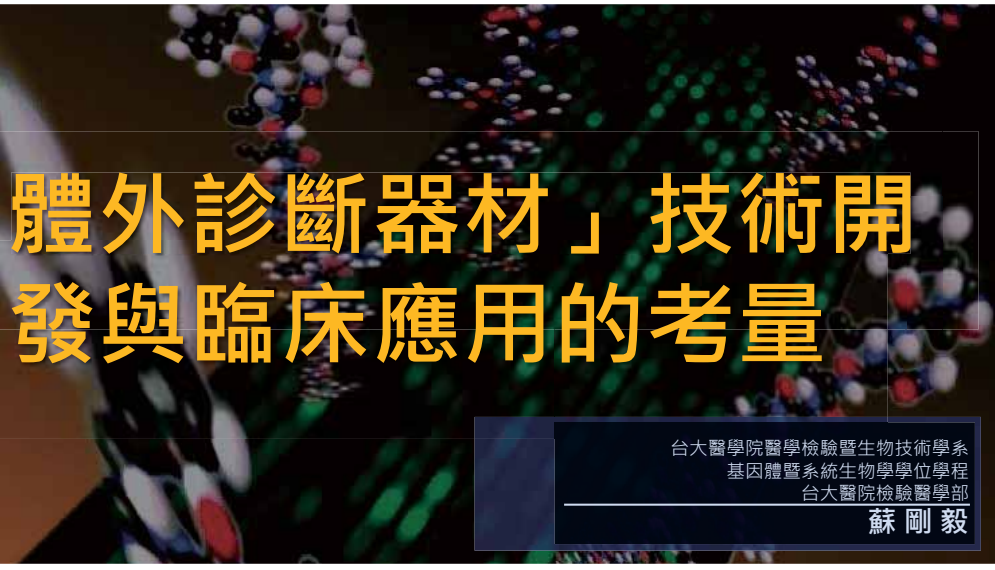


體外診斷器材」技術開發與臨床應用的考量

台大醫學院醫學檢驗暨生物技術學系
基因體暨系統生物學學位學程
台大醫院檢驗醫學部
蘇剛毅

大綱

- 生物晶片醫學上的應用
- 生物晶片技術開發與創新
- 臨床未被滿足之需求與考量
- 體外診斷器開發與確效
- 法規查驗登記與試驗

生物晶片醫學上的應用

生物晶片的應用

- **應用：**致病基因探尋、基因調控、蛋白質功能研究 、新藥開發、法學檢定、軍事偵防等及單一核甘酸多型性(SNP, single nucleotide polymorphism)等 。
- **新藥開發：**找到人體生病細胞上的蛋白質，再開發有效的藥物，以提升新藥開發速度。
- **疾病臨床檢驗：**應用在病人檢體之細菌、病毒、寄生疾病的檢驗。
- **醫療診斷：**病人檢體中萃取核酸，再將目標核酸放大(Amplification)、雜交進而獲得結果
- **親子鑑定：**因此利用生物晶片可作DNA順序(Sequence)檢定
- **環境與食品檢驗：**檢測食物是否受到某種微生物或毒物污染。

生物晶片的優點

- 所需的樣本量極微小
- 分離與分析的操作平行化
- 儀器技術的整合
- 降低製造成本
- 減少試劑用量
- 減低操作成本
- 縮短檢測時間
- 可攜帶

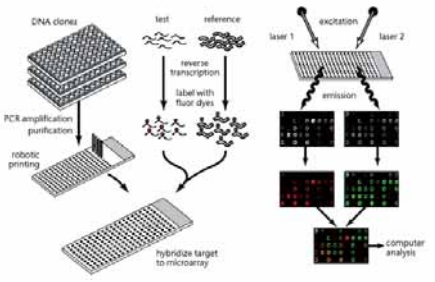
缺少上述優點的生物晶片將失去市場競爭力

生物晶片應用領域

生物晶片產業市場2001-2020 應用領域 市場預估值 (百萬美元)						
應用領域	2001	2002	2005	2010	2015	2020
Biomedical/Gene Research	801	1118	3081	6820	14560	20090
Disease Treatment/Management	27	52	234	1430	3640	6650
Pharmacogenomics	9	13	78	660	1820	3690
Diagnostics/Testing	54	104	390	1760	4420	8200
Agricultural Biotechnology	0	0	39	110	260	410
Environmental Industries	9	13	39	220	520	820
Forensics & Military	0	0	39	110	520	410
Others	0	0	0	0	260	410
Total	900	1300	3900	11110	26000	40680
Source: Helmut Kaiser Consultancy						

生物晶片的演進

物理性雜交



化學性反應

系統性實驗過程

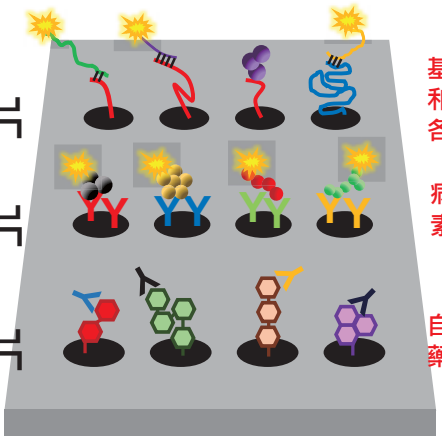
Lab on chip

微陣列晶片種類

核酸晶片

蛋白晶片

抗體晶片

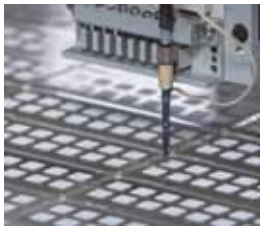


基因突變、基因表現量、單一和苷酸多型性、基因拷貝數、各種基因型鑑定。

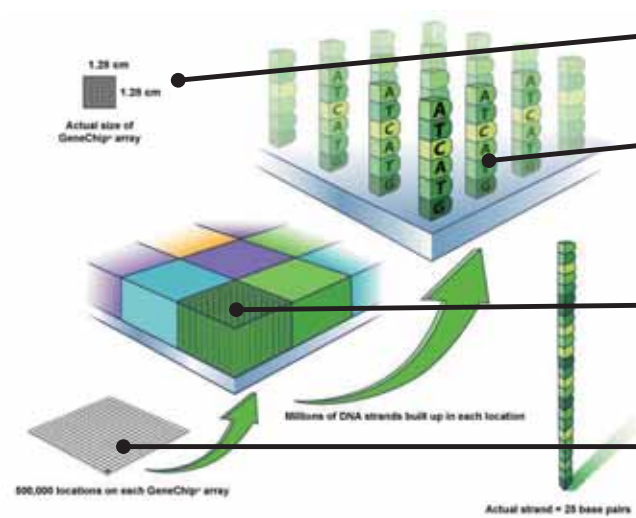
病源抗原鑑、賀爾蒙、細胞激素、環境感染源鑑定。

自體免疫抗體、感染源抗體、藥物抗藥鑑定。

狹義的生物晶片
微陣列晶片如何運作



cDNA微陣列晶片構造



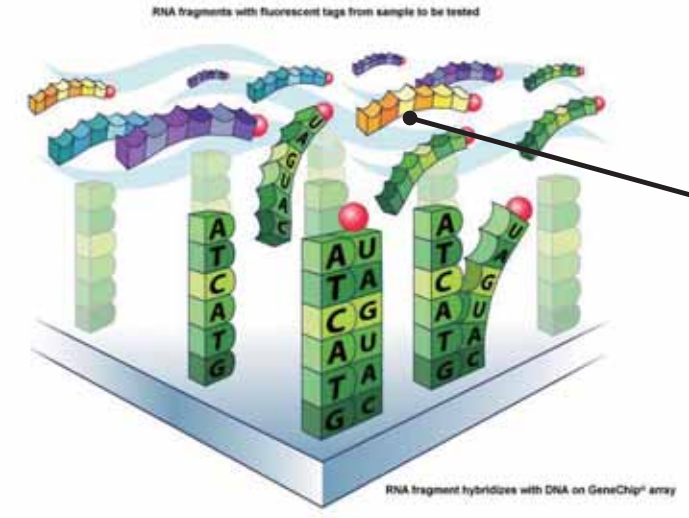
晶片

核酸探針

特定基因探針群

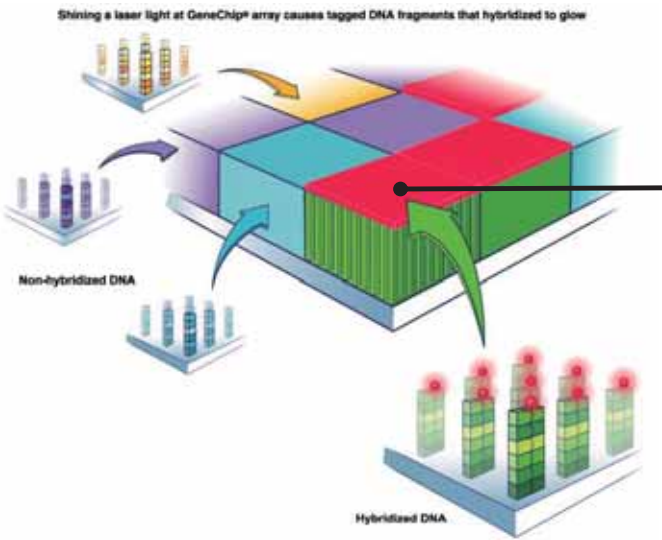
微陣列探針群
(~50000)

cDNA微陣列晶片運作



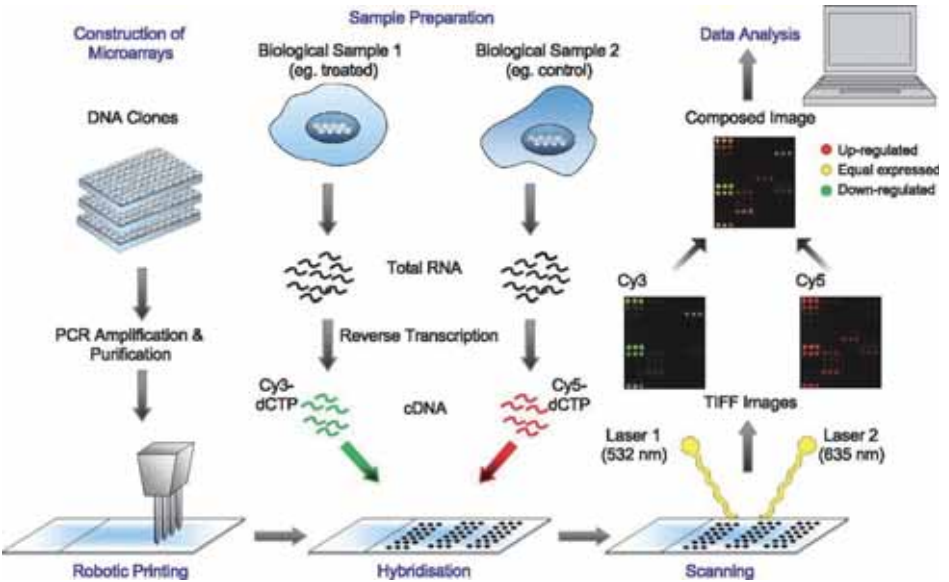
具冷光/螢光標記的樣本
(反應出基因表現的核酸量)

cDNA微陣列晶片偵測



雜交後具有冷光的探針叢
(代表樣本之基因有表現)

微陣列晶片探討基因表現差異

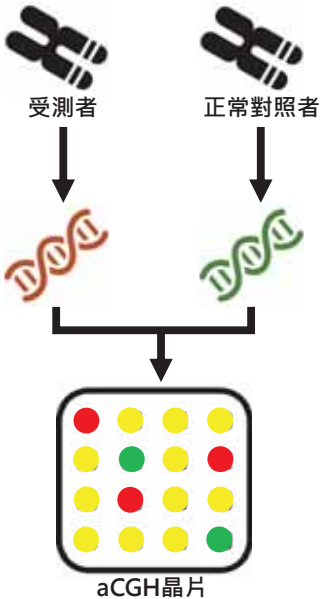


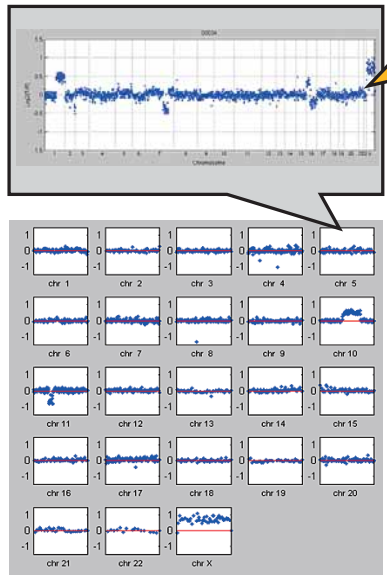
利用aCGH晶片分析染色體異常

染色體DNA萃取

染色體DNA隨機片斷化後標記螢光

與帶有染色體區段探針(門牌)的aCGH晶片雜交





將受測者與對照者的螢光強度進行比較

可得知受測者在23條染色體的何處變多或是缺少

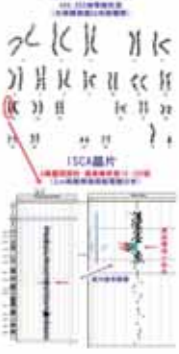
aCGH, array comparative genomic hybridization

aCGH應用


高齡產婦 10年增5倍 物羊水驗基因 防生缺陷兒

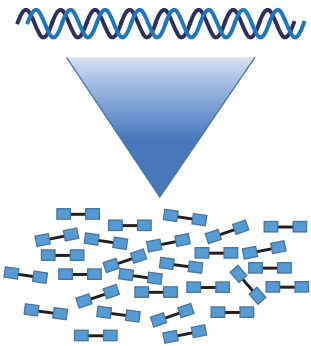

YAHOO! 奇摩

懷孕前需做檢查　可做遺傳諮詢與篩檢



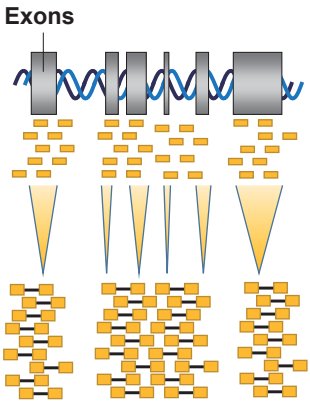

新一代定序儀
Next-generation Sequencer

定序-多少資訊、甚麼資訊?



