GUIDANCE MANUAL FOR ASSURING QUALITY OF THERAPEUTIC MONOCLONAL ANTIBODIES

NATIONAL INSTITUTE OF BIOLOGICALS

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INTRODUCTION

Monoclonal Antibodies **(mAbs)** are immunoglobulin's (novel protein therapeutics) with a defined specificity derived from a monoclonal cell line. Their biological activities are characterised by a specific binding characteristic to a ligand (commonly known as antigen), and may also be dependent on immune effect or function such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).**mAbs** may be generated by recombinant DNA (rDNA) technology, hybridomas technology, B lymphocyte immortalisation or other technologies (e.g. phage display technology, genetically engineered animals).As a result of naturally-occurring molecular heterogeneity, imperfect cellular processing, chemical and enzymatic changes during manufacturing and additional changes upon storage, antibody drugs display a wide variety of minor chemical changes, collectively termed microheterogeneity. Common examples include glycan structural differences, deamidation, oxidation and glycation. Control of microheterogeneity within predefined analytical specifications has been used in quality control laboratories to guarantee consistent product quality during cGMP manufacturing. The recent Quality by Design (QbD) initiative for therapeutic biotechnology products, a joint pilot program between the regulators, academia and the biotechnology industry, is providing new guidance and expectations on QbD approaches in manufacturing for therapeutic proteins. As the name indicates, QbD encourages developers to build quality into the drug from the start. This approach requires significant knowledge of the drug's mechanism of action and how the drug's attributes affect quality. Physical or chemical changes known to affect the safety or efficacy of the drug are considered Critical Quality Attributes or CQAs. Manufacturing is then designed to control the desired levels of CQAs within defined limits, providing a consistent product quality. With this new QbD paradigm, the process is defined by the target ranges for the CQAs which, in turn, provide assurance of consistent product quality.

Of the various classes of antibodies, or immunoglobulin's, IgG1 is the most common immunoglobulin used for pharmaceutical and biomedical purposes, however, recent developments have shown that other immunoglobulin types (e.g., IgG2, IgG4) and mAb-

related products (e.g., Fc-fusion proteins, Fabs, etc.) are also being used for therapeutic purposes. The technological advancement in the evolution of therapeutic antibodies is shown at Figure – 1.

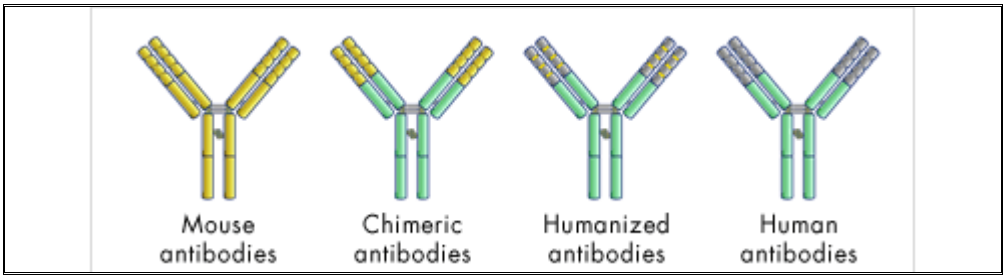


Figure - 1: Various types of mAbs evolved with technological advancement.

2. SCOPE

The guidance manual is aimed at emphasizing on the quality control testing parameters in general for human **mAb** intended for therapeutic and prophylactic use. Parallel to the aforesaid, there is a need felt to meet the requirements of good manufacturing practices, product-lot-testing to ensure product reproducibility, production process verification and quality assurance.

3. Market Dynamics of Biopharma including Therapeutic mAbs

India BioPharma market comprising primarily of vaccines, therapeutic drugs, insulin, animal biologicals, statins and diagnostics, continued to grab the largest share of the total biotech industry revenue of Rs.17, 249.34 crore in 2010-11. The biopharma market accounted for Rs.10, 655 crore, with over 60 % of market share in2010-11. It accounted for Rs.8, 829 crore, taking 62 % market share in 2009-10.

mAbs represent a significant portion of products in the biopharmaceuticals market. **mAbs** have been developed to treat a variety of indications to address significant unmet medical needs, and are generally target-specific and well tolerated with a relatively long half-life, contributing to the success of the molecule class for drug development. While a successful mAb product can generate upwards of a billion dollars or more in sales annually, current estimates indicate that it can cost approximately US $1.5 billion and

take about 10-15 years and in bringing a novel therapeutic from the bench to the market and these estimates are predicted to rise in the future. However, India is in a beneficial position as it can provide the much needed the cost benefits that can attract out-sourced projects. A recent Boston Consulting Group report (2011) had stated that the current cost advantage provided by India is estimated to be approximately 60% and is predicted to remain significant until 2025 at about 20%.

