9th DIA Cell and Gene Therapy Products Symposium in Japan

Development of Next-Generation Cell and Products

-Issues in Development of Existing Products and Prospects for Practical Application of Next Generation Products –

December 11-12, 2024

Hybrid | Nihonbashi Life Science Building & Online

Early consideration: Discussion points of quality control of the product derived from Evs

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DIA

Today’s Agenda

- Basic concept for biopharmaceutical quality control
- Virus safety of EV products
- Comparability of EV products

Today’s Agenda

- **Basic concept for biopharmaceutical quality control**
- Virus safety of EV products
- Comparability of EV products

Pharmaceutical Research
https://doi.org/10.1007/s11095-024-03757-4

REVIEW ARTICLE

Quality and Safety Considerations for Therapeutic Products Based on Extracellular Vesicles

Yoshinobu Takakura[1] · Rikinari Hanayama[2] · Kazunari Akiyoshi[3] · Shiroh Futaki[4] · Kyoko Hida[5] · Takanori Ichiki[6] · Akiko Ishii-Watabe[7] · Masahiko Kuroda[8] · Kazushige Maki[9] · Yasuo Miura[10] · Yoshiaki Okada[11] · Naohiro Seo[12] · Toshihide Takeuchi[13] · Teruhide Yamaguchi[14] · Yusuke Yoshioka[15]

i) Native EVs from genetically non-manipulated cells
ii) Native EVs from genetically modified cells without trans-gene products
iii) EVs as drug delivery systems (DDS) loaded with synthesized chemicals or defined recombinant molecules
iv) EVs from genetically modified cells with trans-gene products

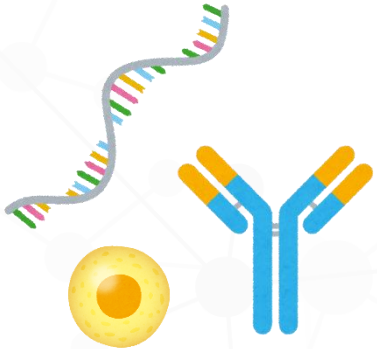
Today's scope

Chemical compound VS Biopharmaceutical

Chemical Compound

- Just one identical molecule
- Clear comparability

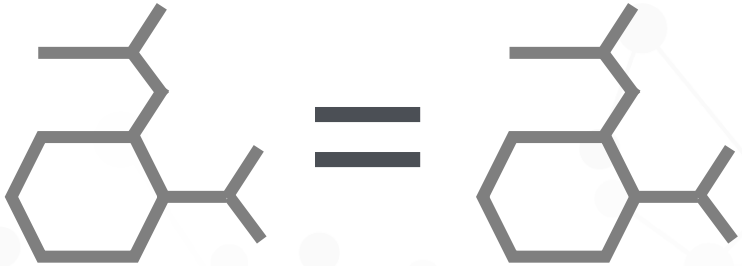
Biopharmaceutical



- A lot of variations (sugar chains, nucleic acid mutations etc.)
- Numerous active ingredient-related proteins/cells/nucleic acid
- Impossible to confirm identical

Comparability???

Chemical Compound

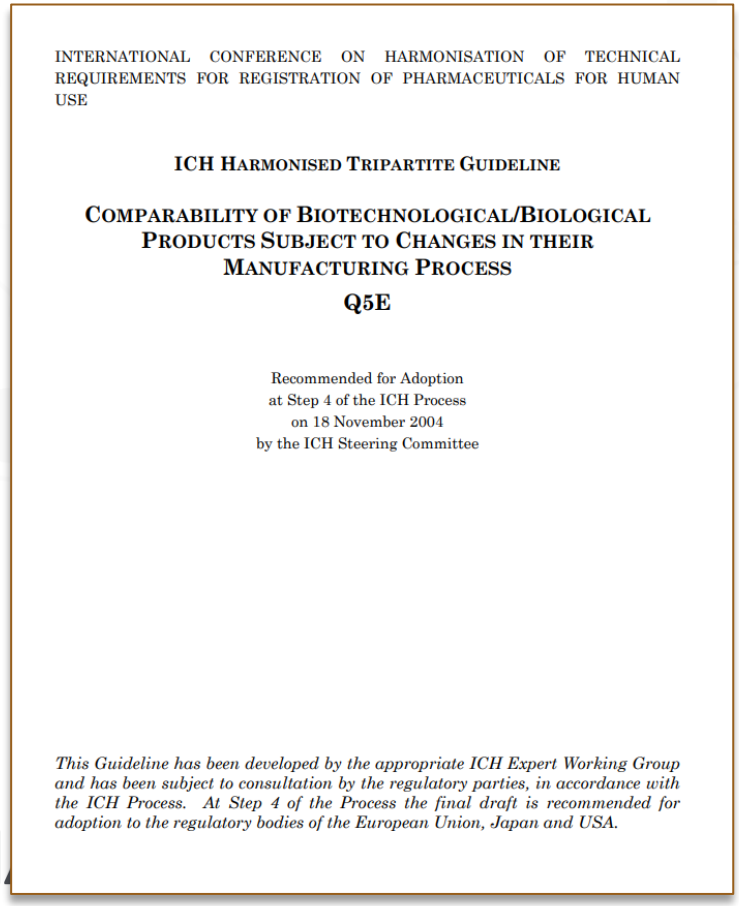


Easy to clarify two compounds are identical.

Biopharmaceutical

A lot of variations. Which are comparable?