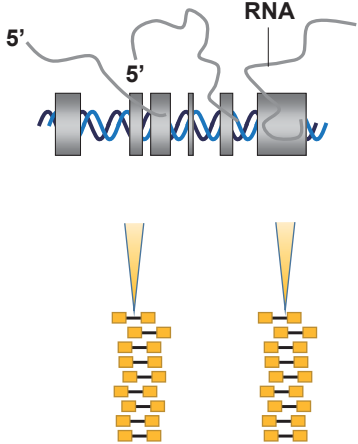
Whole genome

針對23條染色體全部定序



Whole exon

只針對會表現出基因的染色體區域進行定序 (約為whole genome的1%)



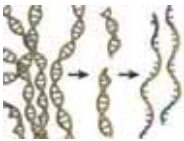
PCR amplicon

只針對常常發生突變的區域進行定序就好

主要新一代定序系統

Company/Platform		Library amplification	Sequencing principle	Nucleotide modifications	Signal detection method	Type of sequencing error	Run-time	Max length/read	Output (Gb)/run
Roche	454 FLX Titanium 454 FLX+ 454 GS Junior Titanium	emPCR on microbeads	Pyrosequencing	None	Optical detection of light, emitted in secondary reactions initiated by release of Ppi upon nucleotide incorporation	Indels in homopolymeric regions	10h	400bp	0.5-1
Illumina	MiSeq	Bridge-PCR on flow cell surface	Reversible terminator sequencing by synthesis	End-blocked fluorescent nucleotides	Optical detection of fluorescent emission from incorporated dye-labeled nucleotides	Substitutions, in particular at the end of the read	5-55h	2x300bp	0.3-13
	HiSeq						11h-11d	2x150bp	15-500
	NextSeq						11-30h	2x150bp	19-120
	HiSeqX						3d	2x150bp	1,800
	NovaSeq						48h	2x150bp	6,000
Ion Torrent	PGM	emPCR on microbeads	Semiconductor-based fluorescent oligonucleotides	None	Transistor-based detection of H+ shift upon nucleotide incorporation	Indels	3-7h	400bp	0.09-1.9
	Proton						4-6h	500bp	12-88
	S5						2.5-4h	400bp	2-16
PacBio	RSII	NA	Single-molecule, real-time DNA sequencing by synthesis.	Phosphor-linked fluorescent nucleotides	Real-time optical detection of fluorescent dye in polymerase active site during incorporation	Indels	2h	3000bp	0.09
	Sequel						0.5-6h	20,000bp	0.08-1.25
Oxford Nanopore	MinION	NA	Changes in electrical current are used to read off the chain of nucleic acid bases.	None	Real-time changes in electrical current caused by bases of nucleic acid flow through.	Indels	1min-48h	10,000bp	44

Roche 454 NGS Workflow



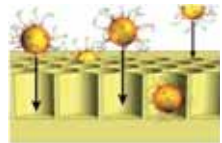
Genome fragmentation. Adapter ligation.

DNA annealing to capture beads.

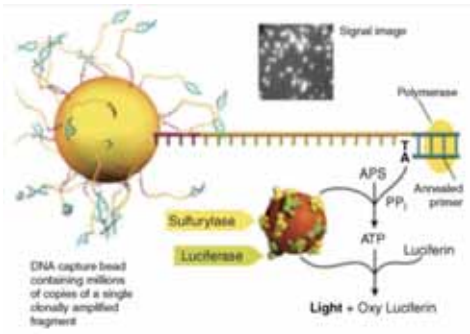
Emulsify beads and PCR reagents in microreactors.

Clonal amplification.

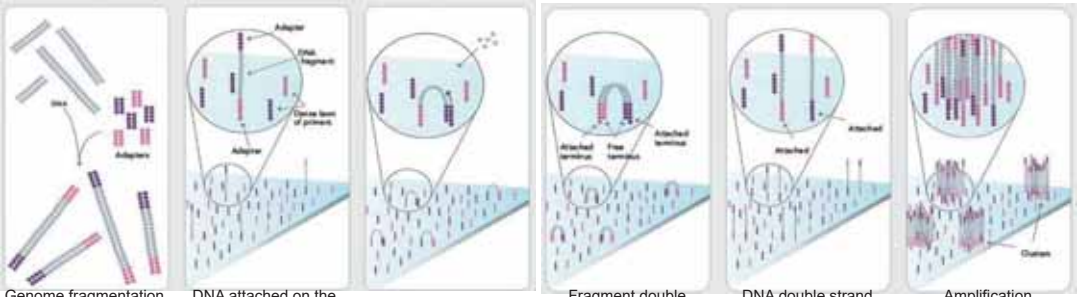
DNA enrichments.



Beads loading on chips.



Illumina NGS Workflow



Genome fragmentation. Adapter ligation.

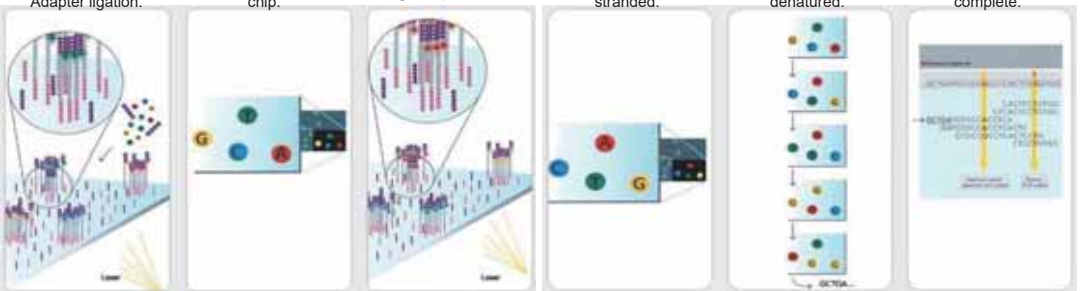
DNA attached on the chip.

Bridge amplification.

Fragment double stranded.

DNA double strand denatured.

Amplification complete.



First base determination.

First base imaging.

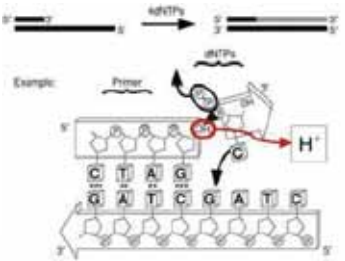
Second base determination.

Second base imaging.

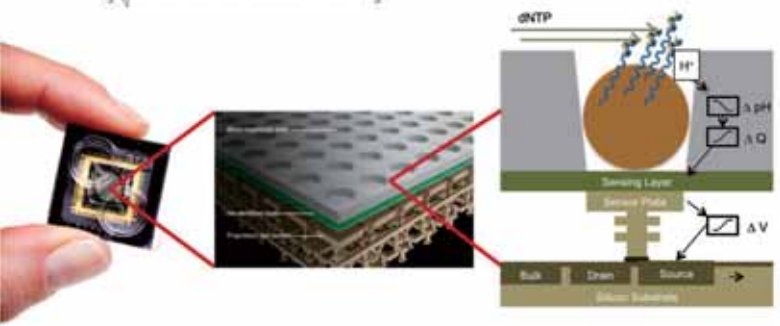
Sequence reads by multiple cycles

Data alignment.

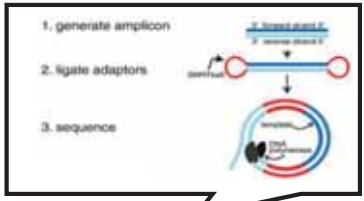
Ion Torrent NGS Workflow

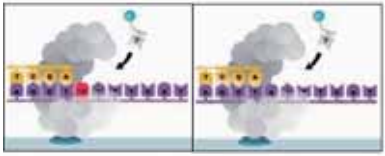


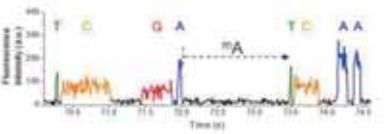
- Procedures and chemistry similar to Roche 454.
- Instead of PPi, measure H+ release (pH change) via semiconductor chip.
- No expensive camera or laser required, no modified nucleotides.

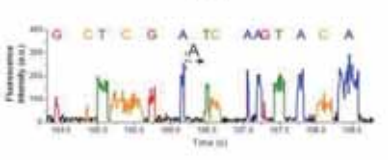


PacBio NGS Workflow



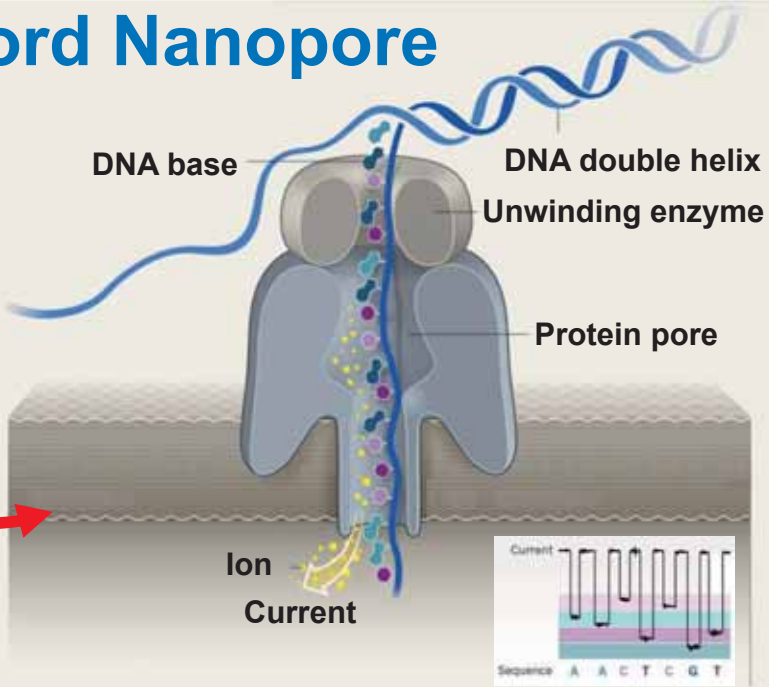






Oxford Nanopore

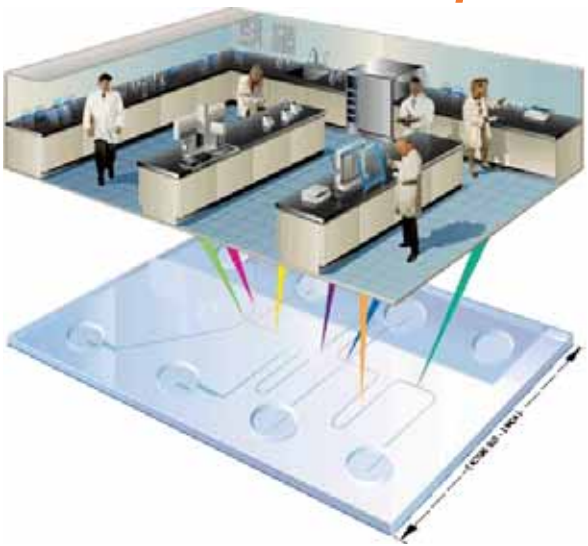
The membrane contains pore-forming proteins with an inner diameter of 1nm.