As the patents for biologics move closer to their expiration dates and the challenging economic situation across the globe forces a decrease in healthcare expenditures, biosimilars emerge as the cost-effective therapeutic alternative across the globe. Market for biosimilar mAbs is on the constant rise.Global sales figures for biosimilars mAbs in 2009 were US $1.23 billion and the market is predicted to reach around US $43 billion in 2020. **Currently accounting for 16% of global pharmaceutical expenditure** and significantly outpacing total branded sales, biologics will continue to out-perform the global market as more innovative products deliver new treatment options for a growing range of indications. Being an affordable yet equally-effective alternative for biologics, they can provide savings that range from 25% to 85% of the original versions. Moreover, the **patent expiry of approximately 48 biologics (worth US $73 billion)** in the next 10 years will also fuel the demand for biosimilars (Table – 1& 2). Despite the resistance from the innovator companies, and the regulatory hurdles involved in their production, biosimilars have immense market potential for both developed, as well as growing economies.

Table – 1: *FDA approved therapeutic mAbs available in the world market.*

Name: Antibody	Target: Antibody Type	Indication	Company	Approval Date	Patent Expiry
***OKT3:** Muronomab-CD3	CD3: Murine, IgG2a	Autoimmune	Johnson & Johnson	1986 (US)	Data Not available
ReoPro: Abciximab	PIIb/IIIa: Chimeric, IgG1, Fab	Homeostasis	Johnson & Johnson	1984 (US)	Data Not available
Rituxan: Rituximab	CD20: Chimeric, IgG1	Cancer	Genentech	1997 (US)	26.11.2017
				1998 (EU)	02.06.2018
***Zenapax:** Daclizumab	CD25: Humanized, IgG1	Autoimmune	Roche	1997 (US)	Data Not available
				1999 (EU)	Data Not available
Simulect: Basiliximab	CD25: Chimeric, IgG1	Autoimmune	Novartis	1998 (US)	Data Not available
				1998 (EU)	Data Not available
Synagis: Palivizumab	RSV: Humanized, IgG1	Infections	MedImmune	1998 (US)	Data Not available
				1999 (EU)	Data Not available
Remicade: Infliximab	TNFa: Chimeric, IgG1	Autoimmune	Johnson & Johnson	1998 (US)	24.08.2018
				1999 (EU)	13.08.2019
Herceptin: Trastuzumab	HER2: Humanized, IgG1	Cancer	Genentech/ Roche	1998 (US)	25.09.2018
				2000 (EU)	28.08.2018
***Mylotarg:** Gemtuzumab ozogamicin	CD33: Humanized, IgG4, immunotoxin	Cancer	Wyeth/ Pfizer	2000 (US)	Data Not available
Campath: Alemtuzumab	CD52: Humanized, IgG1	Cancer	Genzyme	2001 (US)	07.05.2021
				2001 (EU)	06.07.2021
Zevalin: Ibritumomab tiuxetan	CD20: Murine, IgG1, radiolabeled (Yttrium 90)	Cancer	Biogen Idec	2002 (US)	Data Not available
				2004 (EU)	Data Not available
Humira: Adalimumab	TNFa: Human, IgG1	Autoimmune	Abbott	2002 (US)	31.12.2022
				2003 (EU)	08.09.2023
Xolair: Omalizumab	IgE: Humanized, IgG1	Autoimmune	Genentech/ Roche	2003 (US)	20.06.2023
Bexxar: Tositumomab-I-131	CD20: Murine, IgG2a, radiolabeled (Iodine 131)	Cancer	Corixa/GSK	2003 (US)	Data Not available
***Raptiva:** Efalizumab	CD11a: Humanized, IgG1	Autoimmune	Genentech/ Roche	2003 (US)	Data Not available
				2004 (EU)	Data Not available
Erbitux:	EGFR:	Cancer	Imclone/	2004 (US)	12.02.2024

Cetuximab	Chimeric, IgG1		Lilly	2004 (EU)	Data Not available
Avastin: Bevacizumab	VEGF: Humanized, IgG1	Cancer	Genentech/ Roche	2004 (US)	26.02.2024
				2005 (EU)	Data Not available
Tysabri: Natalizumab	a4-Intergrin: Humanized, IgG4	Autoimmune	Biogen Idec	2004 (US)	Data Not available
Actemra: Tocilizumab	Anti-IL-6R: Humanized, IgG1	Autoimmune	Chugai/ Roche	2005(JP) 2010 (US)	Data Not available
Vectibix: Panitumumab	EGFR: Human, IgG2	Cancer	Amgen	2006 (US)	Data Not available
Lucentis: Ranibizumab	VEGF: Humanized IgG1 Fab	Macular degeneration	Genentech/ Roche	2006 (US)	Data Not available
Soliris: Eculizumab	C5: Humanized IgG2/4	Blood disorders	Alexion	2007 (US)	Data Not available
Cimzia: Certolizumab pegol	TNFa: Humanized, pegylated Fab	Autoimmune	UCB	2008 (US)	Data Not available
Simponi: Golimumab	TNFa: Human IgG1	Autoimmune	Johnson & Johnson	2009 (US, EU, CAN)	Data Not available
Ilaris: Canakinumab	IL1b: Human IgG1	Infalmmatory	Novartis	2009 (US,EU)	Data Not available
Stelara: Ustekinumab	IL-12/23: Human IgG1	Autoimmune	Johnson & Johnson	2009 (US)	Data Not available
				2008 (EU)	Data Not available
Arzerra: Ofatumumab	CD20: Human IgG1	Cancer	Genmab	2009 (EU)	Data Not available
Prolia: Denosumab	RANK ligand: Human IgG2	Bone Loss	Amgen	2010 (US)	Data Not available
Numax: Motavizumab	RSV: Humanized IgG1	Anti-infective	Meddimmune	Pending	Data Not available
ABThrax: Raxibacumab	*B. anthrasis*PA: Human IgG1	Anti-infection	GSK	2012(US)	Data Not available
Benlysta: Belimumab	BLyS: Human IgG1	Autoimmune	Human Genome Sciences	2011 (US)	Data Not available
Yervoy: Ipilimumab	CTLA-4: Human IgG1	Cancer	BMS	2011 (US)	Data Not available
Adcetris: Brentuximab Vedotin	CD30: Chimeric, IgG1, Drug-conjugate	Cancer	Seattle Genetics	2011 (US)	Data Not available
Perjeta: Pertuzumab	Her2: Humanized, IgG1	Cancer	Genentech/ Roche	2012 (US)	Data Not available
Kadcyla: Ado-trastuzumab emtansine	Her2: Humanized, IgG1, Drug-conjugate	Cancer	Genentech/ Roche	2013 (US)	Data Not available

