Joint MHLW/PMDA-USP Workshop "Role of Quality in Pharmaceuticals"
June 16 - 17, 2021　Virtual/Hibiya Conference Square

Session 3: Standards for Biologics
General Monographs, New testing for endotoxin

Current trends and future perspectives of JP standards for biologics

Akiko Ishii-Watabe, Hiroko Shibata

PMDA JP Expert committee on Biologicals

& National Institute of Health Sciences

Outline

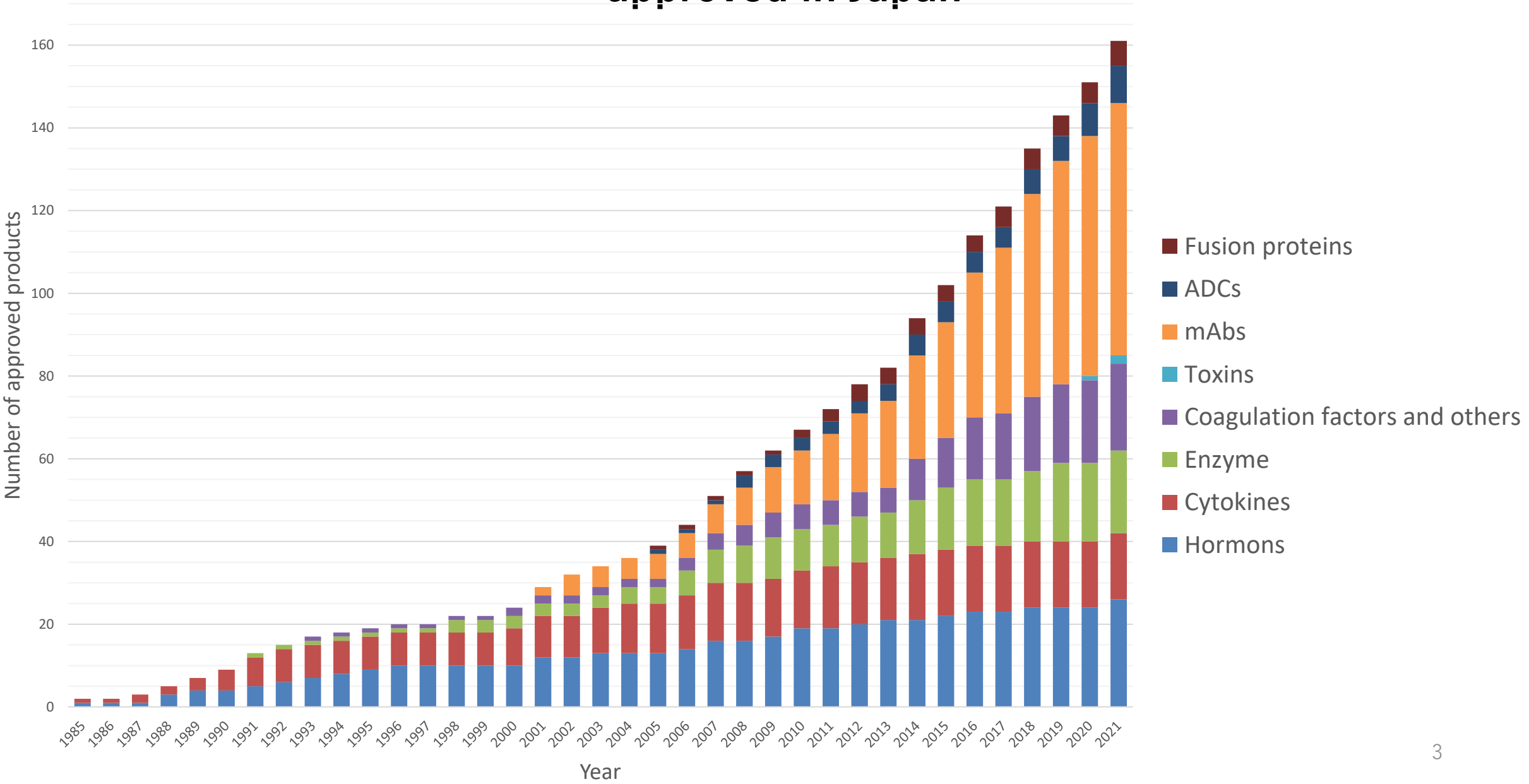
1. Biological Products approved in Japan
   - Drugs including Biological products and Regenerative medicinal products

2. Overview of JP chapters related to Biological products
   - Monographs, General tests, General Informations

3. Recent revision related to Biological products
   - <6.17> Insoluble particulate matter test for therapeutic protein injections
   - New General Information for “Basic concept of the quality control on biotechnological products”

4. Ongoing projects and future plan
   - Activities of AMED research group ~Flow Imaging~ ~Bioassay~
   - Multi-Attribute Method
   - General monograph of mAbs

Biological products (mainly recombinant therapeutic protein products) approved in Japan



Biosimilar products approved in Japan, EU and US

EU

- somatropin
- insulin glargine
- insulin lispro
- insulin aspart
- teriparatide
- epoetin alfa, zeta
- filgrastim
- pegfilgrastim
- ★ follitropin alfa
- infliximab
- adalimumab
- rituximab
- trastuzumab
- bevacizumab
- etanercept

15 original products
(LMWH products are not listed here)

Japan

- somatropin
- insulin glargine BS1,2
- insulin lispro BS1
- insulin aspart BS1
- filgrastim BS1,2,3
- teriparatide BS1
- epoetin alfa BS1
- ★ darbepoetin alfa BS1,2,3
- ★ agalsidase beta BS1
- infliximab BS1,2,3
- adalimumab BS1,2,3
- rituximab BS1,2
- trastuzumab BS1,2,3
- bevacizumab BS1,2
- etanercept BS1,2

15 original products

US

- somatropin*
- insulin glargine*
- Insulin lispro*
- teriparatide*
- filgrastim
- pegfilgrastim
- epoetin alfa
- infliximab
- adalimumab
- rituximab
- trastuzumab
- bevacizumab
- etanercept

13 original products

mAb
TNFR-Fc

*FDC Act 505(b)(2) Follow-on product

Each biosimilar product has its own Japanese Accepted Name (JAN) in Japan.