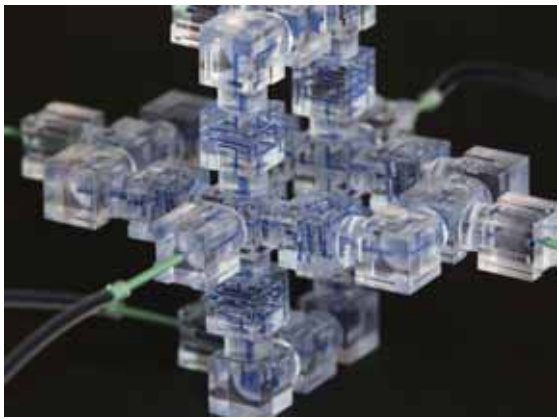
生物晶片技術開發與創新

廣義的生物晶片

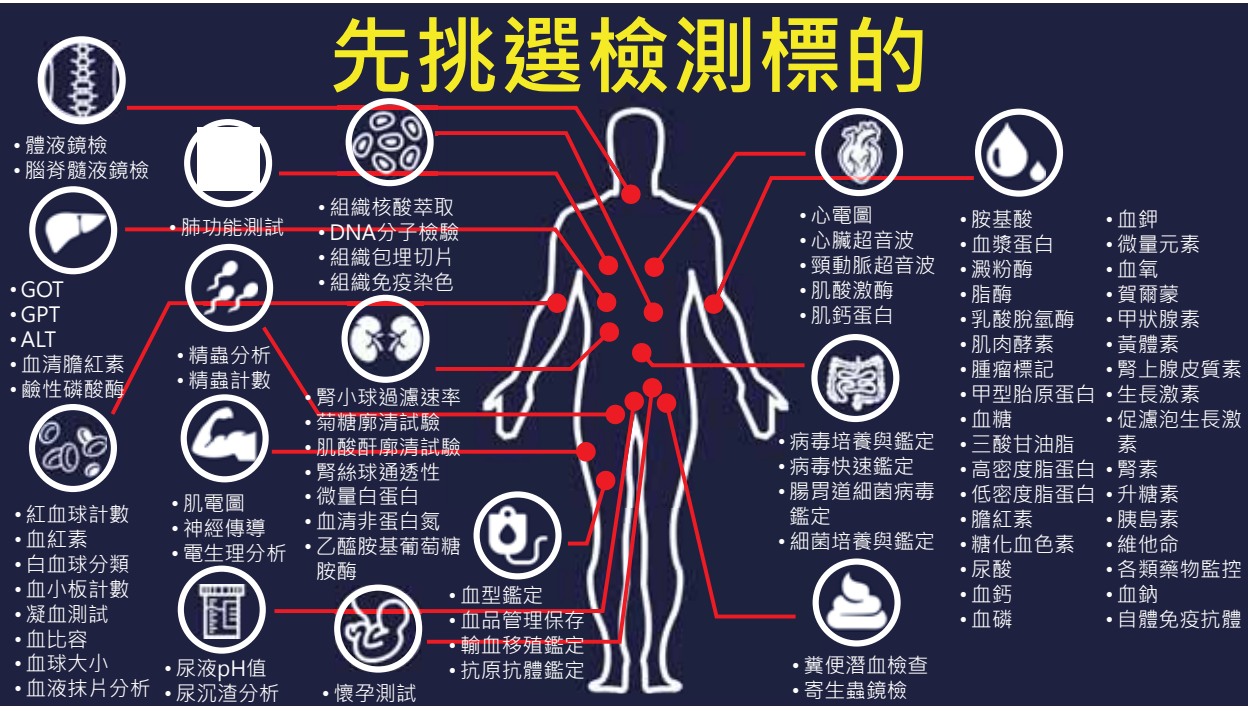
Lab-on-a-chip



Small Lab v.s Large Lab



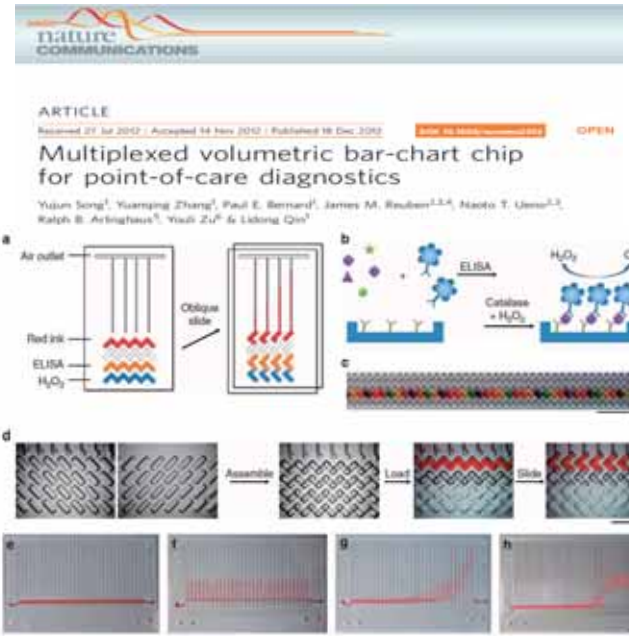
先挑選檢測標的


• 體液鏡檢
• 腦脊髓液鏡檢
• 肺功能測試
• 組織核酸萃取
• DNA分子檢驗
• 組織包埋切片
• 組織免疫染色
• GOT
• GPT
• ALT
• 血清膽紅素
• 鹼性磷酸酶
• 精蟲分析
• 精蟲計數
• 腎小球過濾速率
• 菊糖廓清試驗
• 肌酸酐廓清試驗
• 腎絲球通透性
• 微量白蛋白
• 血清非蛋白氮
• 乙醯胺基葡萄糖胺酶
• 紅血球計數
• 血紅素
• 白血球分類
• 血小板計數
• 凝血測試
• 血比容
• 血球大小
• 血液抹片分析
• 肌電圖
• 神經傳導
• 電生理分析
• 血型鑑定
• 血品管理保存
• 輸血移殖鑑定
• 抗原抗體鑑定
• 尿液pH值
• 尿沉渣分析
• 懷孕測試
• 心電圖
• 心臟超音波
• 頸動脈超音波
• 肌酸激酶
• 肌鈣蛋白
• 病毒培養與鑑定
• 病毒快速鑑定
• 腸胃道細菌病毒鑑定
• 細菌培養與鑑定
• 糞便潛血檢查
• 寄生蟲鏡檢
• 胺基酸
• 血漿蛋白
• 澱粉酶
• 脂酶
• 乳酸脫氫酶
• 肌肉酵素
• 腫瘤標記
• 甲型胎原蛋白
• 血糖
• 三酸甘油脂
• 高密度脂蛋白
• 低密度脂蛋白
• 膽紅素
• 糖化血色素
• 尿酸
• 血鈣
• 血磷
• 血鉀
• 微量元素
• 血氧
• 賀爾蒙
• 甲狀腺素
• 黃體素
• 腎上腺皮質素
• 生長激素
• 促濾泡生長激素
• 腎素
• 升糖素
• 胰島素
• 維他命
• 各類藥物監控
• 血鈉
• 自體免疫抗體

研發評估

遺傳性疾病
產前診斷
血糖監控
懷孕測試
急性心肌梗塞測試
藥效監控
感染源檢測
遺傳基因檢測

多試劑反應居家檢測晶片



擬真活體組織培養晶片

Lab on a Chip

Dynamic Article Links

Cite this: *Lab Chip*, 2012, **12**, 2165–2174

www.rsc.org/loc

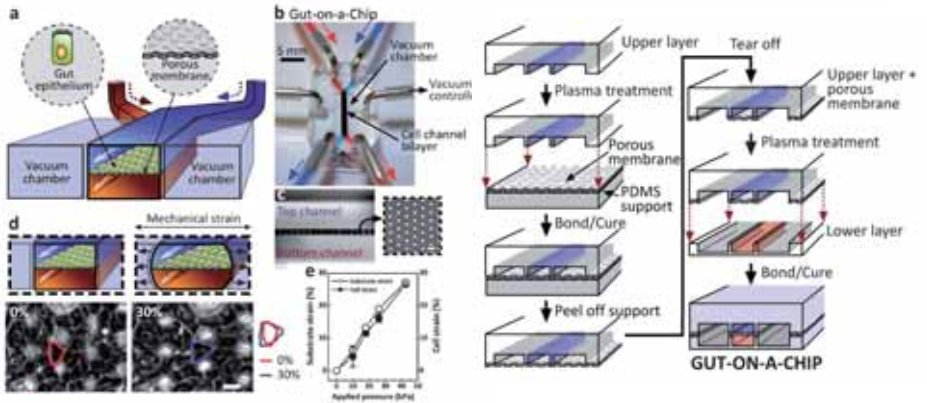
PAPER

Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow†‡

Hyun Jung Kim,[a] Dongeun Huh,[a] Geraldine Hamilton[a] and Donald E. Ingber[*a,b]

Received 18th January 2012, Accepted 5th March 2012

DOI: 10.1039/c2lc40074j



BIOENGINEERING

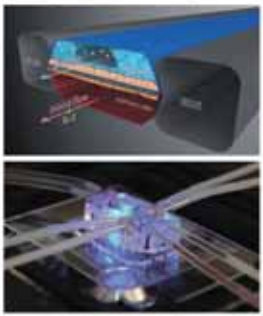
Lung-on-a-Chip Breathes New Life Into Drug Discovery

At first blush, the idea of growing facsimiles of lungs, kidneys, or other human organs in a bioreactor sounds vaguely diabolical. But researchers have been cultivating combinations of tissues for years in hopes that they would imitate working organs, and thereby serve as testing grounds for novel drugs to treat a wide variety of diseases. Now that promise has come a big step closer to reality. In this week's issue of *Science Translational Medicine* (*STM*), a team of academic and drug company researchers shows that an engineered "lung-on-a-chip" can not only faithfully model a serious respiratory ailment known as pulmonary edema, but can also accurately predict the toxicity of a compound that causes the disease and the ability of a new drug to prevent it.

"This really pushes the field to the next level," says Shuichi Takayama, a biomedical engineer at the University of Michigan, Ann Arbor, who has helped pioneer the field with his own lung-on-a-chip system. "People had been asking whether these systems could predict disease. Now it looks promising and we can ask 'How can we do this in the best way?'"

Efforts to incubate multiple cell types together to make organ mimics date back nearly 2 decades. In recent years, researchers have combined cell-culturing advances with microchip-patterning techniques to turn out artificial livers, kidneys, guts, and even brain tissue. Two years ago, a team led by Donald Ingber, a biomedical engineer at Harvard University, went so far as to make an artificial lung device complete with a layer of human capillary cells and lung cells on either side of a porous membrane, together with blood flow below the capillary layer and airflow above the lung cells. This entire assemblage was produced within a clear, flexible plastic material about the size of a computer thumb drive that could expand and contract, reproducing the mechanical motions involved in breathing (*Science*, 25 June 2010, p. 1662).

For their current study, Ingber and his colleagues used their lung-on-a-chip to model pulmonary edema. This life-threatening condition often follows heart failure, because fluid and blood-clotting proteins leak between endothelial cells in capillaries that pass through epithelial cells lining the lung and end up in alveolar pockets in the airways. It's also a common side effect among cancer patients given the chemotherapy drug interleukin-2 (IL-2). To see if their device would reproduce that effect, Ingber's team introduced IL-2 at a clinically relevant concentration into the blood flowing beneath capillary cells in their chip. Not only did the IL-2 cause the fluid leakage to occur, but this leakage increased fourfold when the chip repeatedly flexed to simulate the physical motions involved in breathing.



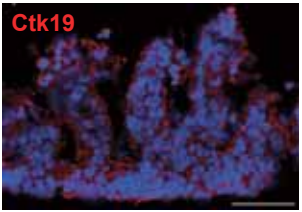
Disease mimic. In a lung-on-a-chip (above), IL-2 in the blood causes fluid to flow (top, white arrows) into the airway.

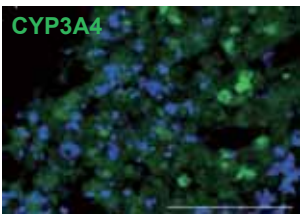
That success prompted Ingber's team to use this edema stand-in to screen drugs that might treat the disease. Previous work by other groups had shown that mechanical strain, such as that caused by breathing, can stimulate activity in TRPV4, a type of ion channel in capillary endothelial cells. This in turn can increase fluid leakage from capillaries into alveoli. Researchers at the pharmaceutical giant GlaxoSmithKline (GSK) had recently developed TRPV4-blocking drugs. Ingber's group partnered with Kevin Thorneloe and Allen McAlexander at GSK, and showed that the new TRPV4 blockers do in fact prevent IL-2's pulmonary edema side effects. In a separate study in the same issue of *STM*, the GSK team documented similar beneficial effects of TRPV4 inhibition in mice models of pulmonary edema caused by heart failure.

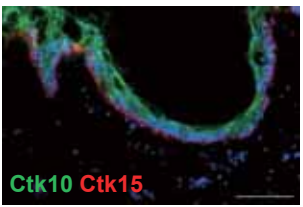
Ingber says the new results are a proof of principle that organs on chips can be a useful tool for researchers looking to screen new drugs and sort out mechanisms involved in disease. Down the road, that could limit the pharmaceutical industry's reliance on testing new drugs on animals. Of the candidate drugs that make it through animal testing, only a paltry 10% work in humans and make it to market. So any improvement could make a big impact.

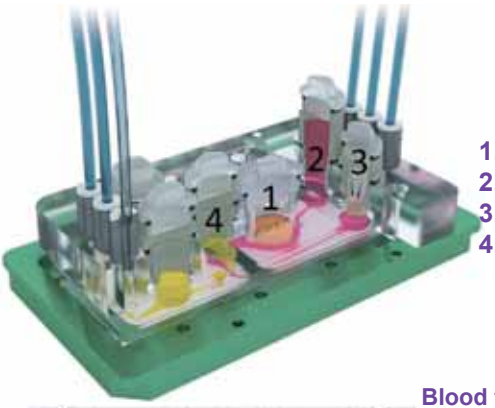
–ROBERT F. SERVICE

多器官晶片



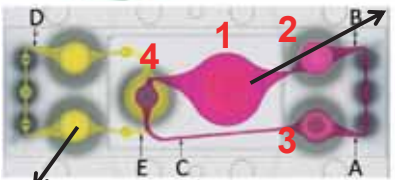






1. Intestine
2. Liver
3. Skin
4. Kidney

Blood flow circuit



Excretory flow circuit

Lab Chip, 2015, 15, 2688-2699

多色奈米晶片檢測感染源

Lab on a Chip

COMMUNICATION

View Article Online

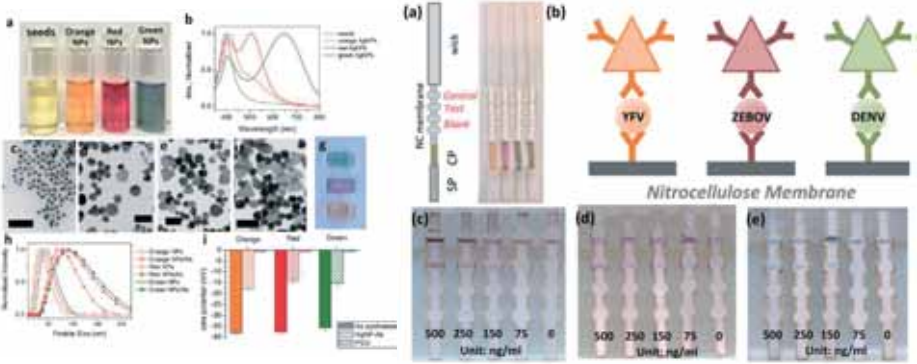
Multicolored silver nanoparticles for multiplexed disease diagnostics: distinguishing dengue, yellow fever, and Ebola viruses†

Chun-Wan Yen,[ab] Helena de Puig,[c] Justina O. Tam,[ab] José Gómez-Márquez,[d] Irene Bosch,[ab] Kimberly Hamad-Schifferli*[ce] and Lee Gehrke*[af]