ICH guideline Q5E Comparability of biopharmaceuticals



INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

COMPARABILITY OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS SUBJECT TO CHANGES IN THEIR MANUFACTURING PROCESS

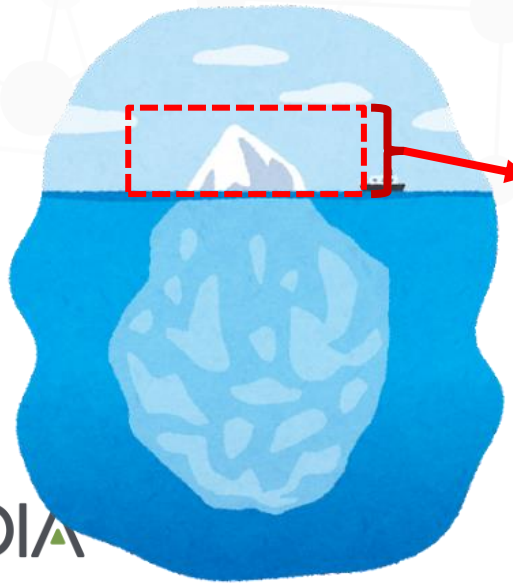
Q5E

Recommended for Adoption
at Step 4 of the ICH Process
on 18 November 2004
by the ICH Steering Committee

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

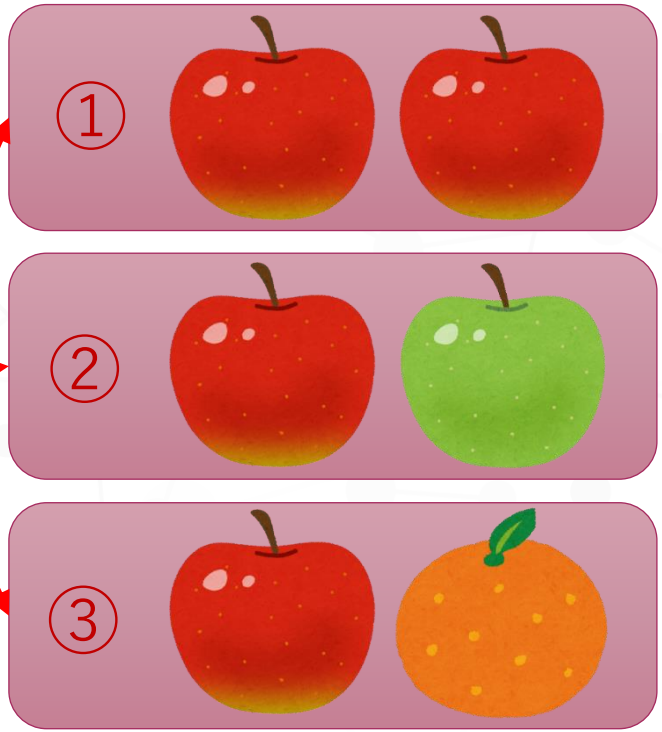
The demonstration of comparability does not necessarily mean that the quality attributes of the pre-change and post-change product are identical, but that they are highly similar and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon safety or efficacy of the drug product.

Are routine Shipping tests enough to evaluate comparability?

