IJBPAS, September, 2025, 14(9): 4915-4923

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

REGULATORY GUIDELINES FOR MONOCLONAL ANTIBODIES AND RELATED PRODUCTS: EMA'S STANDARDS ON DEVELOPMENT, PRODUCTION, CHARACTERIZATION & SPECIFICATIONS

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Received 15th March 2024; Revised 20th April 2024; Accepted 11th Aug. 2024; Available online 1st Sept. 2025

https://doi.org/10.31032/IJBPAS/2025/14.9.9330

ABSTRACT

The comprehensive process of monoclonal antibody development, production, characterization, and specification in the European context are engineered to precisely target various threats and have revolutionized medical approaches against diseases like cancer and infections. In Europe, European Medicines Agency (EMA) plays a prominent role in ensuring quality by establishing stringent guidelines across four distinct stages: development, production, characterization, and specification. The **Development phase** focuses on aligning antibody structure with its intended mechanism, biological activity, and stability. **In Production**, a meticulous manufacturing process description and validation are central aspects. **Characterization** involves a comprehensive evaluation of attributes like physicochemistry, immunochemical properties, biological activity, purity, impurities, and quantity. In the **Specification stage**, maintaining product consistency and compliance is paramount. Finally

harmonized approach across the Different stages ensures that monoclonal antibodies and related products adhere to the highest quality standards. EMA's stringent guidelines provide a robust framework for developing targeted, effective, and safer therapeutic solutions, thereby transforming medical practices and patient care. The revolutionary potential of monoclonal antibodies helps to reshape healthcare needs.

Keywords: EMA, Monoclonal Antibody, Safety, Specification, Characterization, B-lymphocytes, TSE/BSE

INTRODUCTION:

The Monoclonal Antibodies have significant importance in the Current Era. The monoclonal antibodies were not considered as a "new" technology in regulatory affairs, but when we consider the Drugs, Cosmetics, Medical Devices and any other products which will help diagnosis, treatment & prevention of a disease this Monoclonal Antibodies are also having significant importance and regarded as a well Established class of therapeutic agents. We have been continuously using these monoclonal Antibodies from Decades. And their regulatory pathways are well defined by the regulatory agencies like Food and Drug Administration (FDA) and the European Medicines Agency (EMA).

The history of monoclonal antibodies (mAbs) is marked by significant advancements in their development and application in the field of medicine. Over the past several decades, mAbs have become a crucial part of personalized therapy and have emerged as a forefront technology for treating a wide range of diseases. The journey started in 1975, when the hybridoma process was used to produce the first monoclonal antibody. This procedure entailed collecting the B-lymphocytes from the animal's spleen, merging them with immortal myeloma cells, and immunizing a particular species against a target antigen. As a consequence, antibodies that were highly specific for the target antigen were generated by hybridoma cells. The drawbacks of this strategy included its dependency on appropriate myeloma cell lines, challenges with poor yield, and genetic instability. The first monoclonal antibody, Orthoclone OKT3, was approved in 1986 to prevent kidney transplant rejection, but faced limited clinical success due to side effects and human anti-mouse antibody responses. Despite these challenges, monoclonal antibody development continued to expand.

As genetic sequencing and biomedical research advanced, new methods of generating monoclonal antibodies emerged. One of the significant advancements used

for the development of monoclonal antibodies is phage display, a technique that involves isolating B-lymphocytes from human blood, amplifying VH and VL segments through PCR, and inserting them into a bacteriophage vector. This method allowed for the creation of antibody libraries containing diverse sequences. E. coli bacteria could then produce the bacteriophages, displaying the antibody fragments on their surface. This technique offered advantages such as the rapid generation of antibodies without animal immunization and the ability to target toxic antigens that couldn't be used for immunization. The aim of the study is to understand the regulatory Guidelines for Development, production, characterization and specifications for monoclonal antibodies and related products in Europe.