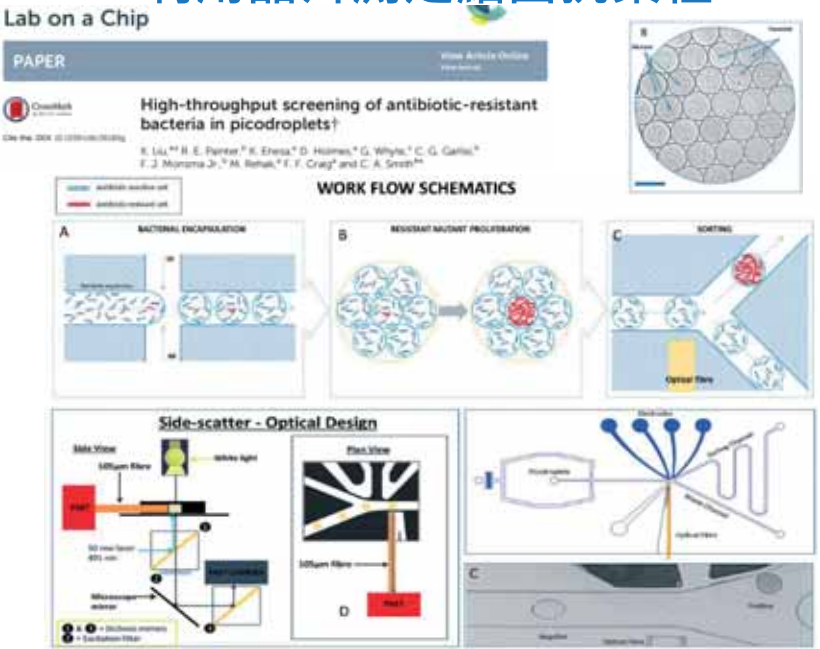
Cite this: Lab Chip, 2015, 15, 3038

Received 15th January 2015,
Accepted 4th February 2015

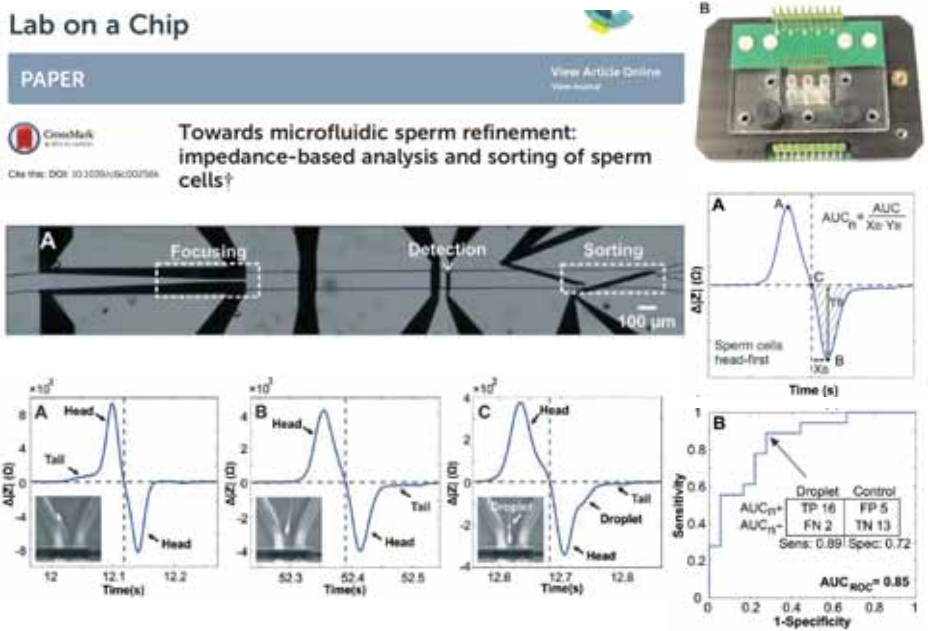
DOI: 10.1039/c5lc00059f



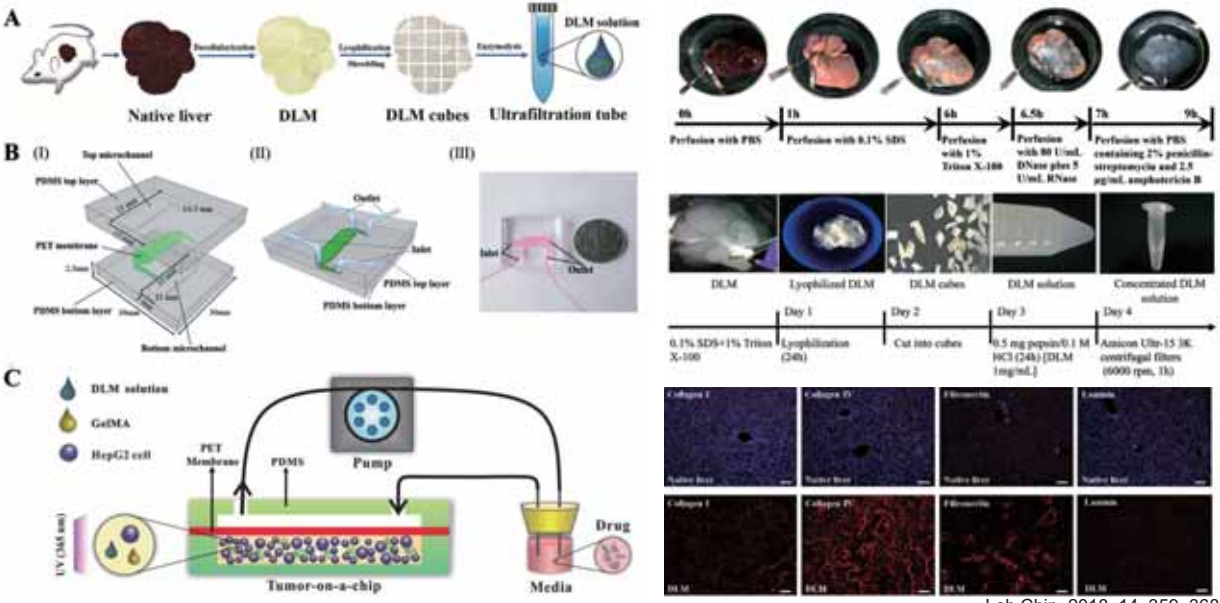
利用晶片測定細菌抗藥性



利用晶片篩選高品質的精子

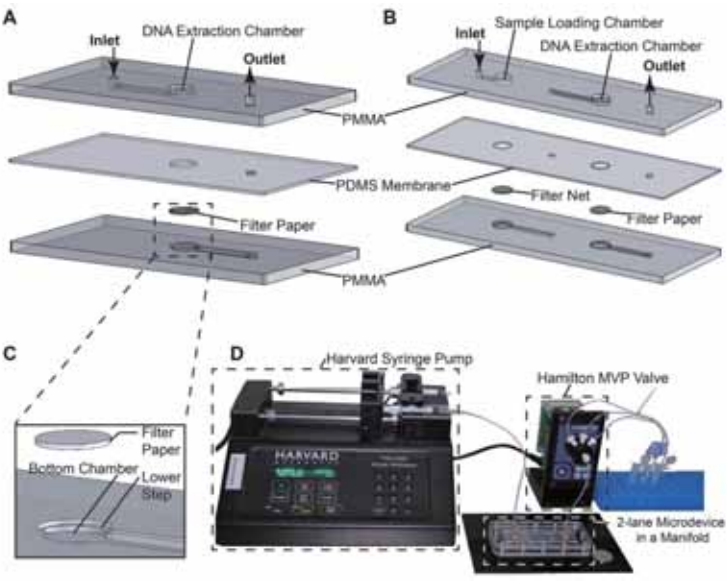


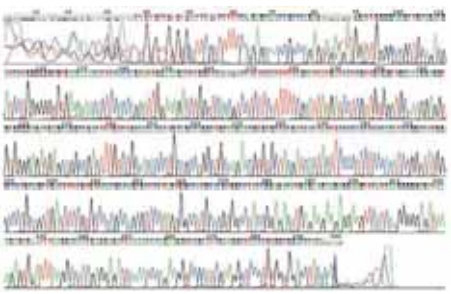
利用晶片建構肝臟環境進行藥物篩選



Lab Chip, 2018, 14, 359–368

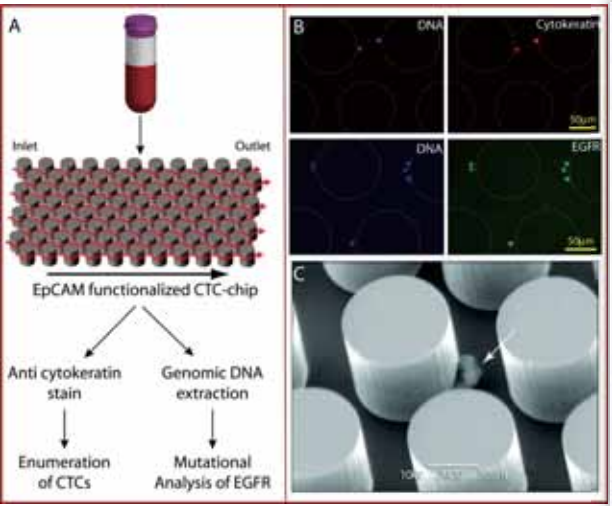
利用濾紙與晶片萃取多種樣本DNA

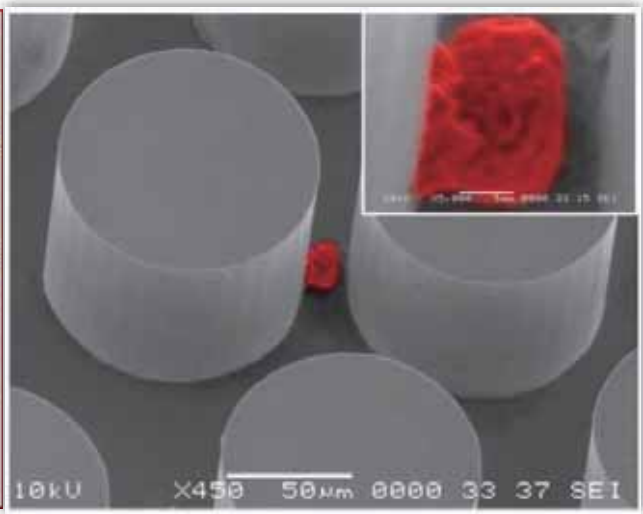




Lab Chip, 2014, 14, 3719-3728

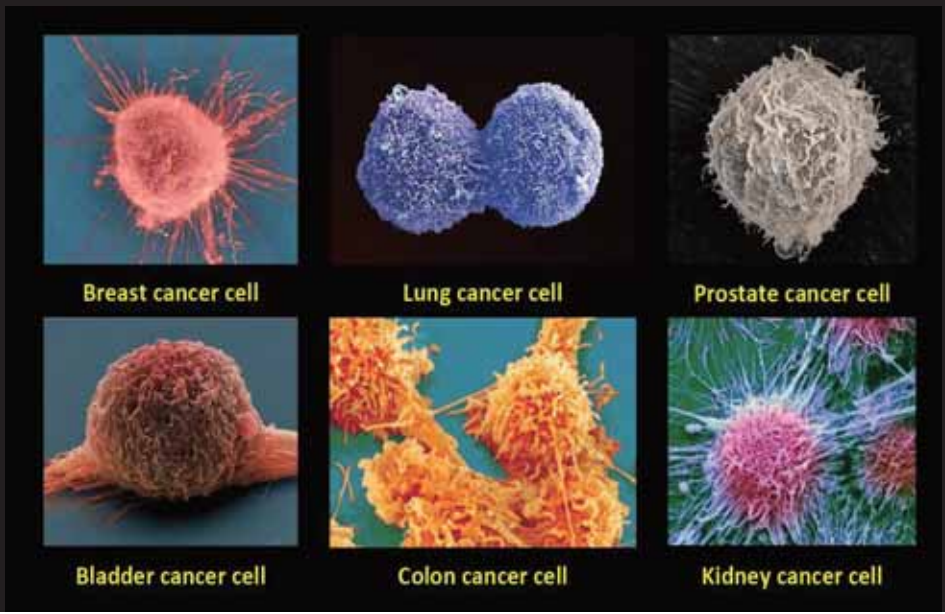
利用特異性抗體分離循環腫瘤細胞



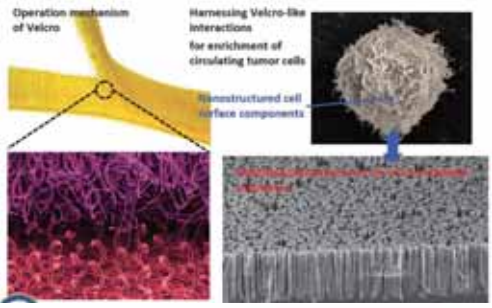


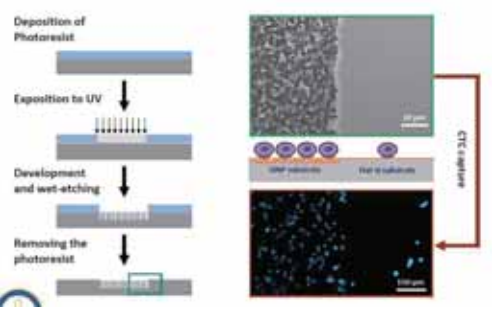
N Engl J Med 2004; 351:781-791

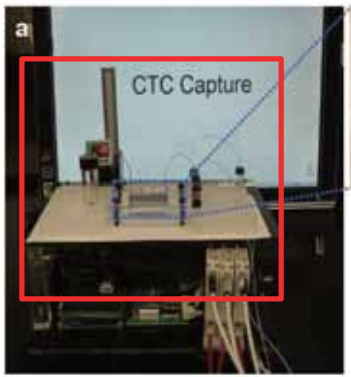
利用物理接觸分離循環腫瘤細胞

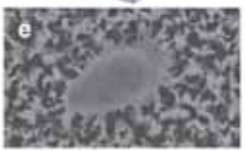


利用物理接觸分離循環腫瘤細胞

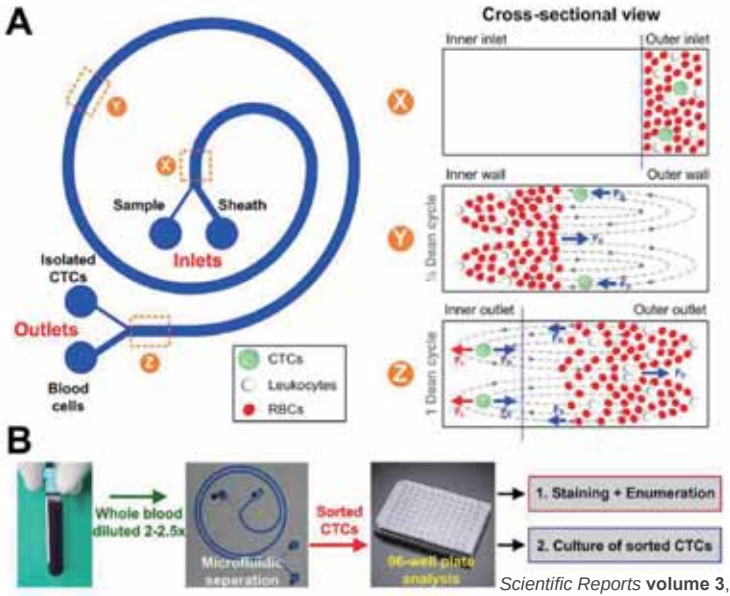






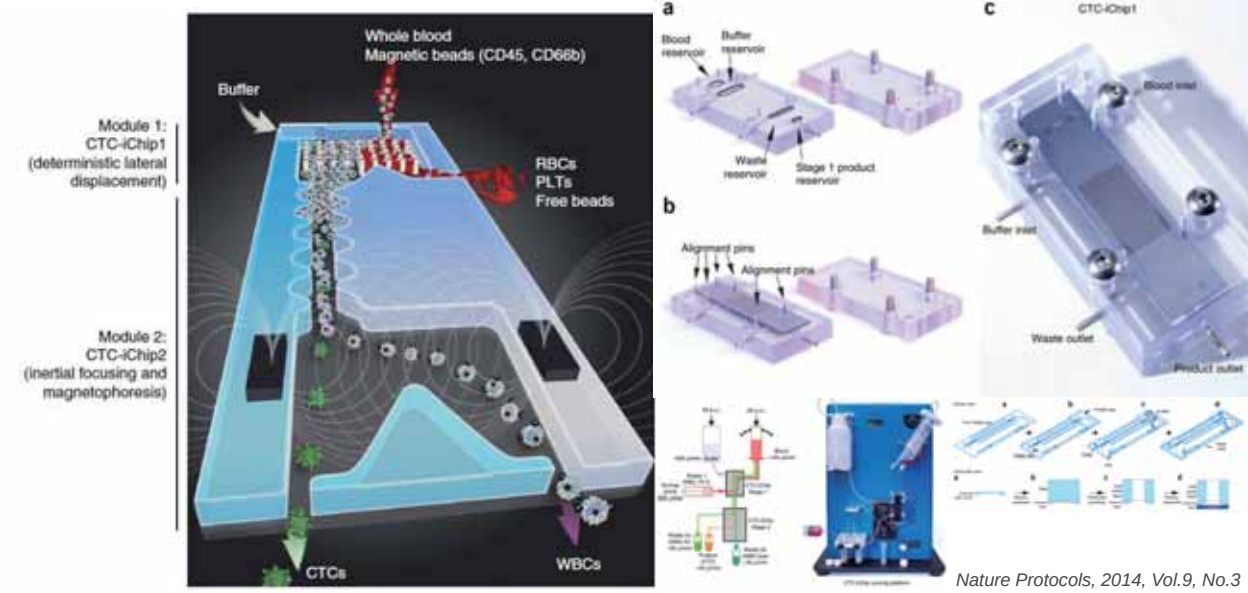


循環腫瘤細胞分離



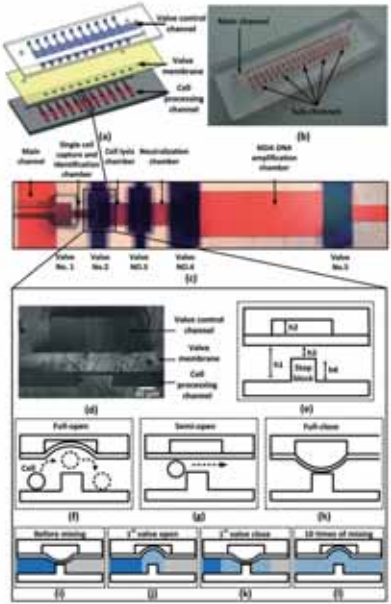
Scientific Reports **volume 3**, Article number: 1259 (2013)