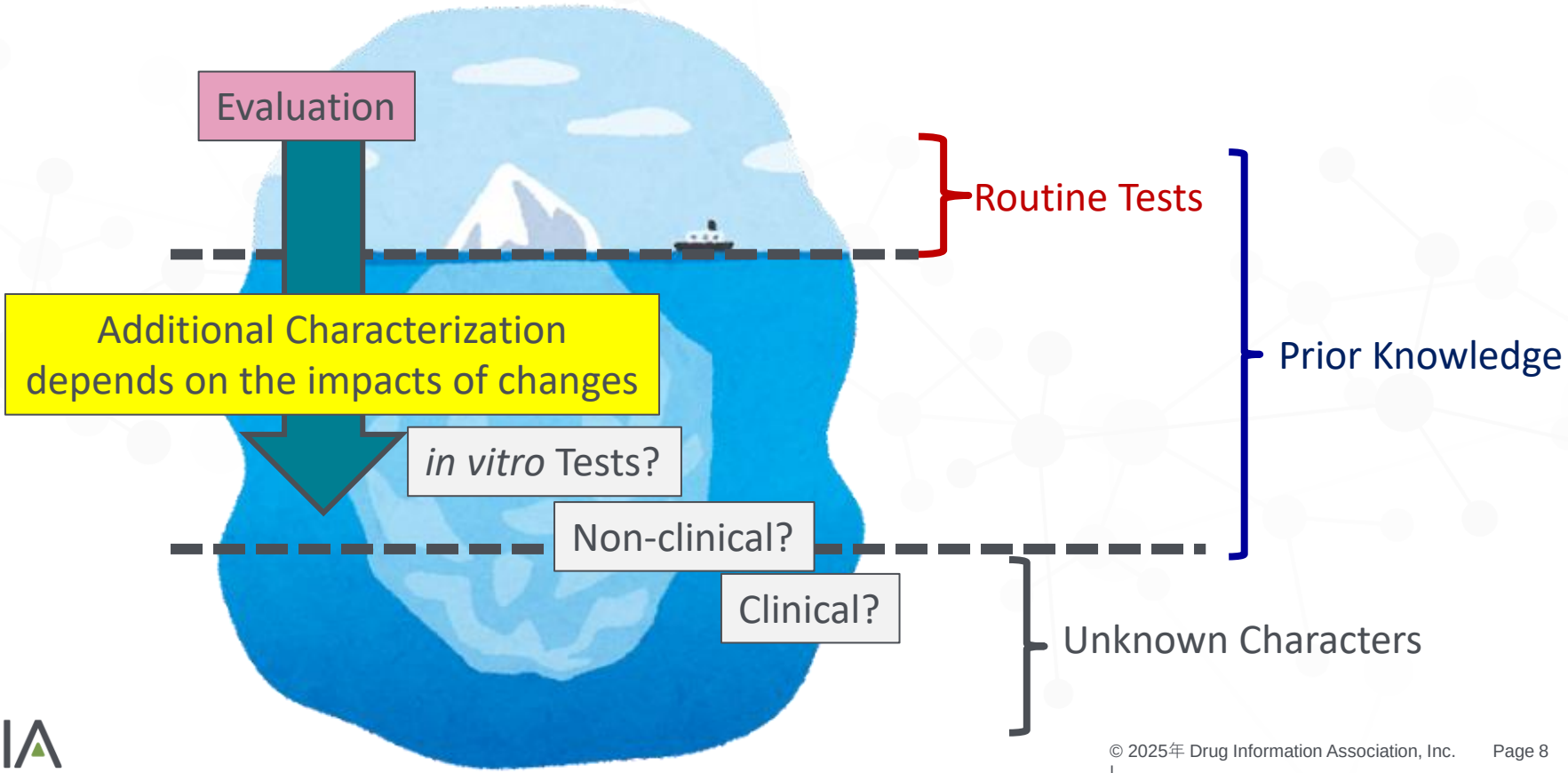


Comparability

【Shipping test】
Sugar Content
pH
Weight



Comparability of Biopharmaceutical



Today’s Agenda

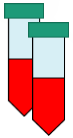
- Basic concept for biopharmaceutical quality control
- **Virus safety of EV products**
- Comparability of EV products

Contamination of infectious agents in Biopharmaceuticals

Easy to come, easy to grow, and hard to remove

Cultured Cells

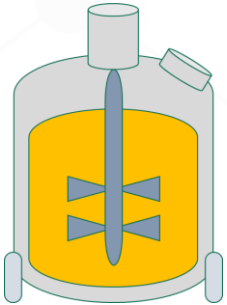
Virus seed



Fetal Bovine Serum

Porcine Trypsin

Raw Materials



Cultivation

Manufacturing Process

Will Collapse the products

× Autoclave
× Dry-air Sterilization
× Gamma ray
× Ethylene Oxide Treatment

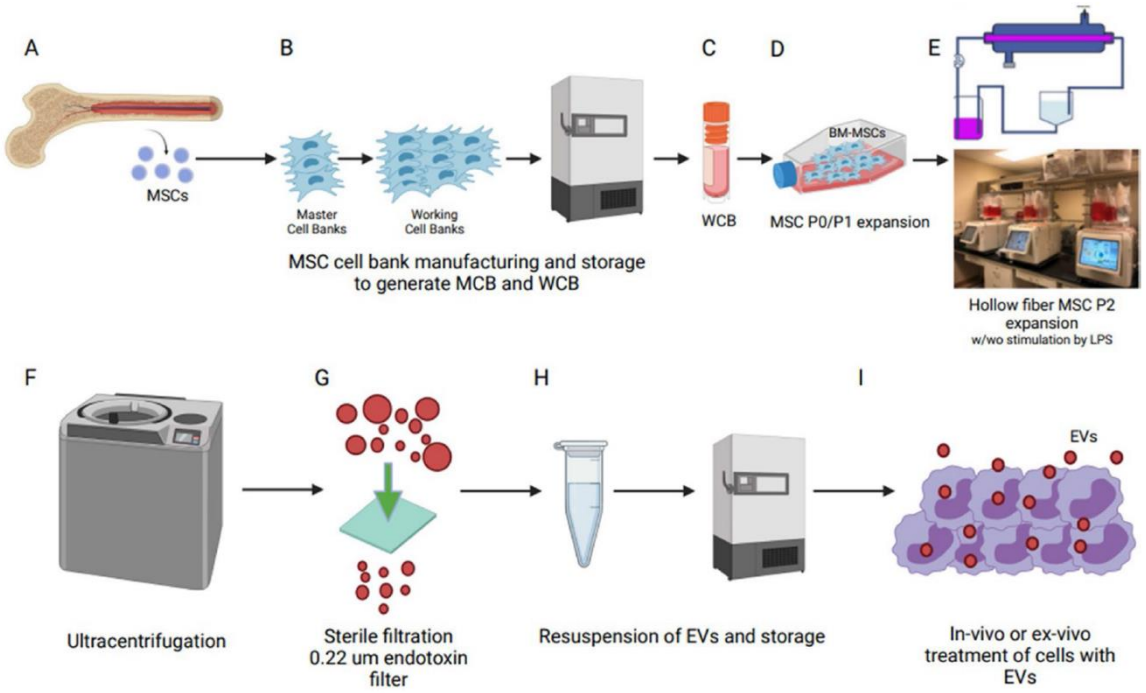
Severe Viral Inactivation

Kink et al. Stem Cell Research & Therapy (2024) 15:72
https://doi.org/10.1186/s13287-024-03688-2

RESEARCH Open Access

Large-scale bioreactor production of extracellular vesicles from mesenchymal stromal cells for treatment of acute radiation syndrome

John A. Kink[1,2], Michael A. Bellio[3], Matthew H. Forsberg[4], Alexandra Lobo[5], Anna S. Thickens[1], Bryson M. Lewis[1], Irene M. Ong[1,2,5,6], Aisha Khan[3], Christian M. Capitini[2,4,7] and Peiman Hematti[1,2,7*]



EXAMPLE OF VIRUS CONTAMINATION IN BIOLOGICAL MANUFACTURE.

nature biotechnology

PERSPECTIVE

https://doi.org/10.1038/s41587-020-0507-2

Check for updates

Viral contamination in biologic manufacture and implications for emerging therapies

