

PREPARATION OF NATIONAL REFERENCE STANDARD FOR INSULIN

Testing program on Collaborative study

NIB National Institute of Biologicals

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AMENDMENT SHEET

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References

- i. ISO guide G-34: 2000: General requirements for competence of Reference material producers.
- ii. ISO guide 17511: 2000: in vitro diagnostic medical devices –Measurement of quantities in biological samples –Metrological traceability of values assigned to calibrators and control materials.
- iii. NABL 162 issue No 4: 24.03.2008 : Guidelines for Proficiency testing program for testing and calibration laboratories (*available on site*)
- iv. NABL 164 issue No 01: 28.04 2005: Guidelines for inter laboratory comparison for Calibration laboratories where formal PT programmes are not available (*available on site*)
- v. NABL 163 issue No 05 28.03.2008: Policies & Procedures for interlab comparisons and / Or Proficiency testing . *available on site*
- vi. ILAC-12: 2000: Guidelines for the requirements for the competence of Reference material producers (*available on site*)
- vii. ISO guide 43: 1997: Proficiency testing by Interlab comparison part 1 development and operation of Proficiency testing schemes

1. Introduction

Preparation of insulin National Reference Standard involves a program for testing through Inter laboratory comparisons. The study assist in the evaluation of competence of laboratories to meet the Proficiency program complying with the NABL *162 Guideline Issue No: 04 2008*. The Purpose of the study is:

- 1.1 To determine the performance characteristics of a method, by identifying inter laboratory differences.
- 1.2 To assign property values to human insulin Active Pharmaceutical Ingredient, (API)and assess their suitability by measurement method based on Calibration hierarchy of the highest metrological order. **Fig 1**

2. Scope

The aim of the Collaborative study is to set out the criteria in which collaborators must meet to be recognized as competent to supply Reference standard of stated property for human Insulin. This applies to:

- 2.1 Manufacturer's, Regulatory bodies. The coded samples provided to each participant laboratory shall remain homogenous so that any results later identified as extreme should not be attributed to any significant sample variability.
- 2.2 The study possess comparison of test results obtained by 5 testing laboratories. NIB as one of the participating laboratories is a controlling and coordinating the functions of calibration performances and characterizations by means of interlaboratory comparison. It consists of two parts viz.

Part-I Calibration for Potency as per requirements in Indian Pharmacopoeia -2007:

- The test for Potency and Loss-on-Drying (LOD) will be performed on Insulin human –API. Minimum of six repeatability tests will be performed for Potency and LOD.
- Calibration for potency determination is done as per Assay by HPLC method. In Each performance the coded sample is used as Analyte and calibrated against the Certified Standard of human Insulin obtained from *EDQM-Ph Eur* and arranged by the collaborating laboratories at their end to determine the potency value. Property value in IU/mg is calculated on **Dried basis** to give an indication of the performance of the individual laboratories. The method for calculation is attached in the study Protocol – **NIB ILC 02, 2009**

Part-II Characterization of the human insulin- API as per requirements in Indian Pharmacopoeia -2007. The data will be generated by NIB for :

- Identification of proteins by Peptide mapping using HPLC
- Impurity for related proteins by HPLC assay
- Impurity for Higher molecular weight proteins by HPLC assay

3. Organisation and Design

FRAMEWORK

3.1 Name and address of the organisation conducting the Collaborative study

National Institute of Biologicals
Ministry of Health and Family Welfare
A-32 Sector 62, Institutional Area

Noida: 201307, E-mail: info@nib.gov.in , drrenujain@gmail.com Fax: 0120-2403014,
0120-2400074

Tel: 0120-2400022,
0120-2400072

3.2 Name and address of the coordinator and other personnel involved

- i. The Coordinator will be National institute of Biologicals with following allocation of responsibility:

Coordinator <i>NIB-Noida</i>	Administrative coordinator <ul style="list-style-type: none"> ▪ Deputy Director-Admn- 	Scientific coordinator <ol style="list-style-type: none"> 1. Deputy Director-Quality Control 2. Lab. Head-Recombinant Product 3. Quality Manager /Deputy Quality Manager
Planning and Operation, <i>NIB-Noida</i>	<ol style="list-style-type: none"> 1. Addl DGHS & I/c Director-NIB 2. Deputy Director-Quality Control 3. Lab. Head-Recombinant Product 	
Sampling and Sample Processing <i>NIB-Noida</i>	<ol style="list-style-type: none"> 1. Lab. Head-Recombinant Product 2. Analyst - Recombinant Product Laboratory 	
Sampling method <i>NIB-Noida</i>	<ul style="list-style-type: none"> ▪ Each Laboratory will be given 25 vials coded for one type of sample, along with MSDS ▪ Each vial will contain 100 mg of human Insulin-API bearing a material code and Lab code ▪ Total content of 2.5 g will be sufficient to carry out test for Potency determination against Certified Reference standard. And LOD determination ▪ Six determination will be performed for potency and LOD 	
Sample Processing method <i>NIB-Noida</i>	<ul style="list-style-type: none"> ▪ Each Participant laboratory , on receipt of coded samples will store the material at -20°C. ▪ The vial content will not be transferred into any smaller aliquots. ▪ The portions of weighing from each vial will be used to perform the test for Potency and characterization. 	