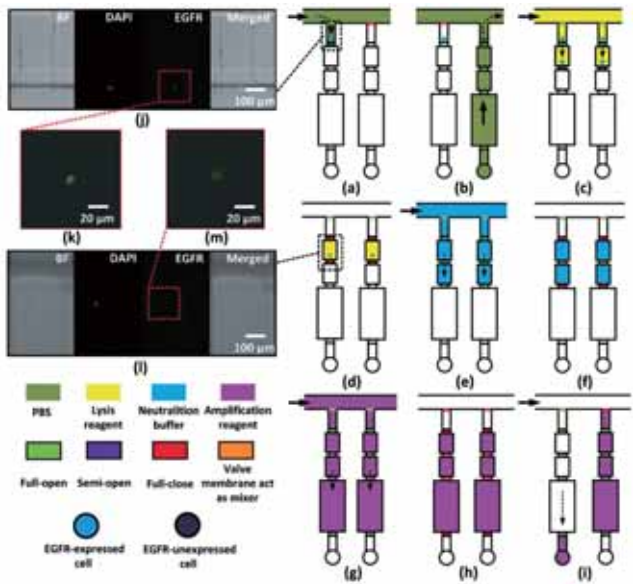
循環腫瘤細胞分離



Nature Protocols, 2014, Vol.9, No.3

利用晶片分離單一細胞檢測突變



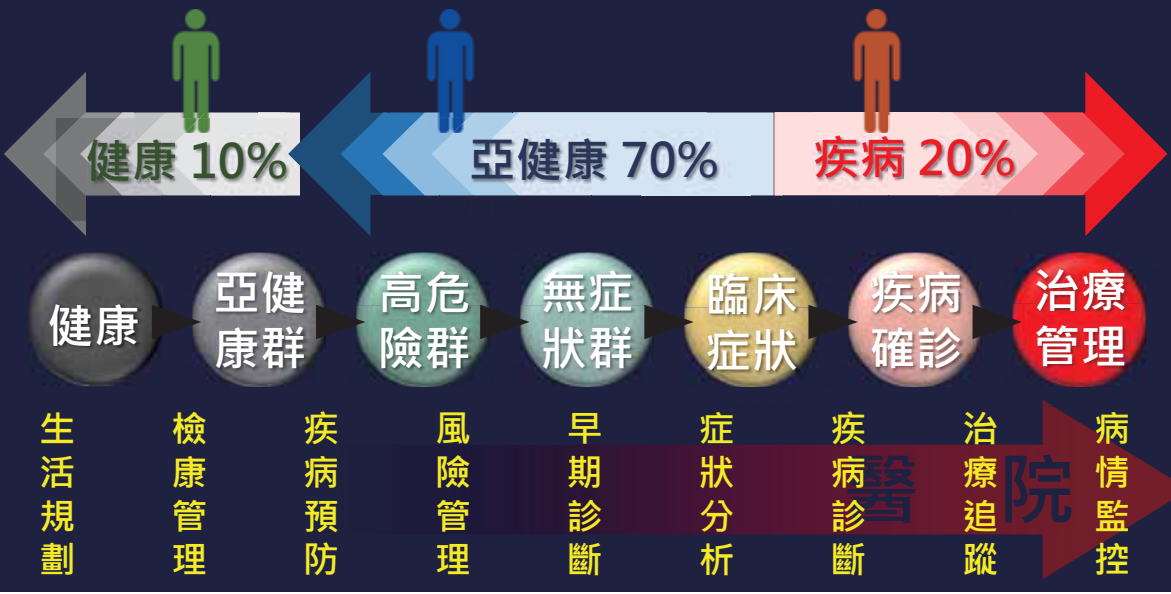


LabChip,2018,18,723–734

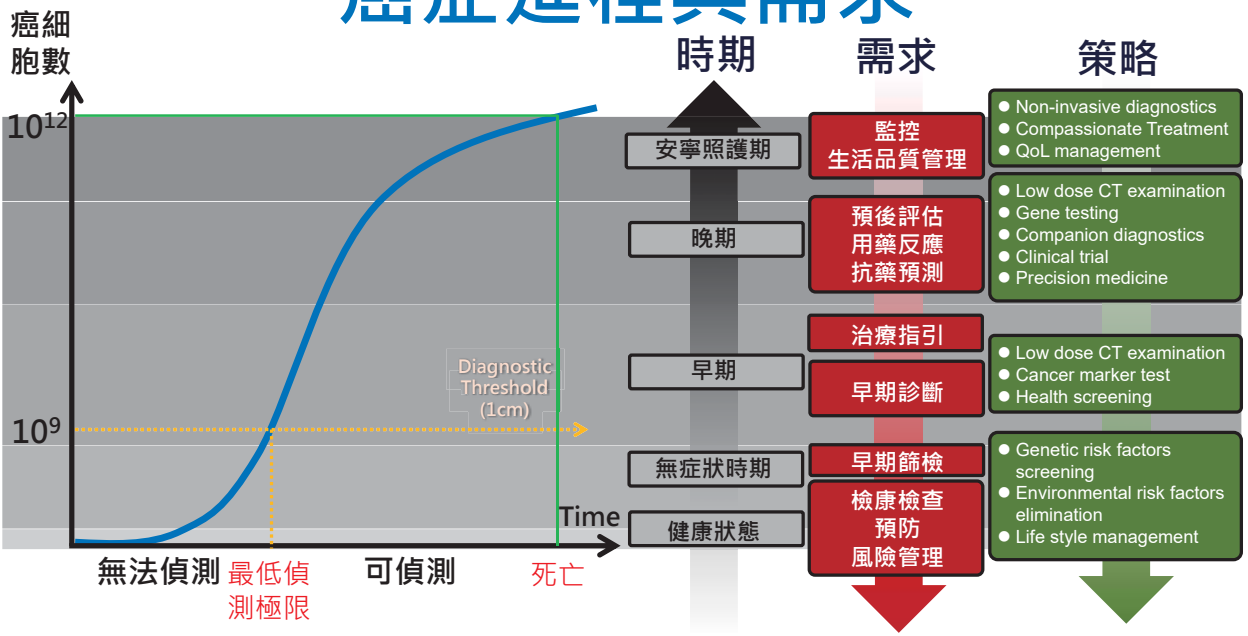
臨床未被滿足之需求與考量


誰來解決未被滿足的需求?
Clinical Physicians
Professional Nurse
Medical Decision
Medical Care
Clinical
Observation/Validation
Genomic
Approach
In vitro
Mechanical Investigation
Researcher
Treatment
Efficiency
Treatment
Innovation
Pharmacist
Pharmacy
In vitro
Diagnostics
In vivo
Validation
Medical Technologist
Treatment
Bioinformatics

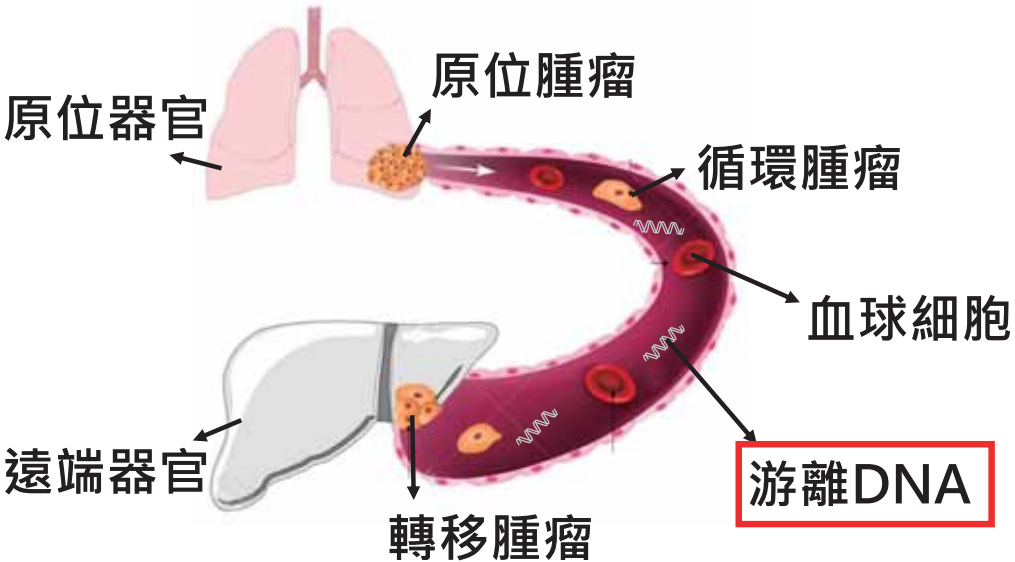
那裡有未被滿足之需求?


健康 10%
亞健康 70%
疾病 20%
健康
亞健康群
高危險群
無症狀群
臨床症狀
疾病確診
治療管理
生活規劃
檢康管理
疾病預防
風險管理
早期診斷
症狀分析
疾病診斷
治療追蹤
病情監控
醫院

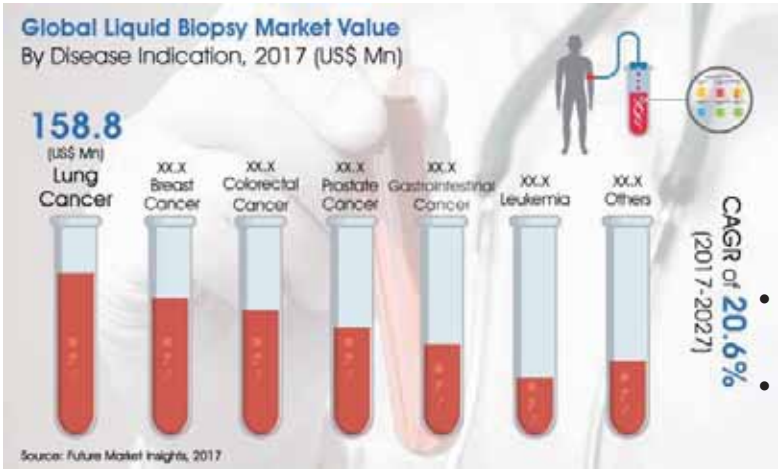
癌症進程與需求


癌細胞數
10^{12}
10^{9}
Diagnostic Threshold (1cm)
Time
無法偵測
最低偵測極限
可偵測
死亡
時期
安寧照護期
晚期
早期
無症狀時期
健康狀態
需求
監控生活品質管理
預後評估用藥反應抗藥預測
治療指引
早期診斷
早期篩檢
檢康檢查預防風險管理
策略
Non-invasive diagnostics
Compassionate Treatment
QoL management
Low dose CT examination
Gene testing
Companion diagnostics
Clinical trial
Precision medicine
Low dose CT examination
Cancer marker test
Health screening
Genetic risk factors screening
Environmental risk factors elimination
Life style management

腫瘤細胞在活體內的分佈

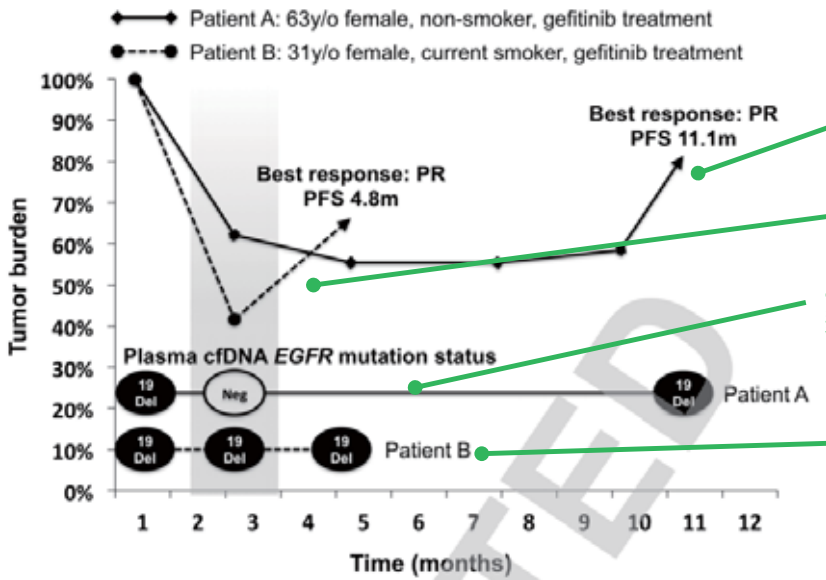


液態檢體市場



- Global liquid biopsy market: US$ 456 M, 2017
- Estimated market at 2027: US$ 3,130.7 M