***Withdrawn by the sponsor**

Table 2: *List of biologics coming off patent from 2012 to 2015.*

Product	Company	Therapeutic Category	Patent Expiry
Enbrel	**Amgen, Pfizer**	**Other anti-rheumatics**	**23-10-2012**
Neupogen	**Amgen**	**Immunostimulants**	**12-03-2013**
Humalog	**Eli Lilly**	**Anti-diabetics**	**07-05-2013**
Avonex	**Biogen Idec**	**MS Therapies**	**30-05-2013**
Epogen	**Amgen**	**Anti-anaemics**	**20-08-2013**
Procrit/Eprex	**Johnson & Johnson**	**Anti-anaemics**	**20-08-2013**
Cerezyme	**Genzyme**	**Other therapeutic products**	**27-08-2013**
Rebif	**Merk KGaA**	**MS Therapies**	**21-12-2013**
Novomix	**Novo Nordisk**	**Anti-diabetics**	**06-06-2014**
NovoRapid/NovoLog	**Novo Nordisk**	**Anti-diabetics**	**07-12-2014**
Rituxan	**Roche**	**Antineoplastic Mabs**	**31-12-2014**
Kogenate	**Bayer**	**Anti-fibrinolytics**	**31-12-2014**
Prevnar	**Pfizer**	**Vaccines**	**01-01-2015**
Lantus	**Sanofi-Aventis**	**Anti-diabetics**	**12-02-2012**
Actemra	**Roche**	**Other Anti-rheumatics**	**07-06-2012**
Gonal-F/Gonalef	**Merk KGaA**	**Fertility agents**	**16-06-2015**
Neulasta	**Amgen**	**Immunostimulants**	**20-10-2015**
Nimotuzumab	**YM BioSciences**	**Anti-neoplastic Mabs**	**17-11-2015**
Norditropin SimpleXx	**Novo Nordisk**	**Growth Hormones**	**15-12-2015**
Helixate	**CSL**	**Anti-fibrinolytics**	**31-12-2015**

4. Indian Market – Therapeutics

The total biologics market in India witnessed a growth of over 35 % and stood at over Rs. 2,000 crore for year ending 2010 as against Rs 1,480 crore for the year ending 2009.There are 20 recombinant therapeutic products approved for marketing in India and Indian companies have established capabilities to manufacture about 15 of these recombinant therapeutic products. Out of these, the products that have a large share in the market include Human insulin, Erythropoietin, Hepatitis-B vaccine (recombinant

surface antigen-based), Human Growth Hormone, Granulocyte Colony Stimulating Factor (G-CSF) and Streptokinase. Close to 50 brands are being marketed in India by Biocon, Shantha Biotechnics, Bharat Serums and Vaccines, Virchow Biotech, Zenotech, Reliance Life Sciences, Bharat Biotech International, Wockhardt, Shreya Life Sciences, Intas Biopharmaceutical's and Shreya Life Sciences-manufacturing an average of five brands in India and about 72 recombinant therapeutic products are at different stages of clinical trials. From just six top Indian companies with manufacturing capabilities in recombinant therapeutic products in 2005–(Table 3) the number has more than doubled to about 15 companies in 2011.