While considering the regulatory aspects of monoclonal Antibodies in The European Medicines Agency and European Directorate for the Quality of Medicines and Healthcare (EDQM) are the main regulatory bodies which currently regulating the Monoclonal Antibodies. Monoclonal antibodies are artificially engineered molecules that replicate the immune system's ability to combat threats like cancer cells and viruses. They target specific antigenic sites on proteins, pathogens, or cell types. These antibodies are crafted from a unique immune cell type called a hybridoma cell, highlighting their precision and controlled nature compared to the broader antibody spectrum generated by the body's immune system. Monoclonal antibodies have revolutionized medicine, research, and diagnostics with advancements in bispecific antibodies, antibody-drug conjugates (ADCs), fc fusion proteins, and antibody fragments. These advancements enable targeted cell eradication, safeguard healthy tissues, and enhance diagnostic efficacy. Monoclonal antibodies also underpin therapeutic vaccines, driving immune responses against specific antigens and driving personalized medicine and targeted immunotherapy. These developments have transformed healthcare and scientific progress, driving innovative disease management and reshaping healthcare and scientific progress.

Table 1: Regulatory department for Monoclonal Antibodies in EU

Country	EU
Regulatory Body	EMA & European Directorate for the Quality of Medicines and Healthcare (EDQM)
Regulatory Classification	Biologics
Regulatory Department	Science Medicines Health

For the EU, the European Medicines Agency (EMA) is in charge of guaranteeing the efficacy, safety, and quality of medicines. It is responsible for the design, creation, evaluation, and specification of monoclonal antibodies and associated goods. These customized proteins are effective tools for treating a variety of disorders because they target particular molecules in the body. For the whole lifetime of these goods, from research and development to production and testing, the EMA offers rules and regulations. The EMA sets strict guidelines to make sure these cutting-edge treatments adhere to the highest quality standards and are secure for patients across the EU **[1, 2]**.

RESULTS AND DISCUSSION:

In Europe the development of Monoclonal Antibodies takes place with 4 Stages:

Table 2: Stages involved in the development of Monoclonal Antibodies

Stages	Focusing Area	Activities
Stage 1	**Development**	• **Mechanism of action, Biological activity and Stability.** • **Cell Substrate selection and Rationale.** • **Specific Development procedures and Information.** • **Immortalization and Concerns**
Stage 2	**Production**	• **General considerations** • **Platform Manufacturing** • **Viral safety and Transmissible spongiform Encephalopathy (TSE).**
Stage 3	**Characterization**	• **Physicochemical Characterization** • **Immunological properties** • **Biological Activities** • **Purity, Impurity & Contaminants** • **Quantity**
Stage 4	**Specification**	• **Identity** • **Purity and Impurities** • **Potency** • **Quality** • **General Tests**

Stage 1: Development:

The justification for a monoclonal antibody's structure must align with its mechanism of action, biological activity, and stability. This involves evaluating immunochemical properties like affinity, cross-reactivity, isotype, and allotype while preserving effector function integrity. Special attention is required for antibodies with low human immunoglobulin homology or immunogenic epitopes, preventing adverse patient responses. For production, a stable monoclonal cell line developed through recombinant DNA techniques is ideal, with rationale provided for its quality over other methods. Cell substrate descriptions should adhere to guidelines, including those for recombinant DNA processes. While specific developmental steps like cell fusion or transformation need not be overly detailed, they should furnish essential data for assessing identity and

purity, especially when influencing safety and efficacy. Justifying B-lymphocyte immortalization for antibody production must be a prerequisite, considering infection risks like vCJD and EBV with human B-lymphocytes. Hybridoma cell lines from B-lymphocyte/myeloma fusion are viable with the necessary documentation of origin, donor health, fusion partner, and exposure history **[3, 5]**.