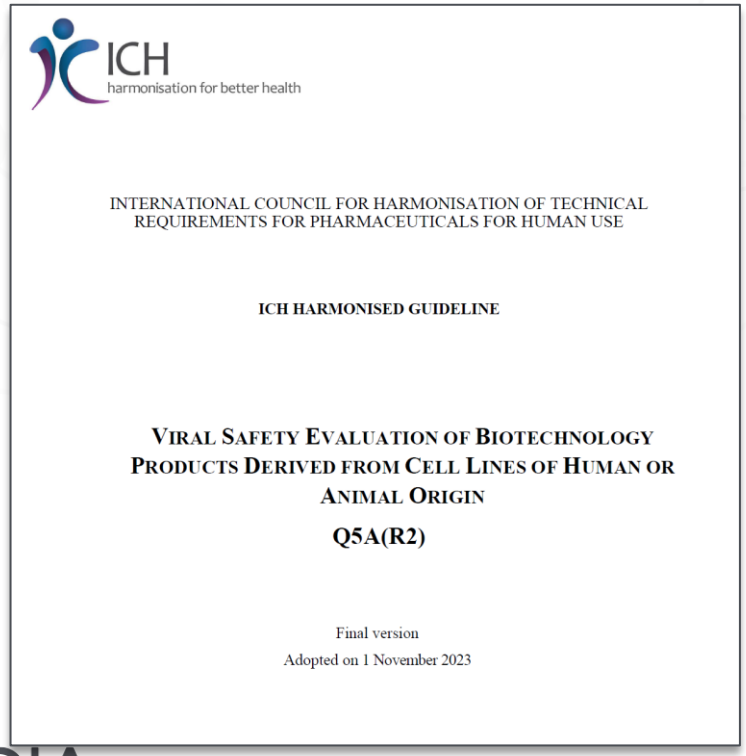
Contamination is not past.

Table 1 | Virus contaminations of mammalian cell culture to produce proteins and vaccines, segregated by year, both publicly reported and contained in the CAACB study

Year of contamination	Contaminations (virus / host cell)	Total
1985–1989	Blue tongue / CHO EHDV / CHO[18,19]	2
1990–1994	Herpesvirus / primary monkey Herpesvirus / Vero MVM / CHO (x2)[20–22] Parainfluenza 3 / MRC5 Reo3 / MRC5 Simian adenovirus / primary monkey	7
1995–1999	CVV / CHO Reovirus / human primary kidney[23] Vesivirus 2117 / CHO[24]	3
2000–2004	CVV / unknown (x2)[25] Human adenovirus / HEK293[26]	3
2005–2010	CVV / CHO MVM / CHO (x2) Vesivirus 2117 / CHO (x3)[27–29]	6
2010–present	MVM / CHO[30] MVM / BHK-21[35] PCV-1 / Vero[31,32]	3
Unknown	MVM / BHK-21[33] Reovirus / Unknown[34]	2

Contaminating virus and host cell line are indicated where known. Note: some contamination events were reported publicly and in more detail to the CAACB. CVV, Cache Valley virus; EHDV, epizootic hemorrhagic disease virus; MVM, minute virus of mice; PCV-1, porcine circovirus type 1; Reo3, reovirus type 3.

Virus Safety of biopharmaceuticals



ICH
harmonisation for better health

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

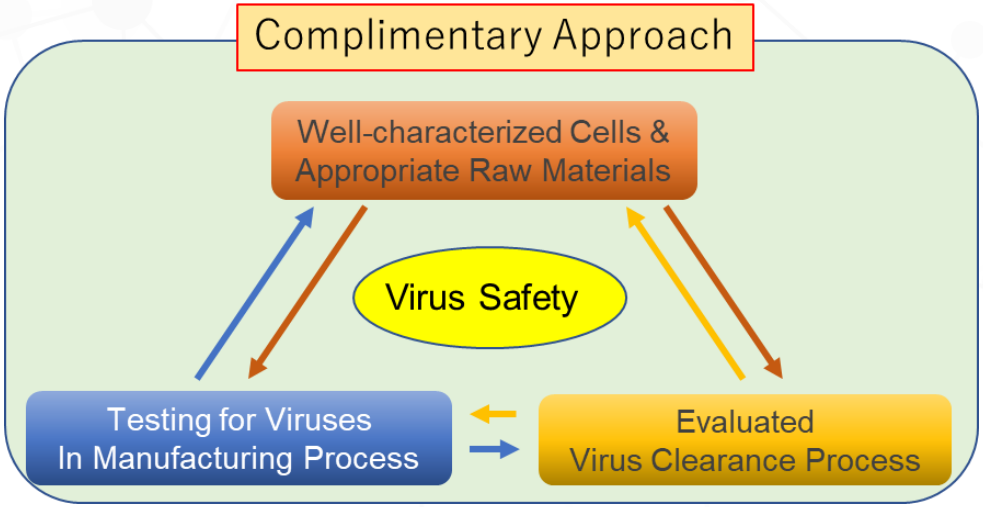
ICH HARMONISED GUIDELINE

VIRAL SAFETY EVALUATION OF BIOTECHNOLOGY PRODUCTS DERIVED FROM CELL LINES OF HUMAN OR ANIMAL ORIGIN

Q5A(R2)

Final version

Adopted on 1 November 2023



Standards for biological raw materials

MHLW Notification No. 210, 2003.

(Latest partial revision on 2018, MHLW Notification No. 37)

Provisional Translation (as of May 2024)*

STANDARDS FOR BIOLOGICAL RAW MATERIALS

Enacted on May 20, 2003 (MHLW Notification No. 210)
Enacted on March 30, 2004 (MHLW Notification No. 157)
Enacted on July 5, 2004 (MHLW Notification No. 262)
Enacted on March 31, 2005 (MHLW Notification No. 177)
Enacted on September 28, 2007 (MHLW Notification No. 310)
Enacted on July 1, 2009 (MHLW Notification No. 343)
Enacted on September 26, 2014 (MHLW Notification No. 375)
Enacted on February 28, 2018 (MHLW Notification No. 37)

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https://www.pmda.go.jp/files/000268474.pdf

III. General Rules for Human-Derived Raw Materials

1. Standards for Human Cell/Tissue-based Raw Materials

(1) The human-derived cell or tissue serving as raw materials, etc. constituting drugs, etc. (excluding blood products) (hereinafter, "human cell/tissue-based raw materials, etc.") must be collected in facilities with sufficient personnel and equipment for necessary sanitation management.

