भारत सरकार
विज्ञान और प्रौद्योगिकी मंत्रालय
बायोटेक्नोलॉजी विभाग
GOVERNMENT OF INDIA
MINISTRY OF SCIENCE & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

सत्यमेव जयते

स्वच्छ भारत
एक कदम स्वच्छता की ओर

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Lodhi Road, New Delhi-110003

No. BT/BS/17/479-2012-PID **Dated: 31.08.2018**

OFFICE MEMORANDUM

Subject: Check list for information requirements in the Applications/Reports on Pre-clinical Toxicity study of Similar Biologics

In Accordance with the Allocation of Business Rules 1961 as enumerated through the Government of India notifications No. CD-172/86 dated 27.02.1986 and No. CD-87/87 dated 31.01.1987 and the powers conferred through Sections 6, & 9 of the Environment (Protection) Act 1986, read with the Central Government Gazette Notification No. GSR 1037(E) dated 05.12.1989, issued by the Ministry of Environment and Forests, New Delhi and based on the recommendations of the Review Committee on Genetic Manipulation (RCGM) in its 165th meeting held 22.05.2018, the Department of Biotechnology has prepared the Check list for information requirements in the Applications/Reports on Pre-clinical Toxicity study of Similar Biologics. This checklist will covers both the applications for conducting Pre-clinical Toxicity (PCT) studies on biopharma products (Form C3) and the Reports on PCT studies conducted on biopharma products (Form C5). All essential information required to be submitted by the applicants in Form C3 &C5 have been indicated in the checklist and this will facilitate the applicants, reviewers as well as regulators in submission, review, processing and approval of the applications/reports of PCT studies on biopharma products. The completed checklist is to be submitted along with the Table of contents, on top of the submission.

As per the provisions of Rules 1989 of EPA 1986, all applicants are required to submit the duly filled checklist along with their submissions in Form C3 & C5 with immediate effect.

(S.R.Rao)
Member Secretary, RCGM &
Scientist-H, DBT

To

The Member Secretaries of all Institutional Biosafety Committees

Checklist and instructions for applications to conduct Pre-clinical toxicity (PCT) study and submission of report for Similar Biologics

Tick **Yes** or **No** for all the parameters to ensure completion of Form C3 PCT protocol/ Form C5 PCT data before submission. If the answer is NO, provide reason(s) just below in the check list.

Proposed check list covers:

1. Information sought in Form C3 for Chemistry, manufacturing and controls and Pre-clinical toxicity (PCT) protocol.
2. Information sought in Form C5 from PCT studies.

Note:

- Avoid duplication of data submission.
- Application must be submitted (with good quality prints) by printing on both sides.
- Submit original Data, figures & graphs for each consistency batch as coloured, interpreted and with proper labelling.
- Applicant should follow Indian Similar Biologic Guidelines, 2016 and other international guidelines referred & current scientific literature on the subject matter.
- Permission for conducting PCT studies shall be granted only after establishing biosimilarity through physicochemical characterization, stability of Drug substance (DS) and Drug product (DP) through functional assays (binding and bioassays).
- Reference Biologic is an innovator's product and a minimum of three lots of RB should be included **in all** physiochemical and biological characterization to establish similarity.
- The DP to be used in PCT studies should contain DS derived from one of three consistency batches for which complete CMC studies are conducted.

S. No.	**Parameters**	**Similar Biologics**	**Page no.**
A	**General**		
A1	The application contained Table of contents, all pages serially numbered, printed on both sides and all cited annexure(s) included	Yes/No	
A2	Mention copies of approval(s) accorded so far for the product under investigation by the RCGM	Yes/No	
A3	Selection of appropriate Reference Biologic (RB): • Reference Biologic approved in India or in ICH countries can be used. • In case RB not marketed in India, it can be imported. • The same RB should be used in all studies supporting similarity, safety, efficacy and quality of the product	Yes/No	
A4	Was the dosage form, strength and route of administration is same for Similar Biologic as compared to RB.	Yes/No	
A5	Background information of RB, in brief, containing mode of action, number of therapeutic indication(s), therapeutic dose is provided. Mention, any known side effects, animal toxicology data, similarity / dissimilarity between the molecule / compound under consideration to be provided*	Yes/No	