利用游離DNA監控病程發展



腫瘤復發

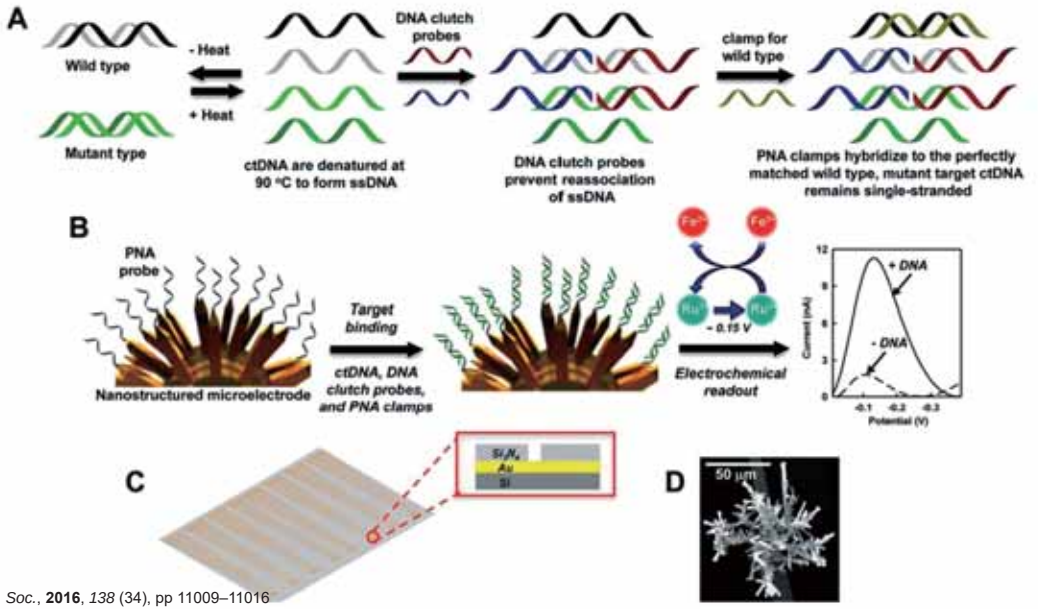
11.1個月才復發的A病人

4.8個月就復發的B病人

血中測不到具有腫瘤突變DNA的A病人

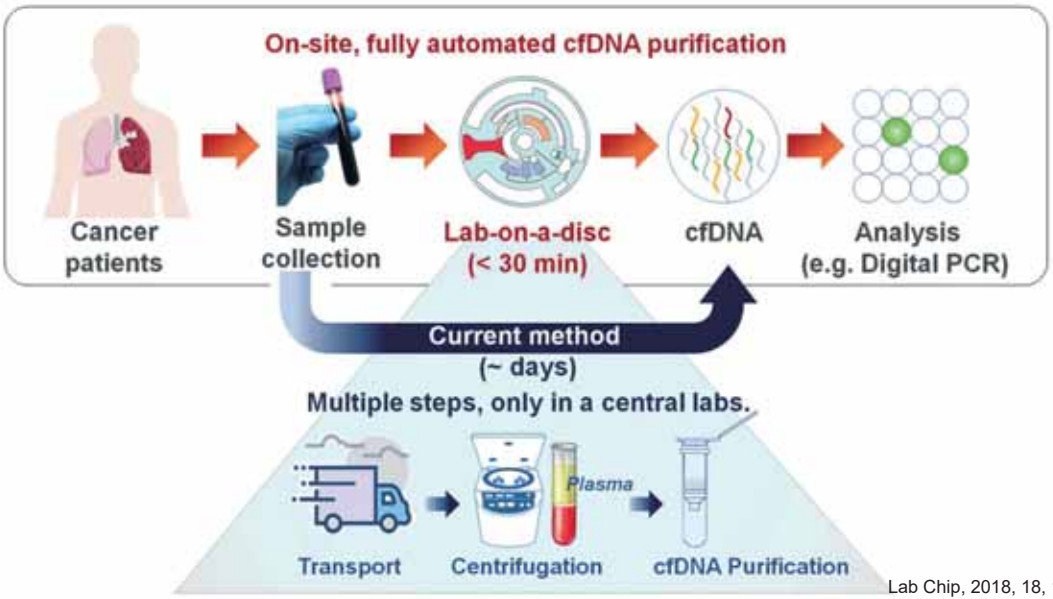
血中很快測到具有腫瘤突變DNA的B病人

利用奈米結構微電極檢測血液中突變



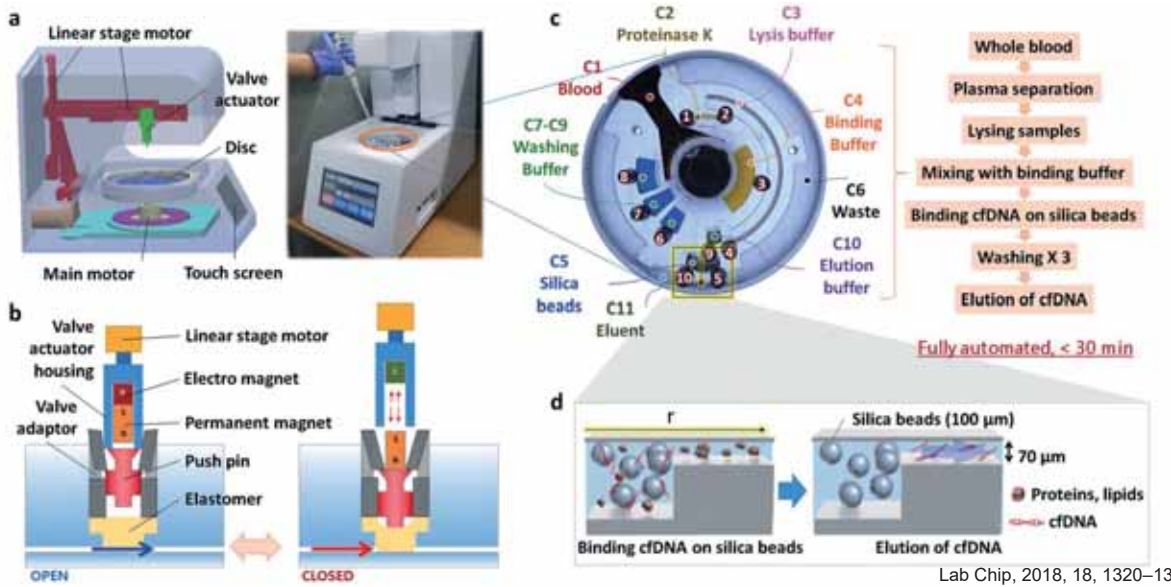
J. Am. Chem. Soc., **2016**, *138* (34), pp 11009–11016

全自動血液游離DNA分離機



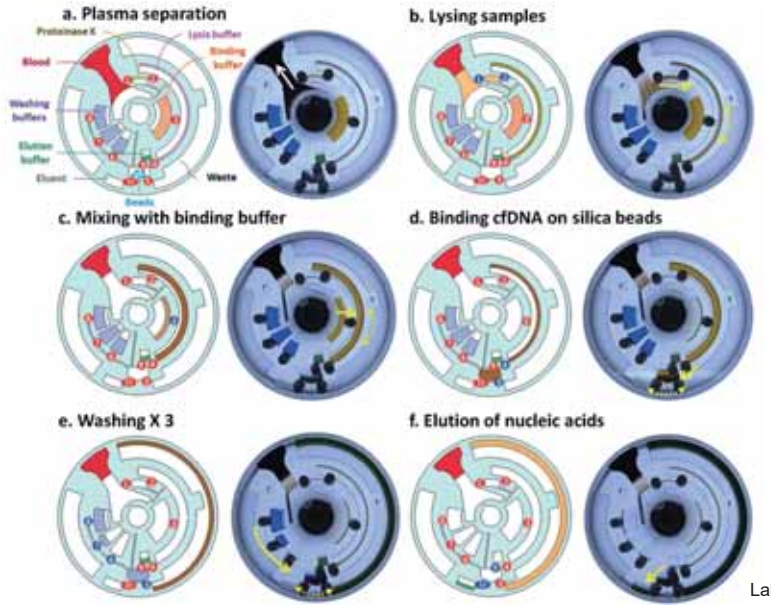
Lab Chip, 2018, 18, 1320–1329

全自動血液游離DNA分離機



Lab Chip, 2018, 18, 1320–1329

全自動血液游離DNA分離機



Lab Chip, 2018, 18, 1320–1329

體外診斷器開發與確效

什麼是實驗室開發方法與體外診斷器材?

實驗室開發方法 (LDT, Lab Developed Test)

An device that is intended for clinical use and designed, manufactured and used within a single laboratory.

FDA does not consider devices to be LDTs if they are designed or manufactured completely, or partly, outside of the laboratory that offers and uses them.

體外診斷器材 (IVD, In Vitro Diagnostic Devices)

An instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including any component, part or accessory which is:

1. Recognized in the official National Formulary.
2. Intended for use in the diagnosis of disease or in the cure, treatment or prevention of disease.
3. Used in laboratories or other health professional settings or for consumers to use at home.

US FDA; Federal Food, Drug, and Cosmetic

美國政府對於LDT的規範

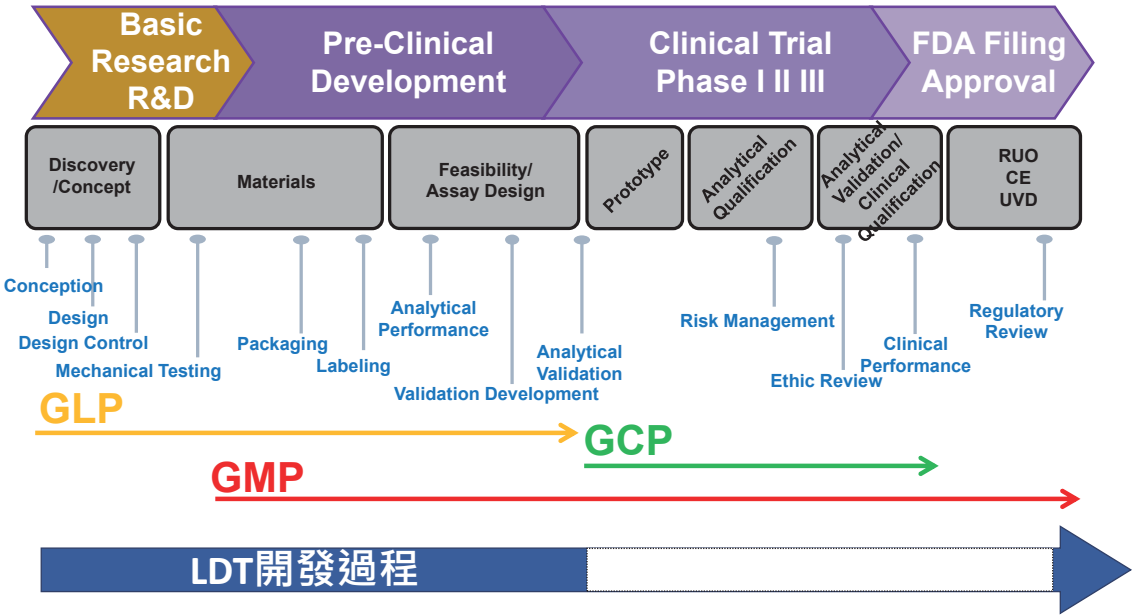
- US FDA自1976起對體外診斷器材有權利管理規範，並謹慎限制LDT的使用。
- FDA於2010年7月舉辦會議，廣納意見，準備制訂規範，達成共識: 需有利害關係人與外部專家參與、需採風險管理與階段進行策略、對罕見疾病和FDA未核可和特定醫院臨床需求限定和經同儕審查過的LDT放寬標準
- 至2012年，尚未有一項基因檢測或NGS技術被FDA核准，即便CLIA、ISO或CAP亦無明確指引。
- 2012年11月US CDC招集專家共識會議，討論分子檢驗及NGS在臨床應用上的確效與彈性。(Nature Biotechnology, Vol.30, Nov.11, 2012)
- 2013年11月US FDA核可NGS在臨床上使用，但書: 必須進行風險管控、確效和品保(Nov. 19, 2013)。(NEJM, 369: 2369-71, 2013)
- 2014年10月3日US FDA釋出Framework for Regulatory Oversight of LDTs。
- 2015年1月美國臨床實驗室協會(ACLA)律師John Conley對2014年FDA的白皮書提出反對LDT歸屬FDA管理。
- 2016年9月美國臨床實驗室協會理事長Alan Mertz表示: LDT是精準醫學的催化劑，一個明確合理的規範才不會阻礙LDT的創新以及臨床醫師與病人的權益。
- 2017年1月US FDA無限期延後LDT規範指引的發行，等待相關領域包括產業界的回饋。

NGS in Clinical Diagnostics

Their commentary hints that such lab-mad tests could come under increased scrutiny: “putting in place an appropriate risk-base regulatory framework is now critical to ensure the validation and quality of tests.”

NEJM, 369:2369-71, 2013

IVD開發過程



階段產出 執行 規範

方法驗證(validation)

準確度 (Accuracy)

讀序與參考序列的一致性；深度、涵蓋率、閥值、正反向讀序(Q值)、高GC含量讀序。

精密度 (Precision)

檢驗重複性(within-run)與再現性(between-run)；3個參考物質進行3-5次的重複性與再現性試驗。

靈敏度 (Analytic Sensitivity)

檢測靈敏度；多少突變比例可檢測?參考物質必須同時包含疾病與非疾病相關的變異點。

特異性 (Analytic Specificity)

檢測特異性；在無核酸變異的參考中，偵測出變異的比例。

可報告區 (Reportable Range)

可信賴知判讀區域；在檢驗過程中符合品質可作為結果判讀的序列(基因)區間。

參考區間 (閥值) (Reference Range)

參考範圍；在可信賴判讀區域中，正常個體可檢測出的變異量(閥值)。

可追溯性 (Traceability)

檢測中所使用的方法或參考物質，是否可以回溯至標準或共識之方法或物值。

檢測過程之流程 (SOP)

檢測過程中由檢體採樣、檢體運送、核酸萃取、檢測、報告等檢驗前、中、後詳細之運作流程。

安定性 (Stability)

檢測方法在各種不同來自於樣本多樣性或環境變化等因素干擾下，結果的重覆性與再現性。

檢測結果 (Reports)

檢測報告中應載明知各項訊息，包括臨床判讀與方法限度等宣告。

CLIA, 衛福部食藥署

Precision 精密度

環境
Variation of the temperature, humidity

Variation of the pipetting, mixing

Repeatability:
Same condition;
Within-run

Reproducibility:
Change conditions;
Between-run

Instability of the instrument

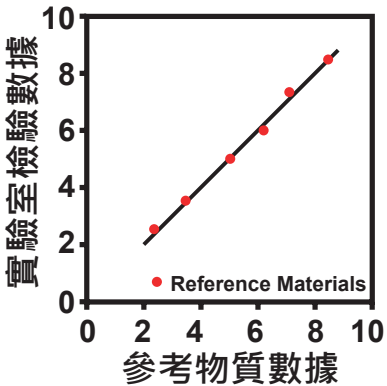
Variation in the reagent or calibrator

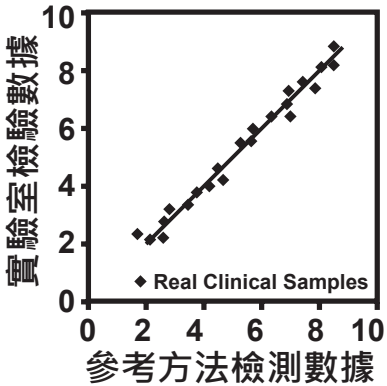
Precision 精密度

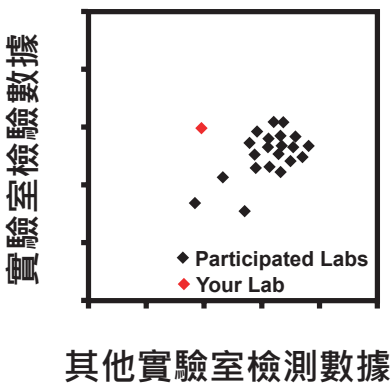
- 應含有至少兩種不同濃度之樣品(low and high concentration)
- Within-run的評估至少20點數據、between-day須累積至少20天數據 (CLSI, Clinical and Laboratory Standards Institute建議)
- 需計算mean、SD、CV等統計數字

Accuracy 精準度

Agreement between the best estimate of a quantity and its true value.