Regulation of biological products and regenerative medicinal products in Japan

Japan MHLW

Drugs (including Biological products)

- chemical entities
- peptide, protein, sugar
- recombinant therapeutic proteins
- vaccines, blood products
- allergens

Regenerative medicinal products

- cell therapy products
- gene therapy products

⇨ Both are regulated under PMD Act
(Pharmaceutical and Medical Device Act)

US FDA

Drugs

- chemical entities

⇨ Regulated under FD&C Act
(Food, Drug, and Cosmetic Act)

Biological Products

- vaccines, blood and blood components
- allergenics
- sugars, proteins, nucleic acids
- recombinant therapeutic proteins
- somatic cells, gene therapy, tissues,

⇨ Regulated under PHS Act
(Public Health Service Act)

What is a biological product?
https://www.fda.gov/about-fda/center-biologics-evaluation-and-research-cber/what-are-biologics-questions-and-answers

JP focuses on Drugs (including biological products)

Regenerative medicinal products approved in Japan

Category	Trade name	Nonproprietary name	Approved year
Cell Therapy	Jacc	Human (auto) cartilage cell	2012
	Temcell	Human (allo) bonemarrow-derived MSC	2015
	HeartSheet	Human (auto) skeletal muscle-derived cell sheet	2015
	Jace	Human (auto)epidermal cell sheet	2016
	Stemirac	Human (allo) bonemarrow-derived MSC	2018
	Kymriah	Tisagenlecleucel	2019
	Nepic	Human (auto) corneal epithelial cell sheet	2020
Gene Therapy	Colategen	Beperminogene perplasmid	2019
	Zolgensma	Onasemnogene abeparvovec	2020

Total 9 products

Usefulness of JP General chapters for Regenerative medicinal products

・Regenerative medicinal products and Drugs are defined as different categories in Japan.

・JP is used for Drugs (including Biological products)

・However, some of JP General Tests and General Informations are useful for testing of regenerative medicinal products.

Examples of analytical methods used in specifications of regenerative medicinal products

HPLC
ICP-MS
UV
Fluorescence
Protein assay
Electrophoresis
ELISA
PCR
DNA analysis
Analytical ultracentrifugation

Flowcytometry
Cell morphology
Cell number
Colony formation

pH
Osmotic pressure
Extractable volume
Foreign insoluble matter
Insoluble particulate matter

Endotoxin
Microbiological contamination
Sterility
Mycoplasma testing
Electron microscopy
Hemagglutination assay
Hemadsorption assay
In vivo virus test

JP monographs of biological products (therapeutic proteins)

Category	Monograph	First adopted issue
Insulins	Insulin Human (Genetical Recombination)	JP14
	Insulin Human (Genetical Recombination) Injection	JP16-2
	Insulin Glargin (Genetical Recombination)	JP16-2
	Insulin Glargin (Genetical Recombination) Injection	JP16-2
	Insulin Aspart (Genetical Recombination)	JP17-1
	Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension	JP17-1
	Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension	JP17-1
Interleuikin-2	Celmoleukin (Genetical Recombination)	JP15
	Teceleukin (Genetical Recombination)	JP15
	Teceleukin for Injection (Genetical Recombination)	JP15
Granulocyte-Colony Stimulating Factors	Filgrastim (Genetical Recombination)	JP16-1
	Filgrastim (Genetical Recombination) Injection	JP16-1
	Lenograstim (Genetical Recombination)	JP16-1
	Nartograstim (Genetical Recombination)	JP16-1
	Nartograstim for Injection (Genetical Recombination)	JP16-1
Erythyropoietins	Epoetin Alfa (Genetical Recombination)	JP16-1
	Epoetin Beta (Genetical Recombination)	JP16-1
Interferons	Interferon Alfa (NAMALWA)	JP17
	Interferon Alfa (NAMALWA) Injection	JP17
Glucagon	Glucagon (Genetical Recombination)	JP18

JP General tests related to biological products

Chapter	Harmonisation in PDG
2.01 Liquid Chromatography	
2.04 Amino Acid Analysis of Proteins	
2.05 Size Exclusion Chromatography	
2.64 Glycosylation Analysis of Glycoprotein	
4.01 Bacterial Endotoxins Test	✔
4.06 Sterility Test	✔
6.05 Test for Extractable Volume of Parenteral Preparations	✔
6.06 Foreign Insoluble Matter Test for Injections	
6.07 Insoluble Particulate Matter Test for Injections	✔
6.17 Insoluble Particulate Matter Test for Protein Injections	

JP General Informations related to biological products

	Chapter	Harmonisation in PDG
〈G3-1-180〉	A basic Concept of the Quality Assurance on Biotechnological Products (Biopharmaceuticals)	
〈G3-2-171〉	Amino Acid Analysis	✔
〈G3-3-142〉	Peptide Mapping	✔
〈G3-4-161〉	Mass Spectrometry of Peptides and Proteins	
〈G3-5-170〉	Monosaccharide Analysis and Oligosaccharide Analysis/Oligosaccharide Profiling	
〈G3-6-142〉	Isoelectric Focusing	✔
〈G3-7-180〉	Capillary Electrophoresis	✔
〈G3-8-170〉	SDS-Polyacrylamide Gel Electrophoresis	✔
〈G3-9-172〉	Host Cell Protein Assay	
〈G3-10-170〉	Surface Plasmon Resonance	
〈G3-11-171〉	Enzyme-linked Immunosorbent Assay (ELISA)	
〈G3-12-172〉	Total Protein Assay	
〈G3-13-141〉	Basic Requirements for Viral Safety of Biotechnological/Biological Products listed in Japanese Pharmacopoeia	
〈G3-14-170〉	Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/ Biological Products	
〈*G3*-3 *15-141*〉	Qualification of Animals as Origin of Animal-derived Medicinal Products provided in the General Notices of Japanese Pharmacopoeia and Other Standards	

General test <6.17> Insoluble Particulate Matter Test for Protein Injections

Listed in JP17-2

Collaborative study by 6 laboratories.