3.3 Nature and the purpose of the program

- i. Indian Pharmacopoeia-IP-2007 has a Monograph on human Insulin. However the National Reference Standard on insulin with any metrological trace ability is not available so far and need is felt.
- ii. The certificate of Analysis on Insulin working standard also varies from one Manufacturer to another as there have been no guidelines and Recommendations laid down for National Reference standard.
- iii. International biological standards are the "Primary standards" against which Secondary standards are calibrated. Which may be a) National standard b) Regional standard, c) Working Reference material .

3.4 Procedure for the manner in which the participants are selected

- i. Meeting was held in *March 20, 2009* to discuss the modalities for preparation of Insulin National reference standard. All the Manufacturer of insulin , indigenous and whose product is being imported in the country were invited to participate in the meeting . The meeting was attended by Dr. G.N. Singh, Secretary-cum-Scientific Director, Indian Pharmacopoeia Commission, Ghaziabad, and the representatives of Drug Controller General (India); M/s Wockhardt Ltd.; M/s Biocon Ltd.; M/s Novo Nordisk and concerned officers of NIB. The meeting notice had been issued to all the manufacturers and importers of insulin. The list of participants is annexed- Annexure 3.4 p17
- ii. The representatives of the manufacturing institutions agreed to participate in the study as availability of national reference standard was a felt need. Minutes of Meeting along with Power point presentation were communicated.
- iii. Manufacturers were sent letters to confirm the cost of 600g of insulin bulk and confirmation of their participation in the collaborative study. The responses were obtained from the Manufacturers confirming their participation and the cost of material. The approval details are Confidential.
- iv. It has been decided that initially 300g of API will be purchased and based on the demand and supply the final packing of Reference standard will be done in 1000 vials of 100 mg per vial.

3.5 Nature of the test item(s)

- i. The test item is a Active Pharmaceutical ingredient of human Insulin prepared by recombinant DNA technology. The requirements of tests for assay by HPLC are described under Insulin human in Pharmacopoeia- IP-2007, EP/BP and USP.
- ii. Calibration by potency test will be done as per Assay described in IP-2007. Test Protocol for Calibration along with the Data Recording Forms **Study Protocol NIB ILC02** , were prepared and communicated & discussed in the meeting held on Oct 27, 2009 at NIB. Annexure Minutes of Meeting

3.6 Description of the manner in which the test items are obtained, processed, checked and transported

- i. The material shall be obtained in dry ice by the Coordinator Lab at National institute of Biologicals –Noida and on receipt shall be transferred at temp -20°C in the walk in cold room. The Certificate of information to be obtained from the producer of human Insulin –API shall be as per the details given to them –Table 1, p13
- ii. Characterization data for NMR, amino acid analysis, Mass Spectrometry and Circular Dichroism will be obtained from the supplier of the human Insulin –API. The representative data of another batch, available with the Manufacturer will be kept as record with NIB.

3.7 Test Item Management : Procedures for sampling, randomising, transporting, sorting, and handling of test items

As per the directions given to the Manufacturer's, the sampling of material done by them shall be homogenous for each test parameter so that all laboratories will receive test samples that do not differ significantly. The procedure is described as under:

Procedure for Sampling / Randomizing

The Producer of human Insulin–API shall sent the material in packing of :

- i. 300 g of API packed into 11 containers and each container will have 25 g.
- ii. One container of 25 g will be aliquoted to contain 100 mg / vial which will be used for coding the material and sending to the participant labs.

Procedure for Handling of test items

- i. NIB will code the vials containing 100 mg / vial. These coded vials will be entered into the records and identity will not be disclosed to anybody. Each Participant lab will be recognized by a unique LAB code assigned to them. Material Safety data sheet (**MSDS**) will be communicated along with the coded samples.
- ii. NIB will ensure proper packaging of the samples to maintain cold chain in Dry ice and able to protect the stability and characteristics of the test items. There may be certain restrictions on transportation such as dangerous goods, regulations or customs requirement which will be duly taken care by reputed Couriers for transportation.
- iii. Appropriate customs declaration forms wherever applicable be completed by the coordinator to ensure those delays in custom clearance are minimized.
- iv. Test and data processing Equipments are adequate to conduct the tests. All Equipment like HPLC, temp controlled equipment are Calibrated to Traceability. The storage and security of data file is also ensured with the use of computer based system. Each of the Five participating laboratories are adequately equipped to carry out the study for Calibration and Characterization of human insulin API as they are the Manufacturer of human Insulin in the country of origin and are the Regulatory testing laboratory under the Drugs and Cosmetics Rule.