Sensitivity and Specificity 敏感度與特異性

- 在所有確認是陽性的樣本中，被測到陽性的比例(真陽性率)即為敏感度。

- 在所有確認是陰性的樣本中，背側到陰性的比例(真陰性率)即為特異性。

Sensitivity and Specificity
敏感度與特異性

	患者 (標準方法或臨床診斷為陽性)	正常 (標準方法臨床診斷陰性)
你的晶片檢出陽性	**A**	**B**
你的晶片檢出陰性	**C**	**D**

敏感度:A/(A+C)

特異性:D/(B+D)

偽陽性:B/(B+D)

偽陰性:C/(A+C)

陽性預測率:A/(A+B)

陰性預測率:D/(C+D)

Sensitivity and Specificity
敏感度與特異性

針對10000個人進行方法的評估

	患者 (標準方法陽性)	正常 (標準方法陰性)
檢出陽性	90 A	990 B
檢出陰性	10 C	8910 D
	100	9900

敏感度:90/100=90%

特異性:8910/9900=90%

偽陽性:B/(B+D)=10/100=10%

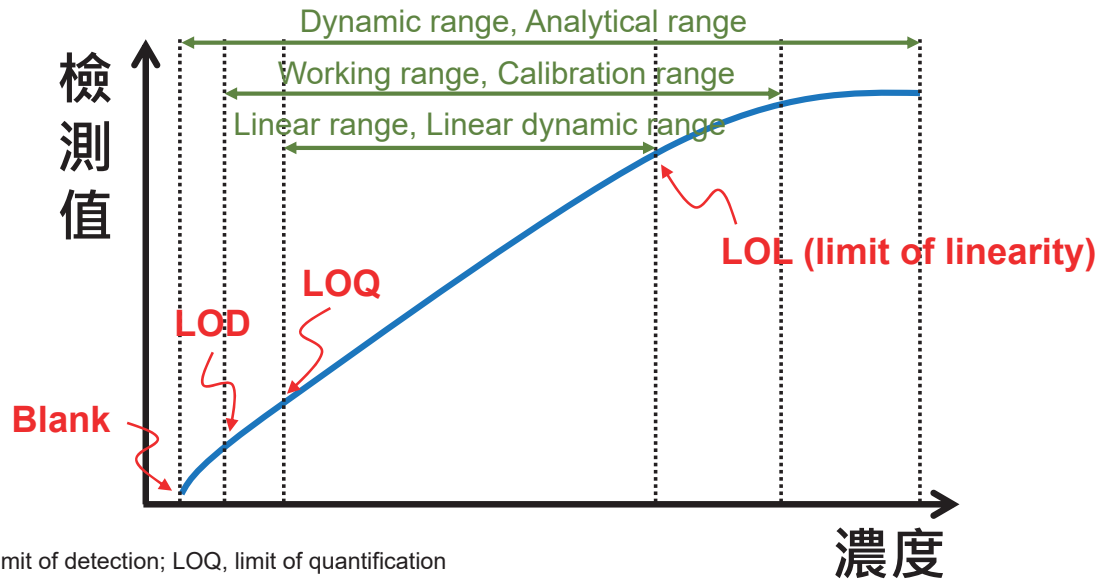
偽陰性:C/(A+C)=1000/10000=10%

陽性預測率:A/(A+B)=90/1090=8.3%

陰性預測率:D/(C+D)=10/9010=99.9%

➡ 盛行率: 100/(100+9900)=1%

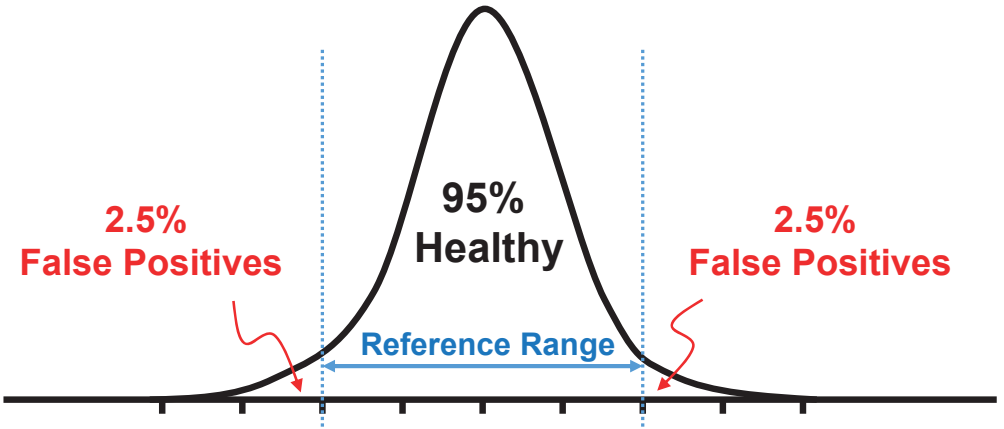
Analytical (Report) Range (檢測範圍)



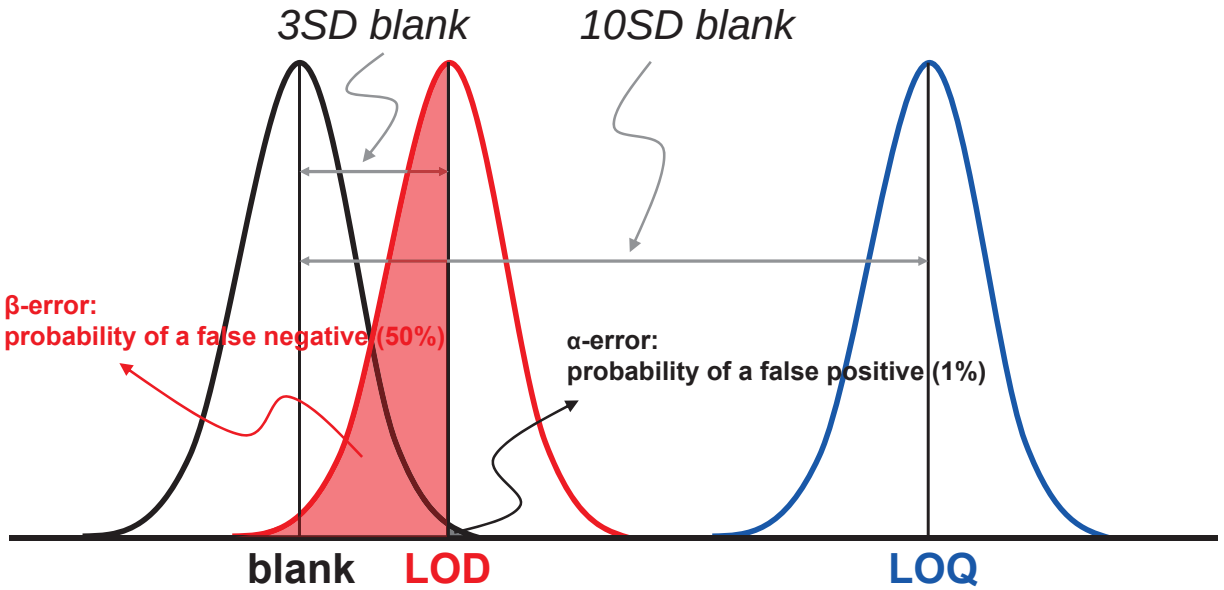
LOD, limit of detection; LOQ, limit of quantification

Reference Range (參考範圍)

A reference range or reference interval is the range of values for a physiologic measurement in health persons.



LOD and LOQ 偵測極限與定量極限



干擾評估(Interferences)

外因性

特定藥物、抗凝劑、環境條件

內因性

高脂血、溶血、受測者生理狀態、膽色素、其他代謝產物

IVD與LDT的比較

	IVD (In vitro diagnostics)	LDT (Lab developed tests)
Development and Manufacturing	by device manufacturing	by single laboratory
Regulatory Agency	FDA	CLIA via CMS; FDA: enforcement authority
Documentation	GLP, GCP (if applicable), GMP, CLIA SOPs/quality system	GCP (if applicable), GLP, GMP, CLIA SOPs/quality system
Analytical Validation	Required	Required
Premarket Review & Approval for Tests	Required	**Not Required**
Clinical Validation	Required	**Not Required**
What is Sold?	Diagnostics	Service

所以即便是實驗是自己開發的技術平台，還是要證明自己測得又準又穩!

臨床確效 (US FDA)

Risk-based development

- ✓ 篩選進行確效的族群應須可代表臨床待檢驗族群。
- ✓ 須考量使用前瞻性試驗(prospective)或回溯性試驗(retrospective)進行評估。
- ✓ 明確定意納入或排除試驗個案之標準。
- ✓ 陽性預測值(PPV)、陰性預測值(NPV)、偽陽性、偽陰性皆需要進行評估。

法規查驗登記與試驗

醫材商品化要素

創新

專利

市場

法規

商業模式

體外診斷醫療器材查驗登記

IVD係指蒐集、準備及檢查取自於人體之檢體，作為診斷疾病或其他狀況（含健康狀態之決定）而使用之診斷試劑、儀器或 系統等醫療器材。

需檢附資料

1. 黏貼或裝釘於標籤黏貼表上之**中文仿單**目錄、使用說明書、包裝及標籤。
2. **臨床前測**試及原廠品質管制之檢驗規格與方法、原始檢驗紀錄及檢驗成績書。
3. 產品之結構、材料、規格、性能、用途、圖樣等 有關資料。
4. 學術**理論依據**與有關**研究報告**及資料。
5. **臨床試驗**報告。
6. 發生游離輻射線器材之輻射線防護安全資 料 。

適用範圍

包含

A、臨床化學及臨床毒理學
B、血液學及病理學
C、免 疫學及微生物學
D、其他相關規定之體外診斷醫療器材。

臨床前測試
Pre-Clinical Testing

1. 精密度/再現性 (Precision/Reproducibility)
2. 準確性 (Accuracy)
3. 靈敏度 (Sensitivity)
4. 特異性 (Specificity)
5. 閥值確認 (Cut-off Value)
6. 安定性 (Stability)
7. 干擾性研究 (Interference Study)
8. 追溯性 (Traceability)
9. 證明符合相關安全性與功效性要求所 需之化學、物理、電力、機械、生物性、電性安全、 電磁相容性、軟體驗證、無菌或微生物限量等內容的 說明資料。
10. 檢附一份製造過程之流程圖及其描述。
11. 檢附一份主成份（ Main Active Ingredient ） 及最終成品之檢驗成績書。

平行比對

- 臨床前測事應選擇國內已核可或美國、日本、加拿大、瑞士、澳洲或歐盟中至少一國核准上市之同類產品進行比對測試。

- 如無，則以新體外診斷醫材管理，需檢附學術理論依據與有關研究報告及資料或臨床評估報告。

預期用途
Intended Use

1.器材的檢測標的。
2.器材是否為自動化。
3.器材的預期用途。
4.器材為定性、半定量或定量。
5.用於特定疾病、狀況或風險因子的檢測、定義或判別。
6.檢體的種類。
7.受檢族群。

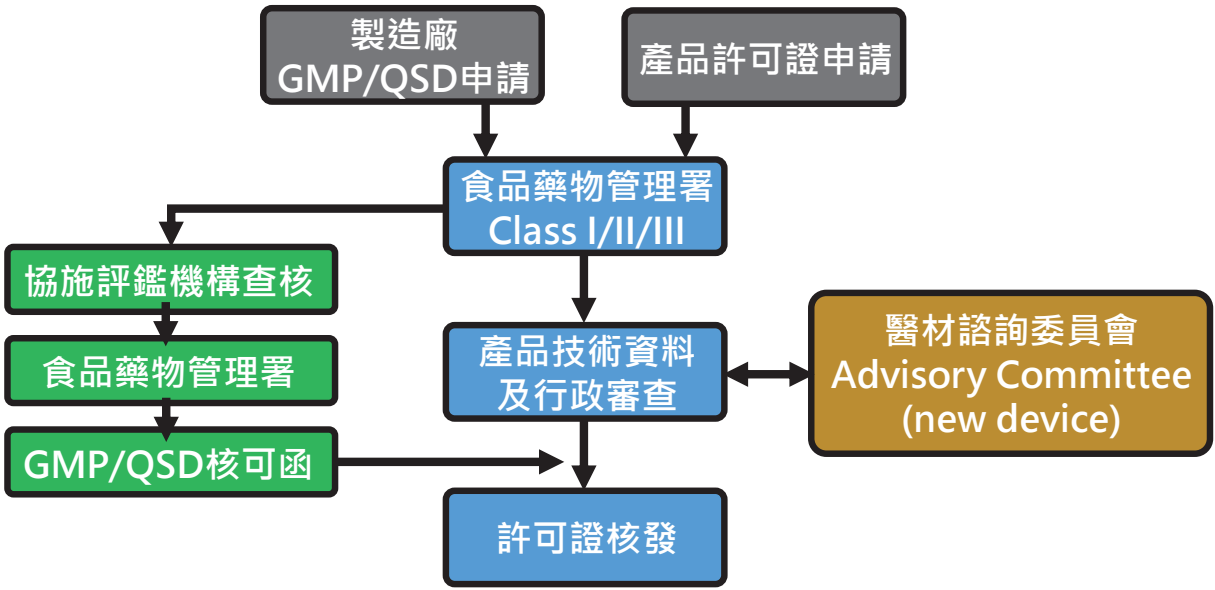
臨床試驗

- 需進行再現性(Reproducibility)、靈敏度(Sensitivity)、特異性(Specificity)、交互反應(Cross Reaction)等臨床評估。
- 需與國內核准上市或十大先進國家核可上市之同類產品進行平行比對。
- 比對有差異怎麼辦?

