基因及細胞治療產品之品管試驗 (簡介)

啓弘生物科技
董事長 阮大同 博士

2018, May 28, 31

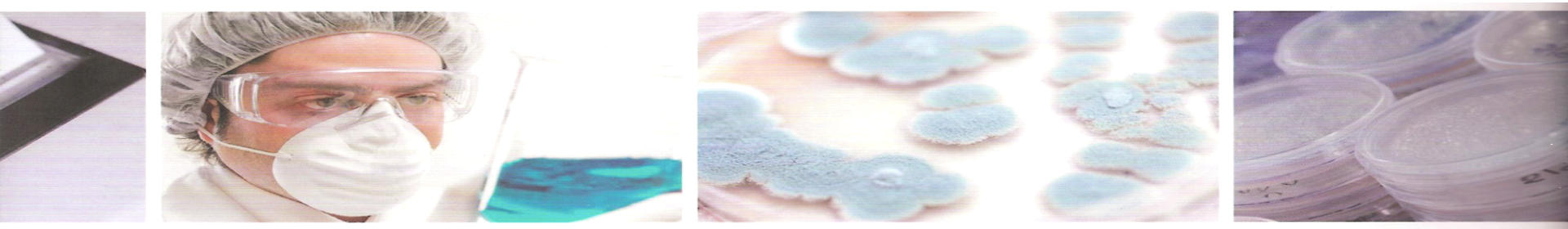
大綱

- 基因及細胞治療產品的興起
- 細胞及基因治療產品法規
  - 人類細胞治療產品查驗登記審查基準(台灣)
- 第二章 品質與製造管控
  - 製造原料
  - 細胞產品的特性分析
  - 最終產品的放行測試
- 結論

啓弘公司簡介

- 啓弘生物科技股份有限公司，由生物技術開發中心前副執行長阮大同博士於2016年創辦

- 從事研發新藥安全性及有效性檢驗，希望建立生技藥品檢驗在亞太地區的領導品牌



Testing Facility for Biological Safety

啓弘的檢驗能力及業務

臨床(前)試驗樣品檢驗方法開發	製程安全性確效	產品批次放行方法開發
臨床(前)試驗樣品檢驗	產品安全性檢驗	產品批次放行檢驗

The World's First Off-The-Shelf Stem Cell Therapy TEMCELL Approved in Japan

February 24, 2016

JCR Pharmaceuticals launched its mesenchymal stem cell product TEMCELL® HS Inj., for the treatment of acute graft versus host disease (aGVHD) in Japan.

2017年核准的細胞治療產品

http://www.onclive.com/web-exclusives/fda-oks-tisagenlecleucel-in-all-as-first-approved-car-tcell-therapy

FDA OKs Tisagenlecleucel in ALL as First Approved CAR T-Cell Therapy

Jason M. Broderick

The FDA issued a historic approval of the first chimeric antigen receptor (CAR) T-cell Therapy, authorizing the use of tisagenlecleucel (Kymriah) for the treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse.

KYMRIAH™ (tisagenlecleucel) Suspension for IV infusion

The New York Times | https://nyti.ms/2zjDAjp

HEALTH

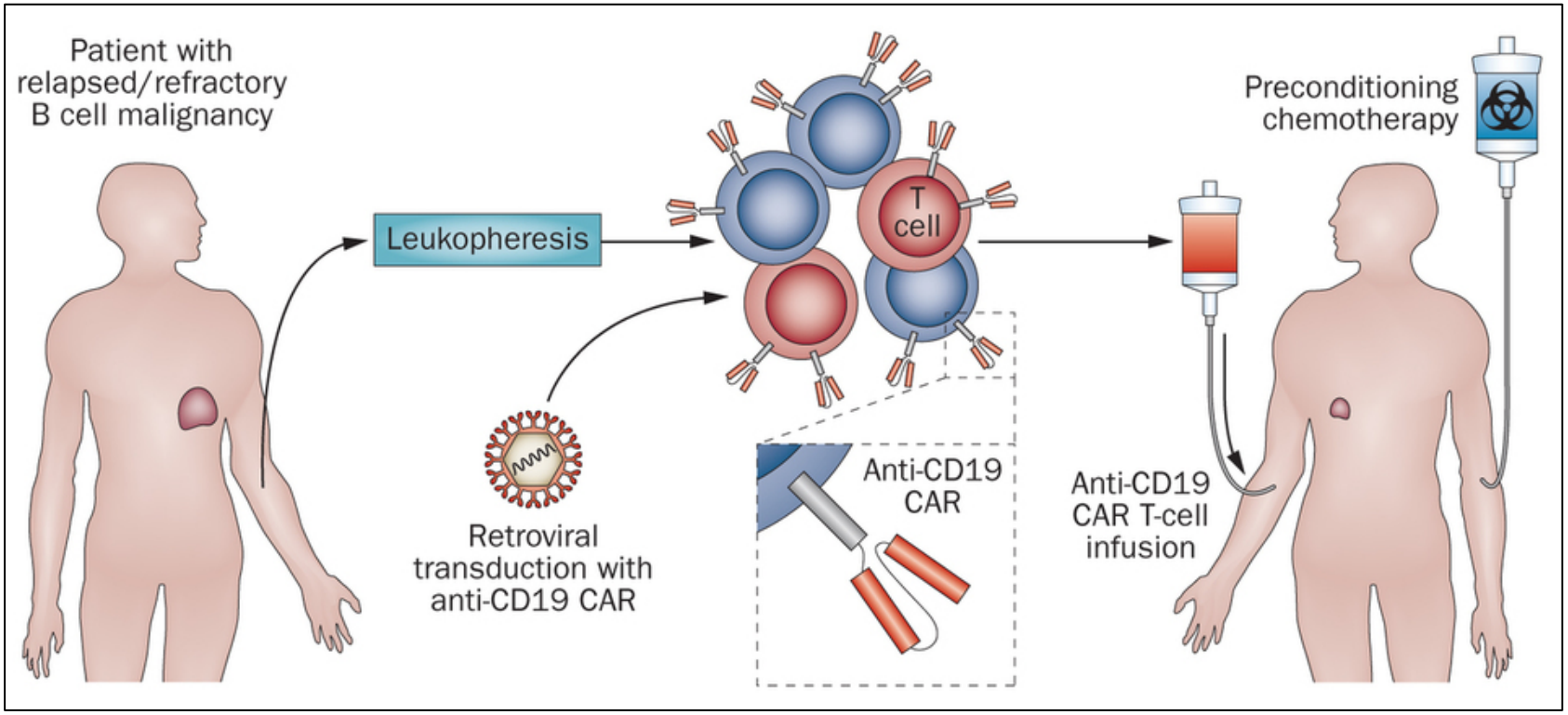
F.D.A. Approves Second Gene-Altering Treatment for Cancer

By DENISE GRADY OCT. 18, 2017

The Food and Drug Administration on Wednesday approved the second in a radically new class of treatments that genetically reboot a patient's own immune cells to kill cancer.

NOW APPROVED
YESCARTA™ (axicabtagene ciloleucel) Suspension for IV infusion

Beginning of Cell/Gene Therapy



- Ex vivo genetically modified cells are considered gene therapy. Issues associated with gene therapy products are addressed in detail in Gene Therapy Products 1047

#DEALS AUGUST 28, 2017 / 6:53 PM / 5 MONTHS AGO

Gilead to buy Kite for promising cancer therapies in $12 billion deal

#DEALS JANUARY 22, 2018 / 7:45 PM / 10 DAYS AGO

Celgene to buy Juno for $9 billion to boost cancer pipeline

Michael Erman, Tamara Mathias

4 MIN READ

2017年核准的基因治療產品

BIOTECH AND PHARMACEUTICALS

HEALTH CARE | HOSPITALS | PHARMA | HEALTH INSURANCE | MODERN MEDICINE

FDA approves Luxturna gene therapy for rare form of inherited vision loss

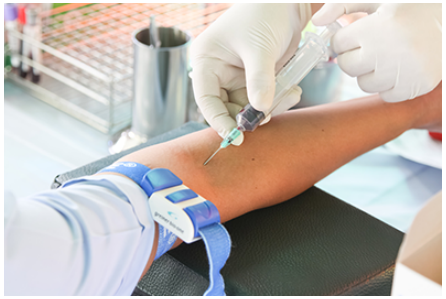
Angelica LaVito

Published 11:37 AM ET Tue, 19 Dec 2017 | Updated 4:01 PM ET Tue, 19 Dec 2017

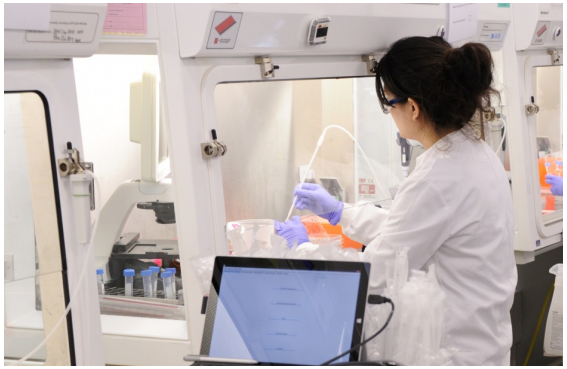
FDA Approved Cellular and Gene Therapy Products (-2017)