A6	Global marketing Status of reference biologic as on date.	Yes/No	
A7	Status of Similar Biologics/Biosimilars already approved in India or elsewhere	Yes/No	
B	**Molecular Characterization of the GMOs/LMOs Used**		
B1	Details of origin of gene(s) coding the molecule under consideration	Yes/No	
B2	Provide Nucleotide and translated protein sequences	Yes/No	
B3	Indicate prominently, if any changes made in the gene(s) sequence leading to change in amino acid sequence in comparison with RB. If so, provide details (sequence alignment in case of similar biologic) of modifications at DNA and protein level. Provide, Genbank Acc. Numbers when available.	Yes/No	
B4	Information about the vector (Include restriction map, Promoter and Terminator used for the expression of recombinant gene, method of transformation, selection agent used, etc.)	Yes/No	
	Is the host/ cell line used for the expression of Similar Biologic/ Reference Biologic is same. If not, provide details and explain the reason(s).		
B5	Description of host organism characteristics.	Yes/No	
B6	Safety of the host organism (indicate Risk Group#).	Yes/No	
B7	Copy number and stability of plasmid in expressing host cell for microbial fermentation before induction and at the time of harvest.	Yes/No	
B8	Provide information on the expression levels of protein	-	
B9	Provide brief note on containment level adopted and biosafety procedures followed during the study.	-	
C	**Standardization of fermentation/production procedures**		
C1	Detailed media composition for pre-inoculum, inoculum and production process (Indicate wherever commercial media used), feeding rate of media (in grams of nutrient/h/L of initial fermentation broth)	Yes/No	
C2	Information on batch size (in terms of liters of fermentation)	Three consistency batches	
C3	Consolidated trend of different parameters fromthree representative batches (such as cell growth, product formation, pH, temperature, dissolved oxygen, nutrient consumption, agitation rate, aeration rate, CO2supplementation) during fermentation.	Yes/No	
C4	Time dependent product profile: 1). Concentration of product/L, yield and volumetric productivity. 2. Consistency of specific protein yield (amount of protein per unit cell mass at different cell concentration during fermentation).	Yes/No	
D	**Downstream process for purification:**		
D1	Purification process (flow chart detailing all major steps involved).	Yes/No	
D2	List of reagents, resins, membranes used in the purification process along with their properties.	Yes/No	

D3	Description of each unit of operation step (batch size) during purification. Chromatograms of three consistency batches.	Yes/No	
D4	In case of recombinant protein forming inclusion bodies, details of refolding process and quality of refolded protein in terms of confirmation of solubility and absence of protein aggregation.	Yes/No	
D5	Quality of the product at each step of purification SDS-PAGE, reducing and non-reducing gels (include suitable MW Marker, Mention loading of DS in µg (e.g., 1µg, 3µg, 5µg etc.) Chromatographic analysis for each purification step(include an overlay of all batches and reference molecule).	Yes/No	
D6	Overall recovery of the product (for each batch) in a tabulated form	Yes/No	
D7	Summary table showing consistent recovery of drug substance (yield at each stage of purification, overall product yield *etc.*)	Yes/No	
E	**Physico-chemical characterization** [*All evaluations must be carried out in comparison with a minimum of three lots of (different age/ timing) Reference Biologic*]. **		
E1	Intact mass analysis	Yes/No	
E2	Peptide mapping (overlay results of all batches) and N-terminus amino acids sequencing data	Yes/No	
E3	Secondary structure data by CD spectroscopy/Near and far UV visible spectra (overlay results of all batches)	Yes/No	
E4	Fluorescence spectroscopy to provide evidence for similarity at high order structure (overlay results)	Yes/No	
E5	Data on disulfide bond presence (when applicable)	Yes/No	
E6	Charge heterogeneity (Data from cation exchange chromatography, Isoelectrofocusing, etc.)	Yes/No	
E7	Carbohydrate/glycan content analysis and details of components, as applicable	Yes/No	
E8	Presence of aggregates (using any suitable method e.g. Size Exclusion Chromatography (SEC), Dynamic Light Scattering (**DLS**) etc.)	Yes/No	
E9	Endotoxin/Pyrogen content (*for each consistency batch)*	Yes/No	
E10	Host Cell Protein content (*for each consistency batch)*	Yes/No	
E11	Host Cell DNA content (*for each consistency batch)*	Yes/No	
F	**Binding and functional bioassay of proposed Similar biologics** Biological assays should be validated against an international or national reference standard, where available. If no such standards are available, an internal reference standard must be established as per the ICH Guidelines.		
F1	**Results on functional assays** (e.g. Binding to receptor, Activation of signal transduction pathways, Tissue specific activities like reduction in glucose, Cytotoxicity: CDC, ADCC,Neutralization, apoptosis, cAMP and anti-proliferative cell assays, *in vivo* assay etc) as applicable be included.	Yes/No	
F2	Presence of any high or low molecular weight product (DS) related to impurities observed during CMC must be discussed and explained.	-	
G	**Formulation and Stability studies of Drug Substance (DS) and Drug Product (DP) batches done as per regulatory requirement**** [#].		