(2) For collection of human cell/tissue-based raw materials, etc., the following measures must be taken:

A. Necessary measures must be taken to prevent contamination with microbial pathogen and other pathogenic agents when collection of human cell/tissue-based raw materials, etc.

B. The collected human cell/tissue-based raw materials, etc. shall be confirmed to be free from contamination with microbial pathogen and other pathogenic agents by appropriate examinations in light of the latest knowledge about infections, if it necessary.

(3) The donor must meet all the following conditions and be sufficiently eligible to donate human cell/tissue-based raw materials, etc. In the case where the donor is the same as the recipient of the drugs, etc., the donor screening may not be always required.

A. Infection of the donor with any pathogens including bacteria, fungi, viruses, etc. is denied by interview, medical examinations, tests, etc. before the human cell/tissue-based raw materials, etc. are collected, according to their intended uses.

B. The test items and test methods used at A. should be appropriate in light of the latest knowledge about infection, etc.

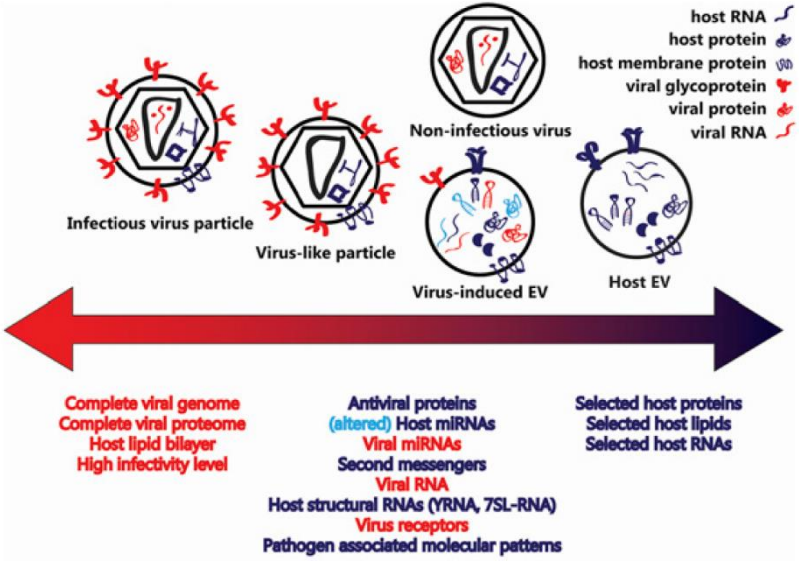
C. The tests or management shall be performed in consideration of the window period: for example, based on the test items and test methods, etc. used at A, re-tests are performed in appropriate timing.

D. In addition to the conditions A-C, the donor eligibility must be determined by conducting interview, medical examinations, tests, etc. for important diseases, and the consideration of experience of blood transfusion or transplantation therapy, etc.

Extracellular vesicles and viruses: Are they close relatives?

Esther Nolte-'t Hoen[a], Tom Cremer[a], Robert C. Gallo[b,1], and Leonid B. Margolis[c]

Edited by Peter K. Vogt, The Scripps Research Institute, La Jolla, CA, and approved June 27, 2016 (received for review April 4, 2016)



www.pnas.org/cgi/doi/10.1073/pnas.1605146113

Viruses **2015**, *7*, 3204-3225; doi:10.3390/v7062770

OPEN ACCESS
viruses
ISSN 1999-4915
www.mdpi.com/journal/viruses

Review

Exosomes and Their Role in the Life Cycle and Pathogenesis of RNA Viruses

Harendra Singh Chahar [1], Xiaoyong Bao [1,2] and Antonella Casola [1,2,*]

Table 1. Viral protein and RNA species present in exosomes derived from RNA virus-infected cells.

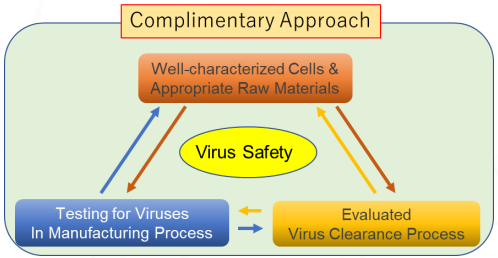
	Protein and RNA Species of RNA Viruses Present in Exosomes
Viral Proteins	**HIV:** Nef and Gag proteins **HCV:** HCV core protein **HTLV-1:** Transactivator protein Tax
Viral RNA and microRNA	**HIV:** HIV-1 transactivating response (TAR) element RNA, microRNAs vmiR88, vmiR99 and vmiR-TAR, unspliced HIV-1 RNA species, **HCV:** HCV genomic RNA **HTLV-1:** HTLV-1 Tax, HBZ, and Env gene mRNA transcripts

Virus safety related process

Conventional Biologics VS EV products

	Conventional Biologicals	EV products
Cells	Well-characterized Cell line	Not Well-characterized MSC, primary cell, etc.
Nanofiltration （15～35nm）	Effective	Remove both EVs and viruses
Low-pH, Detergents, S/D treatment	Effective	Inactivate both EVs and viruses
Affinity Columns	Effective	Buffers will affect EVs
Other purification	Effective	Developing
Concentration Diafiltration	Remove only virus	Concentrate both EVs and viruses

Concept of virus safety for EV products



General concept

STRATEGY OF VIRUS SAFETY FOR EV PRODUCTS

Avoid to use animal-derived raw materials in manufacturing process (as possible)