- ANDEXXA (coagulation factor Xa (recombinant), inactivated-zhzo)
  - Portola Pharmaceuticals, Inc.
- ALLOCORD (HPC Cord Blood)
  - SSM Cardinal Glennon Children's Medical Center
- LAVIV (Azficel-T)
  - Fibrocell Technologies
- MACI (Autologous Cultured Chondrocytes on a Porcine Collagen Membrane)
  - Vericel Corp.
- CLEVECORD (HPC Cord Blood)
  - Cleveland Cord Blood Center
- GINTUIT (Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen)
  - Organogenesis Incorporated
- HEMACORD (HPC, cord blood)
  - New York Blood Center
- Ducord, HPC Cord Blood
  - Duke University School of Medicine
- HPC, Cord Blood
  - Clinimmune Labs, University of Colorado Cord Blood Bank
- HPC, Cord Blood - LifeSouth
  - LifeSouth Community Blood Centers, Inc.
- HPC, Cord Blood - Bloodworks
  - Bloodworks
- IMLYGIC (talimogene laherparepvec)
  - BioVex, Inc., a subsidiary of Amgen Inc.
- KYMRIAH (tisagenlecleucel)
  - Novartis Pharmaceuticals Corporation
- LUXTURNA
  - Spark Therapeutics, Inc
- Plasma Cryoprecipitate (For Further Manufacturing Use)
  - OCTAPHARMA Pharmazeutika Produktionsges.m.b.
- PROVENGE (sipuleucel-T)
  - Dendreon Corp.
- Sterile Cord Blood Collection Unit with Anticoagulant Citrate Phosphate Dextrose Solution USP (CPD)
  - MacoProductions, S.A.S.
- YESCARTA (axicabtagene ciloleucel)
  - Kite Pharma, Incorporated

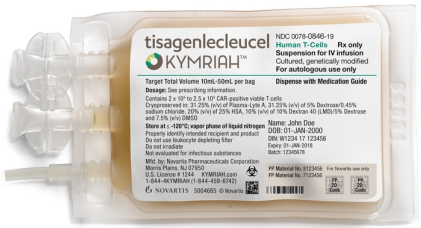
QC Test: Safety? Functionality? Consistency?



Hospital/Clinic

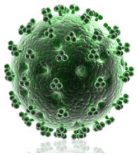


CPC (cell process center)



製藥公司

提供病毒



Cellular & Gene Therapy Guidances

- https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/default.htm
- Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy (PDF - 93KB) 3/1998
- Guidance for Industry; Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors (PDF - 76KB) 11/2006
- Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) (PDF - 173KB) 4/2008
- Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs) (PDF - 184KB) 4/2008
- Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products (PDF - 121KB) 1/2011. (This guidance finalizes the draft document of the same name, dated October 2008.
- Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products (PDF - 165KB) (This guidance finalizes the draft guidance entitled “Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products” dated November 2012) 11/2013
- Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products; Guidance for Industry (PDF - 120KB) 8/2015
- Recommendations for Microbial Vectors Used for Gene Therapy; Guidance for Industry (PDF - 161KB) 09/2016

台灣細胞及基因治療產品法規

- 「細胞及基因治療產品管理法草案」則已於2017年7月25日公告
- 「人類細胞治療產品查驗登記審查基準」2015.07
- 「人類細胞治療產品臨床試驗申請作業及審查基準」, 2014.09
- 「體細胞治療及基因治療臨床試驗計畫申請與審查作業規範(草案)」, 2013
- 「體細胞治療臨床試驗基準(草案)」, 2013
- 「基因治療臨床試驗基準(草案)」, 2013

細胞及基因治療產品管理法草案

- 為確保細胞及基因治療產品之品質、安全性及有效性,防止因使用 該產品而引起傳染病之導入、傳播及擴散,特制定本法。(特參考美國 21 CFR 1271)
- 本法所稱細胞治療產品,指 以診斷、治療或預防人類之 疾病為其目的,對於人體之 細胞施以加工而成之產品。
- 本法所稱基因治療產品,指 以診斷、治療或預防人類之 疾病為其目的,會使人體內 含有重組基因之產品。
- 細胞及基因治療產品推定具備有 關申請之療效性,與確認安全性者,經中央衛生主管機關審查後, 得核發附條件及給予不超過五年效 期之暫時性許可證。

人類細胞治療產品查驗登記審查基準 (2015.07)

- 本基準所稱人類細胞治療產品係指使用取自人類自體(autologous)或同種異體(allogeneic)的細胞,施用於病人以達到 疾病治療或預防的目的。異種異體(xenogeneic)之細胞治療不在 此範圍。
- 細胞種類可為具有自我更新能力之幹細胞、委任的前驅細胞 (committed progenitor cells)或是具有特定功能的分化細胞與組 織細胞;細胞可經過基因修飾;此外,細胞可與生物分子、生物 材料、化學合成之物質或與屬於醫療器材管理的結構材料併用。
- 申請查驗登記的人類細胞治療產品,其細胞或組織檢體的採集和 製造,須符合優良組織操作規範 (Good Tissue Practice, GTP),以及藥品優良製造準則之西藥藥品優良製造規範 (Pharmaceutical Inspection Co-operation Scheme Good Manufacturing Practice, PIC/S GMP)。

人類細胞治療產品查驗登記審查基準 (2015.07)

第一章 總則

第二章 品質與製造管控

第三章 非臨床試驗

第四章 臨床試驗

- 人類細胞治療產品係指使用取自人類自體 (autologous)或同種異體(allogeneic)的細胞 。異種異體(xenogeneic)之細胞治療不在 此範圍。
- 細胞種類可為具有自我更新能力之幹細胞、委任的前驅細胞 (committed progenitor cells)或是具有特定功能的分化細胞與組織細胞;細胞可經過基因修飾;此外,細胞可與生物分子、生物 材料、化學合成之物質或與屬於醫療器材管理的結構材料併用。
- 其細胞或組織檢體的採集和製造,須符合優良組織操作規範 (Good Tissue Practice, GTP),以及藥品優良製造準則之西藥藥品優良製造規範 (Pharmaceutical Inspection Co-operation Scheme Good Manufacturing Practice, PIC/S GMP)。

第二章 品質與製造管控

一.GTP/GMP

二.製造原料
1) 細胞
2) 試劑
3) 賦形劑

三.細胞產品製造與製程管控
1) 細胞之製備
2) 關鍵製程與製程管控
3) 製程確效
4) 儀器設備

四.細胞產品的特性分析
1) 微生物測試
2) 鑑別
3) 純度 ICH Q3
4) 效價 ICH Q6B
5) 存活率
6) 細胞數量/劑量
7) 致瘤性 ICH Q5D

五.最終產品的放行測試
六.批次分析結果
七.參考細胞標準品
八.容器密封系統
九.安定性試驗 ICH Q5C
十.其他議題

1) 細胞 - 細胞庫系統建立與鑑定分析

生物安全性檢測項目	種源細胞庫 (master cell bank)	工作細胞庫(working cell bank)	無細胞庫
Identity & Purity	• 表現型 • 基因型（如分子指紋、染色體安定性和致瘤性） • 細胞組成（預期與非預期細胞之比率） • 存活率 • 細胞活性及細胞分化性 • 細胞的生長動力學 • 族群倍增時間 • 細胞形態 • 繼代培養細胞滿度或細胞密度 • 細胞數目	• 適量鑑別測試（product specific）	
Microbiology	• 無菌性 • 黴漿菌	• 無菌性 • 黴漿菌	

Virus safety	• 體外病毒測試
	• 體內病毒測試
	• 特異性(species-specific)病毒
	  • 14種人病毒
	  • 牛源、豬源病毒
		• 體外病毒測試	• 牛源、豬源病毒

Stability	• 多代後基因型與表現型的安定性 • 細胞經低溫冷凍後的存活率		
Tumorigenicity	• 染色體安定性（核型分析karyotyping評估染色體完整性）		

微生物測試

1. 無菌測試
2. 黴漿菌
3. 外來病原測試

   (1) 體外病毒測試

   - 應在種源細胞庫及最終產品,實施一次體外病毒測試。

   (2) 體內病毒測試

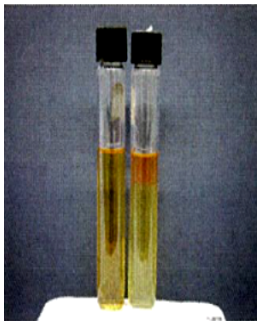
   (3) 對特定物種實施特定外來病毒之測試

   -因為治療產品是使用人類細胞 株,所以應對各種人類病原進行測試。

無菌測試 (Sterility) (1)