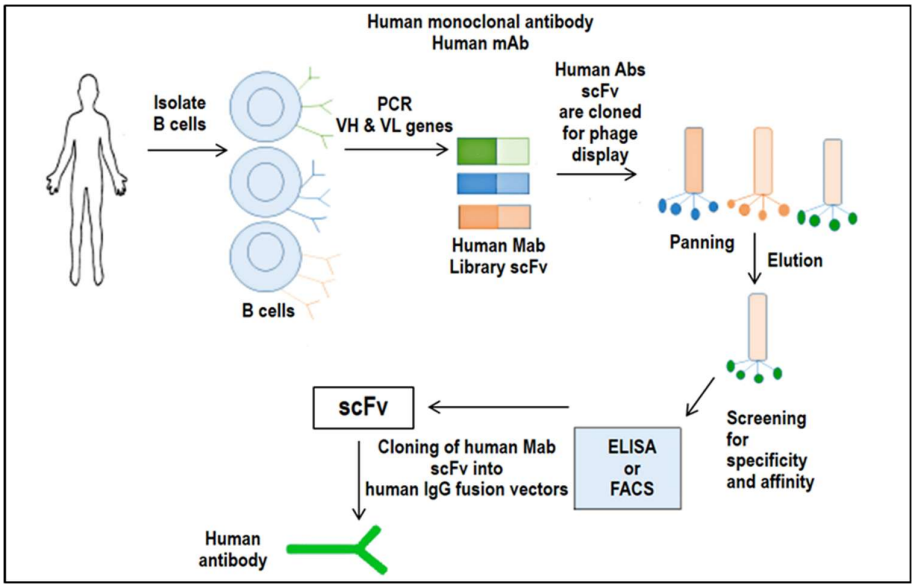

Figure 1: Development of Monoclonal Antibodies

Stage 2: Production:

The production of monoclonal antibodies entails several critical considerations outlined in the guideline. The manufacturing process must be meticulously described and validated, encompassing aspects like consistent quality, process-related impurity elimination, and operational unit performance validation. In-process controls, drug substance specifications, and drug product specifications are essential for monitoring quality attributes and process parameters. When utilizing protein A in purification, source origin and preparation method should be documented, with human IgG quality verified for viral safety. Platform manufacturing, rooted in product and process knowledge, involves validated processes, while process changes demand evaluation of impact. Viral safety conforms to ICH Q5A; with validation studies for viral reduction potentially supported by relevant data. The use of materials from TSE-relevant species necessitates consultation with the respective guidance **[3, 4, 6]**.