- v. Collaborators would utilize the HPLC system already being used in their laboratory. Labs shall use different HPLC systems and HPLC columns as per specifications from different manufacturer's to take into account all the measures of Robustness. Total six determination are performed including different HPLC system / columns
- vi. The estimated potency value (IU/mg) communicated to NIB will not be disclosed to the participants until after the results have been collated.

3.8 Expected initial and target dates or deadlines of the study including the date(s) for the testing to be carried out by the participants

- i. The time lines of the study will be discussed in the meeting schedule to be held in the month of October 2009 at NIB. The expected time frame of the study shall be **6 weeks** from the time of receipt of material by each participating laboratory.
- ii. The participants are responsible for ensuring that test results are submitted within due date prescribed.

4. Collaborators

4.1 The Participant laboratories involved in the program have adequate qualifications and experience as they are holding Senior Positions in the Manufacturing facility and in the National Control Lab.:

S. No	Name of the Participant lab	Participant Coordinator	Designation
1	M/s Biocon-Biopharmaceuticals Pvt. Ltd., Bangalore	Dr. Elango Minnoor	Senior Manager (QC),
		Ms. A.N. Suman	Deputy Manager (QC),
2	M/s M . J. Biopharm-Navi mumabi	Mr H. Pandit	Vice President –Technical services & Regulatory Affiars
3	M/s Novo Nordisk- Pvt. Ltd Bangalore Parent company -Sweden	Sh. Krishnananda A. Nayak	Manager, Regulatory Affairs & Clinical Research
4	M/s Wockhardt Ltd., - Aurangabad	Dr Anand Kumar	Associate Vice President-Quality
		Mr. Mithlesh Thakur	Manager (QC),
5	National Institute of Biologicals -Noida	Dr Renu Jain	Sc-Gr-II & Head-Recombinant Product Lab
		Ms Shalini Tewari	Scientist Gr-III / & Analyst Recombinant Product Lab
		Ms. Gurminder Bindra	
		Ms Richa Barnwal	Technical Assistant Recombinant Product Lab
		Mr Tara Chand	

4.2 There are advisory panel for various functions which includes experts from:

1. Indian Pharmacopoeia Commission
Dr G. N. Singh, Director Cum Secretary
Sector 23, Raj Nagar Ghaziabad
2. Dr Vishwanath Malkar
Chief of Operations , Reliance Life Sciences
Mumbai.
3. Drugs Controller General of India or his representative
FDA Bhawan, New Delhi
4. Dr. Satish K Gaiind
Professor Microbiology, NABL Assessor,
Techno Management Consultant
New Delhi

5. Statistical design ---

5.1 Careful consideration are given to the following matters and their interaction

- i. the precision and trueness of the test(s) involved;
- ii. the smallest differences to be detected between participating laboratories at a desired confidence level;
- iii. the number of participating laboratories;
- iv. the number of samples to be tested and the **number of repeat tests** or measurements to be carried out on each sample;
- v. the procedures to be used to estimate the assigned value. The **Outlier** – Member of a set of values which is inconsistent with the other and members of that set and the **Extreme results** – Outliers and other values which are grossly inconsistent with other members of the data set should be identified while calculating the data.

5.2 Evaluation of performance statistics

a) Calibration laboratories are generally working to different levels of Best Measurement Capability (BMC). Consequently, their performance is not judged by comparing their results with those of the other laboratories. Instead, their results are compared only to the Reference Laboratory's results. Their ability to achieve the BMC is evaluated by calculating the Error normalized (En) ratio (NABL 164 guidelines, & 162 guidelines)

$$En = \frac{\text{LAB-REF}}{\sqrt{U^2_{\text{LAB}} + U^2_{\text{REF}}}}$$

LAB – REF

$$En = \sqrt{U^2_{LAB} + U^2_{REF}}$$

where LAB & REF are equivalent to $\frac{x-X}{S}$ interpreted for calculation in NABL guidelines

LAB is the participating laboratory's result

REF is the Reference Laboratory's result (CRM)

ULAB is the participating laboratory's reported uncertainty

UREF is the Reference Laboratory's reported uncertainty –assigned value

|En| =1 indicates the satisfactory performance of the laboratory

|En| >1 indicates the unsatisfactory performance of the laboratory

b) Commonly used statistics for Quantitative results

i) the simple difference between the participant's result and the assigned value . This may be adequate to determine performance, and is most easily understood by participants.