- Test contaminations of Aerobes, Anaerobes, and Fungi
- Sterility test is frequently performed for cell bank qualification and for product release to establish microbial purity, for cells that require extended culturing.
- Regulations
  - USP *Sterility Tests* 〈71〉
  - EP *Sterility* 〈2.6.1〉

無菌測試 (Sterility) -2

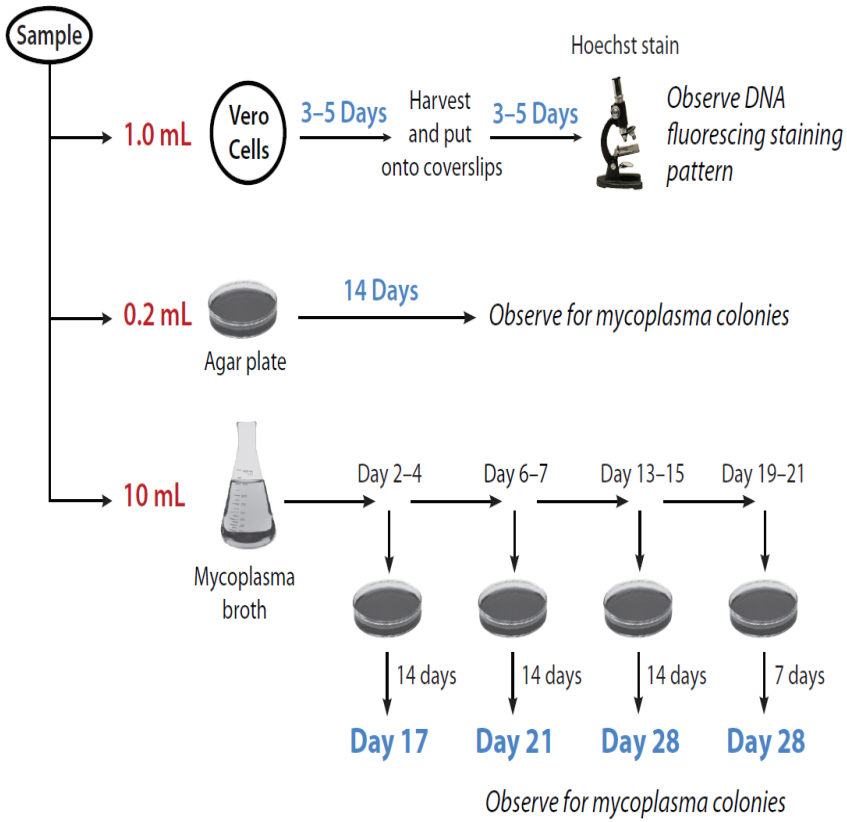


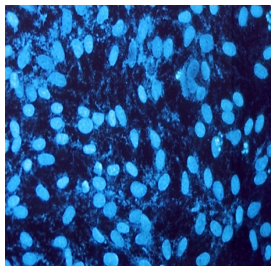
- Media
  - Fluid Thioglycollate Medium: anaerobic bacteria, aerobic bacteria
  - Soybean-Casein Digest Medium: fungi, aerobic bacteria
- separate methods
  - Membrane filtration
  - Direct Inoculation
- Sterility: Incubate **for 14 days**. No growth of microorganisms occurs.
- **Issues**: Many cell--based therapies have short shelf lives and must be delivered to patients before **the 14-day test results** are available.

黴漿菌 (Mycoplasma) -1

- Testing for mycoplasma is recommended for all raw materials derived from a human or animal source, and is required as both cell bank qualification and lot--release assay for cell--based products.
- Directions
  - USP *Mycoplasma Tests* 〈63〉
  - EP *Mycoplasmas* 〈2.6.7〉
- 2 methods
  - Indicator cell
  - Direct cultivation

黴漿菌 (Mycoplasma) -2





Source: BioReliance's Approach to Mycoplasma Testing: Introduction of United States Pharmacopoeia 63 Regulation

Because the classical assay takes ~1 month of testing to complete, alternative methods are being developed and validated for the rapid detection of mycoplasma.

外來病原測試 (Adventitious Agents)

- 體外病毒測試 (In Vitro Detection of Adventitious Virus)
  - 應在種源細胞庫及最終產品，實施一次體外病毒測試。應包括與產品製造所使用同種和同組織型的單層細胞，及易受人類病毒感染的人源，及(或)非人源之靈長類動物細胞株
- 體內病毒測試 (In Vivo Detection of Adventitious Virus)
  - 應對種源細胞庫實施體內病毒分析。成鼠及乳鼠，及雞胚胎蛋。
- 對特定物種實施特定外來病毒之測試
  - 應於製造的各個階段，實施特定外來病毒的測試，及明確敘述使用的測試方法。因為治療產品是使用人類細胞株，所以應對各種人類病原進行測試。測試人類病毒性病原可用聚合酶鏈鎖反應為基礎的測試系統。

In vivo Adventitious Agent Test (1) (Adult mice)

試驗物質	Human derived cell product
樣品製備	於注射之前，由Sponsor提供待測細胞
送樣規格	總共需要提供至少**20 mL** (1×10^7 cells/mL) <不包含留樣>
試驗體系	Adult mice (BALB/c)，5週齡
試驗觀察期	28 days
投予路徑	• IP • IC • PO • IN
試驗組別	**實驗共分成2組：** • 第一組背景組：**Culture medium** or **Isotonic Sodium Chloride Solution** • 第二組實驗組：待測細胞的**cell lysate** (1×10^7 cells/mL)
數據分析	• 動物存活率(至少80%存活) • Body weights (twice weekly)

Ref. *Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications. U.S. Food and Drug Administration (2010)*

In vivo Adventitious Agent Test (2) (Sucking mice, 乳鼠)

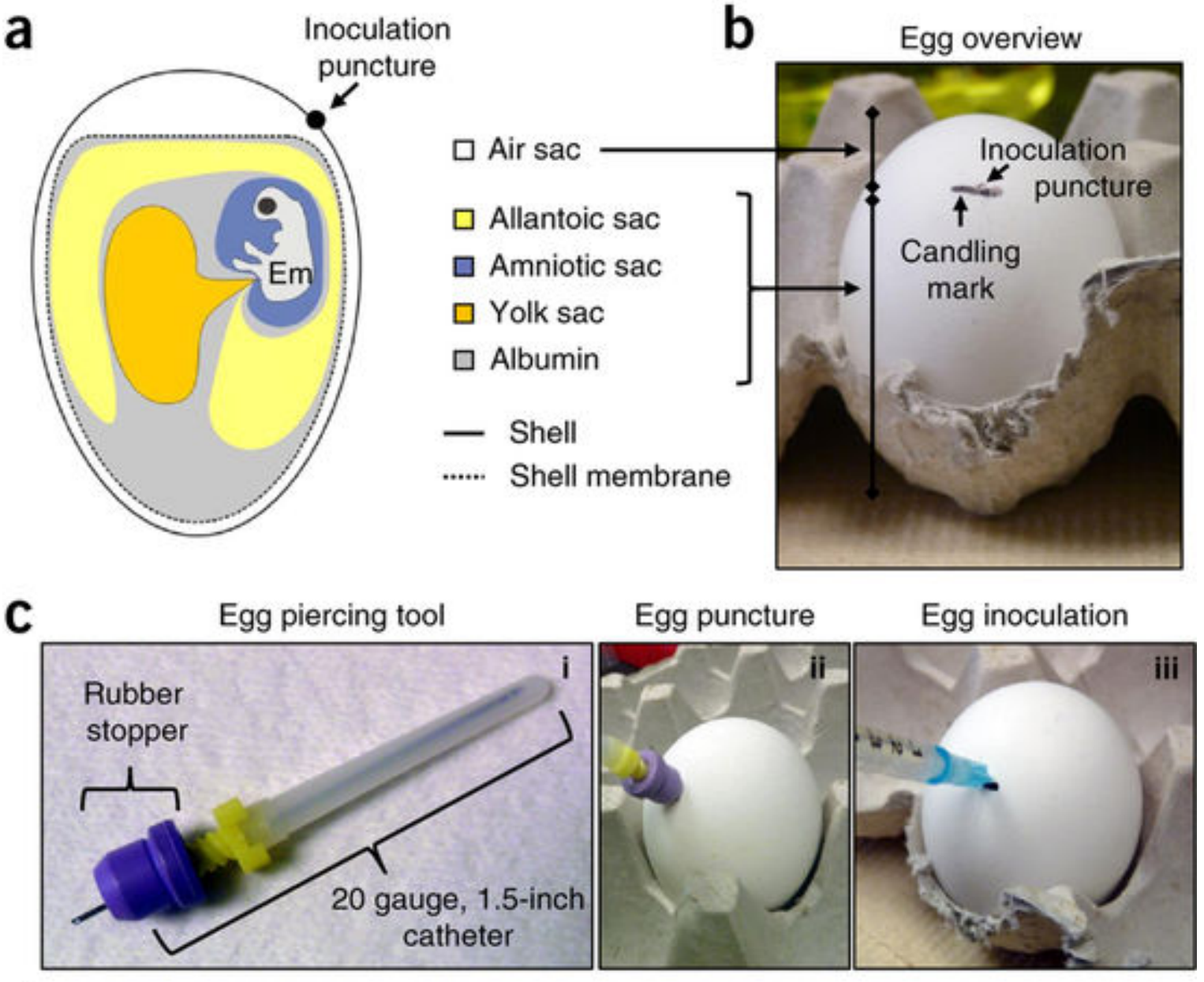
試驗物質	Human derived cell product
樣品製備	於注射之前，由Sponsor提供待測細胞
送樣規格	總共需要提供至少**5 mL** (1×10^7 cells/mL) <不包含留樣>
試驗體系	Sucking mice (CD-1® (ICR))
試驗觀察期	**14 days** (test article inoculation) + **14 days** (tissue homogenate inoculation)
投予路徑及劑量	• IP • IC • PO
試驗組別	實驗共分成4組： • 第一組背景組：**Culture medium** or **Isotonic Sodium Chloride Solution** • 第二組實驗組：待測細胞的**cell lysate** (1×10^7 cells/mL) • 第三組背景繼代組：從**第一組**動物觀察14-day後的tissue homogenate • 第四組實驗繼代組：從**第二組**動物觀察14-day後的tissue homogenate
數據分析	• 動物存活率(至少80%存活)