Table 3: *Biosimilars in India*

Firm	Biosimilar Product
Biocon	**Biomab** (biosimilar nimotuzumab) **Eripro** (recombinant Human Erythropoietin (EPO) **Insugen** (recombinant human Insulin) **Myokinase** (recombinant streptokinase) **Nufil** (recombinant G-CSF)
Dr. Reddy's Lab	**Cresp** (darbenpoitin alfa) **Frafeel** (recombinant G-CSF) **Reditux** (rituximab)
Intas	**Epofit** (recombinant EPO) **Intalfa** (recombinant human Interferon alfa-2b) **Neukine** (recombinant G-CSF) **Neupeg** (PEGylated G-CSF)
Reliance Life sciences	**Reliferon** (recombinant Interferon alfa-2b) **ReliGrast** (recombinant G-CSF) **Relipoietin** (recombinant EPO)
Shantha Biotech	**Shanferon** (recombinant Interferon alfa-2b) **Shankinase** (recombinant Streptokinase) **Shanpoietin** (recombinant EPO)
Wockhardt	**Wepox** (recombinant EPO) **Wosulin** (recombinant Insulin)

Dr Reddy's Laboratories that sells biologic products like Grafeel (generic Filgrastim), Reditux, (genericRituximab) and Peg-grafeel launched in May 2011. Priced at Rs.8, 865, the product represents a breakthrough in the pricing of this complex molecule. It is priced at approximately 25 % of the originator brand in India, and is priced 95 % lower than the US price for pegfilgrastim. This breakthrough in pricing has been enabled by the company's vertically integrated global manufacturing network. Besides, it has another 11 biosimilars in various stages of development and commercialisation. India will see launch of many biosimilar products in the next two-three years, as many biopharma companies have been working on these products for the last few years and are now in different phases of clinical trials. With the launch of these products the Biopharma market has seen a growth of over 25% by 2012.

5. Biosimilar Therapeutics - Indian Scenario

The drug classes for biosimilars in India comprise of human insulin, human growth hormone, granulocyte colony stimulating factor (G-CSF), Erythropoietin, and Streptokinase. Recent estimates indicate that 20 companies in India are already developing biosimilars. There are around 25 recombinant therapeutic available in India and 15 of them are manufactured within the country.

About 50 brands are commercialized in the country by leading companies such as Biocon, Shantha Biotechnics, Reliance Life Sciences, Wockhardt and Intas Biopharmaceuticals among others. About72 recombinant drugs are currently undergoing different stages of clinical trials (Figure – 2) and their launch may cause an increase of 25% in the Indian Biopharma sector.

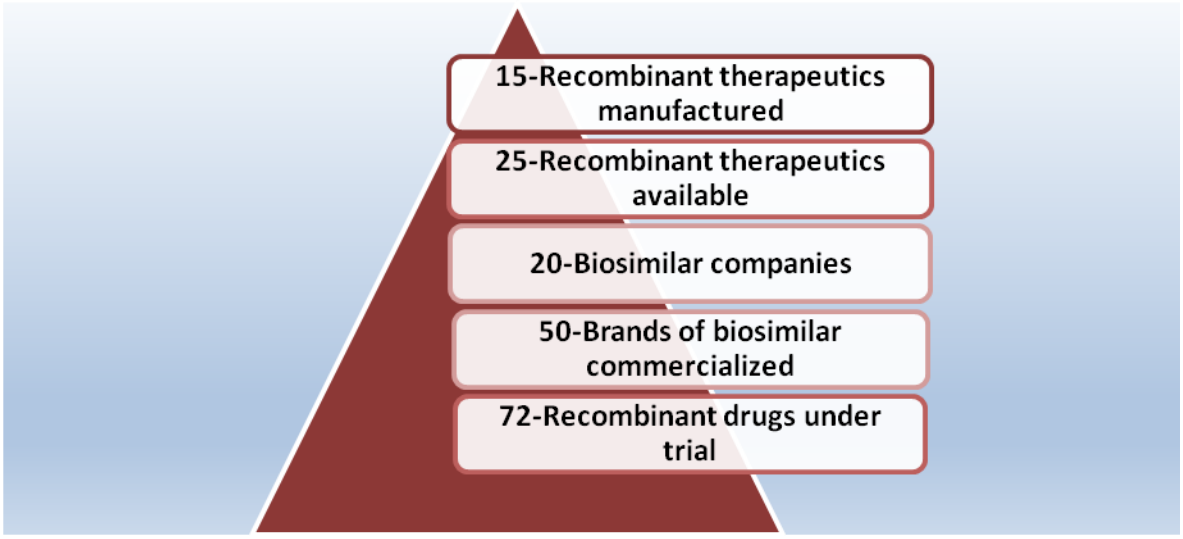
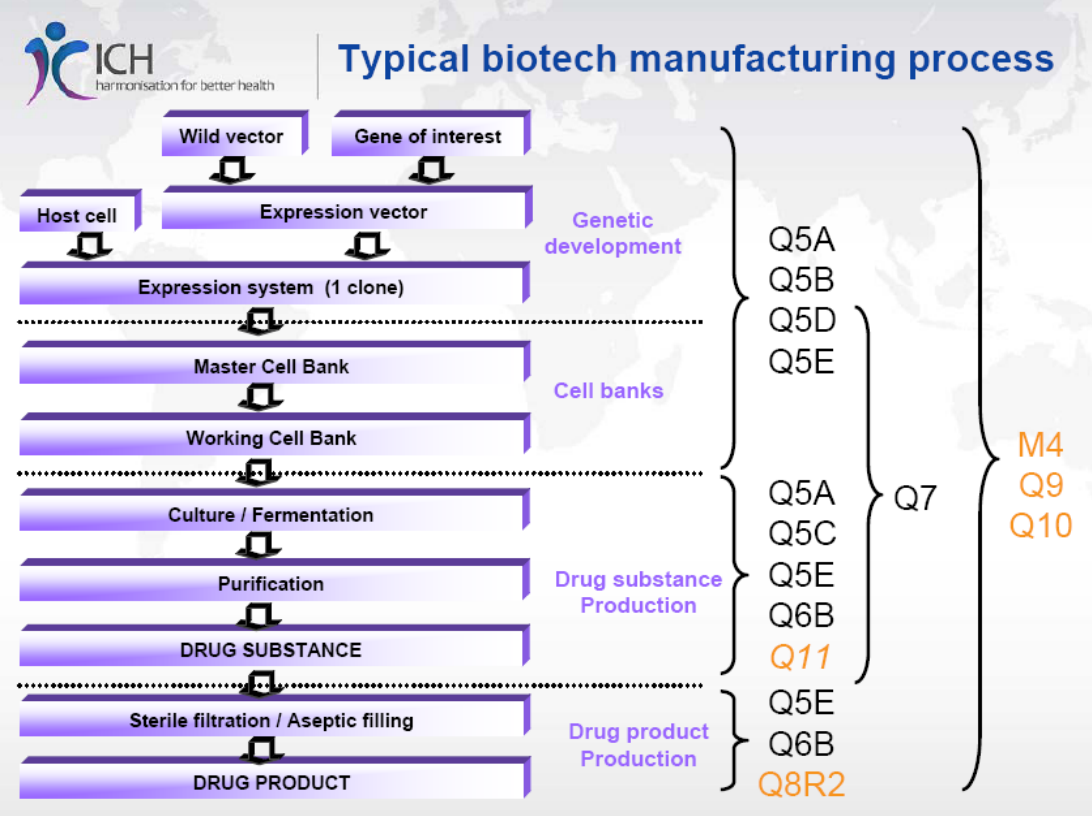