The quantity $(x - X)$ is called the "estimate of laboratory bias"

where "x" is the participant's result and "X" is the assigned value.

ii) z scores, where $z = \frac{x - X}{S}$

s is an appropriate estimate/measure of variability which is selected to meet the requirements of the scheme. This model can be used both in the situation where **X and s** are derived from participants' results or when X and s are not derived from (all) the participant results.

z scores:

$|z| \leq 2$ = satisfactory , $2 < |z| < 3$ = questionable , $|z| \geq 3$ = unsatisfactory

6. Communication with Participants _____

6.1 . Feedback from laboratories are encouraged

- i. Participants are advised immediately of any changes in program design or operation
- ii. Participants will refer to the coordinator if they consider that assessment of their performance in a proficiency testing is in error.
- iii. Participating laboratories are advised to maintain their own records of performance including the results of investigation of any unsatisfactory results and subsequent corrective actions taken by them. If required NIB will depute experts to confirm that corrective actions are effective.
- iv. It should be ensured that the records of performance are maintained by the participating laboratories and are made available to the Assessment team whenever necessary.

7. Confidentiality/Ethical Considerations _____

7.1 Confidentiality

Normally, it is policy of most programs to maintain confidentiality of the identity of individual participants. The identity of participants should only be known to the minimum number of people involved in coordinating a program.

7.2 Collusion and Falsification of Results

There may be tendency among some participants to provide a false impression of their capabilities. NIB will ensure that collusion should not take place between laboratories to submit independent data.

7.3 Testing program has been designed to ensure that there is as little collusion and falsification as possible.

8. Program Reports _____

8.1 The content of program reports will include data on the results from laboratories of individual participants performance. The following information be included in reports:

- i. Name and address of organisation providing the program.
- ii. Names and affiliations of the persons involved in conduct of the program.
- iii. Date of issue of report, Report number and clear identification of study with following information :
 - a) Laboratory participation code and material Code
 - b) Statistical data.
 - c) Details of the traceability (CRM) and uncertainty of estimated value,
 - d) Final estimated value of potency in IU/mg obtained in the laboratory ,
 - e) The characterization results obtained for peptide mapping (along with chromatogram), related proteins and higher molecular weight proteins
- v. Test Format prepared by NIB will be communicated

9. Study conclusion _____

9.1 NIB with the assistance of the technical advisors wherever necessary may provide comments with respect to:

- i. Overall performance versus prior expectations (taking uncertainties into account)
- ii. Variation within and between laboratories
- iii. Variation between methods or procedures, if applicable
- iv. Possible sources of error and suggestions for improving performances
- v. Any other suggestions, recommendations or general comments
- vi. Conclusions on each Participant laboratory

9.2 Study will be concluded with Determination of the assigned value to the coded material for Insulin National Reference standard.

9.3 Declaring the property value to the insulin National Reference standard for Authorization by Indian Pharmacopoeia commission and its Incorporation in the IP human Insulin monograph " RS human Insulin "

