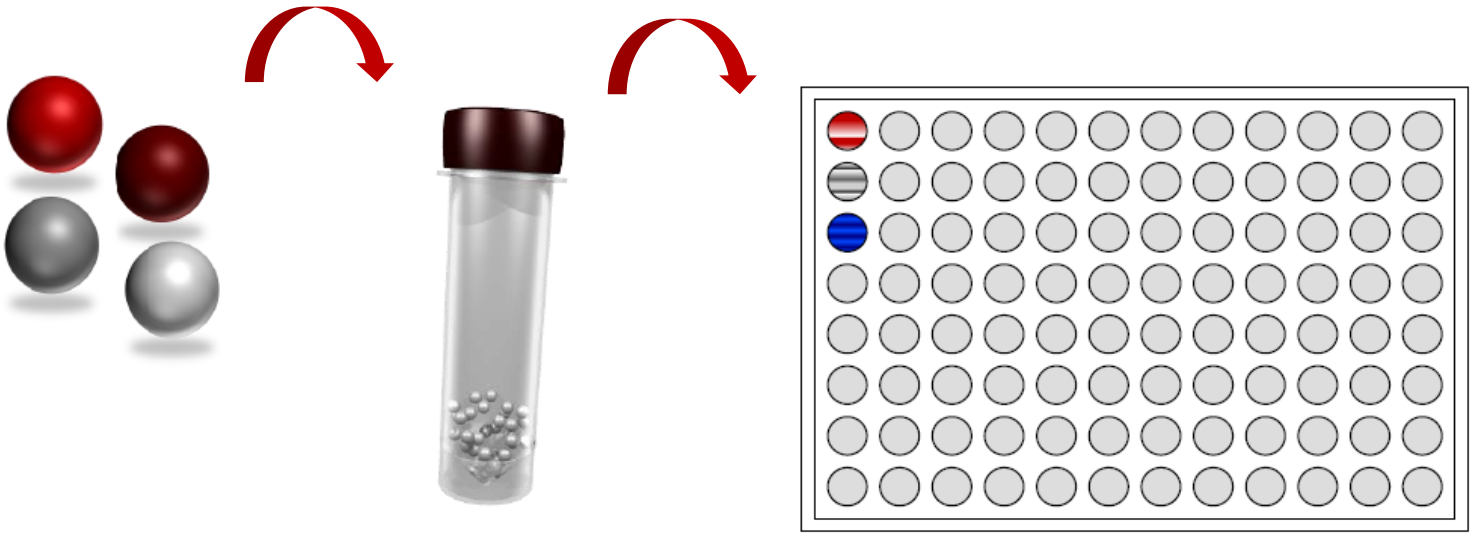


- 基因表達
- microRNA的分析
- 基因分型 (genotyping)
  - SNP
  - CNV
  - 序列測定

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Luminex xMAP Multiplex

- 只需少量檢體，便能從單一well得到多個target的資料

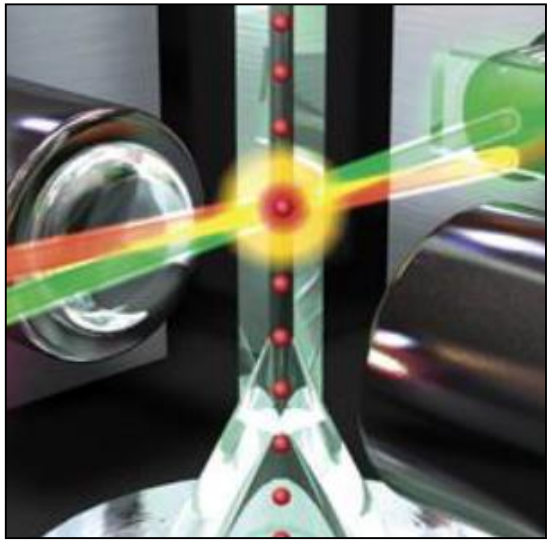


Luminex® 200

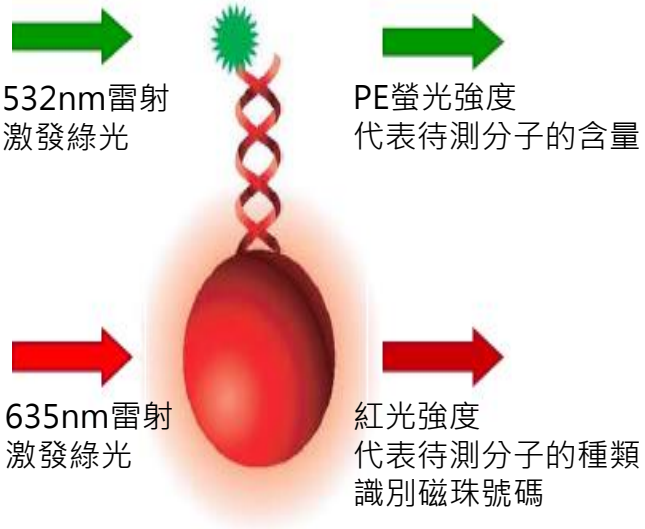


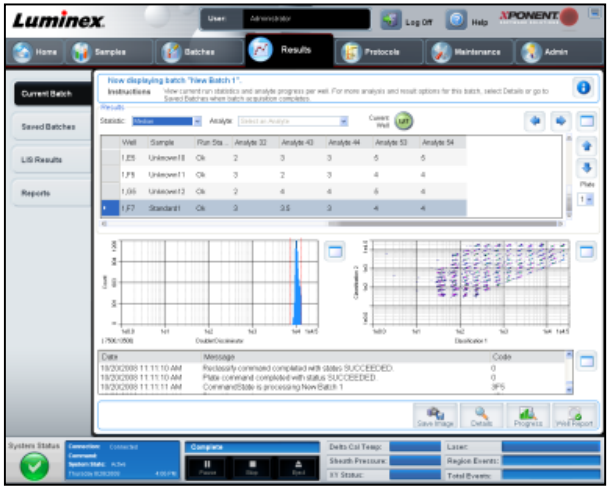
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Luminex® 200偵測原理解說圖

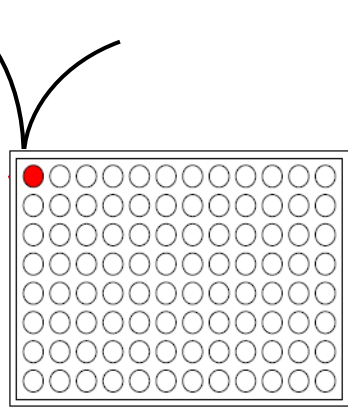


利用Flow Cytometry-based技術使一顆顆微珠通過雷射與檢測器





軟體依照標準品濃度進行帶測分子濃度換算



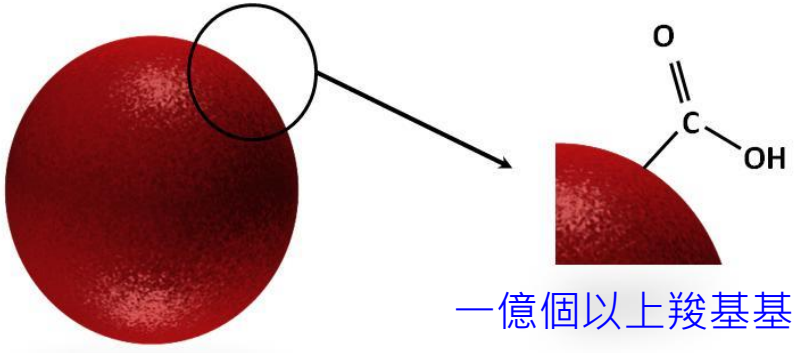
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Luminex xMAP Technology

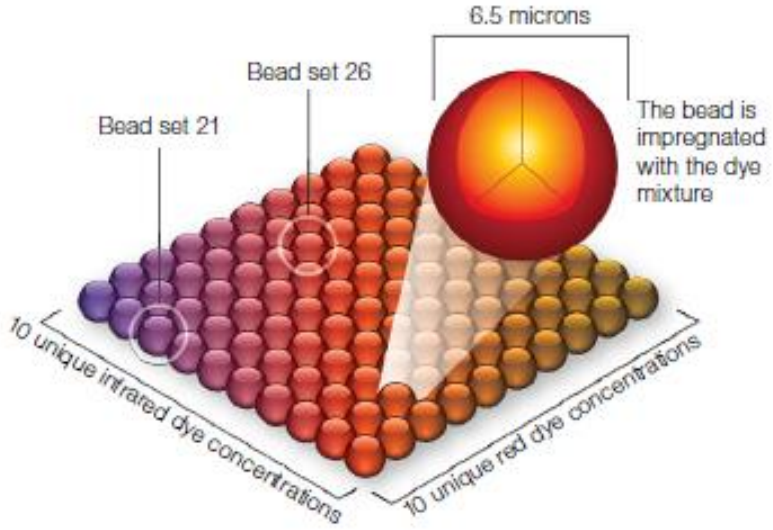
xMAP = Multi-Analyte Profilling

Superparamagnetic particles coated PS beads

MagPlex™ 6.5 µm



一億個以上羧基基團



- 3D 立體結構
- 表面積/體積比大
- 快速液相動力學
- 專利染色技術
- 獨特螢光編碼

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Luminex multiplex assay

- 省時省力, 減少樣本需求量
- 重複數多
  計算至少50個微球的螢光讀值中位數作為數據
  進行統計學分析結果= 50重複！
- 高效率、高靈敏
  液象動力學：碰撞機率增加，靈敏度更優秀！
- 螢光不衰減
  通過雷射後微球以及Reporter螢光才被激發
  螢光強度立即被測定

Luminex multiplex assay

- ProcartaPlex Immunoassays



- MILLIPLEX® Multiplex Assays



ProcartaPlex

產品特色

- **多重偵測技術** 可同時定量100種蛋白質的表現
- **物種多** Human、Mouse、Rat、Porcine、Canine、Non Human Primate
- **結省珍貴檢體** 只需 25ul 。Serum、Plasma、Cell culture supernatant、Cell & tissue lysate 皆適用
- **省荷包** 偵測標的物越多越划算
- **靈敏度高** pg/ml sensitivity
- **產品選擇彈性化** Panel可互相搭配，也提供全客製化組合

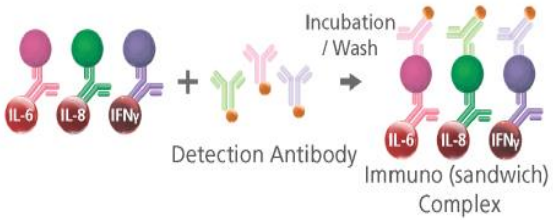
ProcartaPlex workflow



Capture Target Analytes

- Add analyte-specific capture beads coated with target-specific capture antibodies to prepared sample.