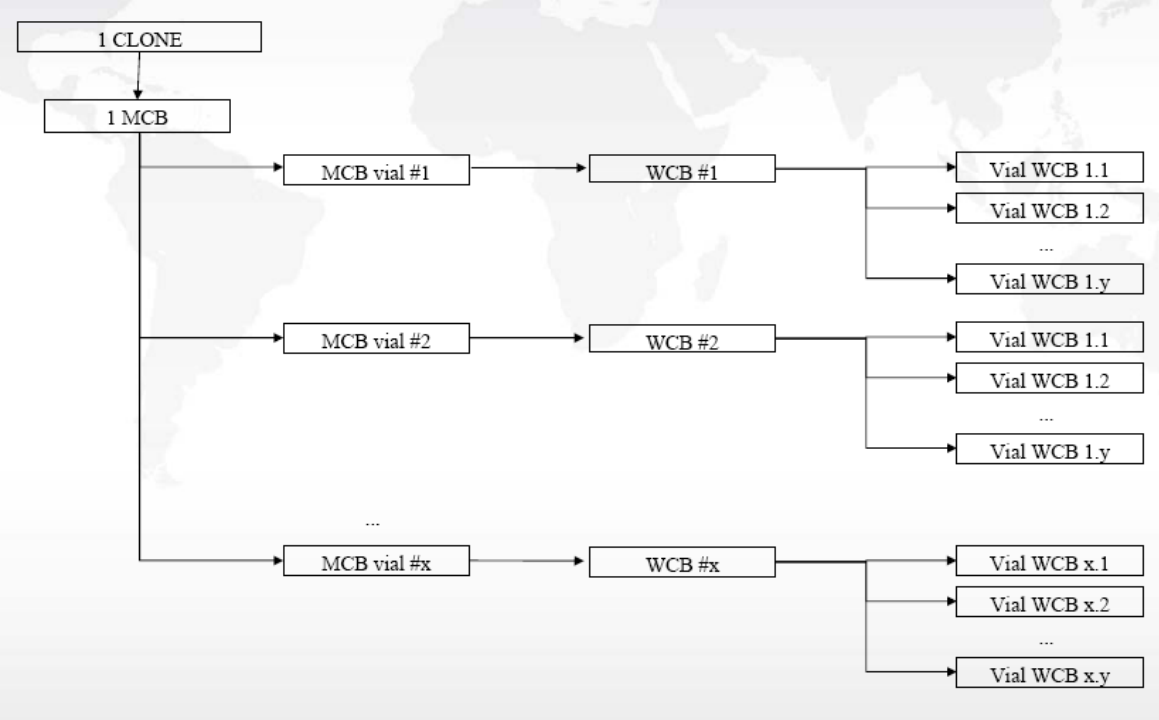
Figure -2. *Global Joint Ventures for Biosimilars*