G1	Submit consolidated batch data [##]	Yes/No	
G2	SDS-PAGE analysis (preferably silver stained &in alignment with MW Marker)	Yes/No	
G3	Overlay of Size Exclusion Chromatography analysis	Yes/No	
G4	Data on bioactivity/bioassays	Yes/No	
G5	Stability data on real time***, accelerated and stress studies of all the pilot batches of drug substance (DS) and drug product (DP) (in comparison with reference biologic in case of DP) at the time points specified in **Table 1**) based on functional bioassay for potency, relative to reference material or reference standard be provided.	Yes/No	
G6	Whether the same excipient/stabilizer as that of reference biologic has been used. If not, specify the stabilizer used and indicate if the same is approved anywhere else globally.	Yes/No	
H	**Acceptability criteria of the formulated material for preclinical safety studies***(Acceptance limits should be set based on RB data and sufficient number of PCT batches)*		
H1	Specifications for DS and DP should be established around critical quality attributes.	Yes/No	
H2	Summary of test resultsof Reference and proposed Similar biologic (SB) establishing biosimilarity.	Yes/No	
I	**Proposed work plan for preclinical toxicity studies-** *(All evaluations must include reference biologic of innovator'sproductfor direct comparison)*		
I1	Whether the representative toxicology batch of DP is one of the RCGM approved consistency batch. If not, generate complete comparativedata of this batch with that of the consistency batch approved earlier by RCGM.	Yes/No	
I2	Provide information on any known toxicity of reference biologic/ innovator, to animals and humans as on date.	-	
I3	List of preclinical toxicity and immunogenicity studies to be conducted.	-	
I4	Selection criteria for animals selected and numbers to be used in each group.	-	
I5	Submit detailed Pre-clinical toxicity & Immunogenicity (sequence specific, non-specific to other proteins and with adjuvant, as applicable0) study protocols. Protocols should include route of administration, dosage to be tested, basis of dose calculation, vehicle, mode of administration.	Yes/No	
I6	Provide address and accreditation status of the facility where studies are to be conducted.	Yes/No	
I7	Explain compliance of containment facility measures.	Yes/No	
I8	Specify decontamination and disposal mechanisms.	Yes/No	
I9	Explain plans in case of any Emergency.	Yes/No	
I10	Attach copies of IBSC approvals of the Sponsor and CRO(s) *(Photocopy of IBSC minutes wherein proposed studies were approved).*	Yes/No	
J	**Undertaking/ Declaration Letter Signatures** *To be signed in original by hand (**Electronic/ scanned signatures not acceptable**).*	Yes/No	

K	**PCT Study report** *(All evaluations must include reference biologicof innovator'sproductfor direct comparison).*		
K1	Copies of IBSC approval of PCT report of the Sponsor & CRO(s).	Yes/No	
K2	IAEC approval for animal use and procedures to be followed.	Yes/No	
K3	QA statement.	Yes/No	
K4	Signatures of Study Director and all investigators involved in the study.	Yes/No	
K5	Study schedule (date of initiation, in life phase experiment, study completion).	Yes/No	
K6	Over all summary of PCT study report.	Yes/No	
K7	Preclinical Protocol deviations and amendments, if any.	Yes/No	
K8	Observations to be recorded - including the Equipment and methods used and units of measurement and expression for each parameter, Time points and Procedures to euthanasia e.g. blood drawing, body weight, etc., Description, organ weights and organs sampled for histopathology.	Yes/No	
K9	Summary data for all quantitative parameters viz., body weight, food consumption, haematological and biochemical parameters and organ weight, comparing with control group should be presented in graphical form. All data should be analyzed to find out statistically significant differences between DP and innovator product. Biological significance of differences, if any, be explained.	Yes/No	
K10	Reference group should be compared with low dose test item group and data should be presented in a separate table.	-	
K11	Bone marrow either examined as an aspirate /smear or on histopathology section.	-	
K12	**Gross Necropsy findings**:$^{\$\$}$ a. **Quantitative:** All major organ weights in a table format with unit of measurement being (i) Grams as absolute weights and (ii) Gms / Kg body weight. b. **Qualitative**: Description of any deviations from normal appearances along with a color photograph of the abnormality with a side by side picture of comparable control or any other group as deemed necessary. c. If test material is given parenterally, then a picture/s of injection sites with any abnormality along with comparable vehicle control of Injection site. d. If there was no abnormality of injection site in any of the groups then one picture of vehicle control and one of the highest dose.	Yes/No Yes/No Yes/No Yes/No	
K13	**Histopathology findings:** ^^ a. Results to be depicted in a tabular form for each of the organs taking in to consideration all the animals of all the groups. b. Specific scientific reason for normal histology without photographs. c. Data on any adverse reaction in organs in high dose group along with thorough examination of same organs in mid	Yes/No Yes/No	