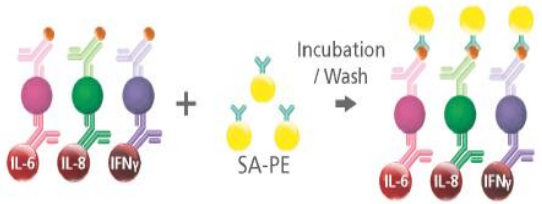
Incubate 1-2 hours and wash



Detect Captured Analyte

- Incubate captured bead/ analytes with biotinylated analyte-specific detection antibody.

Incubate 30 min and wash



Label Detection

- For analyte quantitation, incubate with a fluorescent detection label (SA-PE)
- Wash sample
- Quantitate sample

Incubate 30 min and wash

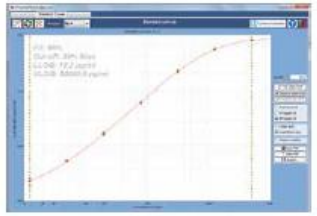


Secreted proteins simultaneously measured using ProcartaPlex Cytokine Assay

Read & Analyze

- Read with Luminex 100/200, Magpix, or Bio-Plex instrument
- Analyze results





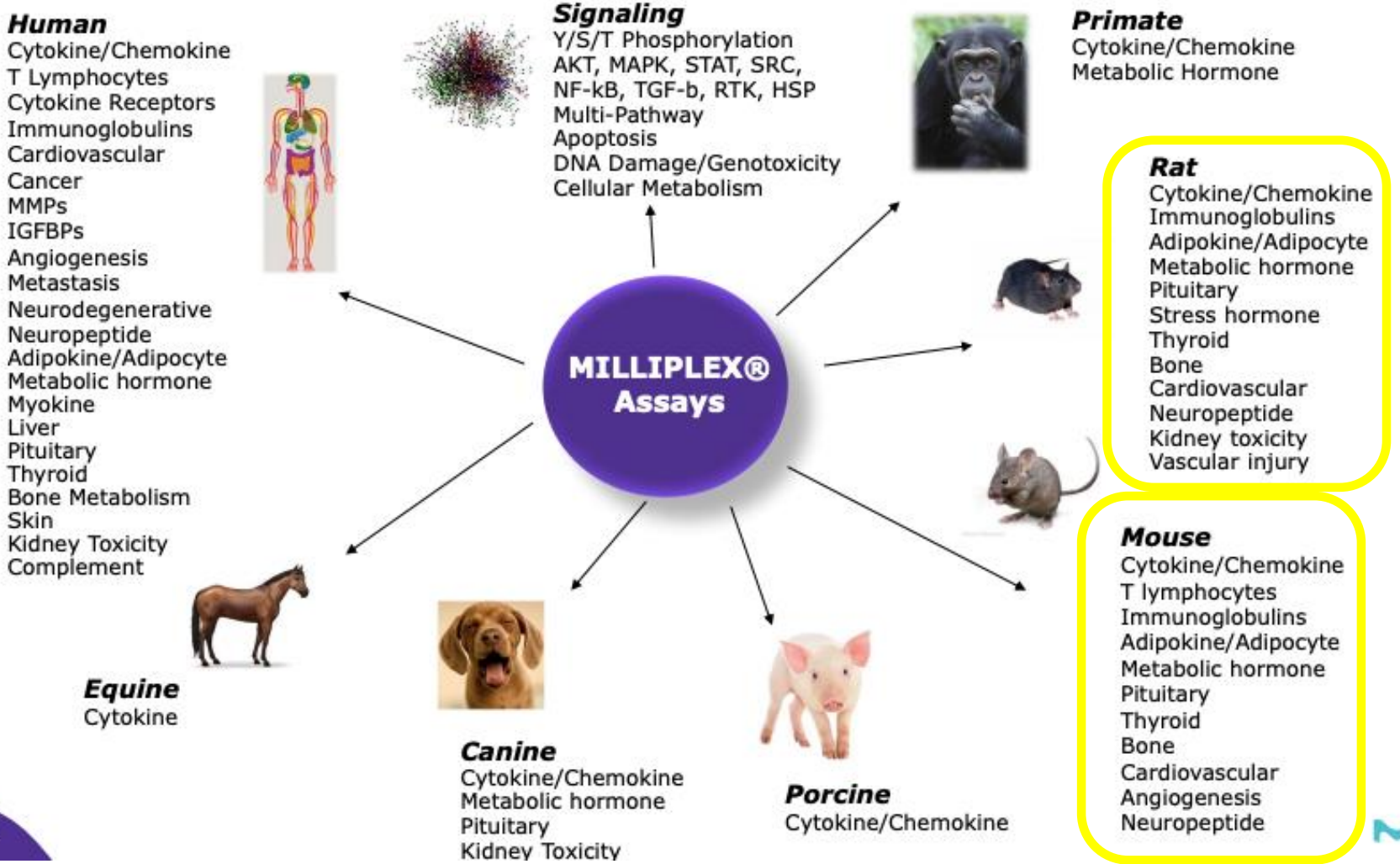
ProcartaPlex Analyte 1.0 Software

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ProcartaPlex product seletion

- **Preconfigured panels**
  原廠預設Panel，針對不同物種的蛋白分類，提供挑選最常用的標的組合而成的產品，共有大於44種組合。
- **Mix & Match**
  客製化產品，任意挑選需要的標的進行預混組合，也解決微球衝突的難題。
- **Simplex Kits**
  單一標的偵測探針，可組合其他單一探針或預設Panel產品，需外加Basic Buffer kit使用，提供大於280種目標探針。
- **High Sensitivity (fg)**
  最新推出其定量偵測 低至fg的產品，解決因為濃度太低無法偵測到樣本蛋白的困擾。

Flexible: The MILLIPLEX portfolio:



MERCK

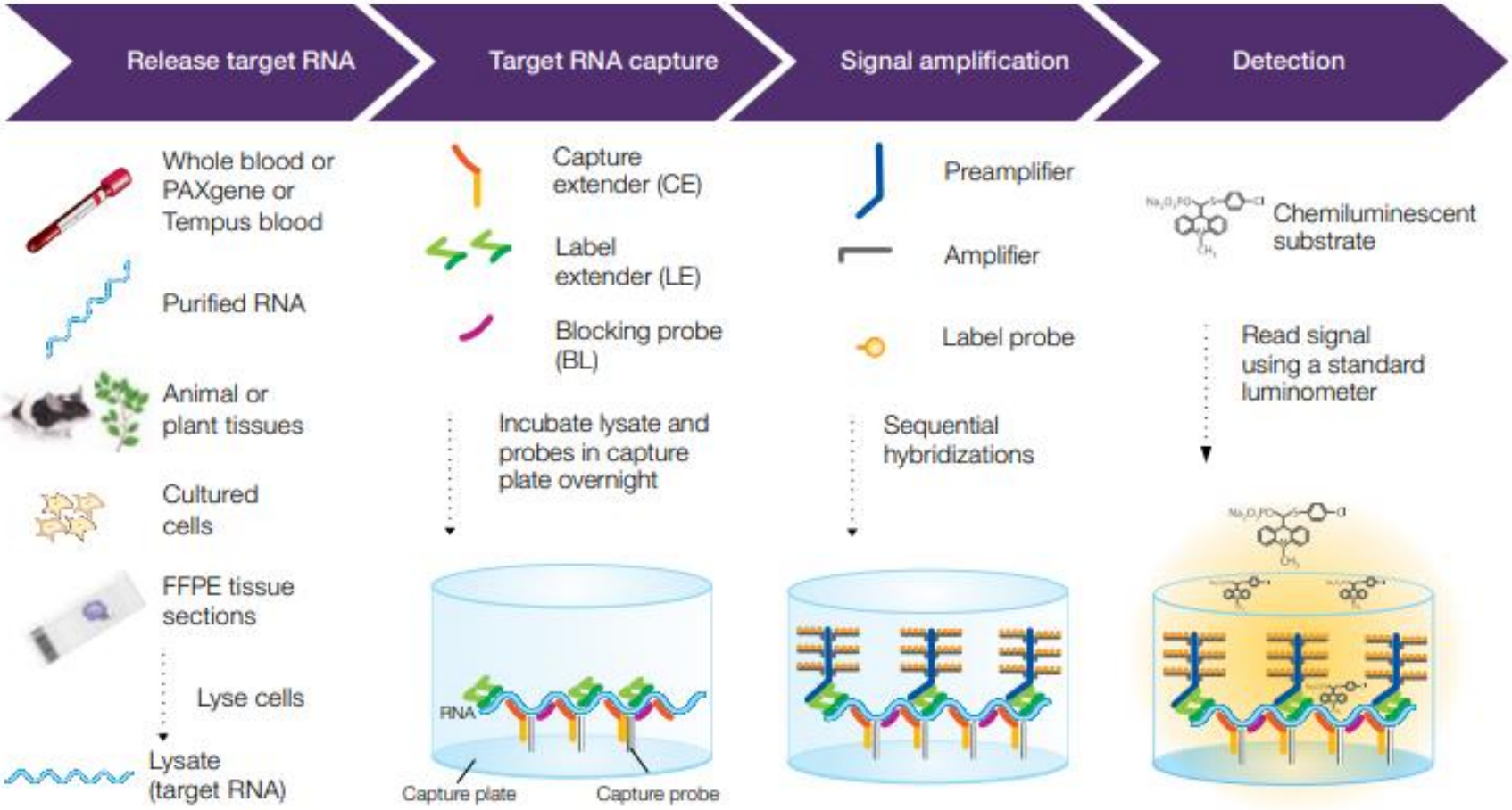
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QuantiGene Plex