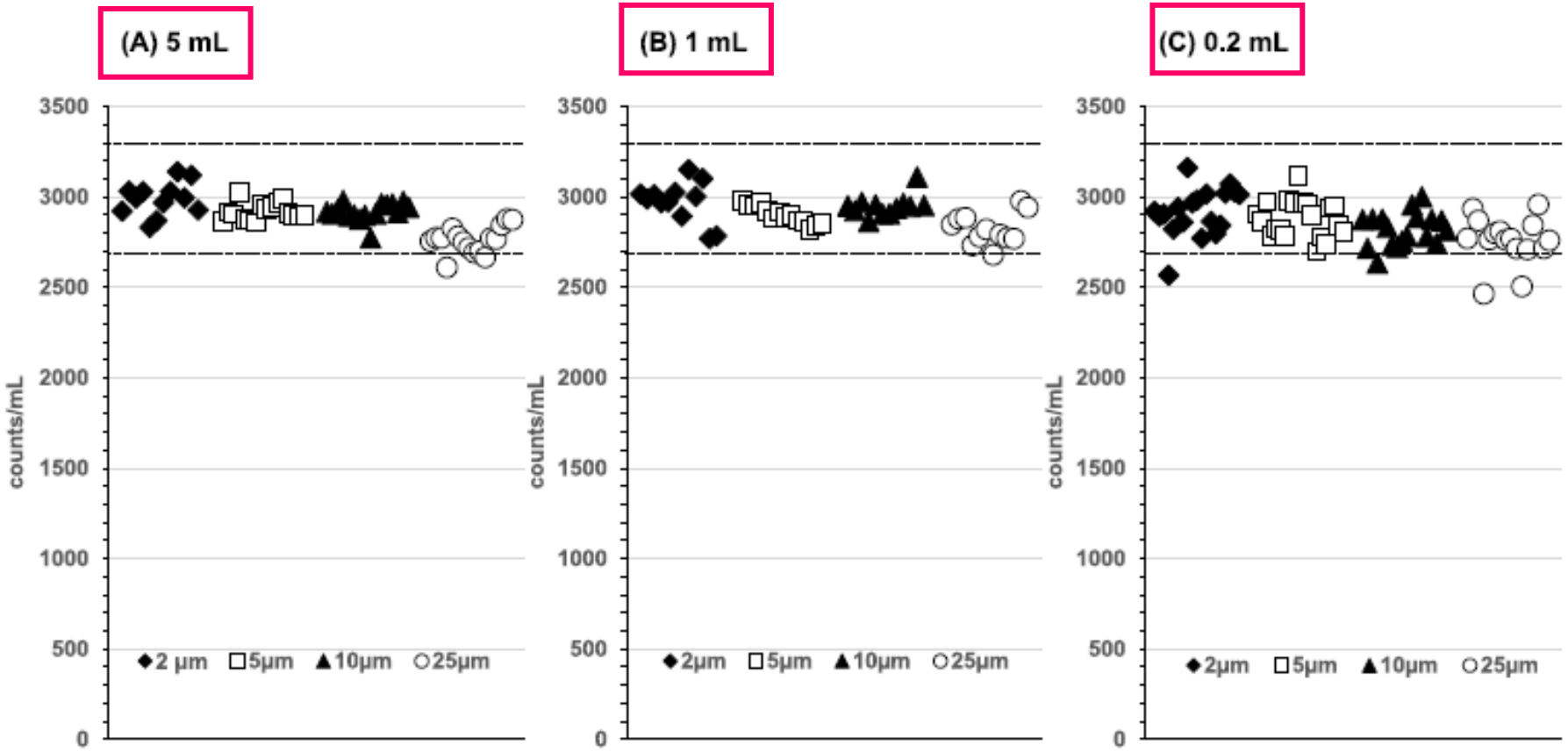


Fig. 1. Influence of measurement volume on particle count of light obscuration method.
The plots show the counts of all tests for 2, 5, 10 and 25 μm polystyrene count standards at a measurement volume of 5 mL (A), 1 mL (B), and 0.2 mL (C).

Harazono A. et al. Biologicals. 2019 Jan;57:46-49. doi: 10.1016/j.biologicals.2018.12.003.

	6.07 Insoluble Particulate Matter Test for Injections	6.17 Insoluble Particulate Matter Test for Therapeutic Protein Injections
Definition of insoluble particulate matter	Extraneous, mobile undissolved particles, other than gas bubbles, that are unintentionally present in the solutions.	Mobile undissolved particles other than gas bubbles in preparations. Extraneous substances, substances derived from manufacturing processes, protein aggregates and so on maybe included.
methods	Method1. Light Obscuration Particle Count Test Method 2. Microscopic Particle Count Test	Light Obscuration
Elimination of gas bubbles	Eliminate gas bubbles by appropriate measures such as allowing to stand for 2 minutes or sonicating.	For elimination of air bubbles, it is recommended to allow a container to stand under ambient pressure or reduced pressure. Other procedures are applicable if confirmed to be appropriate. Sonicating is not appropriate be-cause it may aggregate or denature proteins.
Measurement volume	Remove 4 portions, each of not less than 5 mL, and count the number of particles equal to or greater than 10mmand25mm. the contents of 10 or more units are combined in a cleaned container to obtain a volume of not less than 25 mL	The measurement volume is 1 to5 mL. The measurement volume can be reduced to 0.2 mL when the validity of the reduction is confirmed in consider-ing the property of the sample and the tare volume of the apparatus.
Acceptance criteria	Same (B—Solutions for injection supplied in containers with a nominal content of less than 100 mL. The average number of particles of equal to or greater than 10 μm does not exceed 6000 per container and that of particles of equal to or greater than 25 μm does not exceed 600 per container	

Contribution to Harmonization of PDG Q-09 Particulate Contamination

Recent revision of JP ~Incorporation of ICH Quality guidelines~

✓Quality assurance via process control
✓Harmonization with international standard

JP17（May 2016）

General Notice 12：Manufacture
General Information「Basic Concepts for Quality Assurance of Drug Substances and Drug Products」
←Q6A, Q6B
General Information「Basic Concept of Quality Risk Management」←Q9

JP17-1（Dec 2017）

General Information 「 Basic Concepts for Quality Assurance of Drug Substances and Drug Products 」
revision ← Q8, Q11

JP17-2（June 2019）

General Notice 13： （RTRT）some of the test items in the monograph being performed for the release of a product may be omitted as occasion demands
General Information 「 Basic Concepts for Quality Assurance of Drug Substances and Drug Products 」
Revision ← Q8, Q11
General Information 「Glossary for Quality by Design (QbD),Quality Risk Management (QRM) and Pharmaceutical Quality System（PQS）」 ← Q8, Q9, Q10, Q11