6. ICH documents for biologics:

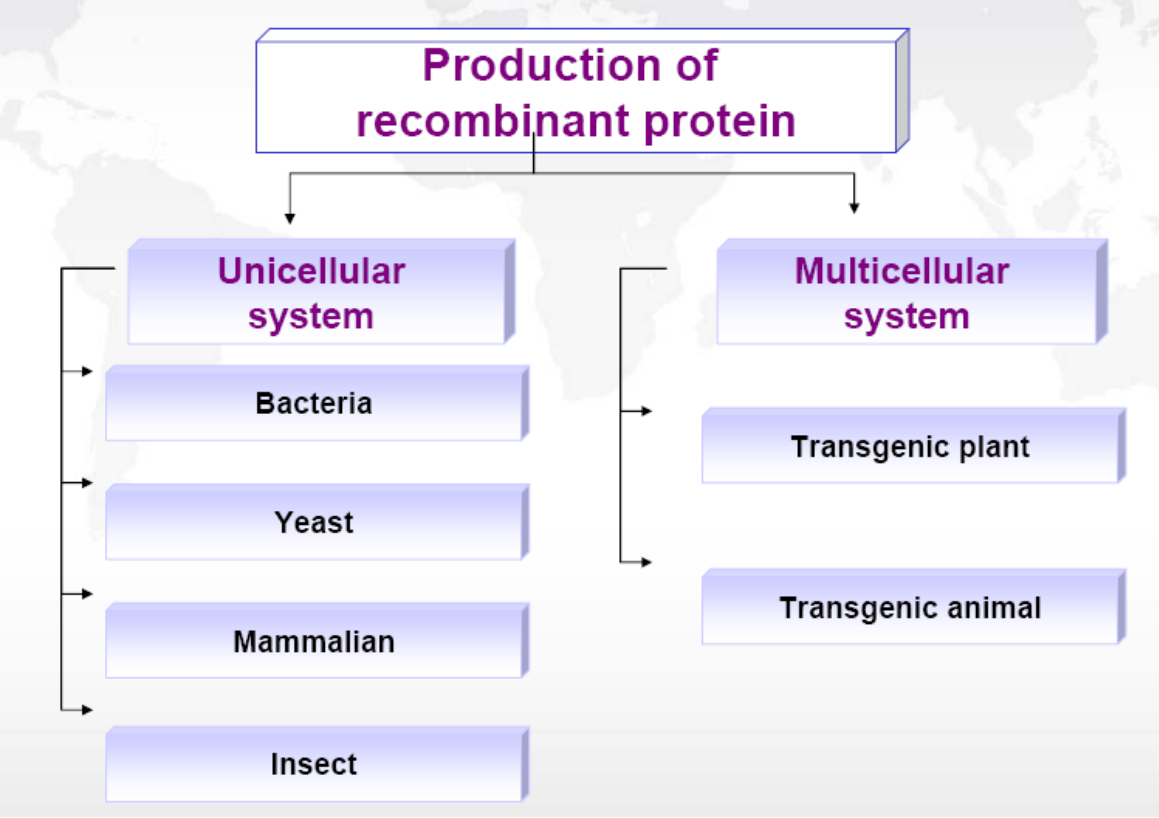
- Q5 A: Viral Safety
- Q5 B: Genetic Stability
- Q5 C: Product Stability
- Q5 D: Cell Substrates
- Q5 E: Comparability
- Q6 B: Specification
- M4 / M2: CTD / e-CTD
- Q7: GMP for APIs
- Q8: Pharmaceutical development
- Q9: Quality Risk Management
- Q10: Pharmaceutical quality system
- Q11: Development and Manufacture of Drug Substances

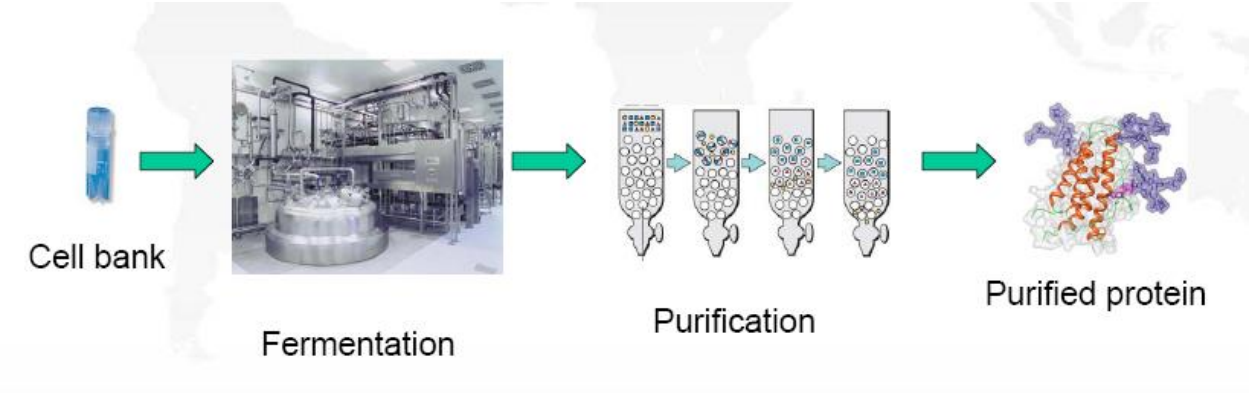
7. Manufacturing Process [Upstream & Downstream]