FIG: 1
CALIBRATION HIERARCHY & METROLOGICAL TRACEABILITY

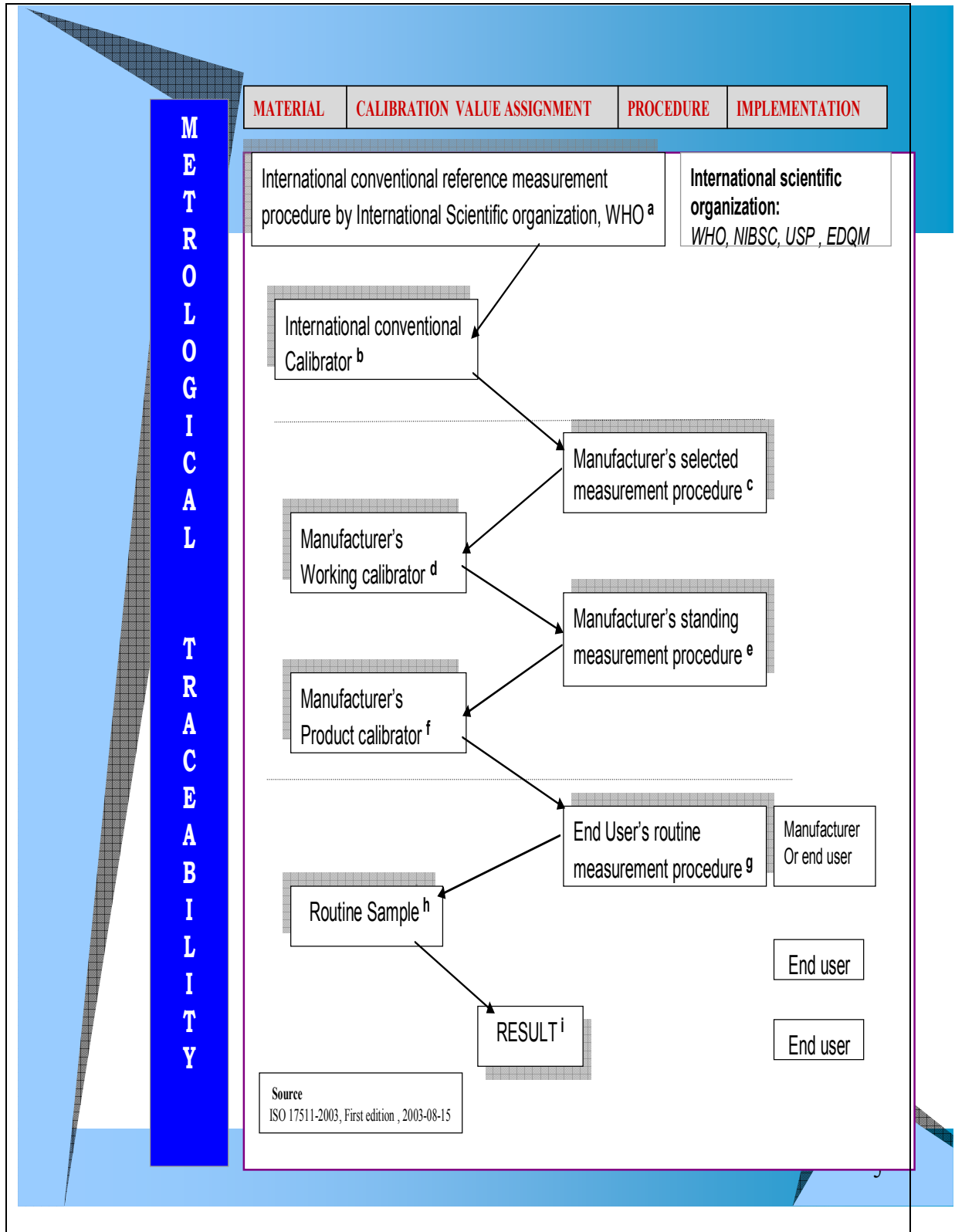


Table: 1

CERTIFICATE INFORMATION OBTAINED FROM
HUMAN INSULIN API- PRODUCERS

S. No.	Logical order for presentation	Information required	Information to be given by the producer	Information given by highest metrological trace ability
1.	The identity of the producer body and identification of the reference material	Name and address of certifying body		√
2.		Title of the document		√
3.		Name of the material		√
4.		Reference material code and batch number		√
5.	A description of the material and its intended use	Description of the material, origin and history		√
6.		Intended use		√
7.		Instructions on the correct use of the material		√
8.		Safety		√
9.		Level of homogeneity		√
10.	The certified values, their Traceability and the period of validity of the certificate	Certified values and their uncertainty intervals at a stated confidence level		√
11.		Traceability		√
12.		Values obtained by individual laboratories or methods		√
13.		Uncertified values		√
14.		Date of certification or assignment of property values		√
15.		Stability		√
16.	Other information	Further information		√
17.		Legal considerations		√
18.		Signatures and names of certifying officer(s) or officer(s) signing an analysis certificate		√ & Leaflet

**MINUTES OF THE MEETING ON TESTING OF INSULIN HELD AT NIB ON
20 MARCH 2009**

1. A meeting was held under the chairpersonship of Dr Jotna Sokhey, Additional Director General of Health Services and Director i/c, NIB on 20 March 2009 to finalize the production protocol in consultation with the manufacturers and regulatory authorities and to discuss issues related to the preparation of national reference standard for rec-insulin. The meeting was attended by Dr. G.N. Singh, Secretary-cum-Scientific Director, Indian Pharmacopoeia Commission, Ghaziabad, and the representatives of Drug Controller General (India); M/s Wockhardt Ltd.; M/s Biocon Ltd.; M/s Novo Nordisk and concerned officers of NIB. The meeting notice had been issued to all the manufacturers and importers of insulin. The list of participants is annexed.