JP18（2021）

General Information（Basic concept of the quality assurance on biotechnological products (biopharmaceuticals)） Q8~11＋Q5 series, Q6B

General Information: Basic concept of the quality control on biotechnological products (biopharmaceuticals)

To be listed in JP18

1. Quality evaluation and control of biopharmaceuticals

1.1Quality evaluation

1.1.1 **Characterization** Q6B

a. Structure and Physicochemical Properties
b. Biological activities
c. Molecular Variants of desired product
d. Process-related impurities

1.1.2. **Identification of CQA** Q8-11

1.2 Construction of quality control strategy

1.2.1. **Raw materials control** Q5B

a. Evaluation and Control of Cell Bank
b. Control of other raw materials Q5D

1.2.2. Manufacturing process control

a. Process Parameter Control
b. In Process Tests Q5A

1.2.3. **Evaluation and control of contaminant**

1.2.4. **Specifications** Q6B

a. Setting Basis of Specification
b. **Description**
c. **Identification test**
d. **Specific physical and/or chemical values**
e. **Purity test**
f. **Biological activities**
g. **Assay**
h. **Tests for preparations**

1.2.5. **Stability testing** Q5C

a. **Conditions of stability testing**
b. **Attributes to be evaluated**

Q5E

2. Comparability of biopharmaceuticals subject to changes in their manufacturing process

2.1 **Considerations for the comparability exercise**
2.2 **Quality considerations**
2.3 **Manufacturing process considerations**

Ongoing projects:
New General Tests and General Informatios for Biological products

General Tests

Mycoplasma test (revised from General Information <*G3-14-170*>)

General Informations

Flow Imaging method for therapeutic protein injections
Flow Cytometry
Bioassay
Analytical procedures of N-glycan analysis
HCP Assay (revision)

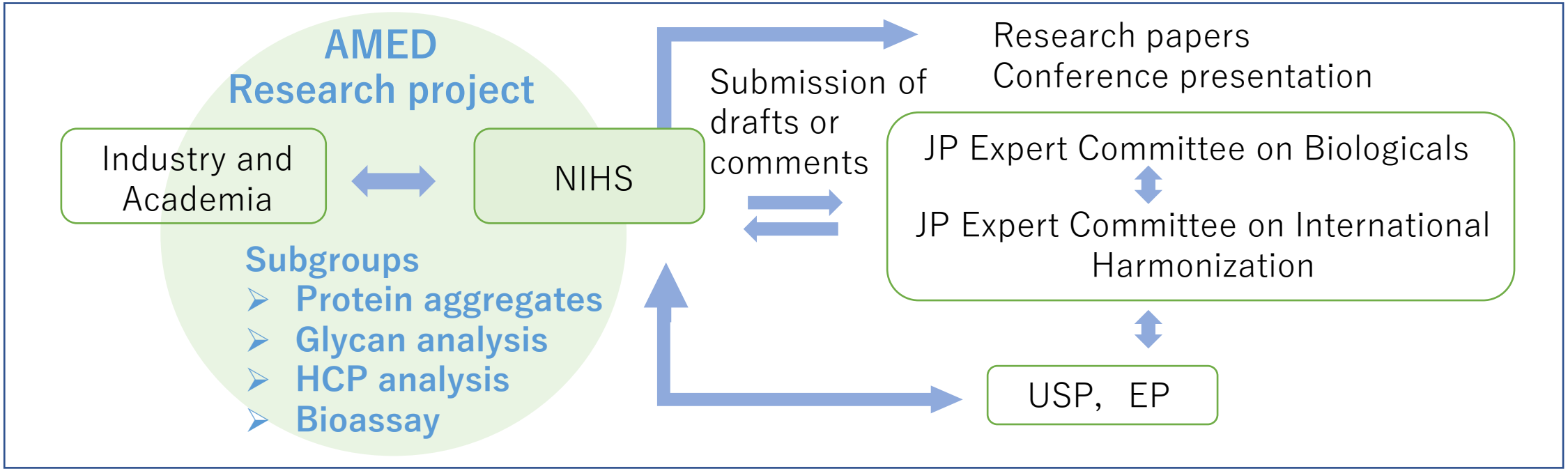
General Monograph

Monoclonal Antibodies

AMED research project
(AMED: Japan Agency for Medical Research and Development)
Japanese biopharmaceutical consortium

Establishment and standardization of fundamental methodologies used for the evaluation of the quality of biopharmaceuticals

unofficial scheme



Previous achievements

General Tests <2.64> Glycosylation analysis of glycoprotein
<6.17> Insoluble Particulate Matter Test for Therapeutic Protein Injections
General Information : Surface Plasmon Resonance, ELISA,
Monosaccharide Analysis and Oligosaccharide Analysis
/Oligosaccharide Profiling , HCP Assay

Research activities related to JP General Chapters for Biological products

Meeting Report of AMED research group in 2020

Journal of Pharmaceutical Sciences 109 (2020) 1652-1661

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journal homepage: www.jpharmsci.org

Special Topic Commentary

Recent Achievements and Current Interests in Research on the Characterization and Quality Control of Biopharmaceuticals in Japan

Akiko Ishii-Watabe [1,*], Hiroko Shibata [1], Hiroyuki Suetomo [2], Yosuke Ikeda [3], Srivalli Telikepalli [4], Masato Kiyoshi [1], Yu Hayashi [5], Takashi Muto [5], Yukako Tanaka [5], Satomi Ueda [5], Takafumi Iwura [2], Satoshi Saitoh [3], Michihiko Aoyama [1], Akira Harazono [1], Masashi Hyuga [1], Yukihiro Goda [6], Tetsuo Torisu [7], Susumu Uchiyama [7]

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Check for updates

National Institute of Health Sciences (NIHS)
Osaka University
AcroScale Inc.
Ajinomoto Co., Inc.
Astellas Pharma Inc.
Chugai Pharma Manufacturing Co., Ltd.
Daiichi Sankyo Co., Ltd.
Immuno-Biological Laboratories Co., Ltd.
Japan Blood Products Organization
JCR Pharmaceuticals Co., Ltd.
Kissei Pharmaceutical Co., Ltd.
Kyowa Kirin Co., Ltd.
Meiji Seika Pharma Co., Ltd.
Mitsubishi Tanabe Pharma Corporation
Mochida Pharmaceutical Co., Ltd.
Nihon Pharmaceutical Co., Ltd.
Nippon Kayaku Co., Ltd.
Ono Pharmaceutical Co., Ltd.
Shimadzu Corporation
Sumitomo Bakelite Co., Ltd.
Sumitomo Dainippon Pharma Co., Ltd.
Takeda Pharmaceutical Company Limited
Terumo Corporation
Toray Research Center, Inc.
Tosoh Corporation
U-Medico Co., Ltd.