直接由樣品偵測定量 miRNA, RNA, DNA

- **Works with difficult sample types**
  適用困難度高的樣本。Ex. Formalin-fixed、FFPE、Whole blood
- **True multiplexing**
  使用多重檢測技術，一個well可檢測 80種RNA、30種DNA
- **Signal vs. target amplification**
  Branched DNA –訊號放大技術
- **Simple workflow**
  No 純化, No 反轉錄, No PCR 擴增反應, 似ELISA方式操作簡便
- **Large inventory of verified genes & Faster customization than other methods**
  超過17,000種基因可任意客製化搭配，亦有>3700 disease-themed panels供選擇

QuantiGene Plex workflow

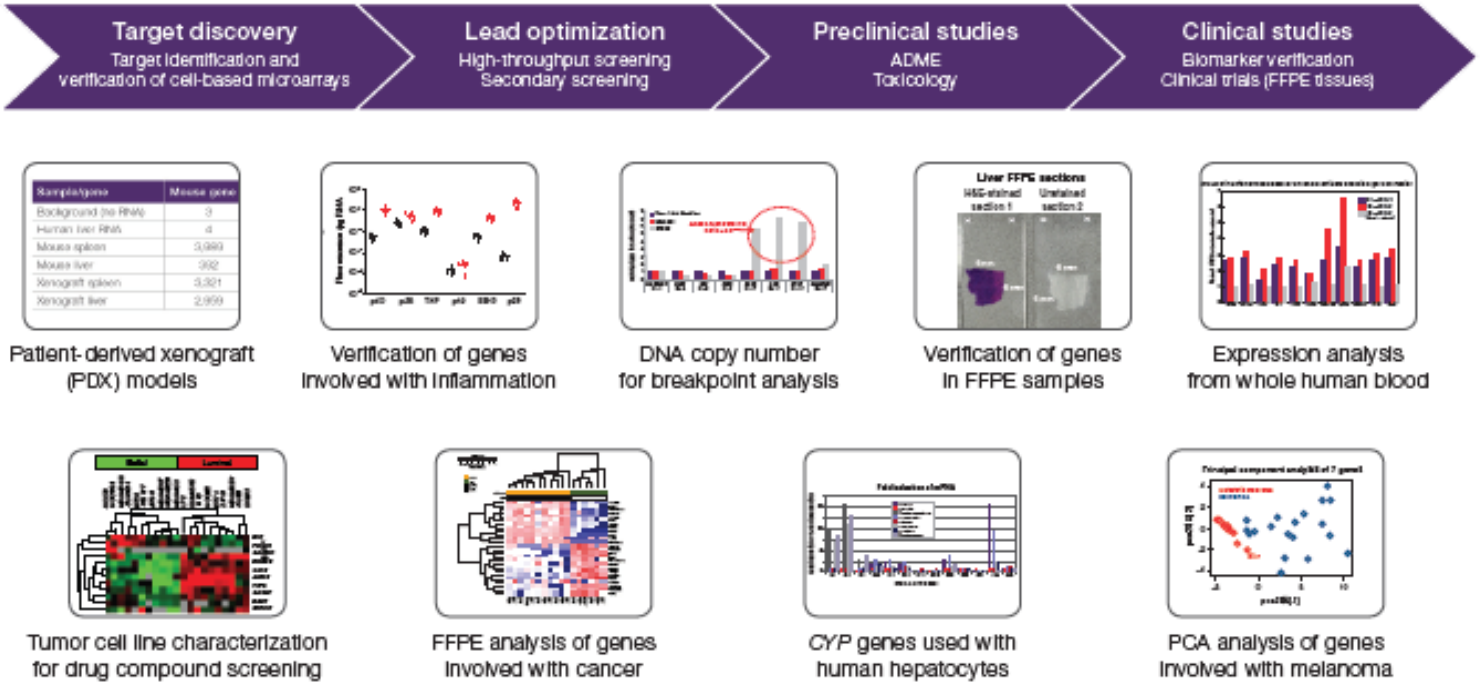


No RNA and DNA isolation
No reverse transcription
No PCR amplification

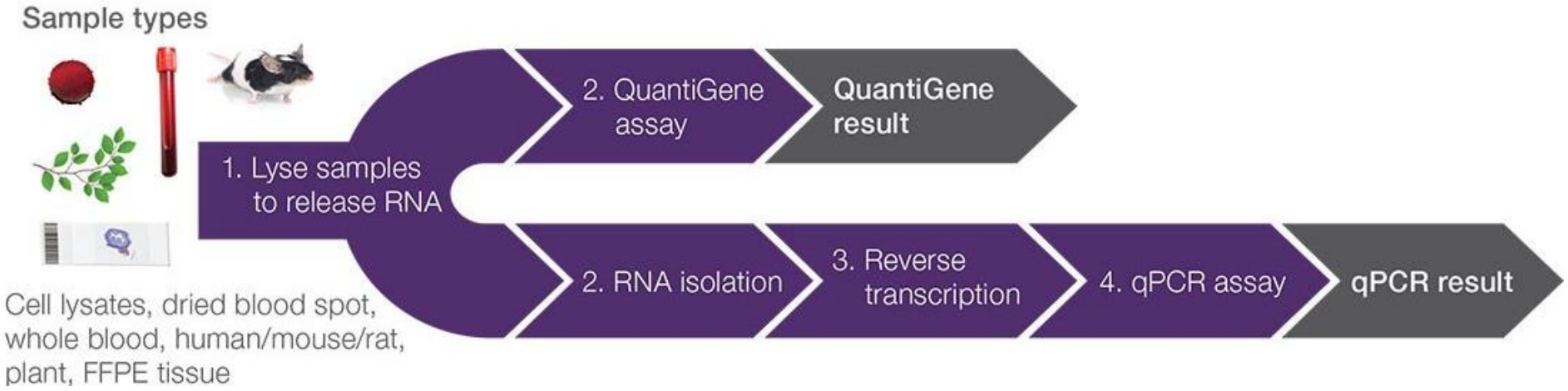
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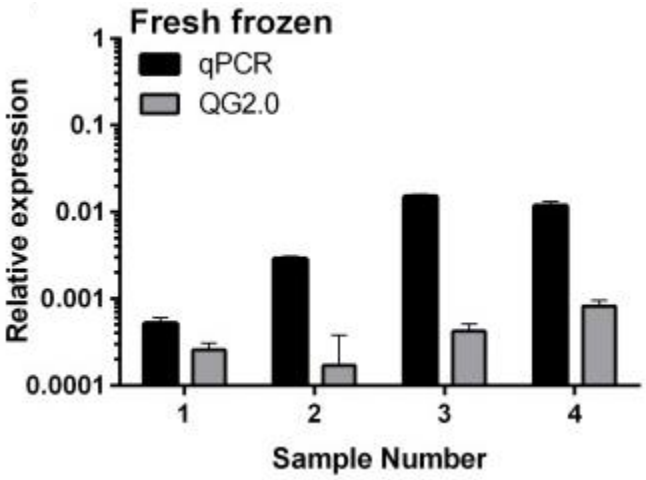
QuantiGene Plex application

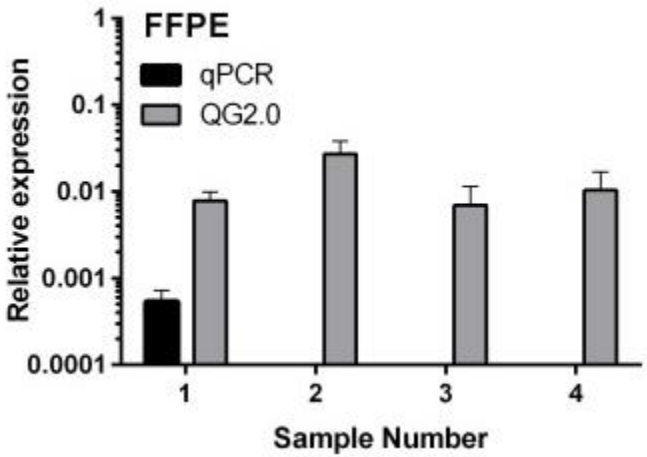
- 確效RNAi 抑制效用/監控off-target效應
- Biomarker 研究
- 第二重標的篩選
- 預測性毒理實驗
- Microarray驗證
- lncRNA 研究
- Xenograft模式研究
- 珍貴臨床樣本研究