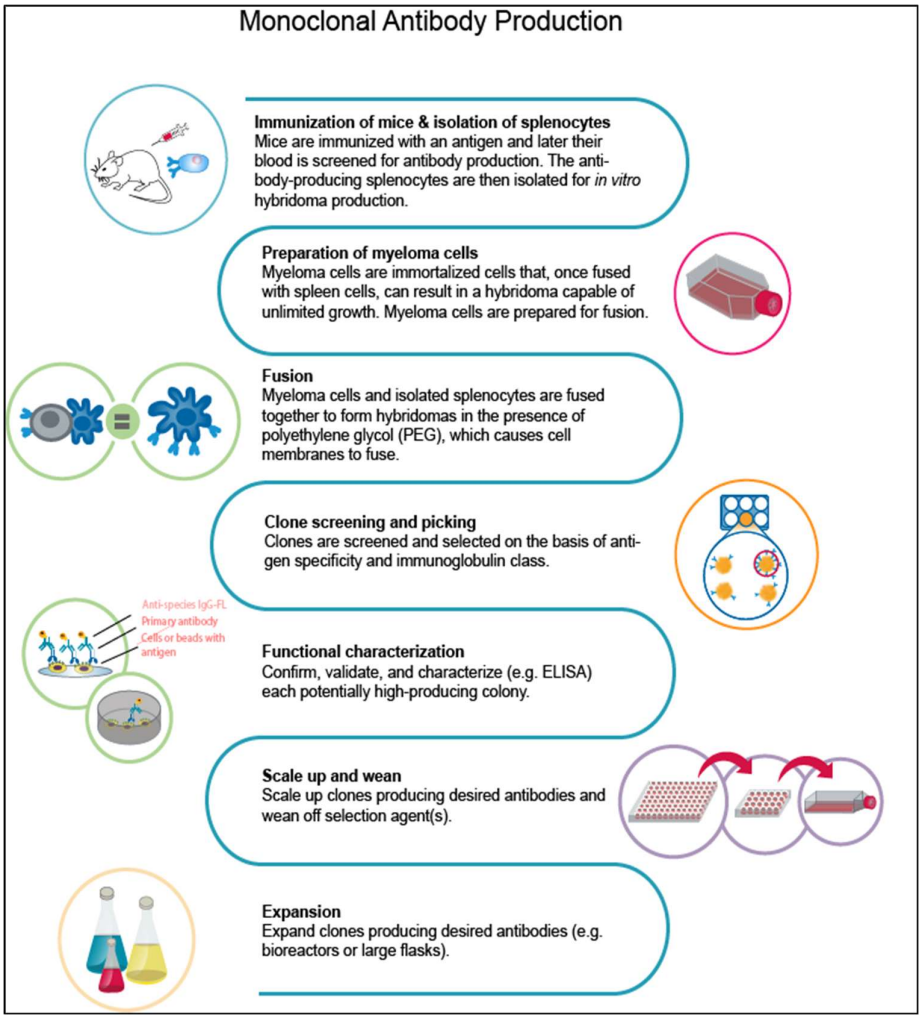

Figure 2: Example for Production of monoclonal Antibodies by using mice

Stage 3: Characterization:

The comprehensive characterization of monoclonal antibodies is essential. This entails evaluating their physicochemical and immunochemical attributes biological activity, purity, impurities, and quantity, following the ICH Q6B guideline. In-house reference materials, appropriately characterized, are vital for lot testing. Physicochemical assessment involves determining class, subclass, light chain composition, primary structure, and carbohydrate content. Variability in terminal amino acids and disulfide bridge integrity are analyzed. Immunological characterization encompasses binding assays, cross-reactivity, unintended reactivity, and complement binding. Biological activity is evaluated through in vitro and, if necessary, in vivo assays, considering its significance for safety and efficacy. Purity analysis involves assessing heterogeneity via orthogonal methods with consideration for relevant variants. Aggregates, particulates, process-related impurities, and contaminants are identified

and quantitatively assessed. Quantity determination correlates with physicochemical and biological assays. This linkage can permit the use of quantity measurement in labeling and manufacturing processes **[4]**.

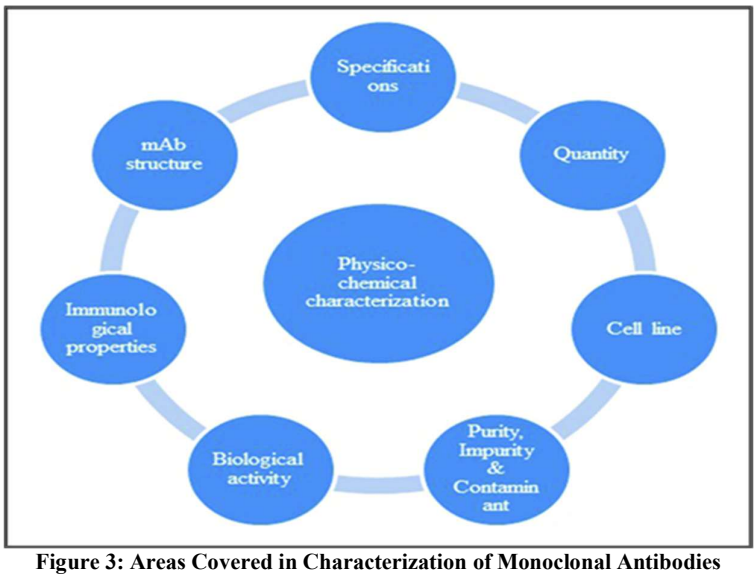

Figure 3: Areas Covered in Characterization of Monoclonal Antibodies

Stage 4: Specification:

Specifications constitute a vital aspect of the quality control strategy, ensuring product consistency and compliance upon testing. Relevant quality attributes from characterization studies guide specification establishment, with tests selected specifically for each product. Acceptance criteria should be set and justified, considering data from preclinical and clinical lots, manufacturing consistency demonstrations, stability studies, and developmental data as per ICH Q6B. Identity tests, such as unique molecular or structural aspects, must be highly specific, potentially requiring multiple assays to distinguish antibodies produced within the same facility. Purity assessment necessitates orthogonal methods for complex profiles with defined criteria for different variants. Glycosylation's impact on pharmacokinetics and immunogenicity should be addressed. Relevant impurities should be controlled, and process-step-specific testing may be appropriate. Potency assays reflecting clinical activity, particularly effector functions, are crucial. Quantity, specific activity, appearance, solubility, pH, osmolality, sterility, endotoxins, stabilizers, water, and particulate matter are general tests that complete specifications, ensuring product quality **[4]**.

SUMMARY AND CONCLUSION:

This study explains the comprehensive development, production, characterization, and specification of monoclonal antibodies

and related products in Europe. These engineered molecules hold great potential for combating various threats by targeting specific molecules, pathogens, or cells. The European Medicines Agency (EMA) plays a pivotal role in ensuring their efficacy and safety, setting stringent guidelines that span four distinct stages. These encompass alignment with the mechanism of action, selection of suitable cell substrates, meticulous characterization, and establishing precise specifications. The harmonization of these stages ensures that these innovative treatments adhere to the highest quality standards, leading to safer and more effective therapies that have revolutionized the medical landscape.

ACKNOWLEDGEMENT:

I express my gratitude to the Sri Adichunchanagiri college of Pharmacy for their unwavering support, as well as to Dr. Vedamurthy Joshi, Mr. Shaniymath and Dr. Abhishek B V for their guidance and help in seeing this article through to completion.

REFERENCES

[1] Liu, J.K.H. (2014), the history of monoclonal antibody development - progress, remaining challenges and future innovations, Annals of medicine and surgery (2012). Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4284445/(Cited on 4 June 2024).

[2] Development, production, characterization and specifications for monoclonal antibodies and related products - Scientific guideline | European Medicines Agency. Available at: https://www.ema.europa.eu/en/development-production-characterisation-specifications-monoclonal-antibodies-related-products

[3] ICH Q5A (R2) Guideline on viral safety evaluation of biotechnology products derived from cell lines of human or animal origin - Scientific guideline | European Medicines Agency. Available at: https://www.ema.europa.eu/en/ich-guideline-q5ar2-viral-safety-evaluation-biotechnology-products-derived-cell-lines-human-animal

[4] ICH Q6B specifications: Test procedures and acceptance criteria for biotechnological/biological products - scientific guideline (no date) ICH Q6B Specifications: test procedures and acceptance criteria for biotechnological/biological products - Scientific guideline | European Medicines Agency. Available at: https://www.ema.europa.eu/en/ich-q6b-specifications-test-procedures-acceptance-criteria-biotechnological-biological-products-scientific-guideline(Cited on 4 June 2024).

[5] Mokhtary, P. et al. (2022) Recent progress in the discovery and development of monoclonal antibodies against viral infections, Biomedicines. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9405509/(Cited on 4 June 2024).

[6] Production of monoclonal antibodies for therapeutic purposes: A Review, International Immunopharmacology. Available at: https://pubmed.ncbi.nlm.nih.gov/37244118/ (Cited on 4 June 2024).