Supported by Japan Agency for Medical Research and Development

Figure 1. Members of the AMED Research Group.

Collaborative Study for Analysis of Subvisible Particles Using Flow Imaging and Light Obscuration: Experiences in Japanese Biopharmaceutical Consortium

(AMED: Japan Agency for Medical Research and Development-HS research group)

Kiyoshi M, Shibata H, Harazono A, Torisu T, Maruno T, Akimaru M, Asano Y, Hirokawa M, Ikemoto K, Itakura Y, Iwura T, Kikitsu A, Kumagai T, Mori N, Murase H, Nishimura H, Oda A, Ogawa T, Ojima T, Okabe S, Saito S, Saitoh S, Suetomo H, Takegami K, Takeuchi M, Yasukawa H, Uchiyama S, Ishii-Watabe A.
J Pharm Sci. 2019

Purpose:
to assess the adequacy/feasibility for the standardization of flow Imaging (FI) technique

Elucidated the analytical performance of FI, mainly focusing on

- ✓ Comparison between methods (LO vs FI)
- ✓ Comparison between manufactures (MFI vs FlowCam)
- ✓ Comparison among laboratories (inter-laboratory variability)

Representative digital images of particles used in the study

NPAS90: IVIG heated at **90 ºC**

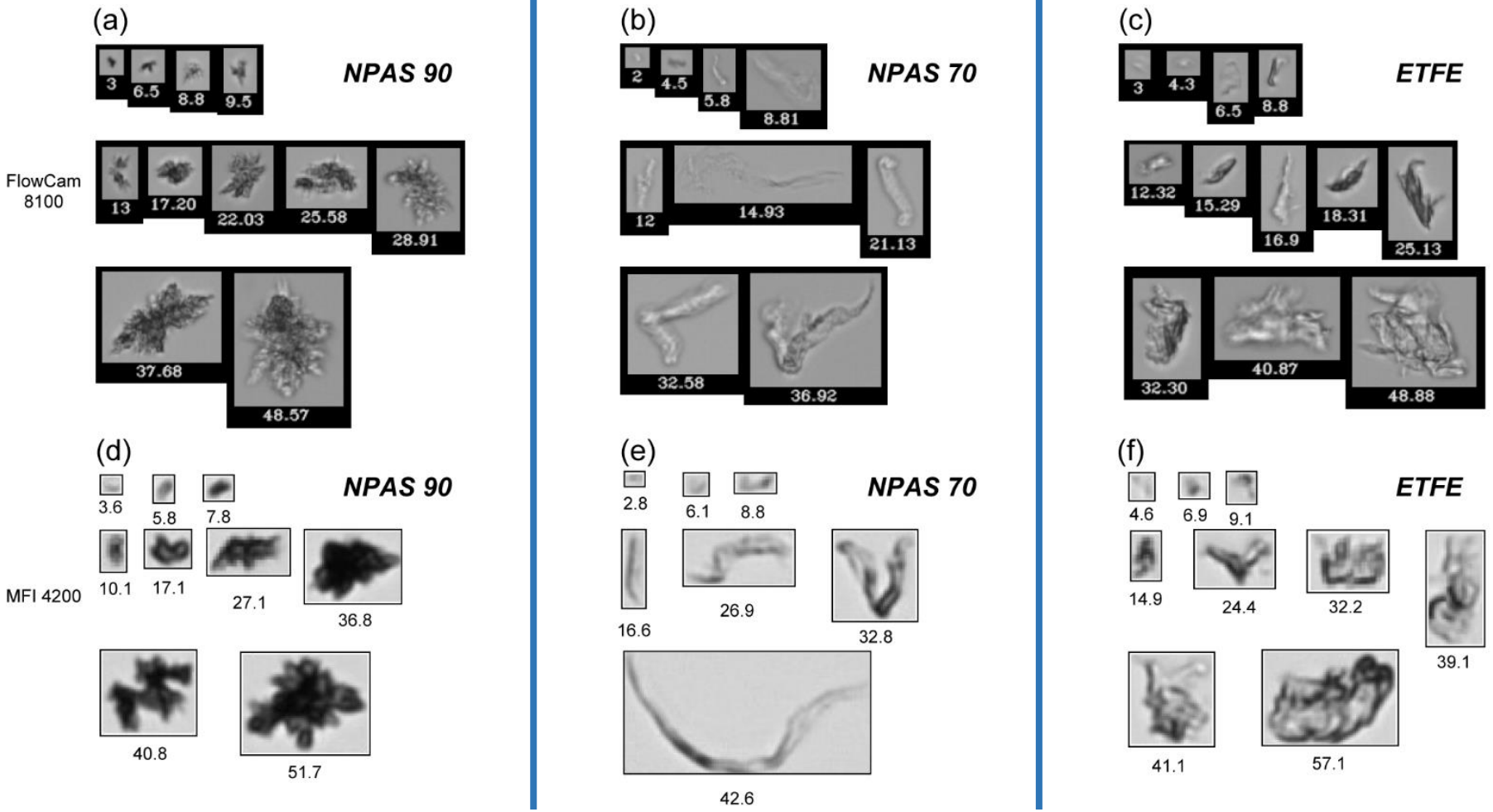
NPAS70: IVIG heated at **70 ºC**

ETFE: Not proteinaceous

Dark particles

Transparent particles

Dark and transparent particles

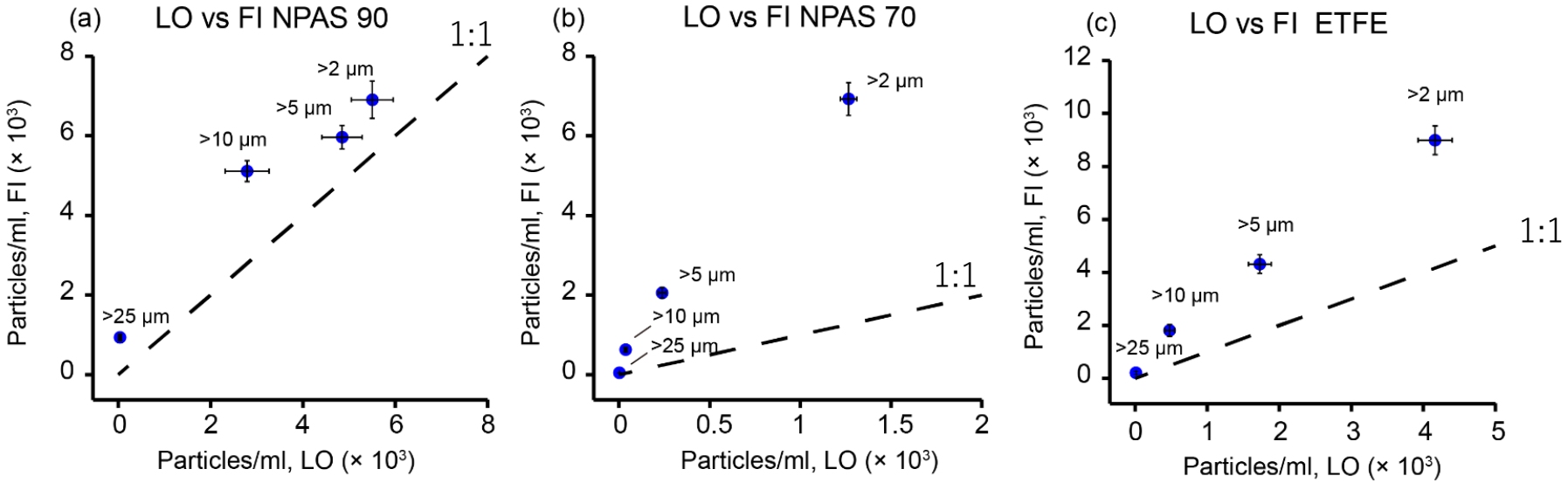


Aspect ratio ：NPAS 90 < ETFE < NPAS 70

Transparency：NPAS 90 < ETFE < NPAS 70

Kiyoshi M, Shibata H. et al. J Pharm Sci (2019)

Comparison of particulate counts (LO vs FI)



Difference in particle counts in the measurement by LO and FI

NPAS 90 < ETFE < NPAS 70

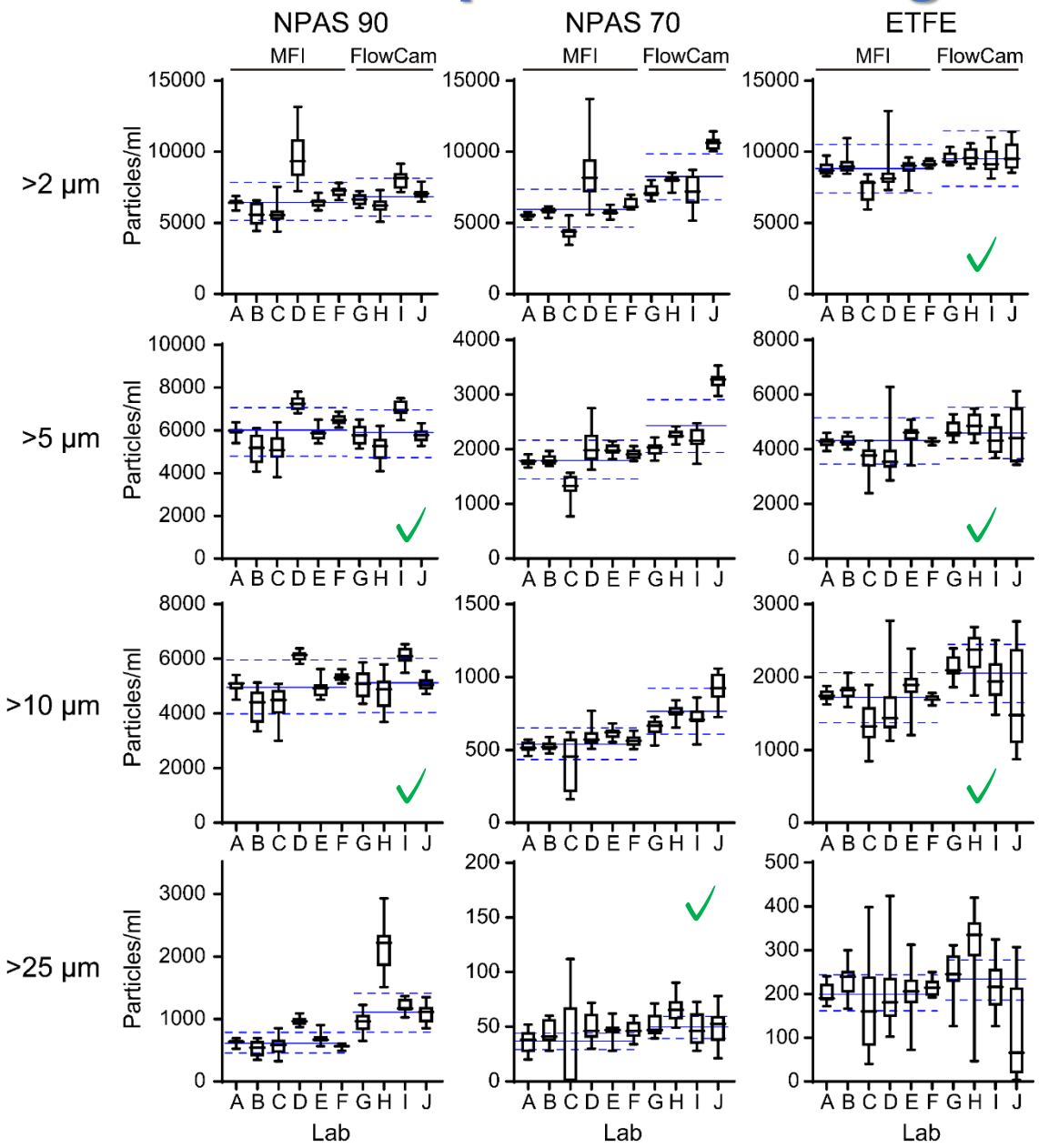
Transparency or Aspect ratio

NPAS 90 < ETFE < NPAS 70

LO underestimated the counts and size, especially for highly transparent and irregularly shaped particles.
FI can detect highly transparent and irregularly shaped particles more precisely.