QuantiGene Plex Vs. qPCR

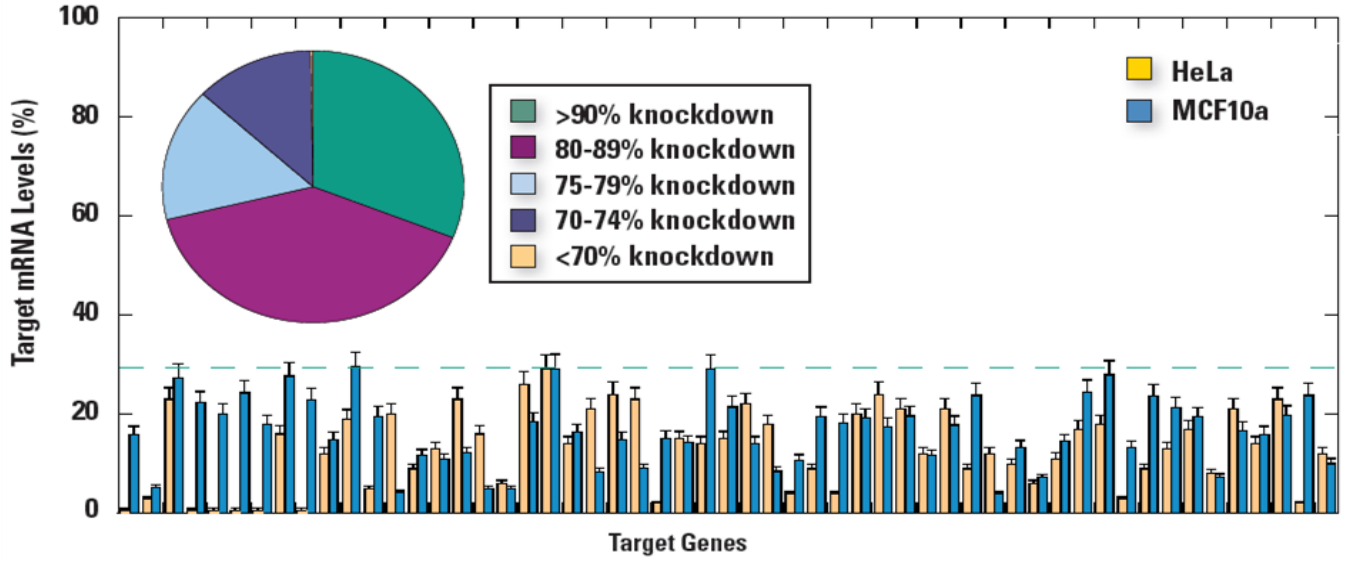






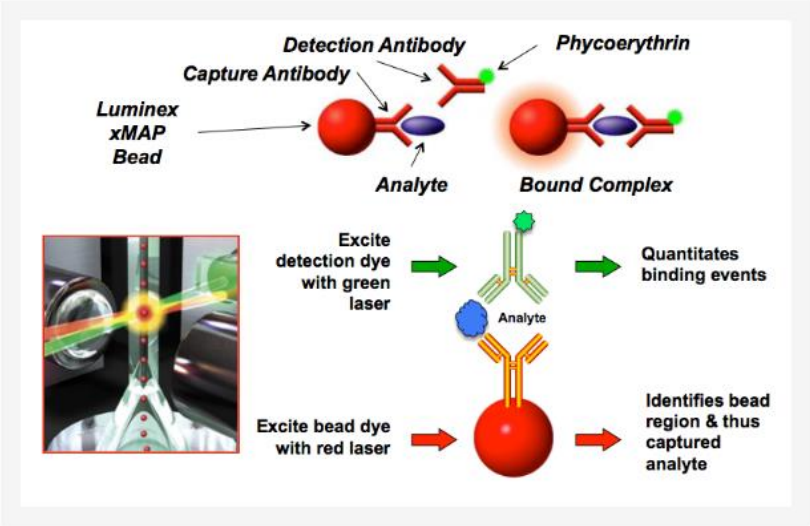
QuantiGene Plex

RNAi silencing application – Dharmacon validation of siRNAs



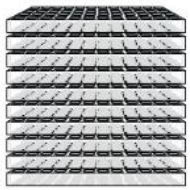
- 為何選擇QuantiGene?
  精準、沒有放大誤差、不需純化、高再現性，可進行多重偵測
  - Dharmacon使用QuantiGene 2.0 Assay驗證siRNA的效率 ，也因此有現成探針可偵測 Dharmacon siRNAs
  - 其他廠商同時也使用QuantiGene產品驗證
    Roche, Pfizer, Alnylam, Rxi, Tekmira, Kirin

xMAP技術應用-Protein detection



10 proteins
40 samples

Example: Human Cytokine kit





	ELISA	xMAP® Technology
Number of plates required		
Total time to result	10 hours	3 hours
Results per plate	80	800
Total sample used per panel	4 ml	50 µl
Internal controls possible?	NO	YES
Dynamic Range	10-2500 pg/ml	1-10,000 pg/ml
Lower limit of detection	~1 pg/ml	~1 pg/ml

LX200 保養校正校驗介紹

儀器組成



分析儀本體

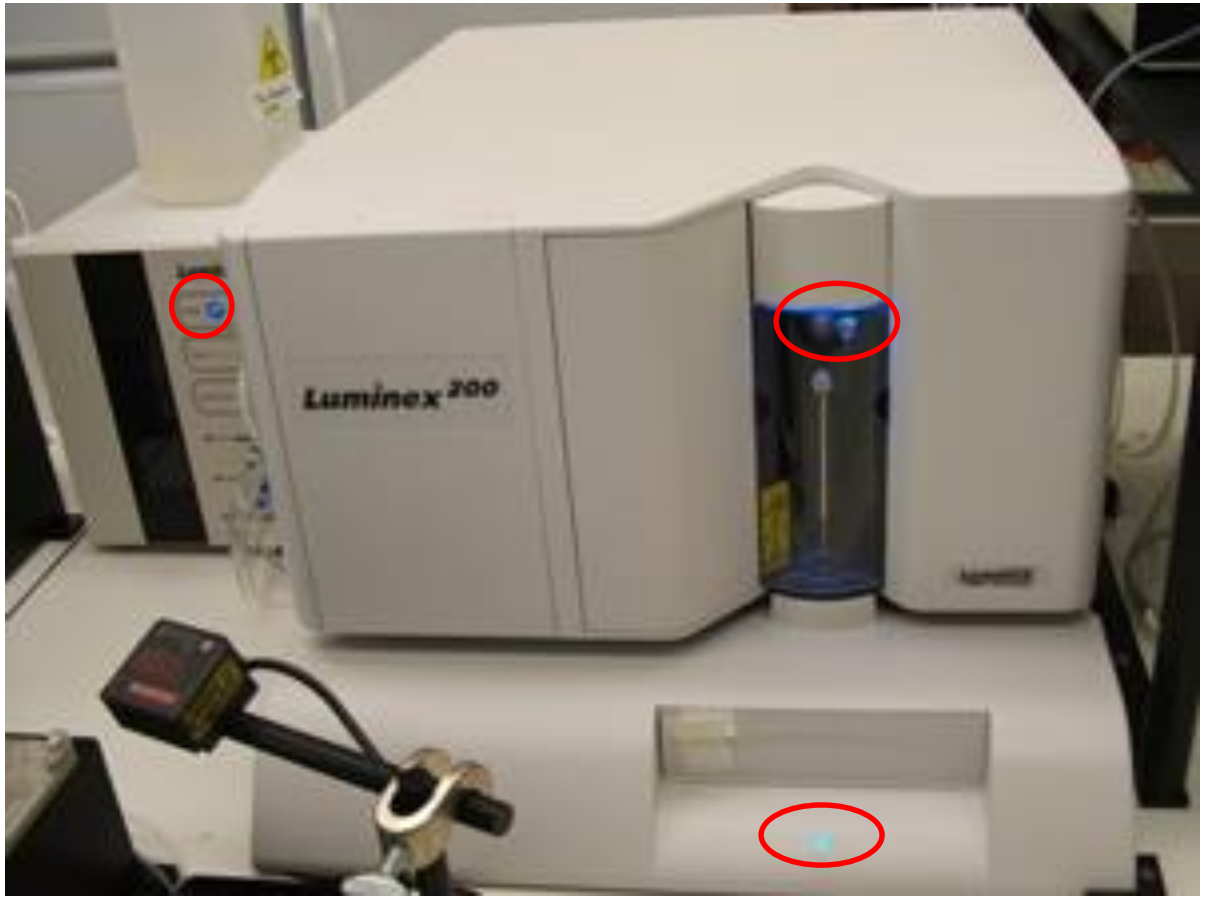
系統水系統

XY 平台

Daily Mantenance

- 開機檢視儀器燈號
- 觀察幫浦、syringe、風扇是否動作
- 執行Daily start up
- Warm up 30分鐘
- 上機
- 執行Daily shut down
- 移除試劑槽內液體
- 關閉軟體、電腦及電源