Strictly conduct virus tests in Cell Banks and manufacturing process

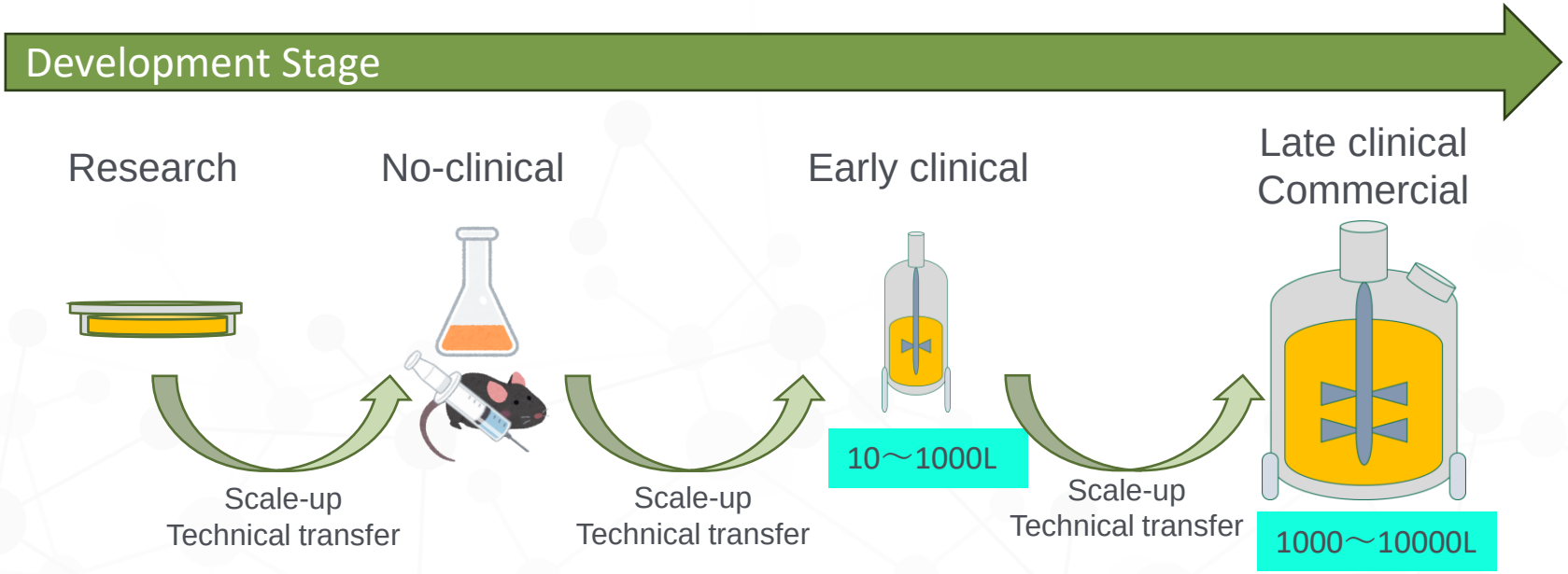
Today’s Agenda

- Basic concept for biopharmaceutical quality control
- Virus safety of EV products
- **Comparability of EV products**

Comparability is critical in pharmaceutical development

- Research-scaled products VS Mass-scaled products
- Non-clinical products VS Clinical trial products
- Clinical trial products VS Commercial products

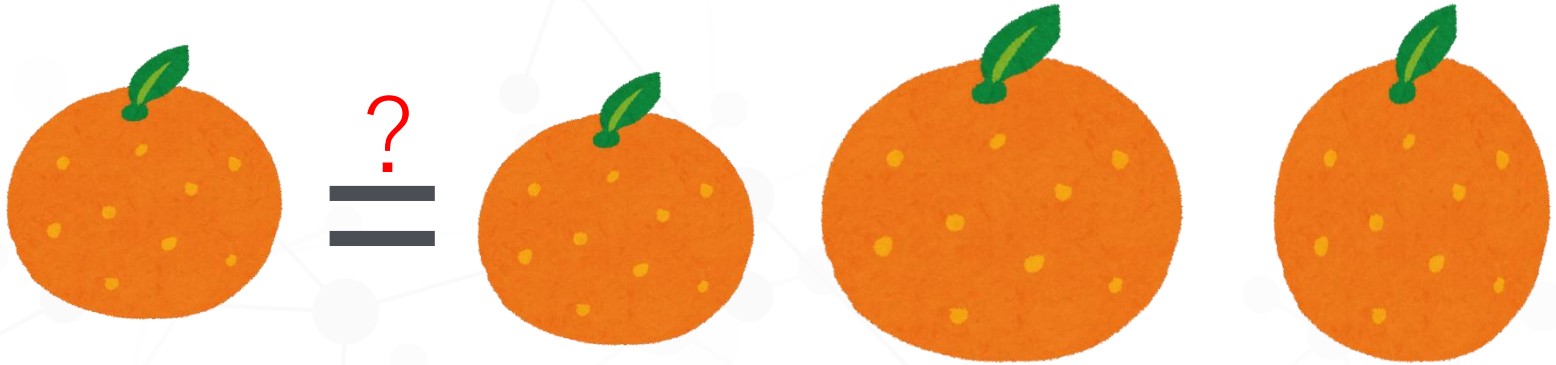
If comparability is broken, the development should be rewound!



Scale-ups and technical transfers will impact the quality of EVs.