Kiyoshi M, Shibata H. et al. J Pharm Sci (2019)

Comparison among laboratories: FI



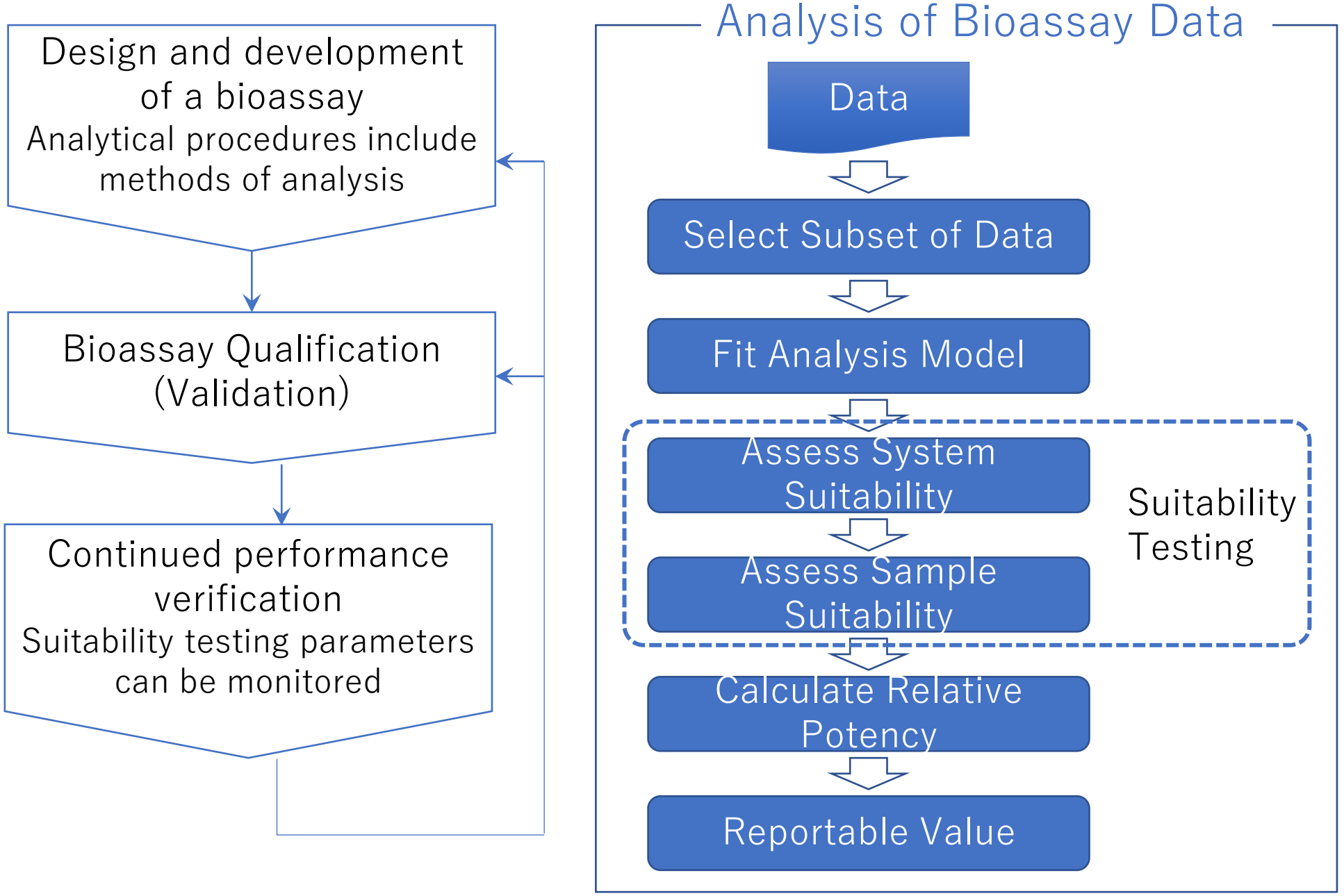
Overall, most of the medians of particle count in each laboratory were within 80-120% of the Lab A-F (MFI users) or Lab G-J (FlowCam users).

✓ Consistent

Considering our data, setting FI as a release test would be feasible to precisely analyze the sizes and counts of particles in therapeutic protein injections.

Kiyoshi M, Shibata H. et al. J Pharm Sci (2019)

Lifecycle of Bioassay



General Information for Bioassay (DRAFT)

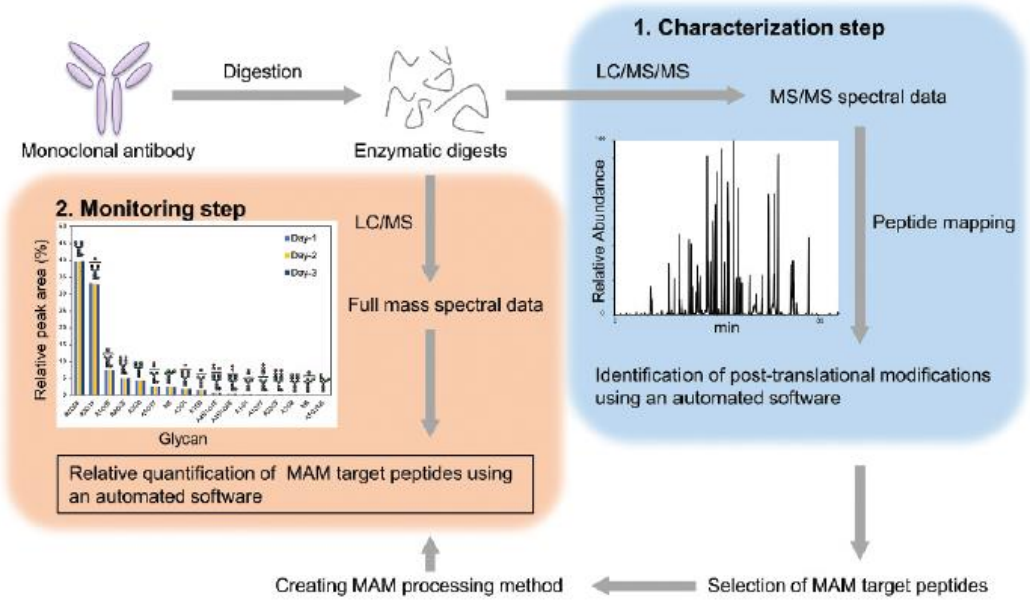
1. Introduction
2. Analysis models
   2.1 Nonlinear model
   2.2 Parallel-line model
   2.3 Slope-ratio model
3. Validation of bioassay
4. Suitability testing
   4.1 Setting of suitability testing
   4.2 Assessment of similarity
   4.3 Examples of suitability testing
5. Statistical tools
   5.1 Outliers
   5.2 Confidence interval and combining results
6. Design and development of bioassay
   6.1 Assay layout
   6.2 Assay development
   6.3 Data analysis during assay development
   6.4 Control of standard materials and critical reagents