燈號



Syringe

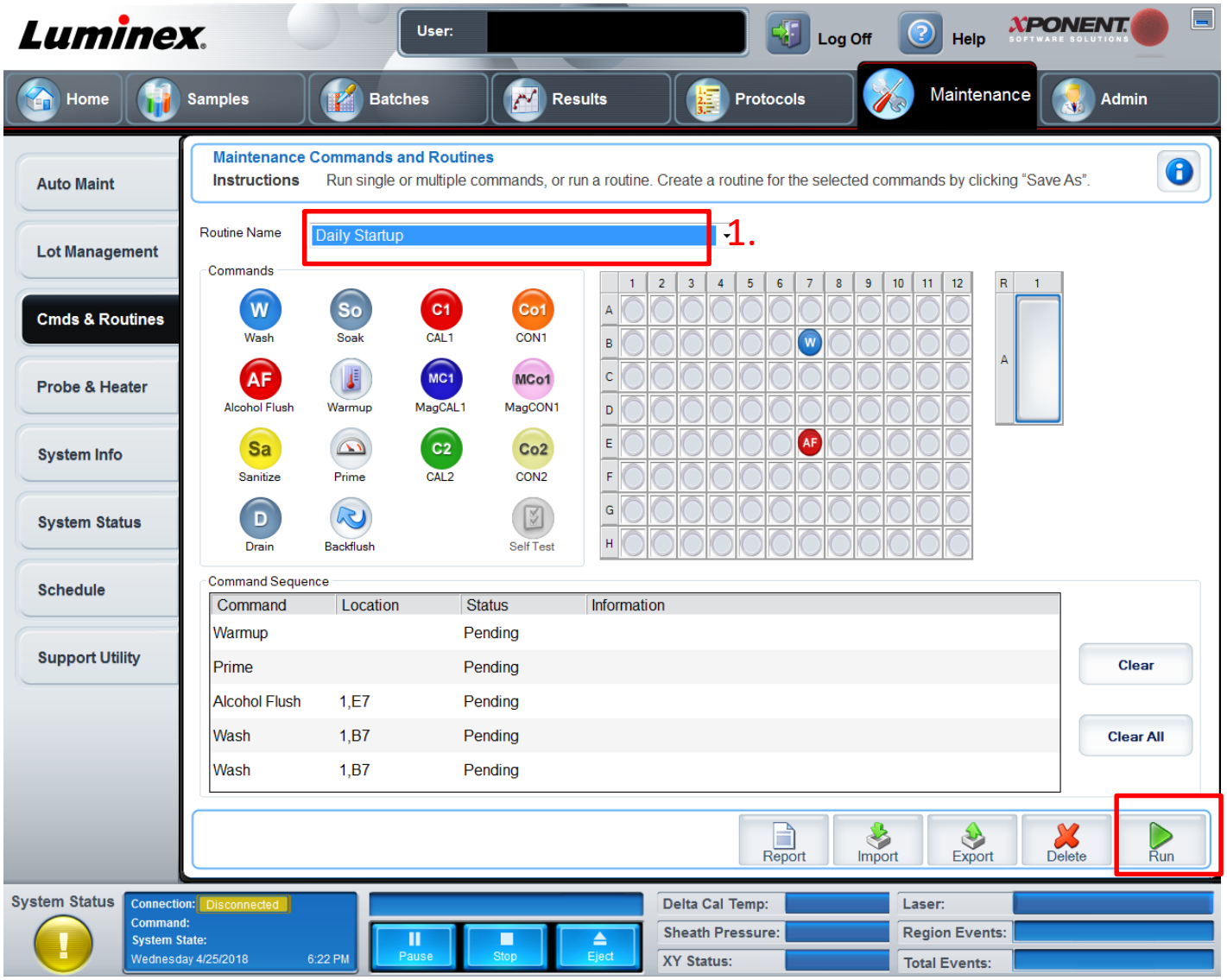


*注意有無氣泡或是漏液

Daily Startup

- 需準備ddH2O, 70%Alcohol
- Automaintenance plate
- 至Maintenance分頁
- 使用Cmds & Routines內下拉選單選擇
- 依螢幕指示放入試劑
- 按下Run

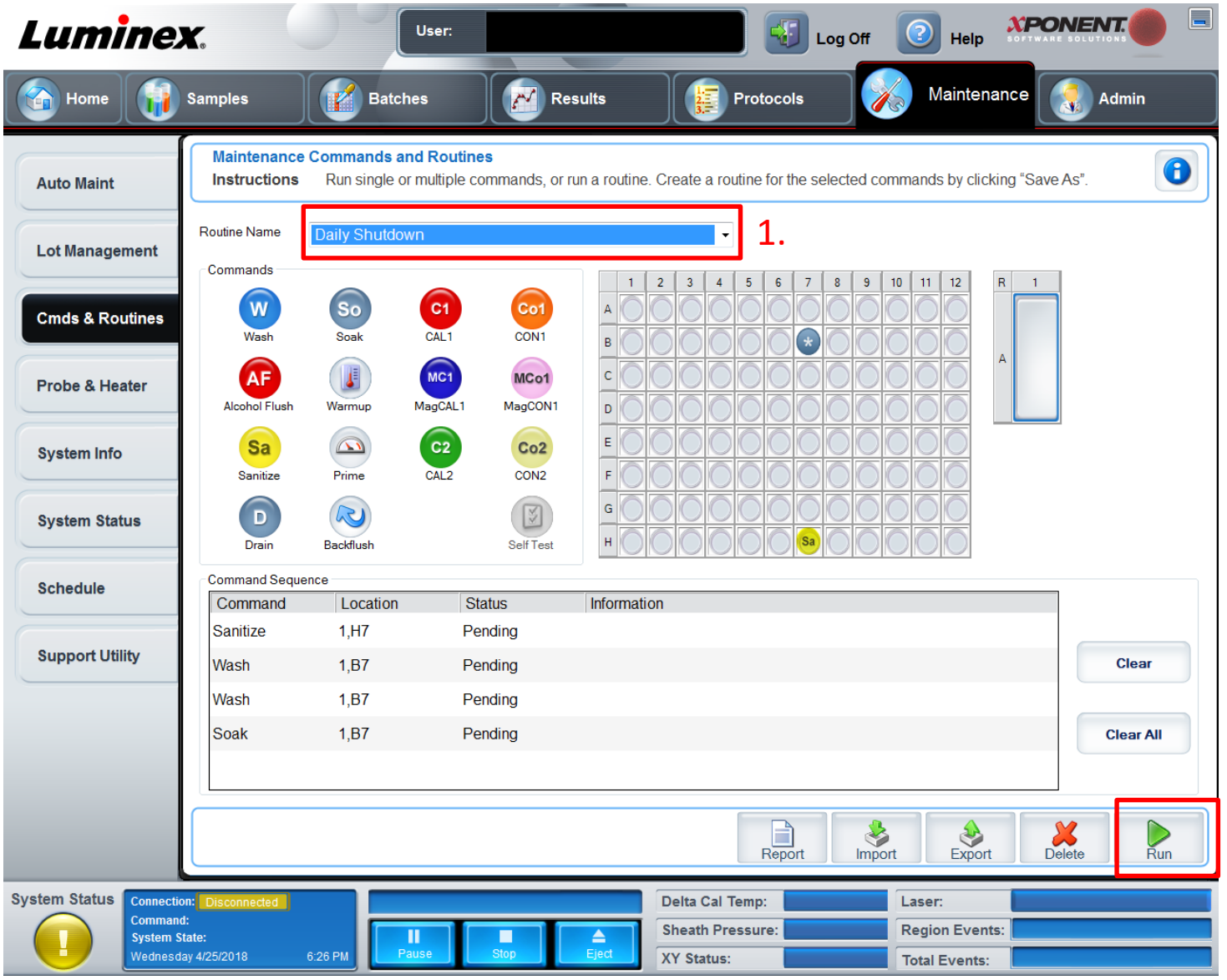
Daily Startup



Daily Shutdown

- 需準備ddH2O, 家用漂白水
- Automaintenance plate
- 至Maintenance分頁
- 使用Cmds & Routines內下拉選單選擇
- 依螢幕指示放入試劑
- 按下Run

Daily Shutdown



Probe Height Adjustment



Standard Flat Bottom	2 Large Discs
Filter Bottom	3 Large Discs
Half Volume Flat Bottom	2 Small Discs
Round or U Bottom	2 Small Discs
Conical	1 Sphere

Calibration Kit

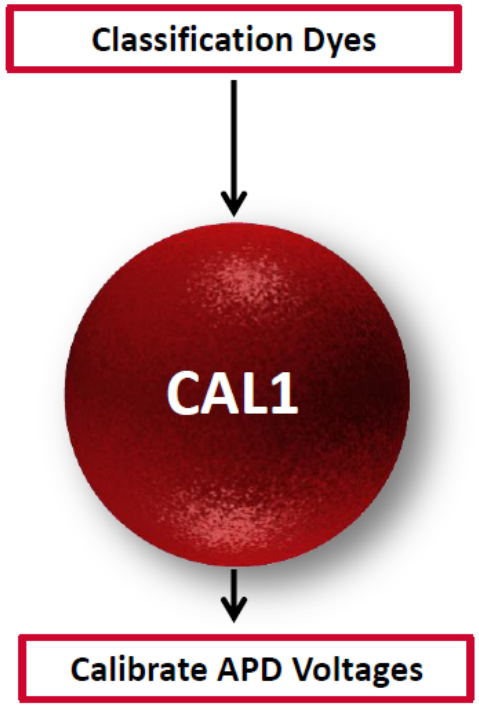


雷射校正與驗證原理

What is CAL1?

- Set of beads with a specific *Target Value* for instrument Calibration
  - »Targets vary from lot to lot
  - »Set CL1, CL2, and DD APD Voltages to classify beads correctly