Ref. *Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications. U.S. Food and Drug Administration (2010)*

In vivo Adventitious Agent Test (3) (Embryonated Egg, 雞胚胎蛋)

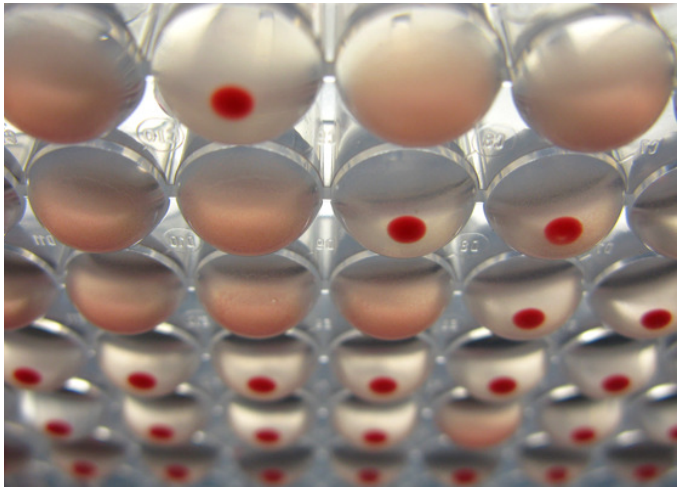
試驗物質	Human derived cell product
樣品製備	於注射之前，由Sponsor提供待測細胞
送樣規格	總共需要提供至少**3 mL** (1×10^7 cells/mL) <不包含留樣>
試驗體系	SPF Embryonated Hen's Eggs
試驗觀察期	**3 days** (test article inoculation) + **3 days** (allantoic/Yolk sac fluid inoculation)
投予路徑及劑量	• Allantoic • Yolk sac
試驗組別	**實驗共分成8組：** • 第一組背景組(Allantoic)：**Isotonic Sodium Chloride Solution** • 第二組實驗組(Allantoic)：待測細胞的**cell lysate** (1×10^7 cells/mL) • 第三組背景繼代組(Allantoic)：從**第一組**雞胚蛋觀察3-day後的allantoic fluid • 第四組實驗繼代組(Allantoic)：從**第二組**雞胚蛋觀察3-day後的allantoic fluid • 第五組背景組(Yolk Sac)：**Isotonic Sodium Chloride Solution** • 第六組實驗組(Yolk Sac)：待測細胞的**cell lysate** (1×10^7 cells/mL) • 第七組背景繼代組(Yolk Sac)：從**第一組**雞胚蛋觀察3-day後的Yolk sac fluid • 第八組實驗繼代組(Yolk Sac)：從**第二組**雞胚蛋觀察3-day後的Yolk sac fluid
數據分析	• 雞蛋存活率(至少80%存活) • Hemagglutination Test

Ref. *Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications. U.S. Food and Drug Administration (2010)*


a
Inoculation puncture
Em
Air sac
Allantoic sac
Amniotic sac
Yolk sac
Albumin
Shell
Shell membrane
b
Egg overview
Inoculation puncture
Candling mark
c
Egg piercing tool
Egg puncture
Egg inoculation
i
ii
iii
Rubber stopper
20 gauge, 1.5-inch catheter

Hemagglutination (HA) Test

	Components	Interaction	Microtiter Results
A	RBCs		No Reaction
B	Virus + RBCs	=	Hemagglutination
C	Virus + Antibody + RBCs	=	Hemagglutination Inhibition



In vitro Adventitious Agent Test

試驗物質	Human derived cell product
樣品製備	於注射之前，由Sponsor提供待測細胞
送樣規格	總共需要提供至少**20 mL** (1×10^7 cells/mL) <不包含留樣>
試驗體系	Cell-based
試驗觀察期	**14 days** (CPE observation) + **7 days** (HAD observation)
指示細胞	Vero, MRC-5 and HEK293 cell
試驗組別(CPE)	實驗分組： • 第一組陽性標準組(PC)：Bovine parainfluenza virus type 3 (BPIV-3), Encephalomyocarditis virus (EMCV) and Measles (MeaV) • 第二組陰性標準組(NC)：Culture medium only • 第三組試驗組(TA)：待測細胞的**cell lysate** (1×10^7 cells/mL) • 第四組試驗+陽性標準組(PC+TA)：PC+待測細胞的**cell lysate**
數據分析(HAD)	• 4 °C • Room Temperature

Bovine (牛) Virus Test

試驗物質	Human derived cell product
樣品製備	於注射之前，由Sponsor提供待測細胞
送樣規格	總共需要提供至少**20 mL** (1×10^7 cells/mL) <不包含留樣>
試驗體系	Cell-based
試驗觀察期	**14 days** (CPE observation) + **7 days** (HAD & IFA observation)
指示細胞	BT & Vero cell
試驗組別(CPE)	**BT cell實驗** **Vero cell實驗**
數據分析(HAD)	• 4 °C • Room Temperature
數據分析(IFA)	• BPV, BAV, BRSV, BVDV, BTV, Rabies, Reovirus

Porcine (豬) Virus Test

試驗物質	Human derived cell product
樣品製備	於注射之前，由Sponsor提供待測細胞
送樣規格	總共需要提供至少**20 mL** (1×10^7 cells/mL) <不包含留樣>
試驗體系	Cell-based
試驗觀察期	**14 days** (CPE observation) + **7 days** (HAD & IFA observation)
指示細胞	BT, ST, PK15 & Vero cell
試驗組別(CPE)	**BT cell實驗** **Vero cell實驗** **ST cell實驗** **PK15 cell實驗**
數據分析(HAD)	• 4 °C • Room Temperature
數據分析(IFA)	• **PPV, Reovirus, TGEV, PAV, BVDV, Rabies**

Human viral detection

Viruses						
HIV-1	HIV-2	HAV	HBV	HCV	HCMV	HAdV
HTLV-1	HTLV-2	EBV	B19	HHV-6	HHV-7	HHV-8

Assay Duration: 5~7 weeks (3 virus/week, 3 samples/study)

Sample request: at least 500 μL/virus (concentration: 1E7 cells/mL)

Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications. FDA, 2010.

Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products. EMEA/CHMP/BWP/398498/2005

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use. CBER, FDA, 1997

WHO Expert Committee on Biological Standardization. WHO TRS 941, 2007

Study include extraction and interference controls

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二.製造原料
1) 細胞
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四.細胞產品的特性分析
1) 微生物測試
2) 鑑別
3) 純度 ICH Q3
4) 效價 ICH Q6B
5) 存活率
6) 細胞數量/劑量
7) 致瘤性 ICH Q5D

五.最終產品的放行測試
六.批次分析結果
七.參考細胞標準品
八.容器密封系統
九.安定性試驗 ICH Q5C
十.其他議題

- 應對最終細胞進行特性分析

1) 微生物測試

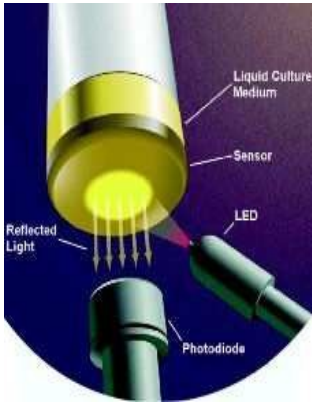
A. 藥典之無菌試驗法

如果細胞須在取得無菌試驗結果的 14 天前供病人使用時, (A)48 至 72 小時 無菌試驗結果,及(B)快速偵測微生物的檢驗方法, 來做為放行標準。此測試也應持續實施14 天的無菌試驗的結果

B. 替代之無菌試驗法

可以其他快速偵測微生物法來偵測細菌及黴菌之生長(例如:自動血液培養儀器 BACTEC、 BacT/ALERT、Rapid Milliflex 等)的檢驗結果替代藥典的無菌試驗法之檢驗結果

Growth based: BacT/ALERT® (bioMérieux)



- BacT/ALERT® 3D- bioMérieux
- Based on the metabolic activity of growing organisms as they produce CO_2
- $CO_2 + H_20 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$ (free H^+ lowers pH)
- Sensor changes color from blue-green to yellow
- The system is suitable for sterility testing of blood, body fluid, for rapid sterility testing of tissue and cell-based product. (the 14-day sterility test was reduced to just **three days**.)