2. The Addl DGHS & Director i/c, NIB welcomed the participants to the meeting. She said that NIB was ready to start the testing of recombinant insulin and the meeting had been convened to clarify matters related to the receipt of samples for testing, finalize production protocol which is to be submitted with each batch sent for testing and also to work out the modalities for the preparation of national reference standard for rec-insulin. She requested the manufacturers and regulatory authorities to send all the necessary documents along with the samples to enable NIB to test the products expeditiously and avoid delays in seeking clarifications etc. She assured the manufacturers that the Institute would monitor turnaround time and make all efforts to send the test reports within 45 working days or earlier. The turnaround time would start after all documents and required number of vials had been received and therefore it would be in the interest of the party sending the samples to see that the laid down criteria were met. She informed that the Institute had also taken steps to prepare national reference standards and thanked the manufacturers for sending bulk material. Since the preparation of the standard required collaborative studies and cooperation of regulatory authorities and the manufacturers, the modalities and timelines would be discussed during the course of the meeting.

3. Dr GN Singh appreciated the steps taken by NIB. He said that the national reference standard of insulin was a felt need. He assured the full cooperation of IPC in the efforts being made in this regard.

4. Dr AK Tahlan briefly reiterated the criteria for the acceptance of samples for testing at NIB. He said that the information in this regard had already been circulated by DCG(I) to all state drug controllers, regional drug control authorities and others. He said that care should be taken to lift samples randomly so that the entire batch was covered. He said that the batch should be interpreted as given in the Drugs and Cosmetics Act, 1940 and Rules there under (amended 2006). If the filling was done on the same day it was considered as one batch; however if filling was done on different days, the same should be reflected in the production protocol and sterility tests recorded. The criteria laid down at NIB for the acceptance of samples and communicated to all concerned were:

- Name and designation of the person sending the sample should be on a letterhead and clearly mentioned
- Box containing the samples should be sealed and intact
- Production protocol and other related documents such as valid manufacturing/ import license, insert/leaflet should accompany the samples
- Required number of vials/ampoules/pre-filled syringes
- At recommended storage and transportation temperatures
- Within 3 months of date of manufacture for indigenous products and imported products to have at least 60% shelf life

5. Dr. Tahlan mentioned that for imported products, import licence, CoA of the country of origin, name of the countries where this product was being used and production protocol with results of necessary tests conducted should be provided.

6. NIB had indicated that 14 vials of insulin in 10 ml vials and 28 prefilled syringes (3 ml) were needed for testing and an equal number was required to be kept as retained samples. The manufacturers, however, felt that the number may be increased to 18 vials if sterility test was also to be done according to their experience in the QC department.

7. Shri Kukraty said that the information provided in the production protocol was very important to assess the quality of the product. He suggested that information on flow process and critical steps should be included in the protocol. He mentioned that the detailed information should be provided in a dossier which should be submitted only once. If any changes were made, the production protocol should reflect these changes. Otherwise, the production protocol should include a statement that 'no deviations from standard/ established procedures were made during production or filling of the formulation'. He appreciated that the Institute was monitoring the turnaround time and agreed that delay could be minimized if the samples were sent as per requirement and that the production protocols, insert/leaflets, valid license etc were sent with the samples and unnecessary correspondence avoided. He said that necessary instructions had been issued to the regional offices and others concerned and that the CDSCO would also monitor and ensure that the guidelines were followed.

8. Dr Renu Jain, Scientist Grade II and Head of the Recombinant Laboratory requested the participants for feedback on the draft production protocol which had been circulated along with the notice of the meeting. She said that attempt had been made to keep the protocol simple and include only the critical information needed for assessing the production steps and results of various tests during production and in the finished product. The suggestions made during the meeting were minor and incorporated. She said that that the revised protocol would be circulated and posted on the website.

9. The criteria for acceptance for biphasic isophane insulin preparations ie. 30:70 and 50:50 as per IP 2007 and EP was raised. Ms Suman said that acceptance limits followed at Biocon are 5% \pm of 30% and 50%. Since the acceptance criteria had not been mentioned in IP 2007 this may be agreed upon in consultation with CDSCO and IPC.

10. Shri Mithlesh enquired about the need for Rabbit Bio-identity test for bulk as per USP. He was advised that the mandatory tests given in the concerned pharmacopoeia were required to be done. If there were other validated alternate tests, the matter for revision in the IP 2007 could be taken up with the IPC along with the relevant data.

11. Dr Jain thanked the manufacturers for sending bulk material which had been received from 3 manufacturers in powder form and from one in liquid form. She said that the tests performed for calibration using certified reference material showed that the results were in conformance to the COA provided by the manufacturers. She said that to produce national reference standard, it was necessary that the international guidelines were followed. She described these requirements in brief. Copy of the power point presentation is attached.

12. The modalities were discussed in detail. The representatives of the manufacturing institutions agreed to participate in the study as availability of national reference standard was a felt need. It was suggested that NIB may follow up with the other manufacturers also who could not be present in this meeting. It was agreed that 10 gm would be sufficient for calibration and characterization by five collaborating laboratories. The national reference standard would be

prepared from one lot. The total quantity would be 5000 vials x 50/100 mg. It was considered necessary that the bulk material required for preparing the national reference standard (500-600 gm) be obtained from each manufacturer participating in the collaborative study to ensure that adequate quantities of the bulk material was available after characterization and calibration had been completed as this may take a few months. NIB will code the material and send it to the collaborating centres along with the protocol.