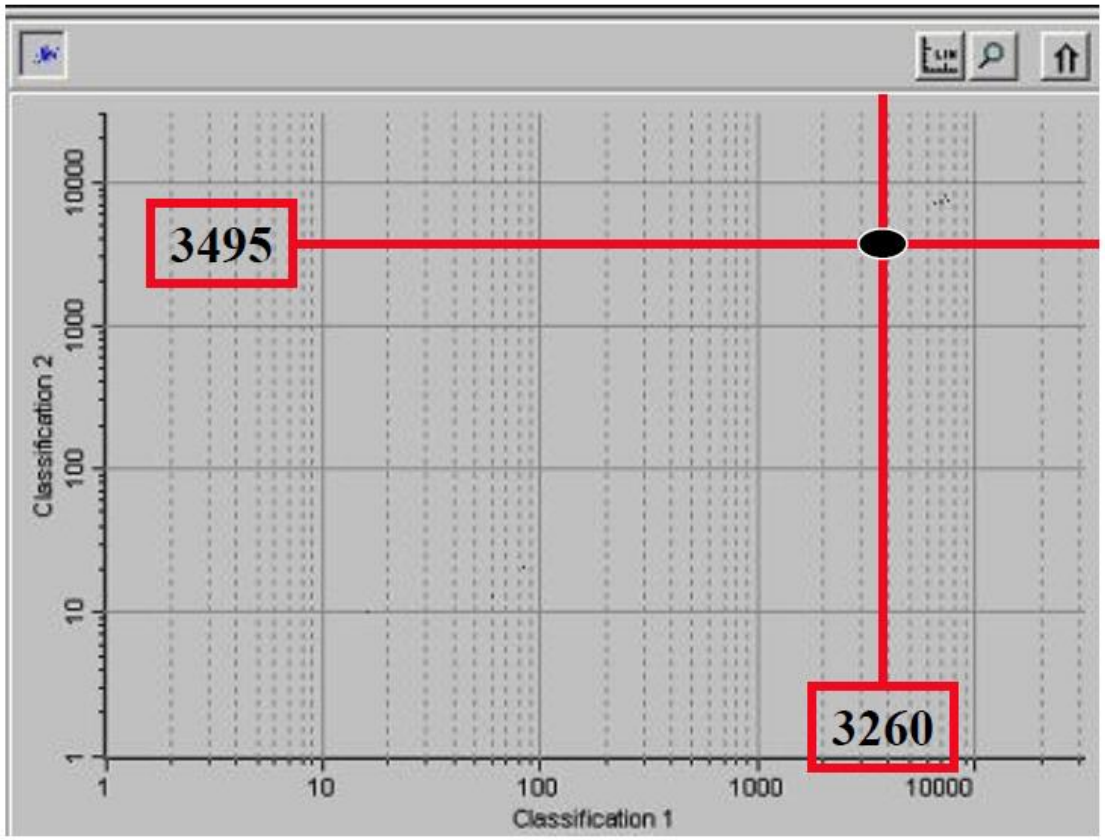




How does calibration work?

- **CL1 target value = 3260**
- **CL2 target value = 3495**

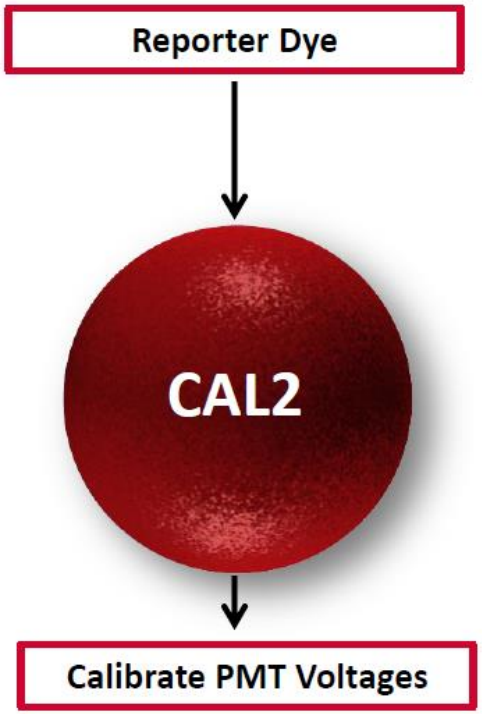
» Voltages for CL1 and CL2 APDs adjust until the beads are at the proper coordinates on the plot



What is CAL2?

- Set of beads with a specific *Target Value* for instrument Calibration
  - »Targets vary from lot to lot
  - »Sets PMT Voltages to accurately report MFI signal

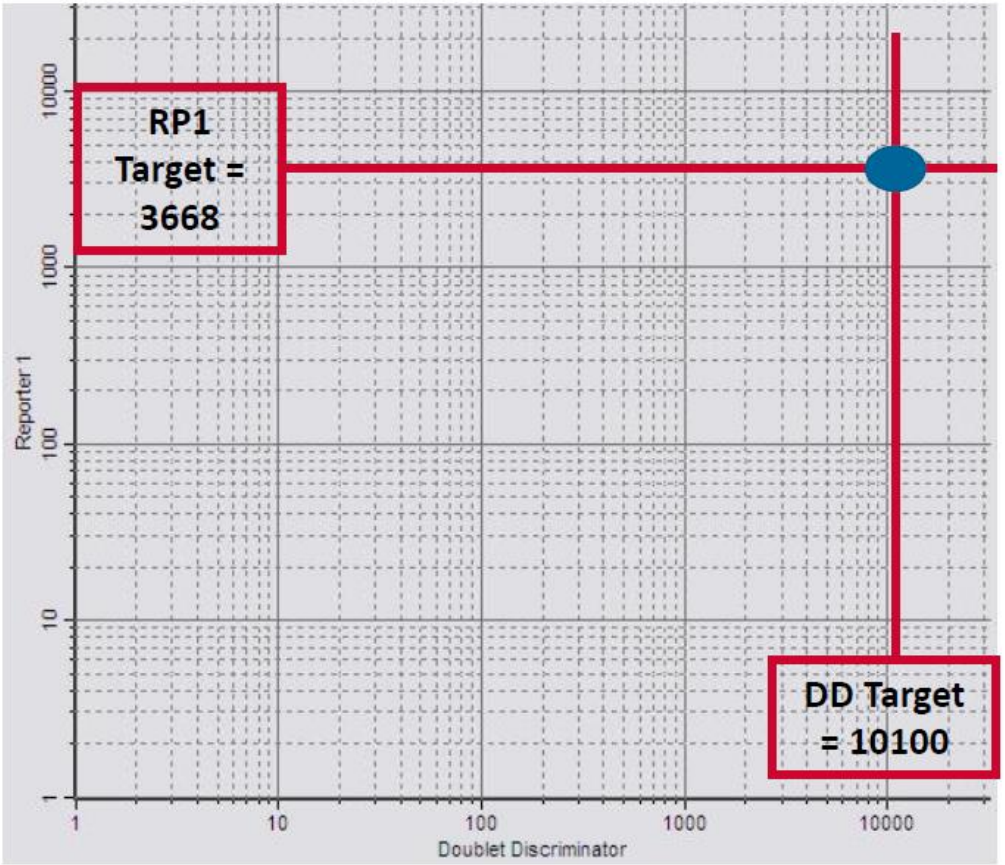




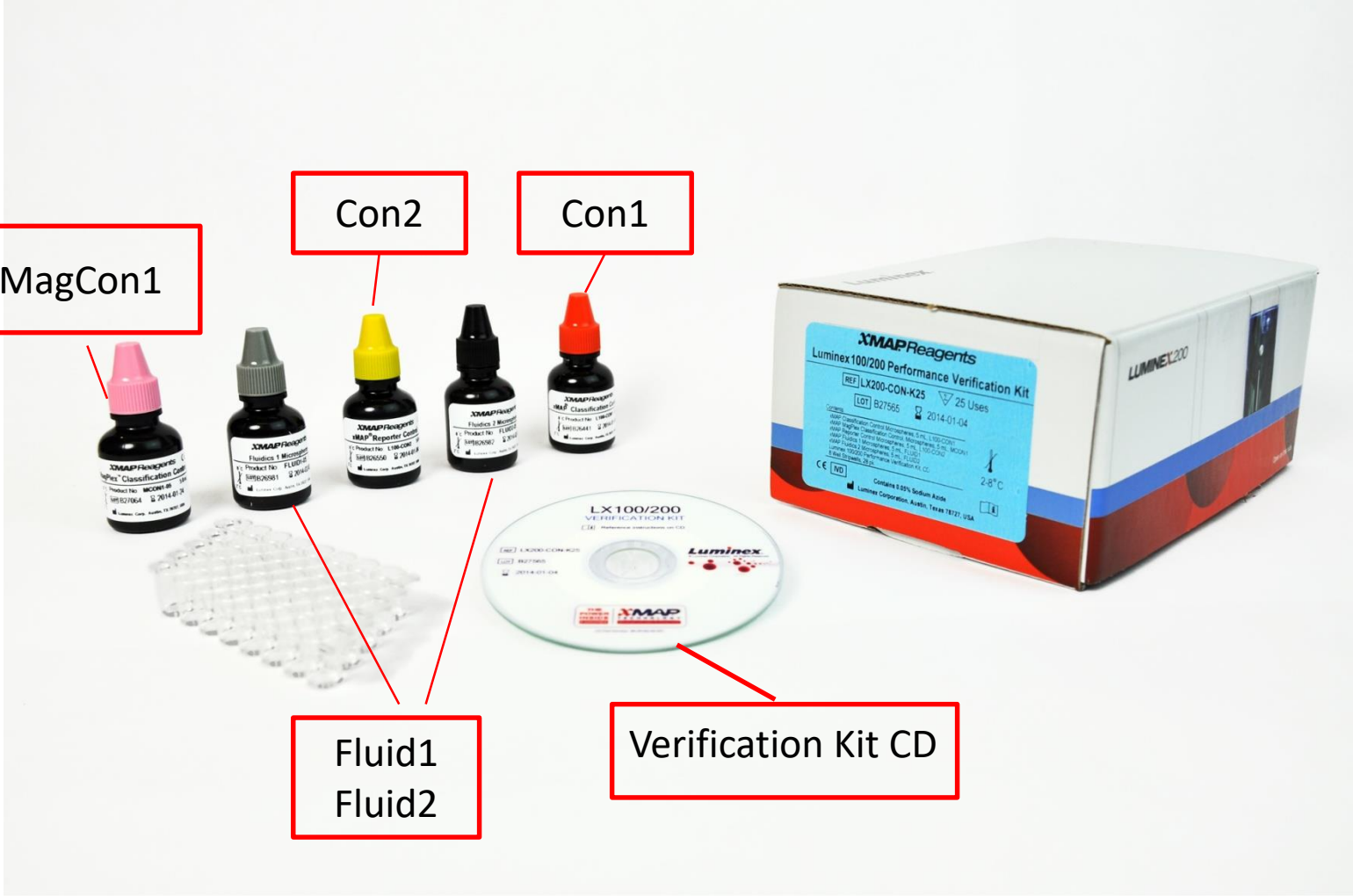
How does calibration work?