	dose and low dose groups and should be compared with control and reference item as well. d. Wherever abnormal findings are observed, this should be supported with a photomicrograph along with the control (2 pictures).	Yes/No	
K14	In case of premature death or morbidity, Necropsy and detailed examination of all the tissues is to be included in the report.	Yes/No	
K15	Animal feed and animal health certifications.	Yes/No	
K16	Discussion on results and conclusions.	Yes/No	

* **Details about the proposed Similar Biologic and the Reference Biologic:** Name, active ingredient(s), therapeutic indication(s), existing treatments for the proposed indications, mode of action, strength, formulation, dosages, route of administration and known side effects, if any, along with appropriate references.

Follow the link below to determine Risk Group of host cell/organism and containment level to be followed. (http://www.dbtindia.nic.in/wp-content/uploads/Regulations-Guidelines-for-Reocminant-DNA-Research-and-Biocontainment-2017.pdf)

**Original Data (Tables, figures in colour wherever appropriate & graphs) with proper labelling and appropriate interpretationmust be submitted.Figures with overlay data should be submitted for to facilitate direct comparison.

***Out of a minimum of 6 months Real time stability data of DS and DP required, applicant is required to submit a minimum of 2 months data at the time of Form C3 submission and 3rd month data at the time of RCGM presentation. 6th month/complete stability data (as per Table 1, see below) should be submitted at the time of toxicity report (Form C5) submission. Comparative accelerated and stress stability data of Similar Biologic and RB drug product should be provided at the time of Form C3 submission.

Data: (i.e. batch size, date of initiation & completion of fermentation, purification, formulation and stability studies) and formulation details along with excipients.

SS **Gross pictures** should be taken with sufficient shadowless white light and printed on photo quality , glossy , color ink jet paper and there should be a dimension marker (scale) included in the picture below the organ.

^^**Histopathology pictures** shall be submitted as per specifications mentioned for gross pictures. In addition, the stain used and the magnification at which the picture was taken should also be given in the photographs.

Table 1. STORAGE CONDITIONS AND STABILITY DATA REQUIREMENTS for pilot batches of Similar biologics (as per ICH Q5C, 1995 and ICH Q1A(R2) 2003 and referred in Biosimilar guidelines 2016)

Temperature: Since most finished biotechnological/biological products need precisely defined storage temperatures, the storage conditions for the real-time/real-temperature stability studies may be confined to the proposed storage temperature.

Accelerated and Stress Conditions : *While the ICH tripartite guideline on stability describes the conditions of the accelerated and stress study of* ***pharmaceutical drugs****, the applicant should note that those conditions may not be appropriate for biotechnological/biological products.* ***Conditions should be carefully selected on a case-by-case basis for biotechnological/biological products.***