Constrained vs Unconstrained

Difference test vs Equivalence test

Research on Multi-attribute method (MAM) for the characterization and quality control of mAbs

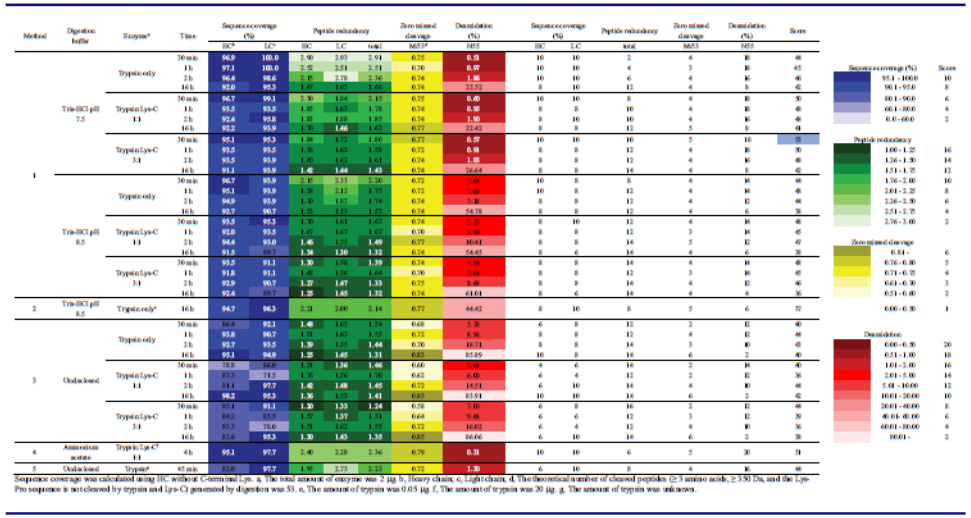
Tajiri-Tsukada M., Hashii N.*, Ishii-Watabe A.: Establishment of a Highly Precise Multi-attribute Method for the Characterization and Quality Control of Therapeutic Monoclonal Antibodies. ***Bioengineered***, 2020; 11(1) : 984-1000.



- The multi-attribute method (MAM) has garnered attention as a new quality control method of therapeutic monoclonal antibodies (mAbs).
- MAM analysis allows multiple relative quantifications of several structural attributes of therapeutic mAbs; however, some issues remain to be addressed in its procedures especially for sample preparation.

Optimization of pre-treatment procedures enabled highly precise analysis.

Parameter	Method 1 (Proposed method)	Method 2	Method 3	Method 4	Method 5	Reference method
Denaturing reagent	GuHCl	GuHCl	SDC and SLS	GuHCl	Not applicable	GuHCl
RCM (temp.)	DTT/MIA (room temp.)	DTT/MIA (65°C)	DTT/MIA (room temp.)	TCEP/IAA (37°C)	(After digestion) DTT/MIA (57°C)	DTT/MIA (room temp.)
Treatment after RCM	Desalting/ buffer exchange by NAP5 column	Desalting by PD10 column and lyophilization	Dilution	Dilution	Not applicable	Desalting/ buffer exchange by NAP5 column
Enzyme	Trypsin:Lys-C (3:1)	Trypsin	Trypsin:Lys-C (3:1)	Trypsin:Lys-C[a] (1:1)	Immobilized trypsin	Trypsin
The amount of enzyme per unit weight of protein	0.4	0.01	0.4	4	unknown	0.04
Digestion buffer (pH)	Tris-HCl (7.5)	Tris-HCl (8.5)	ABC (8.5 ~)	Ammonium acetate (5.5–7.0)	Unknown (7.0)	Tris-HCl (7.5)
Digestion temp.	37°C	37°C	37°C	37°C	70°C	37°C
Digestion time	30 min	16 h	16 h	4 h	45 min	30 min
Required time (days)	1	3	2	1	1	1



Study on MAM was done by AMED research outside JP.

General Monograph for mAb (DRAFT)

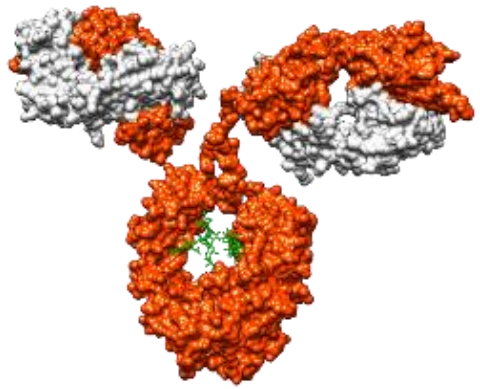
(1) Characteristics and control strategy of mAbs

Brief overview of mAbs

Characterization of mAbs

Risk assessment and Critical Quality Attributes of mAbs

Control strategy of mAbs
- Raw material
- Process control
- In-process tests
- Specifications



(2) Example of specifications

Origin
Manufacture
Description
Identification
Charge variants
Glycosylation profile
Purity (1) aggregates
Purity (2) low molecular weight species
Biological activity (1) antigen binding
Biological activity (2) Fc γ R IIIa activation
Assay
Storage
Reagents and test solutions

Perspective on the role of JP in the field of Biological products

JP is the public standard document showing the standard test methods and concepts

+

Most of the biological products are developed and used globally.

Establishment and harmonization of General tests and General Informations

Contribution to ensuring the quality of innovative biological products used worldwide.

Acknowledgements

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JP Committee on Biologicals

Akira Harazono, Noritaka Hashii, Masashi Hyuga, Minoru Tada

Thank you for your attention!