- **RP1 target value = 3668**
- **DD target value = 10100**

» Voltages for PMT adjust until the beads are at the proper coordinates on the plot (DD vs. RP1)

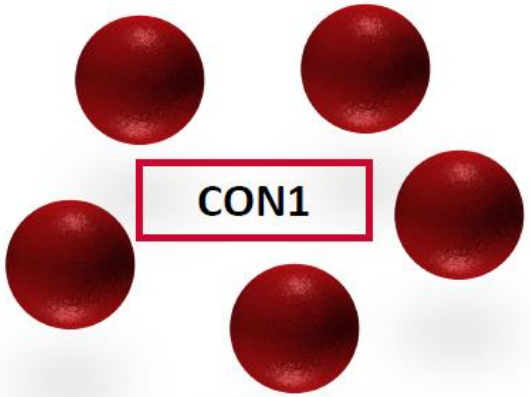


Performance Verification Kit



What is CON1?

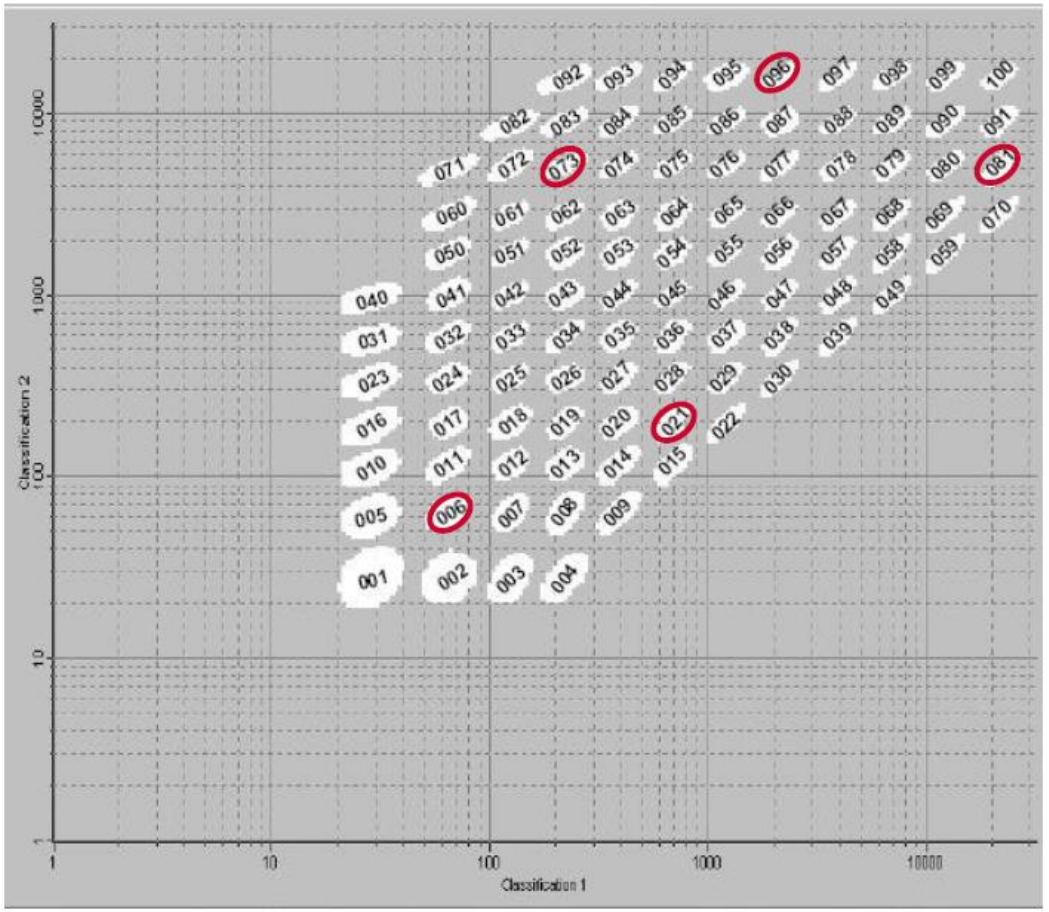
- 5 separate bead regions from the 100 region bead map
  - »Tests integrity of optical system
  - »Ensures proper calibration of the APD voltages
    - *Note: CON1 can be run as an assay if problems with the optics are suspected.*





How does verification work?

- **Checks that the beads fall within their respective regions on the bead map**
  - » **Mean & Scatter of each population (CVs)**
  - » Regions
    - *6, 21, 73, 81, 96*



What is CON2?

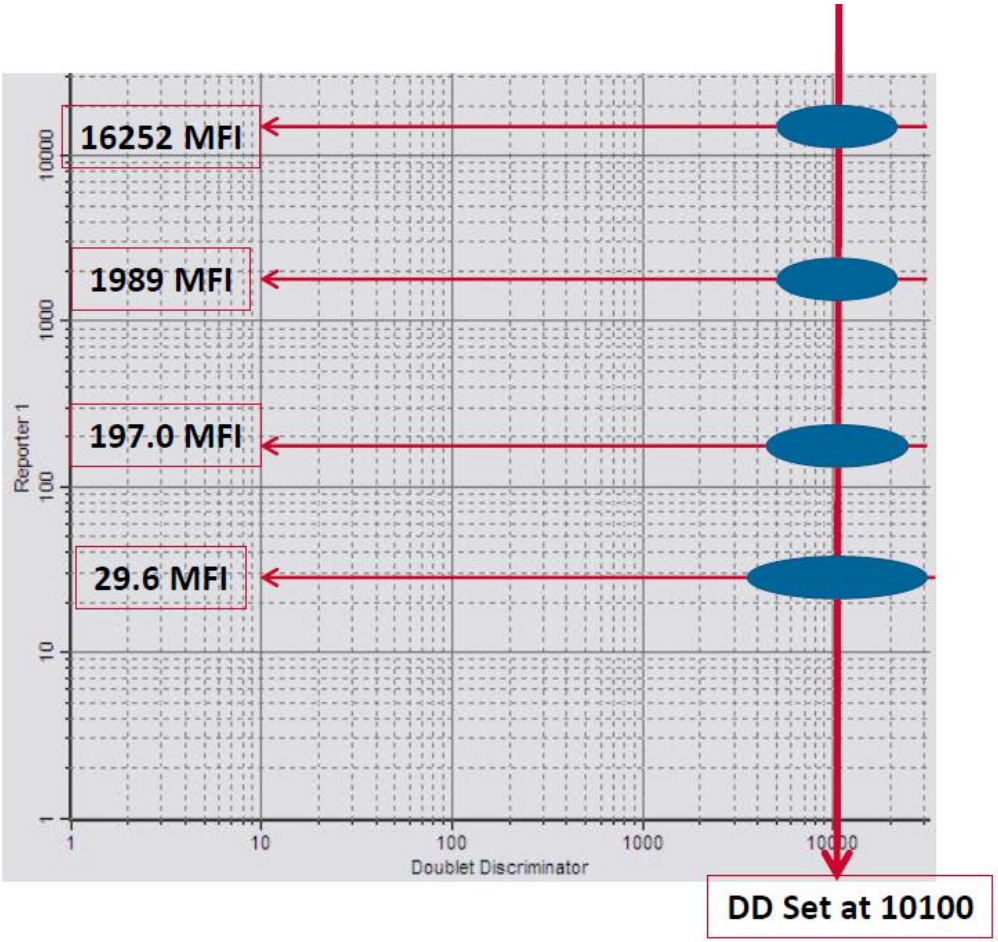
- 4 beads dyed with different reporter concentrations
  - »Tests integrity of optical system
  - »Ensures instrument calibrated voltages of the PMT properly
    - *Note: CON2 beads do not belong to regions of the 100 region bead map*



How does verification work?

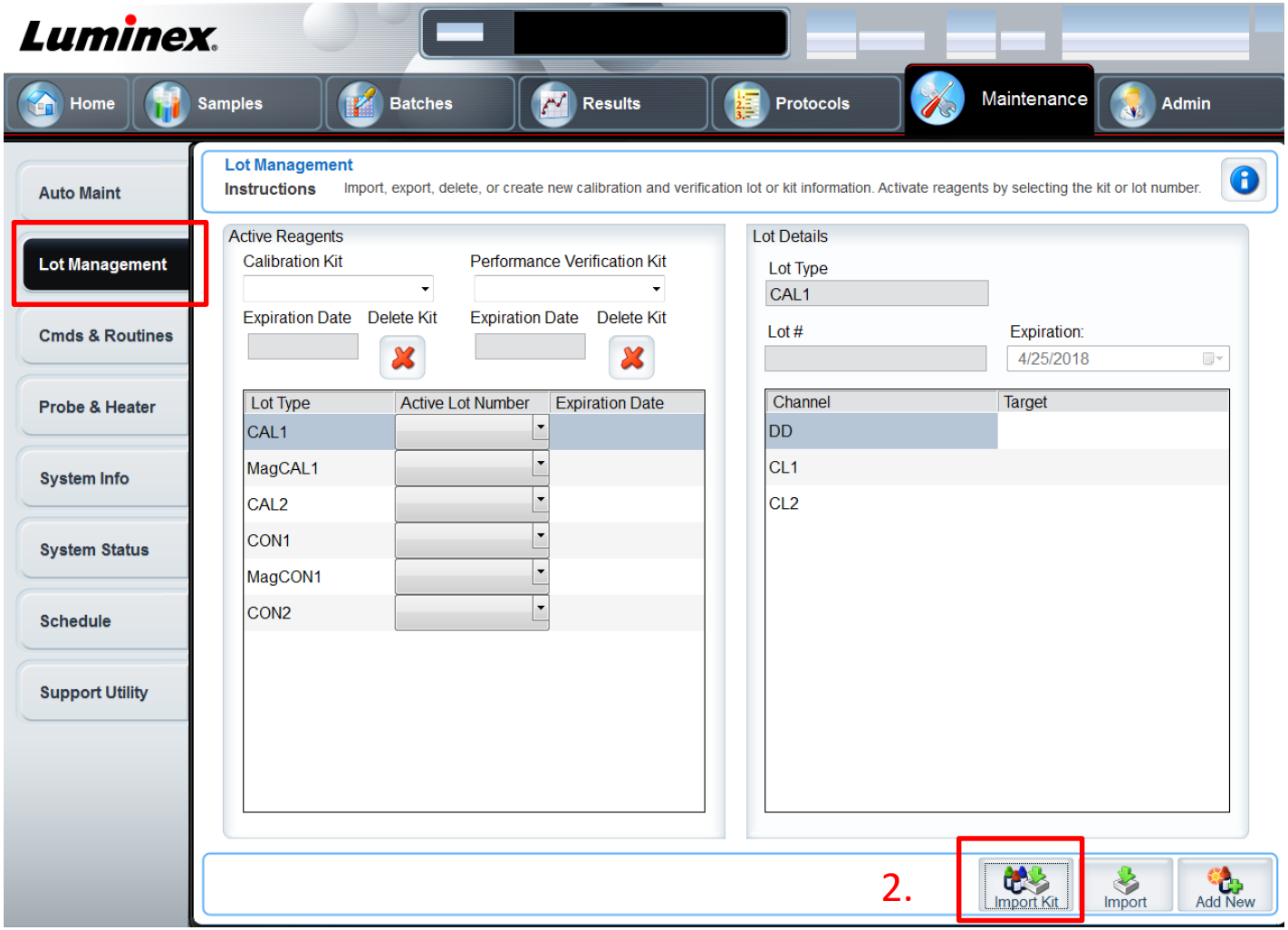
- Each “region” has an MFI value according to the lot information of CON2.