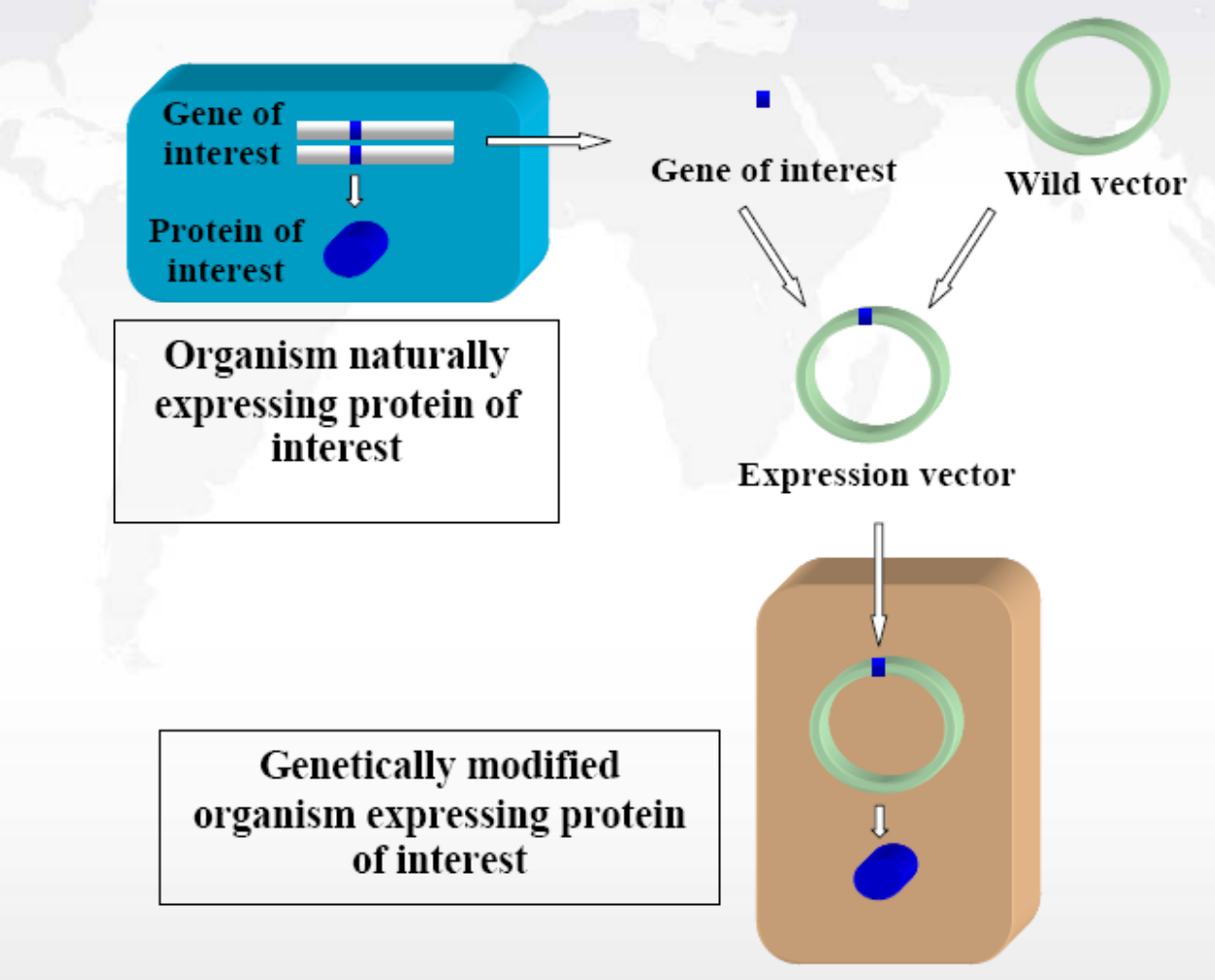




Stages of Master Cell Bank (MCB) and Working Cell Bank (WCB).


Production of recombinant protein
Unicellular system
Bacteria
Yeast
Mammalian
Insect
Multicellular system
Transgenic plant
Transgenic animal


Cell bank
Fermentation
Purification
Purified protein


Gene of interest
Protein of interest
Organism naturally expressing protein of interest
Gene of interest
Wild vector
Expression vector
Genetically modified organism expressing protein of interest

8. Major Manufacturers of Therapeutic (mAbs) [India]

- **Indian Companies with product in market:**
    1. Biocon Ltd: Mabpharm EGFR (Anti-cancer)
    2. Dr. Reddy Lab: Rituximab (Anti arthritic)
- **Companies with mAb's in Pipeline:**
    1. Reliance life science, Mumbai
    2. Biocon ltd, bangalore
    3. Dr. Reddy Labs, Hyderabad
    4. Avesthagen, Bangalore
    5. Bharat serum and Vaccines ltd, Mumbai
    6. Serum Institute of India, Pune
    7. Intas Biopharmaceuticals ltd, Ahmedabad
    8. Zydus research centre, Ahmedabad
    9. Lupin biotech research, Pune
    10. Natco pharma – Hyderabad
    11. Mabpharm - Goa
- **Major International companies in Indian market:**
    1. Roche lab (Genentech): Rituximab, Herceptin, Avastin
    2. Amgen/ Wyeth Labs: Enbrel (Etanercept)
    3. Abbott Labs : Humira