Mycoplasma檢測替代方法

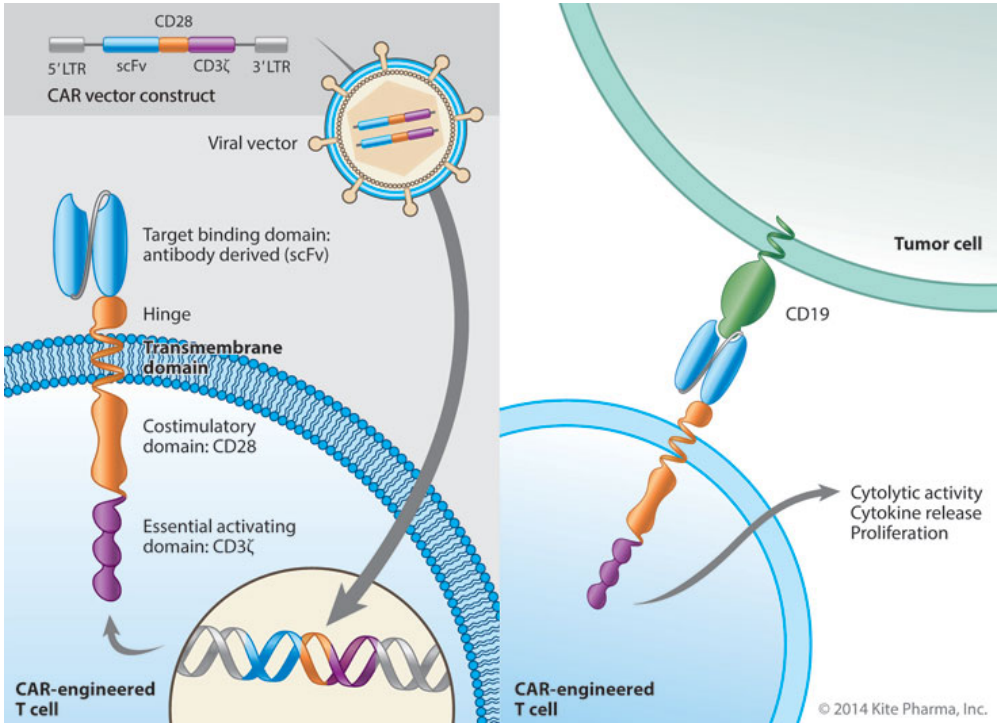
- 產品放行替代之黴漿菌試驗法 -人類細胞治療產品查驗登記審查基準(2015.07)
  - 當細胞治療產品之貯架期有限，致培養基為基礎的**培養基培養法(culture method)以及指示細胞培養法(indicator cell culture method)**為放行測試在實施上不可行時，可以接受以聚合酶鏈鎖反應(PCR)為基礎的黴漿菌分析，然應執行確效試驗來證明所使用的聚合酶鏈鎖反應測試法，有足夠的敏感度與精確度。
- *European Pharmacopoeia, 2.6.7. Mycoplasmas*
  - Broth/agar and indicator cell assay but **also allows PCR assays** as long as of equivalent sensitivity and specificity
- *US FDA Guidance for Industry*: Characterization and Qualification of Cell Substrates and Other Biological Starting Materials used in Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases, 2010
  - "**PCR-based assays may be used** to detect mycoplasma, provided that such an assay can be shown to be comparable to the agar and broth procedure and the indicator cell culture procedure."

2) 鑑別

- 應依據細胞族群以及來源鑑別其細胞基因型以及表現型。
- 細胞表現型可用適當且經確效的生物標記來進行分析，例如： 細胞抗原、生物化學活性等。
- 對於附著性細胞可以細胞形態來鑑別。
- 對於異體來源細胞的鑑別分析，應包含組織相容性標記(histocompatibility markers)以及基因多型性(genetic polymorphisms)分析。

CAR-CD19 T

- % of viable T cells
- Identity by CAR qPCR
- Determination of CAR expression by flow cytometry
- Release of IFN-γ in response to CD19-expressing target cells

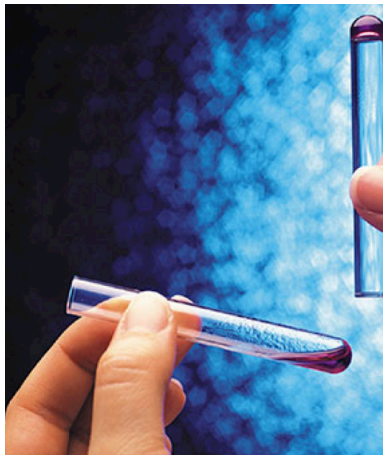


3) 純度

- 細胞治療產品中與療效相關之特定的細胞族群及其活細胞數目直接影響療效與安全性，因此與效能相關的細胞族群、其他細胞汙染物、活細胞/死細胞比例，應列入細胞治療產品放行規格中，並訂定可以接受之標準。若為幹細胞治療產品，應檢測其細胞分化比例。
- 包括：
  - 熱原性/內毒素（LAL）
  - 製程相關不純物 - 應測試對人類細胞治療產品實施的純度，包括：製造時使用的胜肽、蛋白質及試劑
  - 細胞相關不純物 - 細胞治療產品會有細胞來源的不純物，例如：產品本身的細胞聚集、死亡細胞、細胞降解的碎片、未預期的細胞表現型等。

Endotoxin: The LAL gel-clot method

- The most widely used methods for endotoxin detection employ Limulus Amebocyte Lysate (LAL), which is isolated from the blood of the horseshoe crab (Limulus polyphemus,鱟).
- Equal volumes of LAL reagents are mixed with undiluted or diluted test article,incubated for **1 hour at 37°C, and observed for gel formation.**
- A firm gel that remains intact on inversion of the tube indicates a **positive** reaction. A **negative** reaction is indicated by the absence of a solid gel after inversion.
- The sensitivity of this assay is 0.06 EU/ml.



7) 致瘤性

- 當細胞治療產品在體外繼代培養時,可能導致基因的不穩定 性,而產生致瘤性。幹細胞治療產品進行細胞擴增或分化 時,其分化的效率無法達到百分之百,或純化步驟無法有效 移除未分化之細胞,這些未分化的幹細胞或未分化完全的細 胞植入體內,有潛在的致瘤性風險。因此細胞經由細胞培養 程序或於最終細胞培養代數時,應評估其染色體完整性及致瘤性。

- Tumorigenicity & Oncogenicity
  - Tumorigenicity- in vitro (Soft agar)
  - Tumorigenicity- in vivo (Adult nude mouse)
  - Oncogenicity- in vivo (Newborn nude mouse)
  - ICH Q5D(R1)「Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products」

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五, 最終產品的放行測試

- 應以表格方式列出所擬定的最終產品規格，內容應包括：測試項目、測試方法及允收標準。測試項目應包括：微生物測試、鑑別、純度、效價、細胞存活率等
- 若在用於病人前細胞治療產品無法取得完整的放行試驗結果(例如：藥典的無菌試驗結果)，製造商應清楚指出在產品放行前無法取得結果的測試項目，應提供因應方案。
- 放行測試之分析方法應執行方法確效，以確認該方法能確切分析出該細胞治療產品之特性。

細胞安全性檢測項目

生物安全性檢測項目	MCB	WCB	最終細胞產品	放行測試	無細胞庫
Identity & Purity	• 表現型 • 基因型（如分子指紋、染色體安定性和致瘤性） • 存活率 • 細胞組成（預期與非預期細胞之比率） • 細胞活性及細胞分化性 • 細胞的生長動力學 • 族群倍增時間 • 細胞形態 • 繼代培養細胞滿度或細胞密度 • 細胞數目	• 適量鑑別測試（product specific）	• 表現型 • 基因型 • 細胞數目 • 存活率 • 其他適量鑑別測試（product specific） • 熱原性/內毒素	• 適量鑑別測試（product specific） • 熱原性/內毒素	
Microbiology	• 無菌性 • 黴漿菌	• 無菌性 • 黴漿菌	• 無菌性（替代法） • 黴漿菌（替代法）	• 無菌性（替代法） • 黴漿菌（替代法）	