  » Example Lot Info
  - *Reference 1:* ***29.6***
  - *Reference 2:* ***197.0***
  - *Reference 3:* ***1989***
  - *Reference 4:* ***16252***



Cali&Veri數值輸入

- 放入光碟
- 至Lot Management 點選匯入>選擇光碟的檔案
- 可至Auto Main或至Cmds & Routines單獨選擇要執行的試劑

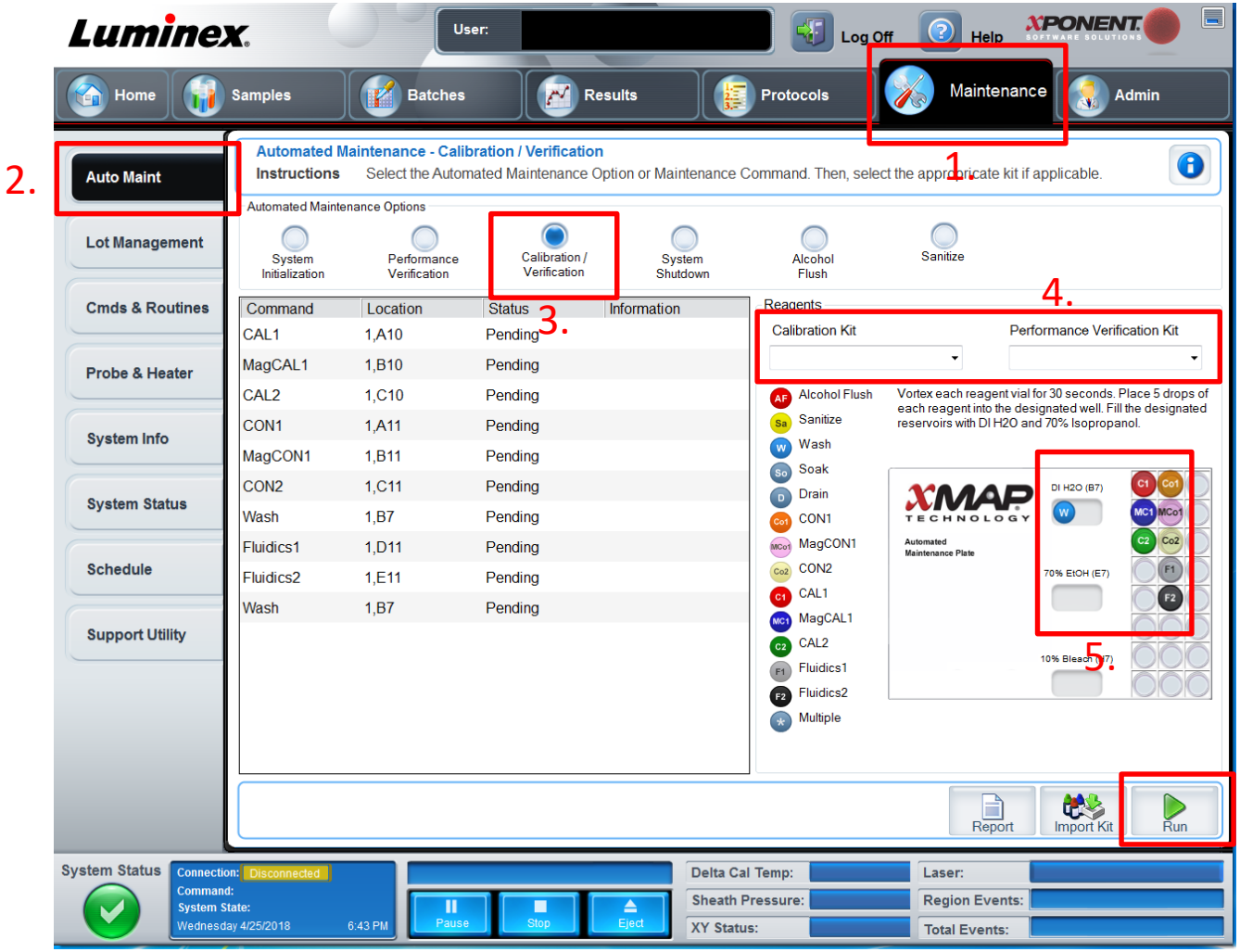

Luminex
Home
Samples
Batches
Results
Protocols
Maintenance
Admin
Auto Maint
Lot Management
Cmds & Routines
Probe & Heater
System Info
System Status
Schedule
Support Utility
Lot Management
Instructions
Import, export, delete, or create new calibration and verification lot or kit information. Activate reagents by selecting the kit or lot number.
Active Reagents
Calibration Kit
Performance Verification Kit
Expiration Date
Delete Kit
Expiration Date
Delete Kit
Lot Type
Active Lot Number
Expiration Date
CAL1
MagCAL1
CAL2
CON1
MagCON1
CON2
Lot Details
Lot Type
CAL1
Lot #
Expiration:
4/25/2018
Channel
Target
DD
CL1
CL2
Import Kit
Import
Add New

1.

2.

進行Calibration&Verification

- 將kit回溫至室溫
- 至Maintenance→AutoMain →Calivbration/Verification
- 確認所選批號與所拿批號相符
- Vortex 20sec
- 依照軟體指示位置放入水及滴5滴校正液或校驗液到strip孔位內
- 按下run


Luminex
User:
Log Off
Help
XPONENT
Home
Samples
Batches
Results
Protocols
Maintenance
Admin
1.
2.
Auto Maint
Lot Management
Cmds & Routines
Probe & Heater
System Info
System Status
Schedule
Support Utility
Automated Maintenance - Calibration / Verification
Instructions
Select the Automated Maintenance Option or Maintenance Command. Then, select the appropriate kit if applicable.
Automated Maintenance Options
System Initialization
Performance Verification
Calibration / Verification
System Shutdown
Alcohol Flush
Sanitize
3.
Command
Location
Status
Information
CAL1 1,A10 Pending
MagCAL1 1,B10 Pending
CAL2 1,C10 Pending
CON1 1,A11 Pending
MagCON1 1,B11 Pending
CON2 1,C11 Pending
Wash 1,B7 Pending
Fluidics1 1,D11 Pending
Fluidics2 1,E11 Pending
Wash 1,B7 Pending
4.
Reagents
Calibration Kit
Performance Verification Kit
Alcohol Flush
Sanitize
Wash
Soak
Drain
CON1
MagCON1
CON2
CAL1
MagCAL1
CAL2
Fluidics1
Fluidics2
Multiple
Vortex each reagent vial for 30 seconds. Place 5 drops of each reagent into the designated well. Fill the designated reservoirs with DI H2O and 70% Isopropanol.
XMAP TECHNOLOGY
Automated Maintenance Plate
DI H2O (B7)
70% EtOH (E7)
10% Bleach (A7)
5.
Report
Import Kit
Run
6.
System Status
Connection: Disconnected
Command:
System State:
Wednesday 4/25/2018 6:43 PM
Pause
Stop
Eject
Delta Cal Temp:
Sheath Pressure:
XY Status:
Laser:
Region Events:
Total Events:

常見故障排除

- 開啟後電源指示燈不亮

-檢查電源線與保險絲，若無法排除請聯絡工程師。

- 軟體沒有連上儀器

-重啟電腦與儀器，若無法排除請聯絡工程師。

- 壓力不足

-檢查管線是否漏氣或是幫浦有無運作，若無法排除請聯絡工程師。

常見故障排除

- 壓力過高

-廢液管是否連接妥當或是探針使否堵塞。

- 液體洩漏

-Syringe密封圈洩漏或是電磁閥故障，請聯絡工程師。

- 檢體探針卡住

-移除上方LED蓋，取出探針，重新調整高度

常見故障排除

- 校正或驗證失敗

-檢查每秒顆數是否有達到250 顆，檢查探針高度，再校正與驗證一次。若無法排除請聯絡工程師。