13. Each laboratory will generate data on potency by calibration using certified reference material (CRM) of the highest metrological traceability. The time line for testing and generating data would be 6 weeks. The test method would be as per IP 2007. NIB will prepare the protocols and communicate to all the laboratories participating in the study. A meeting will be organized at NIB to discuss the results of the study and selection of the candidate reference material.

14. The representatives of the manufacturers said that they did not have the required infrastructure to freeze dry and final filling of small quantities of insulin to be used as national reference standard. They suggested that NIB may explore with institutions engaged for this specific purpose.

The meeting ended with a vote of thanks to the Chair.

LIST OF PARTICIPANTS

1. Dr. Jotna Sokhey Addl. DGHS & Director i/c, NIB Chairperson
2. Dr. G.N. Singh Secretary-cum-Scientific Director,
Indian Pharmacopoeia Commission,
Ghaziabad.
3. Sh. Arvind Kukraty ADC(I), O/o Drugs Controller General of India,
FDA Bhawan, New Delhi.
4. Sh. Somnath Basu Technical Officer, CDSCO HQ,
O/o Drugs Controller General of India,
FDA Bhawan, New Delhi.
5. Dr. Elango Minnoor Senior Manager (QC),
Biocon Biopharmaceuticals Pvt. Ltd., Bangalore.
6. Ms. A.N. Suman Deputy Manager (QC),
Biocon Biopharmaceuticals Pvt. Ltd., Bangalore.
7. Mr. Mithlesh Thakur Manager (QC),
Wockhardt Pvt. Ltd., Aurangabad.
8. Sh. Krishnananda
A. Nayak Manager, Regulatory Affairs & Clinical Research, Novo
Nordisk (I) Pvt. Ltd., Bangalore.
9. Dr. A.K. Tahlan Joint Director & Specialist Grade I,
Central Research Institute, Kasauli.
9. Dr. Renu Jain Scientist Grade-II & Head, Recombinant Product Lab.,
NIB, Noida.

MINUTES OF THE MEETING TO FINALISE THE STUDY PROTOCOL FOR PREPARATION OF INSULIN HUMAN NATIONAL REFERENCE STANDARD

1. A meeting on the Inter Laboratory Collaboration for preparation of Insulin Human National Reference Standard was held under the Chairpersonship of Dr. Jotna Sokhey, Addl. DGHS & Director, NIB on 27.10.2009 at 10.00 a.m. The representatives of leading manufacturers of Insulin, Indian Pharmacopoeia Commission, Directorate General of Health Services, NABL expert and concerned officers of NIB participated in the meeting. No representative from CDSCO attended. The list of participants is annexed.

2. Dr Sokhey welcomed the participants and said that the preparation of the reference standard for insulin was truly participatory as it involved all concerned in the manufacture, formulating guidelines by Indian Pharmacopoeia Commission and in quality assessment of Insulin. She said that care had been taken to follow accepted international guidelines and to ensure that the ISO standards were met. She said that the protocols of study design had been discussed earlier at the meeting held in March 2009. The purpose of this meeting was to finalize the study protocols, the material details of the coded Insulin Human-API and the time schedule for the collaborative study. The documentation to be maintained in the "Data Recording Forms" for study would also be discussed and finalized. The revised study protocol with a number assigned to it will be communicated to all the labs.

3. Dr Renu Jain, Scientist Grade II and Head of the Recombinant Laboratory gave a brief presentation of the salient points of the protocols and the time schedule of activities. She said that API had been procured by NIB and would be sent to the collaborating laboratories in the first or second week of November by courier in dry ice.

4. Four out of the five collaborating laboratories, which had agreed to collaborate in the study, attended the meeting. Consent forms were signed by the representatives of these laboratories. NIB would write to the fifth laboratory to seek their consent before coded material is shipped. The participating laboratories are :

- a. M/s Biocon- Biopharmaceuticals Pvt. Ltd., Bangalore
 - b. M/s Novo Nordisk- Pvt. Ltd Bangalore (Parent company –Sweden)
 - c. M/s Wockhardt Ltd., -Aurangabad
 - d. National Institute of Biologicals –Noida
 - e. M/s MJ Biopharm-Navi Mumbai *
- *did not attend the meeting

5. The document (ILC-01) for collaborative testing program prepared by NIB as per NABL guidelines and requirements of ISO standards was finalized. It was decided that for calculation of measurement of uncertainty ISO standard 98-3 Part 1, Part 3 and for inter laboratory comparisons NABL guideline 163 and ISO Guide 43 will be consulted and communicated to the collaborators. The revised document ILC-01 will be communicated to all the labs.