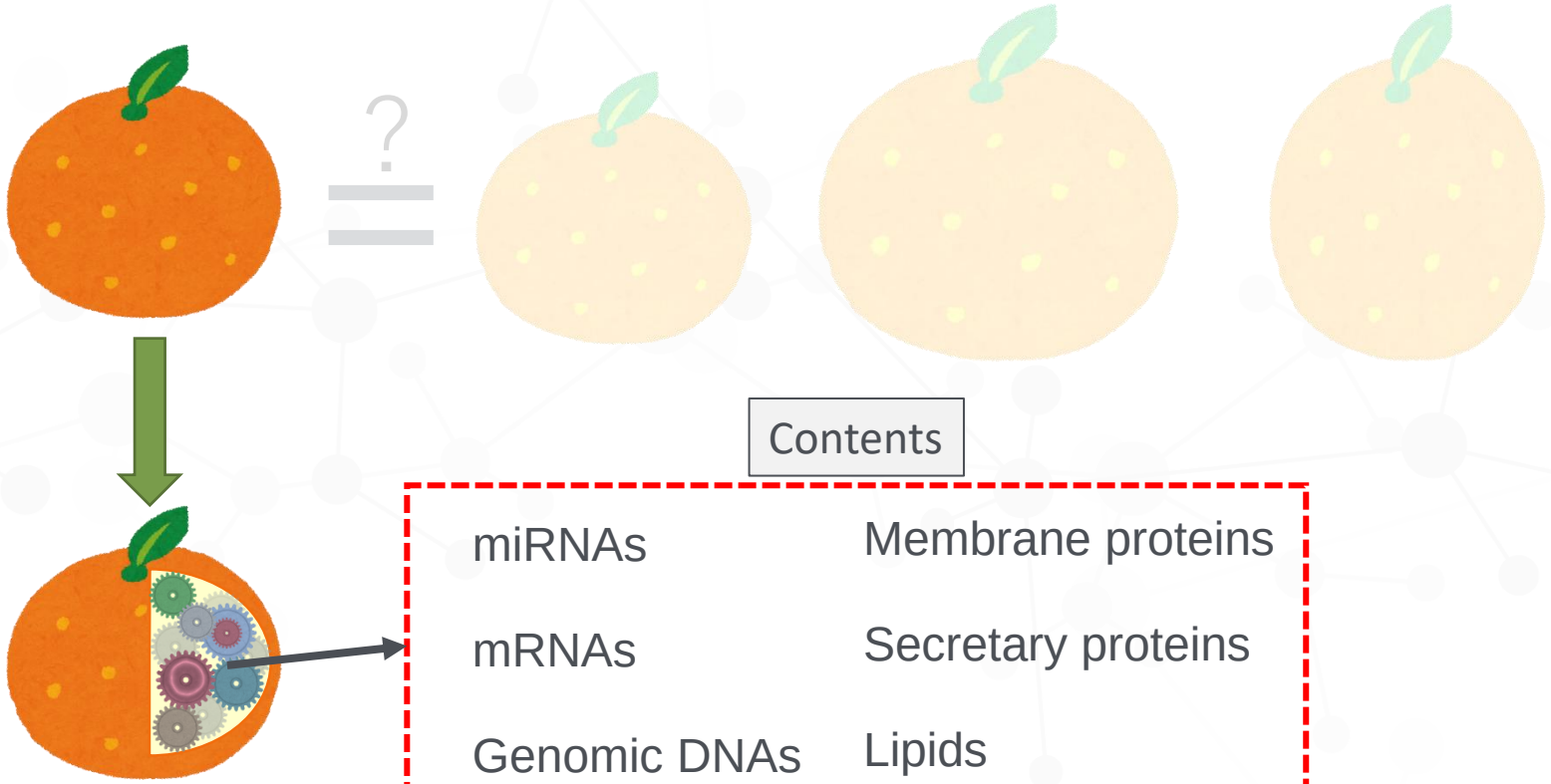
Comparability evaluation should be conducted on each stage.

ALL ARE “EV”S. BECAUSE EV MARKERS ARE POSITIVE!



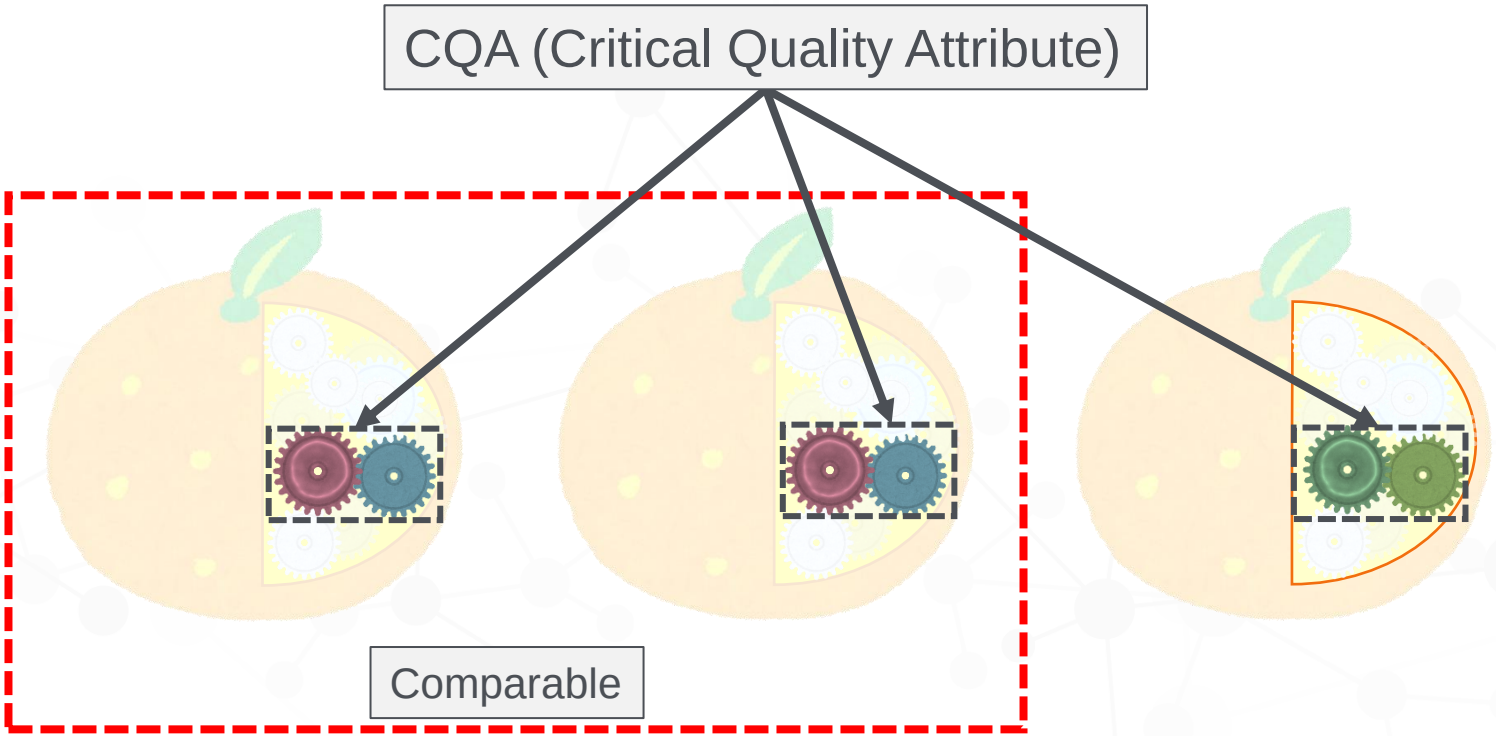
Can we judge which EVs are comparable?