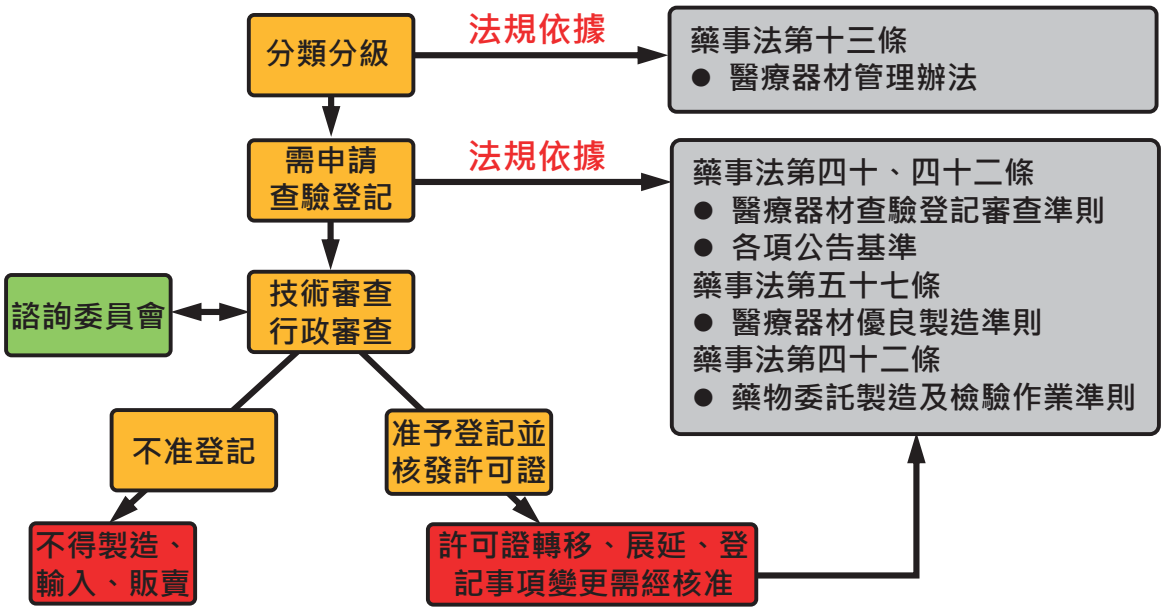
臨床平行比對有差異

- 以另一測試系統評估不一致檢體。
- 使用其他替代方法或標的物。
- 檢視病人狀態。
- 後續檢體追蹤。

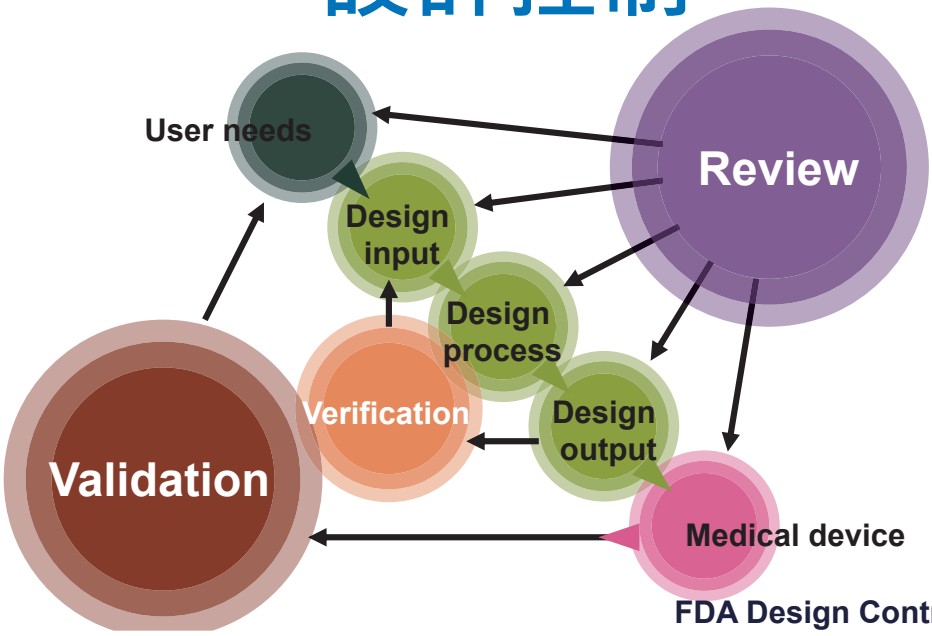
醫材上市前審查申請流程



上市前審查


分類分級
法規依據
藥事法第十三條
● 醫療器材管理辦法
需申請
查驗登記
法規依據
藥事法第四十、四十二條
● 醫療器材查驗登記審查準則
● 各項公告基準
藥事法第五十七條
● 醫療器材優良製造準則
藥事法第四十二條
● 藥物委託製造及檢驗作業準則
諮詢委員會
技術審查
行政審查
不准登記
准予登記並
核發許可證
不得製造、
輸入、販賣
許可證轉移、展延、登
記事項變更需經核准

設計控制



FDA Design Control Guidance

醫療器材＆體外診斷器材

- **醫療器材(Medical Device)**

本法所稱醫療器材，係用於診斷、治療、減輕、直接預防人類疾病、調節生育，或足以影響人類身體結構及機能，且非以藥理、免疫或代謝方法作用於人體，以達成其主要功能之儀器、器械、用具、物質、軟體、體外試劑及其相關物品。

(藥事法第十三條)

- **體外診斷醫療器材(In Vitro Diagnostic Device, IVD)**

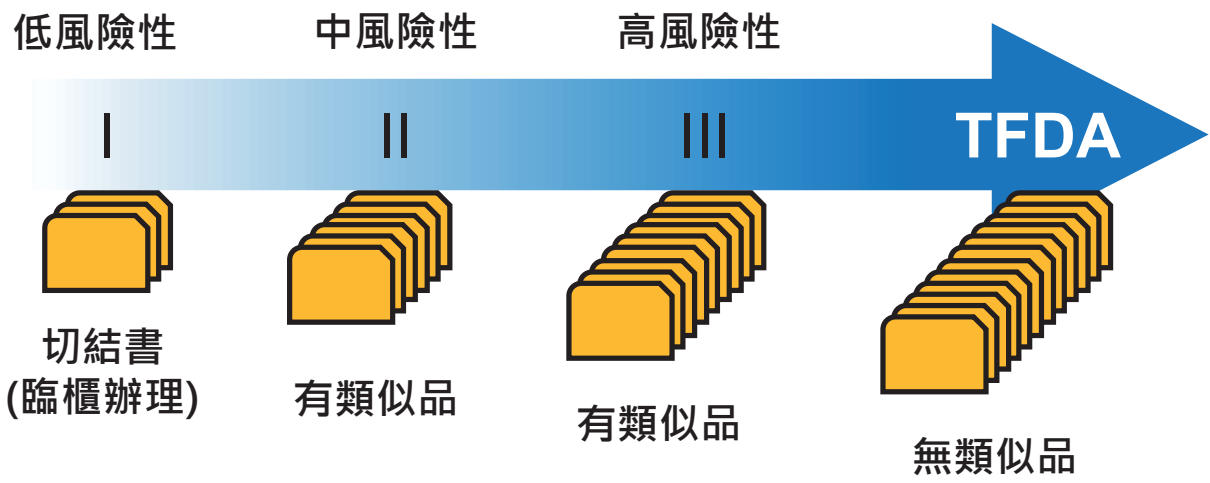
係指蒐集、準備及檢查取自於人體之檢體，作為診斷疾病或其他狀況(含健康狀態之決定)而使用之診斷試劑、儀器或系統等醫療器材。

(醫療器材查驗登記準則第九條)

器材分級

依風險程度分三級:

依照醫療器材管理辦法第二條



臨床前試驗

- **臨床前測試**

目的：瞭解此產品之性能與安全性，並可用以做為後續設計臨床試驗(評估)之參考依據。

原則：模擬實際使用狀況，進行產品安全性與功能試驗。

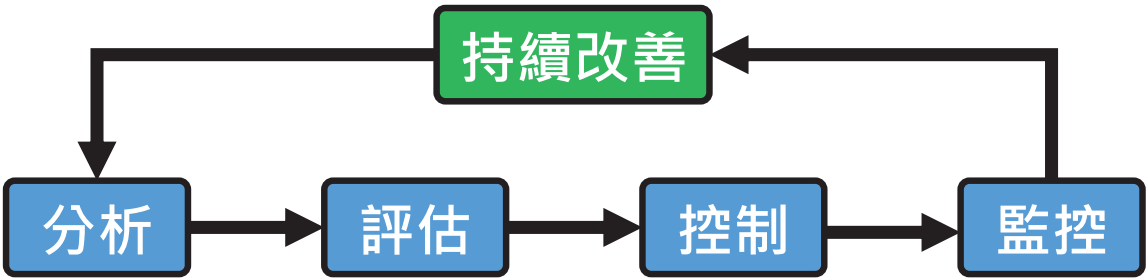
- **安全性測試：**

風險管理ISO 14971
電性安全IEC 60601-1
電磁相容性IEC 60601-1-2
生物相容性ISO 10993
滅菌確效ISO 11135, ISO 11137
軟體確效IEC 62304
可用性分析IEC 62366
動物試驗

- **功能性測試**

ISO, IEC, ASTM, AAMI, CLSI, 自訂...

醫材風險管理 ISO14971



識別風險

進行定性與定量風險管理

規劃風險應對計劃

控制風險

風險機率衝擊矩陣

風險值 Risk Value P*I		衝擊 Impact		
		1	2	3
機率 Probability	1	1	2	3
	2	2	4	6
	3	3	6	9

風險評等低者: 列入觀察清單，持續監測或增加應變準備。
風險評等高者: 需採取積極應對策略。

風險機率衝擊矩陣

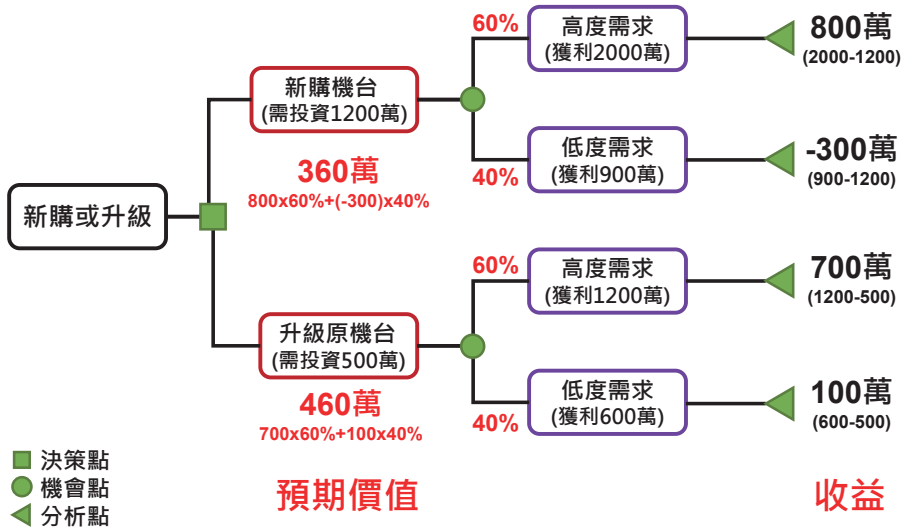
威脅 (Threat) 負面風險	機會 (Opportunity) 正面風險	風險等級
規避 Avoid	開拓 Exploit	高
減輕 Mitigate	提高 Enhance	中高
移轉 Transfer	分享 Share	中低
接受 Acceptance	接受 Acceptance	低

- ✓ **風險主動接受:** 風險發生前先發展應變準備及計畫(Contingency Reserve/Plan)
- ✓ **風險被動接受:** 風險發生後才發展權變措施(Workaround Plans)

風險定量分析技術

決策樹(Decision Tree)

將檢驗中特定決策(改變)造成的預期價值變動進行定量風險分析



電器類醫材相關標準

ISO 14971---風險管理

IEC 60601 系列---醫電設備之安全通用規範

IEC 61010 系列---適用於體外診斷設備、實驗室設備

ISO 10993 系列---生物相容性

IEC 60695 系列---防火試驗

IEC 60529---防水防塵試驗

IEC 60417---標示標記

生物相容性 ISO10993

接觸方式

表面接觸
- 皮膚
- 黏膜
- 裂開或受損表皮

外部通連器材
- 間接血液通道
- 組織/骨骼/牙質連通
- 血液循環系統

植入式器材
- 組織/骨骼
- 血液

接觸時間長短

有限暴露
- 單次或多次暴露/接觸，期間不會超過24小時之器材

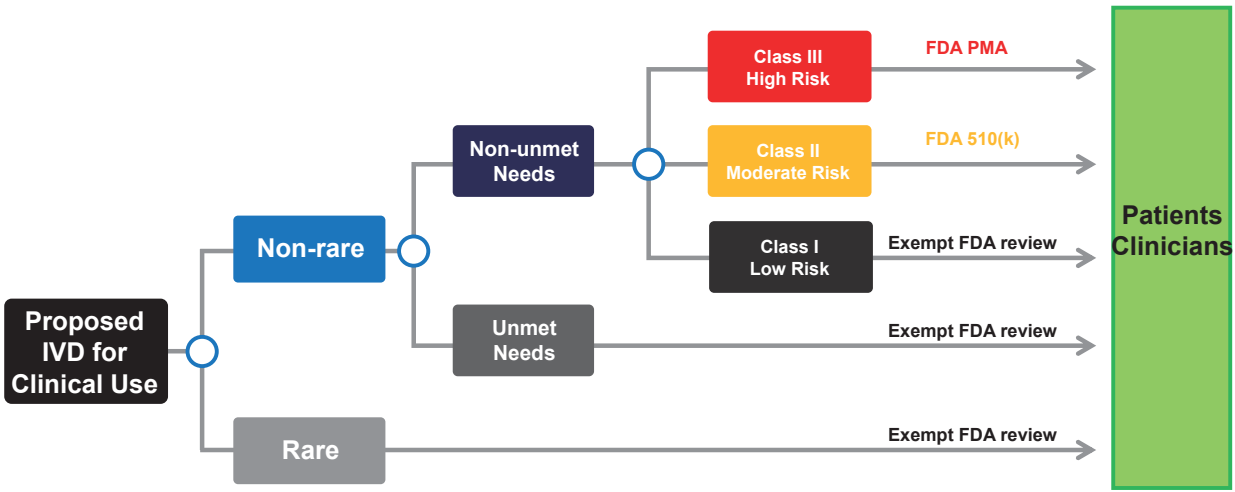
延長暴露
- 超過24小時但不超過30天之器材。

永久接觸
- 超過30天接觸以上之器材。

生物相容性 ISO10993

- 細胞毒性試驗(ISO 10993-5)
- 皮膚敏感性試驗(ISO 10993-10)
- 皮膚刺激性試驗(ISO 10993-10)
  - 眼刺激試驗
  - 皮內刺激性試驗
- 基因毒性試驗(ISO 10993-3)
  - 沙門氏菌回復突變試驗
  - 體外染色體變異分析試驗
  - 囓齒類週邊血液微核試驗
- 生殖毒性試驗(ISO 10993-3)
- 致癌性(ISO 10993-3)
- 血液相容性性試驗(ISO 10993-4)
- 植入試驗(ISO 10993-6)
- 熱原試驗/rabbit or LAL(ISO 10993-11)
- 系統毒性試驗(ISO 10993-11)
  - 急性毒性試驗
  - 亞急/亞慢毒性試驗
  - 慢性毒性試驗

FDA Proposed Risk-based Framework




Q.
A.