**MINUTES OF THE MEETING TO FINALISE THE STUDY PROTOCOL FOR
PREPARATION OF INSULIN HUMAN NATIONAL REFERENCE STANDARD
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6. After discussions, the following decisions were taken:
- i. Potency assay would be done as per IP 2007 given under the monograph of human insulin. Collaborators would utilize the HPLC system already being used in their laboratory. Labs shall use different HPLC systems and HPLC columns as per specifications from different manufacturer's to take into account all the measures of Robustness.
 - ii. Minimum of 6 repeatability tests will be performed for potency determination.
 - iii. The Certified Reference Standard of Insulin (Human) used for the Potency assay shall be from the traceable sources of EDQM which will be arranged by the Collaborating labs at their end. The value reported for human Insulin standard of highest metrological order is in %, the sample which is calibrated against it will be finally reported in IU/mg.
 - iv. The calculation of estimated Potency values will be on the dried basis for which the test for Loss- on-Drying (LOD) will be determined on the coded sample. Six repeatability performances will be done. Once the value of insulin human API is established the LOD need not be performed subsequently.
 - v. Characterization study on coded sample need not to be performed by all the collaborating labs. The data for characterization on human insulin –API will be obtained from the laboratory from where the API is sourced and also be generated by NIB and tests carried out shall be: a) protein identification by peptide mapping, b) purity for related proteins and c) purity for higher molecular weight proteins (HMWP). All the three tests will be performed by HPLC.
 - vi. Characterization data for NMR, amino acid analysis, Mass Spectrometry and Circular Dichroism will be obtained from the supplier of the human Insulin –API. The representative data of another batch, available with the Manufacturer will be kept as record with NIB.
7. NIB will aliquot the Insulin human-API into 1000 vials each vial containing 100 mg. Out of these 25 vials will be labeled for material code and lab code and sent to each participant collaborator along with the MSDS. Total quantity of 2.5 g will be sufficient to carry out the potency assay and test for loss on drying with repeatability of six performances.
8. NIB will arrange to send coded samples in dry ice to the laboratories by courier service.
9. Collaborative laboratories will send data to NIB on a pre set format of Certificate of analysis. The format of Report will be prepared by NIB and communicated.

The meeting ended with a vote of thanks to the Chair.

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LIST OF PARTICIPANTS

Directorate General of Health Services		
1	Dr. Jotna Sokhey	Addl. DGHS & Director, NIB, Chairperson
2	Dr. D. C. Jain	Deputy Director General (NCD), DGHS, Nirman Bhawan, New Delhi
Participating Laboratories		
3	Dr. Elango Minnoor	Senior Manager (QC), Biocon Ltd., Bangalore
4	Mr. R. Ranjith	Senior Executive- QC Biocon Ltd., Bangalore.
5	Sh. Krishnananda A. Nayak	Manager, Regulatory Affairs & Clinical Research, Novo Nordisk (I) Pvt. Ltd., Bangalore
6	Dr. Raman Shetty	Medical Director Novo Nordisk (I) Pvt. Ltd., Bangalore
7	Dr Anand Kumar	Associate Vice-President-Quality, Wockhardt Pvt. Ltd., Aurangabad
NABL Expert		
8	Dr. S. K Gaind	Professor Microbiology, NABL Assessor, Techno Management Consultant New Delhi
Indian Pharmacopoeia Commission		
9	Dr. Jai Prakash	Principal Scientific Officer, Indian Pharmacopoeia Commission, Sector 23, Raj Nagar Ghaziabad
National Institute of Biologicals-Noida		
10	Dr. R. N. Prasad	Deputy Director Quality Control
11	Dr. Renu Jain	Scientist Grade-II & Lab Head, Recombinant Product lab
12	Dr. J. P. Prasad	Scientist Grade-II & Lab Head, Enzyme & Hormone lab and Blood Product lab
13	Sh. A. K. Sahu	Scientist Grade-III, In-charge Sterility Test Lab,
14	Sh Ashis Barua	In-charge Sample Receipt & Report dispatch
15	Ms. Shalini Tewari	Scientist Grade-III, Recombinant Product lab
16	Ms Gurminder Bindra	Scientist Grade-III, Recombinant Product lab
17	Ms Richa Barnwal	Scientist Grade-III, Recombinant Product lab
18	Sh Tara Chand	Technical Assistant, Recombinant Product lab
19	Ms E. Madhu	Deputy Quality Manager
20	Ms Sudha V	Deputy Technical Manager