9. Quality Control:

9.1 Reference Material

Part of a lot that has been fully characterised and evaluated in studies in humans shall be retained as an "in-house" reference material under conditions that maintain its stability for use in assays of subsequent lots, this is of particular importance if international reference materials are not available for testing / reference. The criteria for establishing manufacturer's reference material shall be approved by the national control

authority. In the event of mAb preparations having a short validity period (radiolabelled mAbs), the reference material may consist of an unlabeled immunoglobulin material. These in-house reference or original innovator product are essentially required for head to head comparison with test products

9.2 Lot release testing of mAbs (Drug substance / Drug product)

Once the mAb is purified and formulated, the resulting drug substance must be tested prior to lot release (Table–4). A set of tests and acceptance criteria are established based on mAb characterization and prevalent regulatory requirements in order to ensure drug quality. These tests typically include appearance, identity, purity, protein concentration, potency of the molecule, microbial limits or bio-burden, bacterial Endotoxins, Host cell protein and residual DNA etc.

IEC / SEC are the most frequently used lot release methods for purity for mAbs. Test for immunogenicity of recombinant derived drug is performed by the manufacturer. Stability studies may be performed on the mAb drug substance (e.g. frozen bulk for storage) and drug product, if required (e.g. final vial) according to prevalent regulatory guidelines. EMEA 2006 & 2007 guidelines are available for characterisation of similar biological medicinal products containing biotechnology derived proteins as active substances. WHO (2009) guidelines have been published on evaluation of similar biotherapeutic products. These have been reviewed recently and draft document is ready for comments of manufacturers, academia and stake holders and same is expected to be published in this year. Similarly, guidelines on similar biologics; regulatory requirements for marketing authorization in India have been published in the year 2012 by the DBT & CDSCO, Govt. of India. Indian pharmacopoeia commission is in the process of finalisation of monograph on Rituximab and other recombinant drugs.

Table – 4. *Commonly used tests found on a Certificate of Analysis for lot release of; a selected subset is used for stability testing of mAbs.*

QC Parameter	Test Name
Appearance	Color, Opalescence and Clarity
Identity	Peptide mapping by RP-HPLC (Reverse Phase HPLC), or
	MALDI (Matrix – assisted deionisation) Mass spectroscopy, or
	UV spectroscopy (2nd derivative)
Purity	Limulus Amebocyte Lysate (Endotoxin)
	Size exclusion chromatography (SEC)
	CE-SDS (Capillary electrophoresis-Sodium dodecyl sulphate)
	IEC (Ion exchange chromatography) or icIEF (Imaged capillary isoelectric focusing)
	Glycosylation profile
	Peptide mapping by RP-HPLC
Potency	Potency (ELISA/Cell-Based Assay)
Strength	UV Spectroscopy
General Tests	Osmolality
	pH
	Protein concentration
	Surfactant Concentration (e.g. Polysorbate 20)
Safety	Sterility testing
	Bacterial Endotoxin Test

Critical Quality tests such as Identity, Purity, Potency and Safety are performed head to head with the “Reference therapeutic mAb” by using test methods suitably approved by a national control authority.

9.3 Test for Identity:

The drug product under testing is tested for its isotype composition and its immunological reactivity with the target antigen. The result must meet the product specification set by the manufacturer.

9.4 Test for Potency:

The biological activity of the mAb must be determined by either an in vivo or an in vitro test method. The result must meet the product specification set by the manufacturer.

9.5 Test for Safety:

The test shall be performed by using suitably sensitive test method such as Bacterial Endotoxin test. The result must meet the product specification set by the manufacturer.

9.6 Test for Protein Content:

The total protein content must be determined by suitable method using ultraviolet spectrophotometry. The result must meet the product specification set by the manufacturer.

Other tests such as Appearance, pH, Osmolality, Extractable volume, Sterility, Bacterial Endotoxins, Moisture content, should be assessed wherever appropriate as per the prevalent regulatory guidelines / pharmacopoeia.These tests are performed in head to head comparison with original innovator product or in-house reference prepared by the manufacturer wherever required. Once these tests are performed and the results meet the established acceptance criteria, a Certificate of Analysis (COA) – Table 4, along with the test results obtained is generated and the lot is released for use.

National Institute of Biologicals (NIB) has already standardised tests like pH, protein content, osmolality, reducing SDS page, size exclusion chromatography, bacterial endotoxin test, extractable volume and sterility for Rituximab. Potency of the drug by using WIL2 S cell line is under process of standardisation.

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IX. WHO guideline – “Guidelines for national authorities on quality assurance for biological products” – WHO TRS 822 (Annex 2) – 1992.

X. WHO Guidelines on Biosimilars evaluation of Similar Biotherapeutic products (SBPs) – 2009.

XI. WHO informal consultations on the revision of guidelines on the Quality, Safety, Efficacy of Biological & Medicinal products prepared by rDNA technology- June 2012.

Disclaimer:

This guidance represents the current views of National Institute of Biologicals (NIB's) on assuring Quality of Therapeutic (MAbs). It does not create or confer any rights for or on any person and does not operate to bind NIB or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.