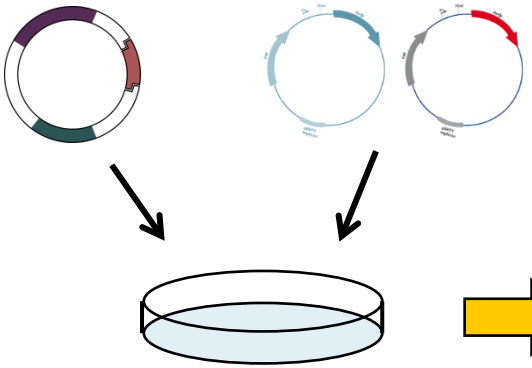
Virus safety	• 體外病毒測試
	• 體內病毒測試
	• 特異性(species-specific)病毒
	  • 14種人病毒
	  • 牛源、豬源病毒
		• 體外病毒測試	• 體外病毒測試
			• 特定外來人病毒
					• 牛源、豬源病毒

Stability	• 多代後基因型與表現型的安定性 • 細胞經低溫冷凍後的存活率		• 細胞經低溫冷凍後的存活率		
Tumorigenicity	• 染色體安定性（核型分析karyotyping評估染色體完整性）		• 染色體完整性（karyotyping） • 致瘤性		

Process control for virus production

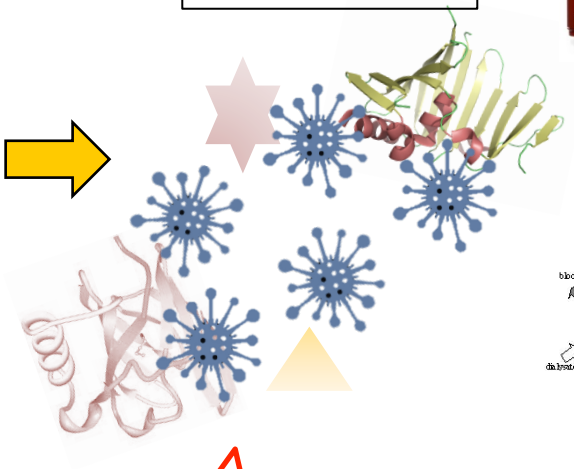
Transfer plasmid

Packaging plasmid



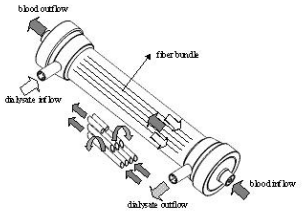
Host cells

Intermediate



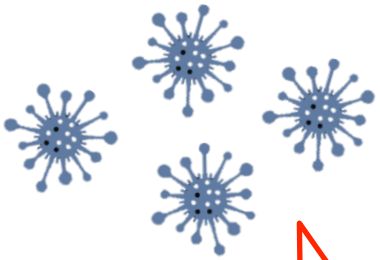
In-process control
- Safety
- Potency

Chromatography : Purification



Tangential flow fiber : Concentration

Product



Product release
- Identity
- Purity
- Quantity
- Safety
- Potency

QC Test for AAV

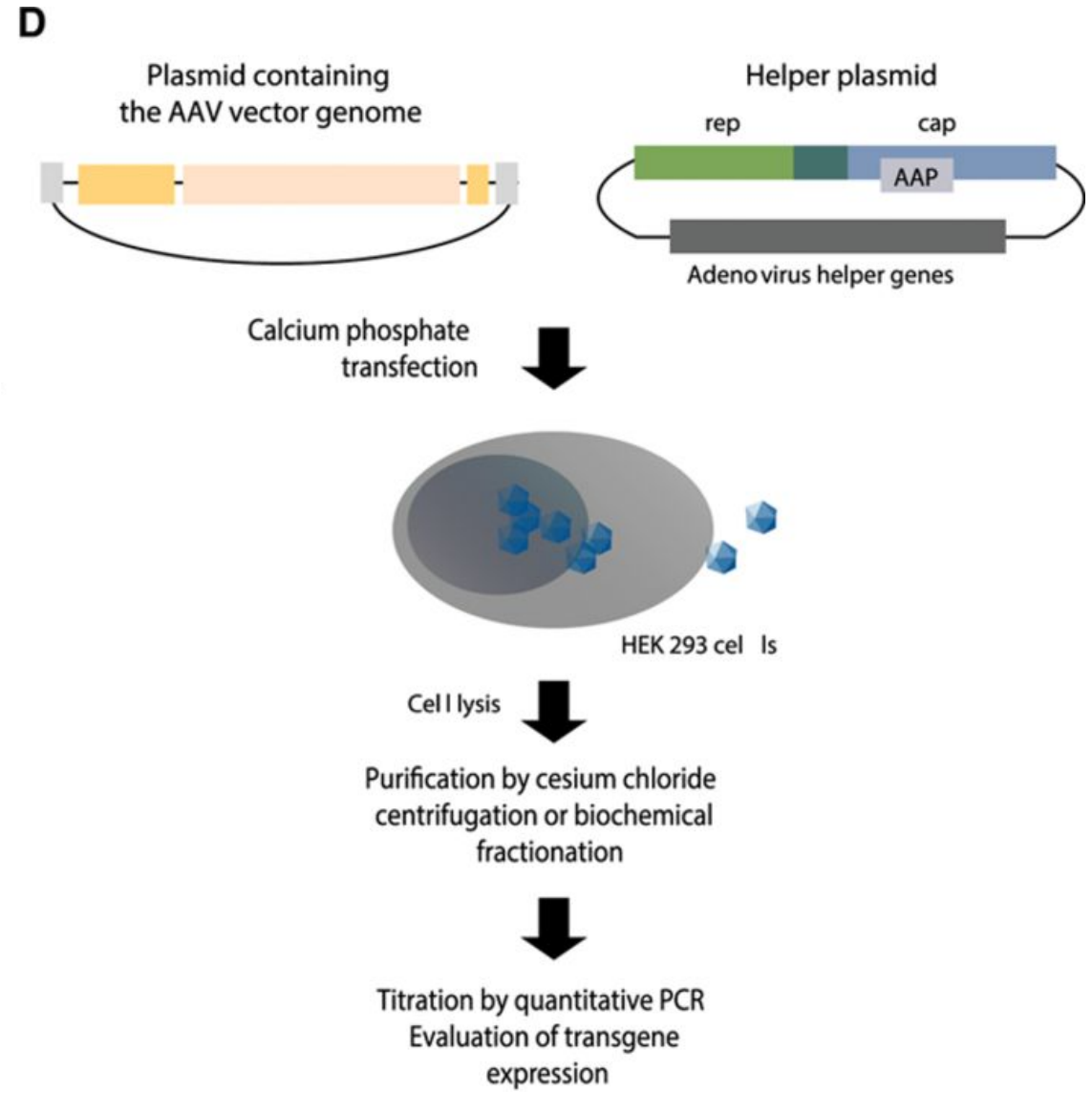
Attributes of AAV	Characteristics	Pre-IND (lot only)	cGMP grade (batch and lot)
Identity	Appearance	V	V
	pH	V	V
	Osmolality	V	V
	Genetic composition/integrity		V
Purity	Host cell proteins/viral proteins/ proteins from the cell medium		V
	Host cell DNA/Residual plasmid DNA		V
	Process derived impurities		V
Quantity	Viral genomes titer	V	V
	Infectivity titer	V	V
	Protein content	V	V
Safety	Endotoxin	V	V
	Sterility/mycoplasma	V	V
	Replication-competent AAV		V
	Testing for adventitious viruses		V
Potency	Depends	V	V

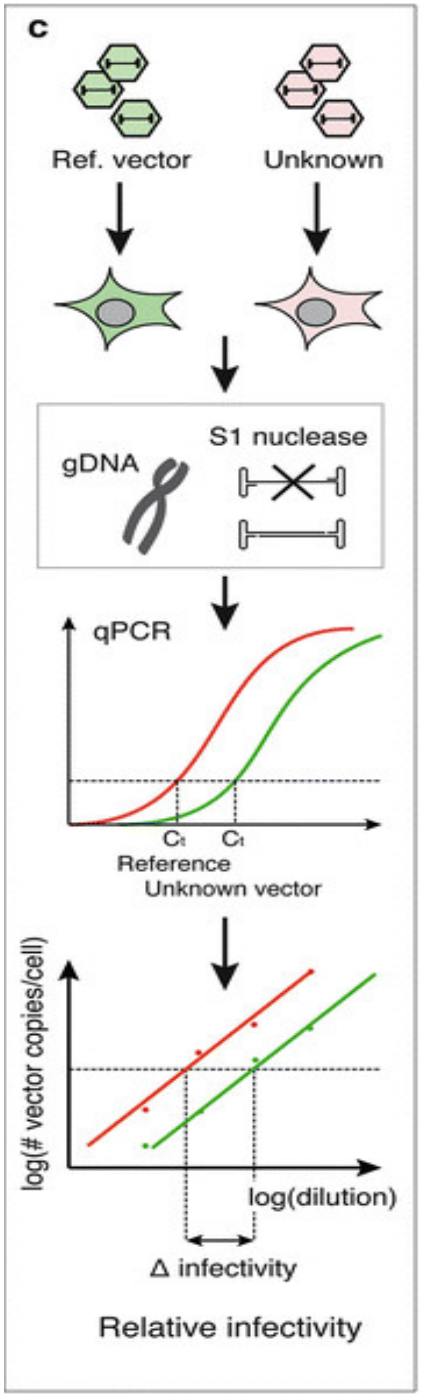
*Potency assay depends on your product. Please contact us to discuss your needs.