THE CONTENTS OF EVS ARE CRITICAL!



CRITICAL CONTENTS SHOULD BE IDENTIFIED!

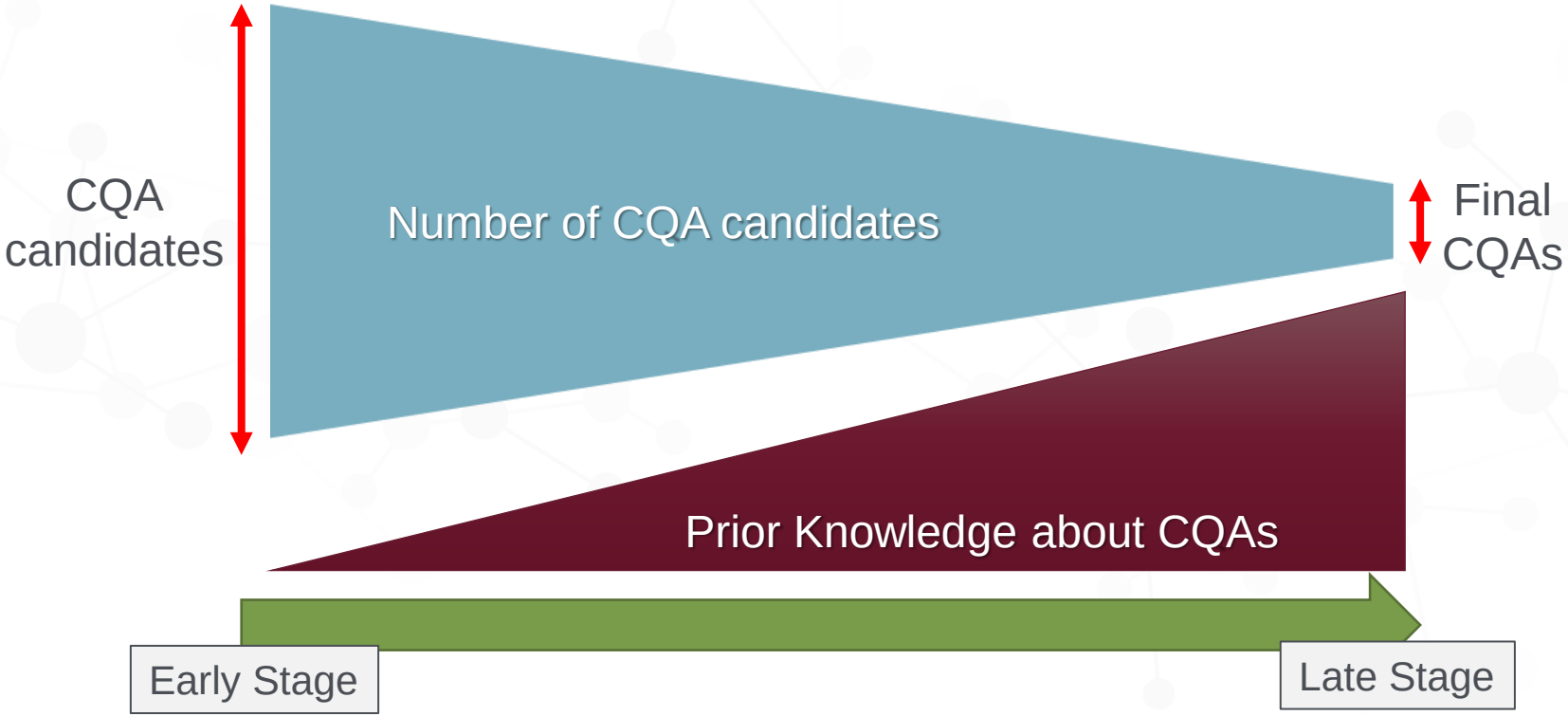
But, it is limited to clarify all contents affect the efficacy and safety….



High priority : Identification of CQA related to efficacy and safety.

CQA

Data of Candidate CQA should be collected since early stage.



Conclusion

- Variations of EVs are more than conventional those of biopharmaceuticals。

- The characters of EVs are similar to viruses,
  so it is hard to separate EVs and viruses in manufacturing process.
- Virus safety strategy weighs on raw materials and virus tests.

- Very difficult to clarify the comparability.
- CQA candidates should be collected since early stage.

Questions?

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