Drug type	Storage condition	Test period/Shelf life of DS/DP	Type of study	Minimum time period to be covered[¥](months)	Testing frequency/Remarks
Drug substance	5±3 °C	1 year or less	**Real time**: At actual storage temperature	12	To be performed at real time storage conditions for at least 3 months at a monthly interval (0, 1st, 2nd, 3rd month) and at 3 month intervals thereafter (6th, 9th, 12th month).
			Accelerated:30°C ± 2°C/70± 5% R.H[#]	3	30°C ± 2°C/70± 5% RH[#] for 3 months (0, 1, 3 months)
			Stress: 40/50/60°C ± 2°C/60± 5% R.H	4 weeks	40± 2°C/ 60 ± 5% RH for (0, 2, 4 weeks).
		Greater than 1 year	**Real time**: At actual storage temperature	12	To be performed at real time storage conditions at a 3 months interval for one year (0, 3rd , 6th, 9th, 12th month) and every 6 months over the second year, and annually thereafter through the proposed re-test period
			Accelerated:30°C ± 2°C/70 ± 5% R.H[#]	3	30±2 °C /70 ± 5% RH[#] for 3 months (0, 1, 3 months)
			Stress: 40/50/60°C ± 2°C, 60 ± 5% R.H	4 weeks	40°C ± 2°C/ 60 ± 5% RH for (0, 2 and 4 weeks).
	-20±5 °C	1 year or less	**Real time**: At actual storage temperature	12	To be performed at real time storage conditions for at least 3 months at a monthly interval (0, 1st, 2nd, 3rd month and at 3 month intervals thereafter (6th, 9th,

					12th month).
			Accelerated: 5±3°C	3	5±3 °C for 3 months (0, 1, 3 months)
			Stress: 30± 2°C, 70± 5% R.H.	4 weeks	30±2 °C, 70% ± 5% R.H# for 0, 2 and 4 weeks.
		Greater than 1 year	**Real time**: At actual storage temperature	12	To be performed at real time storage conditions at 3 months interval for one year (0, 3rd , 6th, 9th, 12th month) and every 6 months over the second year, and annually thereafter through the proposed re-test period
			Accelerated: 5±3°C	3	5°C±3 °C for 3 months (0, 1, 3 months)
			Stress: 30± 2°C, 70% ± 5% R.H.	4 weeks	30°C±2 °C, 70±5% R.H#, for 0, 2 and 4 weeks.
Drug product	5±3°C	1 year or less	**Real time**: At actual storage temperature	12	To be performed at real time storage conditions for at least 3 months at a monthly interval (0, 1st, 2nd, 3rd month and at 3 month intervals thereafter (6th, 9th, 12th month).
			Accelerated: 30 ± 2°C/70 ± 5% RH#	3	30±2 °C/70 ± 5% RH# for 3 months (0, 1, 3 months)
			Stress: 40/50/60°C ± 2°C/65 ± 5% RH	4 weeks	40±2 °C, 75±5% RH# for 0, 2, 4 weeks.
		Greater than 1 year	**Real time**: At actual storage temperature	12	To be performed at real time storage conditions for at least 3 months at a monthly interval (0, 1st, 2nd, 3rd month)and at 3 month intervals thereafter (6th, 9th, 12th month)and every 6 months over the second year, and annually thereafter through the proposed re-test period.
			Accelerated: 30 ± 2°C/70 ± 5% RH#	3	30±2°C for 3 months (0, 1, 3 months)
			Stress: 40/50/60°C ± 2°C/65 ± 5% RH	4 weeks	40±2°C, 75±5% R.H#, for 0, 2, 4 weeks.
	-20±5°C	1 year or less	**Real time**: At actual storage temperature	12	To be performed at real time storage conditions for at least 3 months at a monthly interval (0, 1st, 2nd, 3rd month)and at 3 month intervals thereafter (6th, 9th, 12th month).
			Accelerated: 5±3°C	3	5±3°C for 3 months (0, 1, 3 months)
			Stress 30 ± 2°C, 70 ± 5% R.H.	4 weeks	30±2 °C, 70±5 % R.H, for 0, 2 and 4 weeks.

		Greater than 1 year	**Real time**: At actual storage temperature	12	To be performed at real time storage conditions for at least 3 months at a monthly interval (0, 1st, 2nd, 3rd month)and at 3 month intervals thereafter (6th, 9th, 12th month)and every 6 months over the second year, and annually thereafter through the proposed re-test period.
			Accelerated: 5±3°C	3	5±3°C for 3 months (0, 1, 3 months)
			Stress: 30 ± 2°C, 70 ± 5% R.H.	4 weeks	30±2 °C, 70±5% R.H#, for 0, 2 and 4 weeks.

¥ Minimum or period till the significant change occurs as per ICH Q1A (R2) (Significant change for a drug substance is defined as failure to meet its specifications.

* Comparative data with innovator Reference Biologic is required

Recommended for countries that fall in the climate zone IVb and India fall in this zone.

For those DP whose stability data requirements fall outside the scope of storage conditions listed in the Table 1, applicant shall refer Indian Similar Biologics guidelines 2016 and ICH Q5C, 1995 and ICH Q1A(R2) 2003.