效價

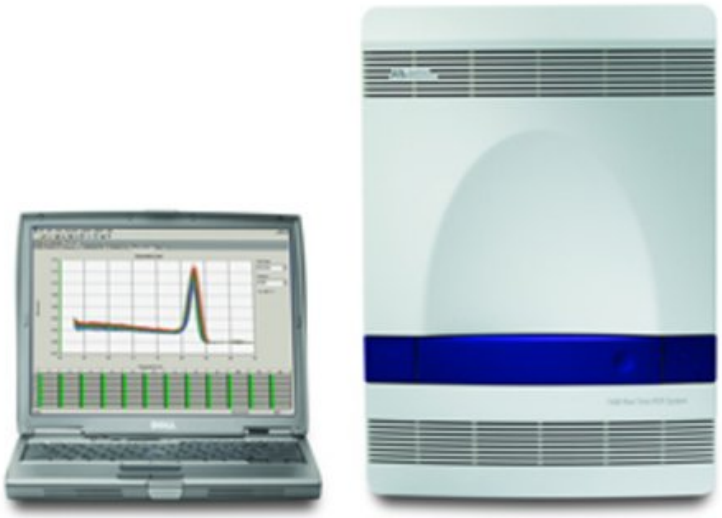
- 細胞治療產品的效價，可由臨床試驗結果來提供支持性證據，其應有細胞活性與臨床療效間的關連性。
- 包括：
  - 生物性測試
  - 非生物性測試
  - 多重測試

AAV genome titration (效價)

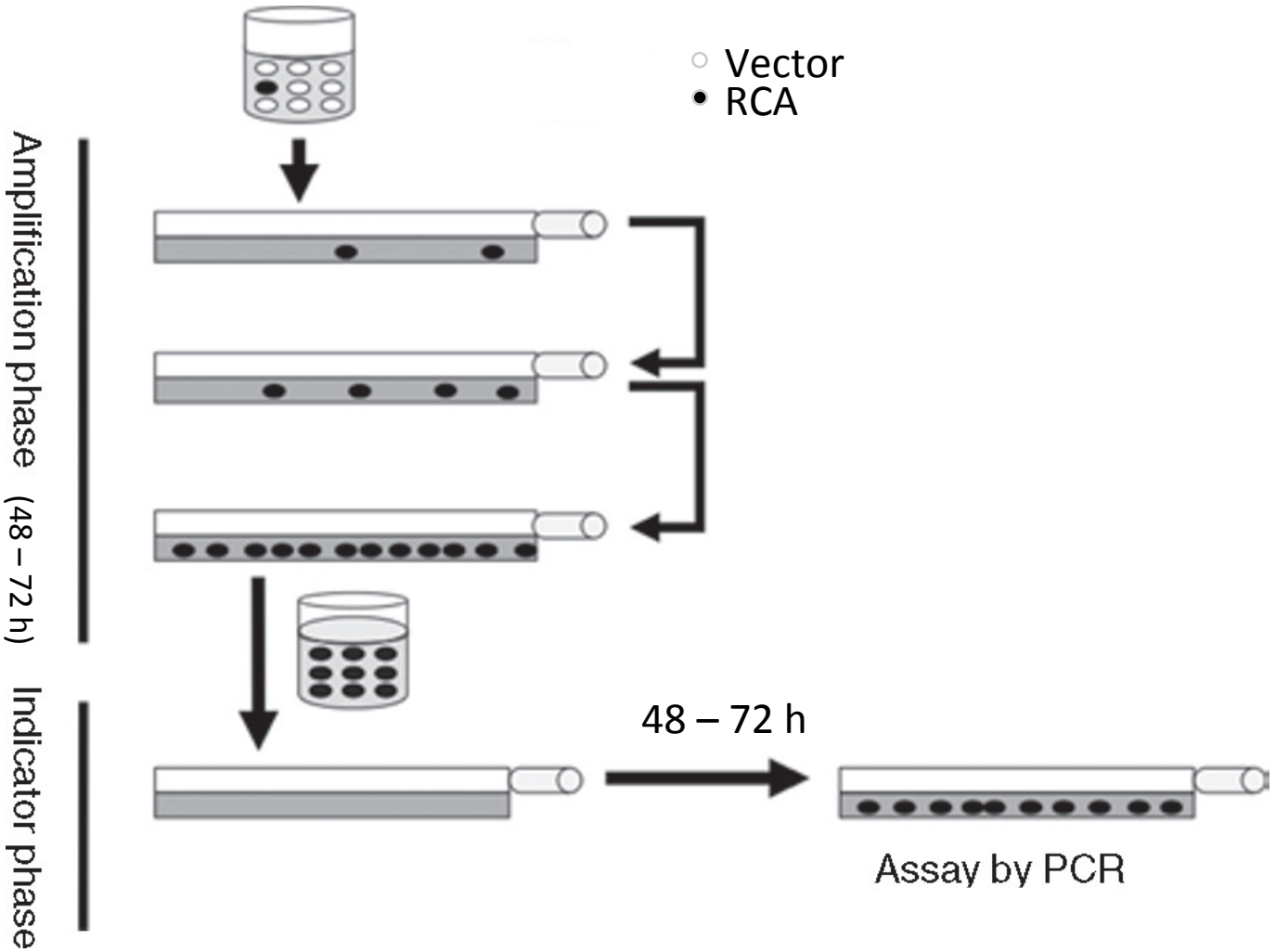




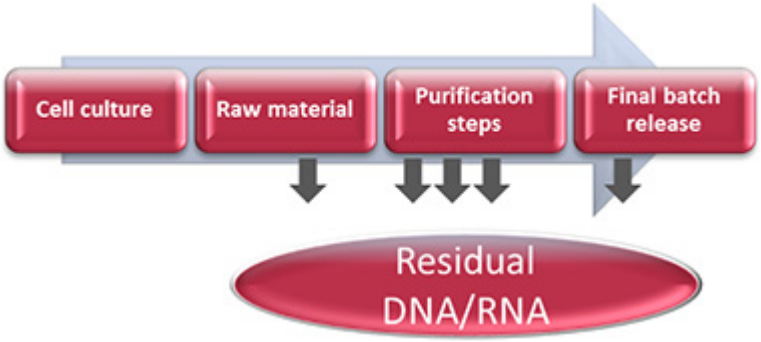
AAV infectivity titer (效價)

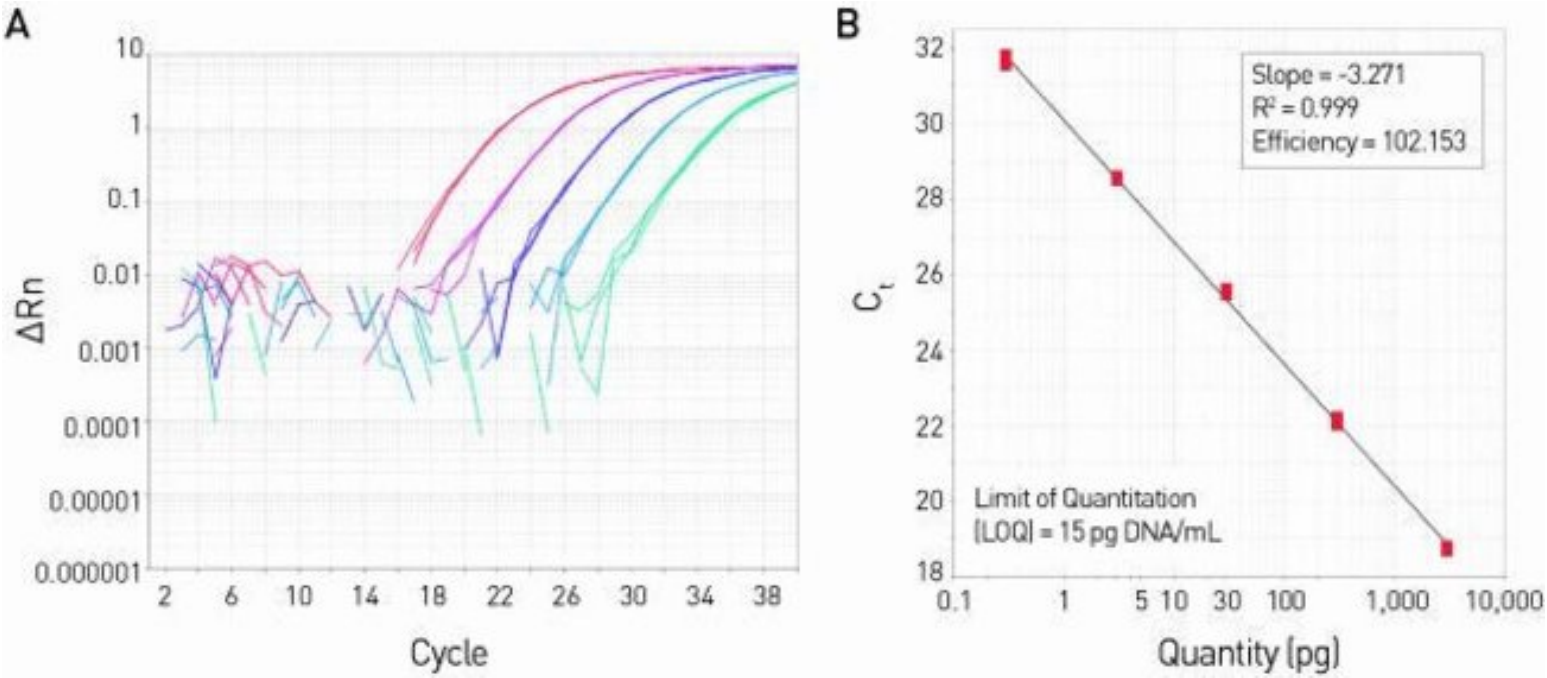


Replication-competent AAV (RCA) (純度)

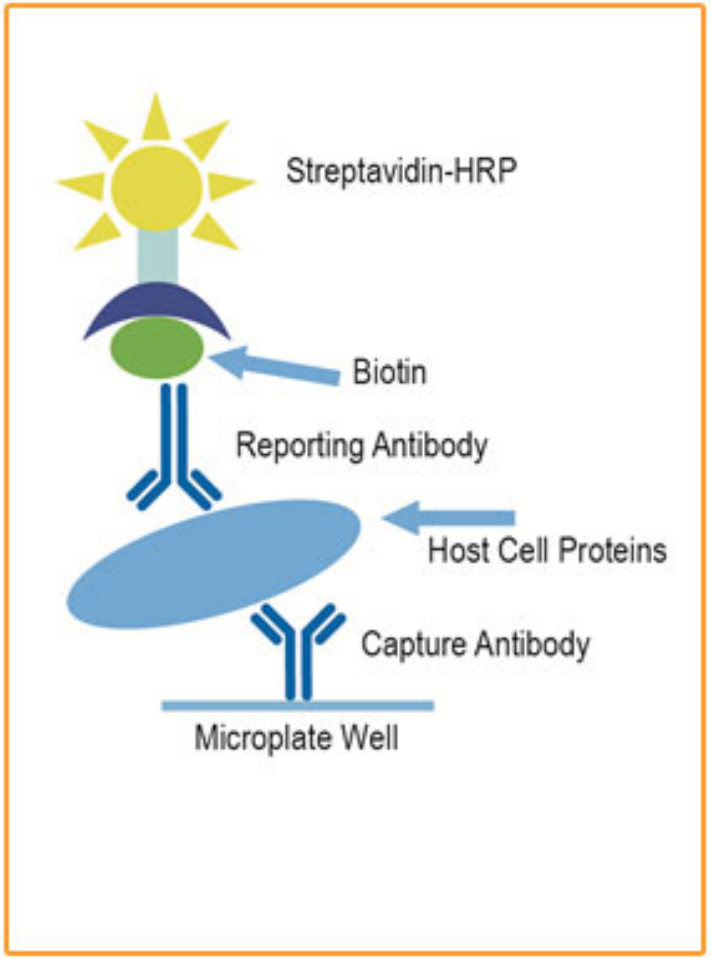


Residual host cell DNA(純度)





Residual host cell protein (純度)



- ELISA for measurement of HEK 293 host cell proteins

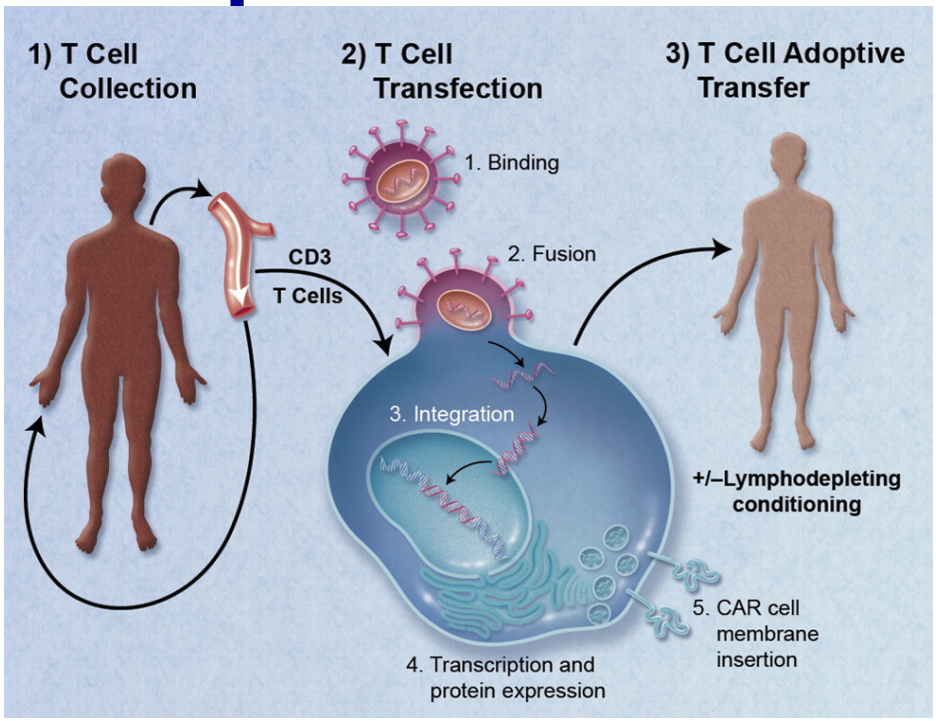
QC Test for CAR-T

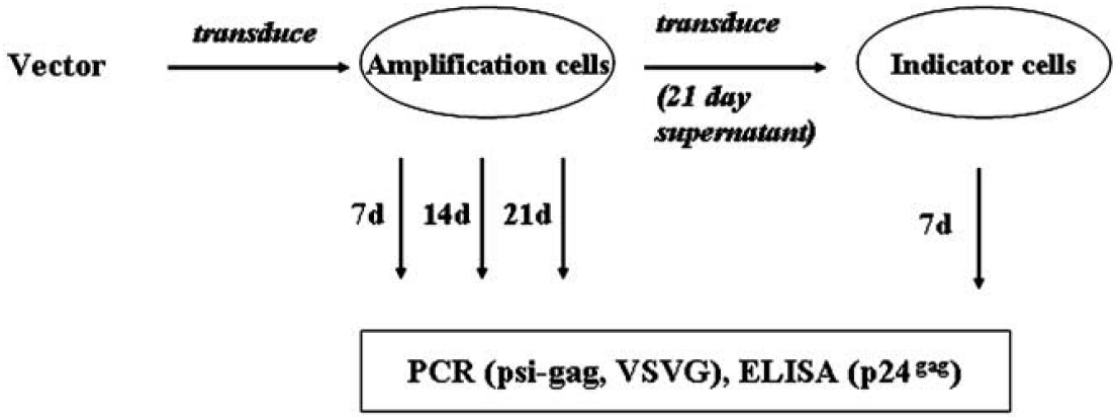
Table 1. KYMRIAH Lot Release Specifications

Test
Appearance
Identity by CAR q-PCR
Percentage of viable T cells
Determination of transduction efficiency by CAR-q-PCR
Cell viability
Determination of residual beads by microscopy
Percentage of viable CD19+ B cells
Total cell count[4]
Number of viable cells (calculated)
Dose (calculated)
Determination of CAR expression by flow cytometry
Release of IFNγ in response to CD19-expressing target cells
Bacterial Endotoxins
Sterility
Mycoplasma
Determination of VSV-G DNA by quantitative PCR (qPCR)

Reference: Novartis

CAR-T: VSV-G DNA by Q-PCR, replication competent lentivirus (RCL)





結論

- 基因及細胞治療產品的研發才剛起步, 但其應用於治療疾病的可能性已經得到臨床的驗證
- 此領域的產品研發, 製造, 及檢驗在往後幾年應會蓬勃發展
- 因為產品的複雜性, 藥檢單位的審查標準尚充滿"實驗性"及"妥協性", 與其充分的溝通是必要的
- 品管技術尚有極大的改進機會及空間

Thanks

QC Test for Lentivirus

Attributes of Lentivirus	Characteristics	Pre-IND (lot only)	cGMP grade (batch and lot)
Identity	Appearance	V	V
	pH	V	V
	Genetic composition/integrity		V
Purity	Host cell proteins/viral proteins/ proteins from the cell medium		V
	Host cell DNA/Residual plasmid DNA		V
	Process derived impurities		V
Quantity	Viral genomes titer	V	V
	Physical titer (p24)	V	V
	Transducing titer (depends)	V	V
Safety	Endotoxin	V	V
	Sterility/mycoplasma	V	V
	Replication-competent lentivirus		V
	Testing for adventitious viruses		V
Potency	Depends	V	V

*Potency assay depends on your product. Please contact us to discuss your needs.