Research Article

An Inter Laboratory Comparison Study on Biological Products for Bacterial Endotoxin Test

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ABSTRACT

In a regulatory set up for biological products the symbiotic relationship for assuring the quality of test results is required to be addressed for a step wise laboratory improvement towards accreditation or inspection of facility compliance with current Good Manufacturing Practices. Pharmaceutical laboratories play a pivotal role in clinical or public health domain by providing accurate and reliable results for the quality attributes of the drug under consideration. Various strategies are adopted by testing laboratories to assure that the test results are accurate, precise, and reproducible. National Institute of Biologicals (NIB) functions as Central Drug Laboratory in India and responsibly assures and reviews the quality of number of biological products available through domestic manufacturers and imports. In this report we present the results of an inter-laboratory comparison (ILC) study coordinated by the Quality Management Unit of NIB during January-September 2014 for assuring the quality of results of bacterial endotoxin test (BET) by gel clot method. Eight laboratories participated in the ILC study and six laboratories provided satisfactory results while results from two laboratories were unsatisfactory in terms of errors in calculation of maximum valid dilution and expression of final results indicating the need for improvement in the technical competence of testing laboratories for assuring the quality of safety test parameter by BET.

Keywords: Assuring quality, bacterial endotoxin test, inter-laboratory comparison, quality control

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INTRODUCTION

Pharmaceutical laboratories play an important role in drug regulation by providing test results of investigation on active pharmaceutical ingredients, pharmaceutical products and excipients. Therefore, it is important that the results generated by these laboratories are accurate, precise, and reproducible. Many approaches are in use for assuring the quality of test results produced by testing laboratories [2]. These include but not limited to: use of certified reference material and quality control samples during testing, participation in inter-laboratory comparison (ILC) and proficiency testing, repeat testing and retesting of samples, and use of quality control charts. ILC studies are conducted to assess the quality of test results produced by laboratories [1]. Participation in ILC provides laboratories with an objective of assessing and demonstrating the reliability of the results they produce. ILC participation also provides independent verification of the competence of a laboratory and shows commitment to the maintenance and improvement of performance [2]. ILC covers the entire process in a laboratory including receipt and storage of test samples, the experimental procedures carried out in the laboratory, interpretation and transcription

of the data, production of reports and the conclusions drawn from the reports.

In a regulatory environment the quality of medicines is assured in accordance with available guidelines from regulatory authorities and state legislations. National Institute of Biologicals (NIB), an autonomous Institute under the Ministry of Health and Family Welfare (Government of India), is a premier scientific organization to ensure quality of biologicals and vaccines in India [3]. The Institute responsibly assures and reviews the quality of biological products available through domestic manufacturers and imports. As a quality control laboratory, NIB has the mandate to develop linkages with other institutions and keep abreast of world-wide scientific research and technological developments in quality control of biologicals with a view to advice on the suitability of their adoption [3]. Quality control testing of biologicals includes a series of tests for identification, potency, purity and safety [4]. The bacterial endotoxin test (BET) is an important safety test that is adopted in the pharmacopoeia to detect or quantitate endotoxins of Gram-negative bacterial origin. BET may be conducted by the gel-clot or photometric (turbidimetric and colorimetric) techniques and is a highly sensitive method for the determination of endotoxins in parenteral drugs *in lieu* of the *in vivo* pyrogen test using rabbits [5]. With this focus, Quality Management Unit (QMU) of NIB undertook an initiative for an ILC study on qualitative parameter of BET by gel clot method to assess the performance of participants and to analyze the deficiencies, if any, observed in test results.

MATERIALS AND METHODS

Study Design

The proposal of the ILC study on BET and the consent form for participation was circulated by email in January 2014 to sixteen laboratories in India who have been the potential stakeholders of the biotherapeutic manufacturers, pharmacopeia laboratories, control laboratories and academia laboratories. Study was planned in 3 phases and process flow steps of the ILC study are shown in (**Fig. 1**). Phase 1 of the study included confirmation by participating laboratories for the ILC. In phase 2, study samples were dispatched by NIB and participating laboratories reported their results after performing the BET. During phase 3 results were compiled and report was prepared and communicated to all the participants.

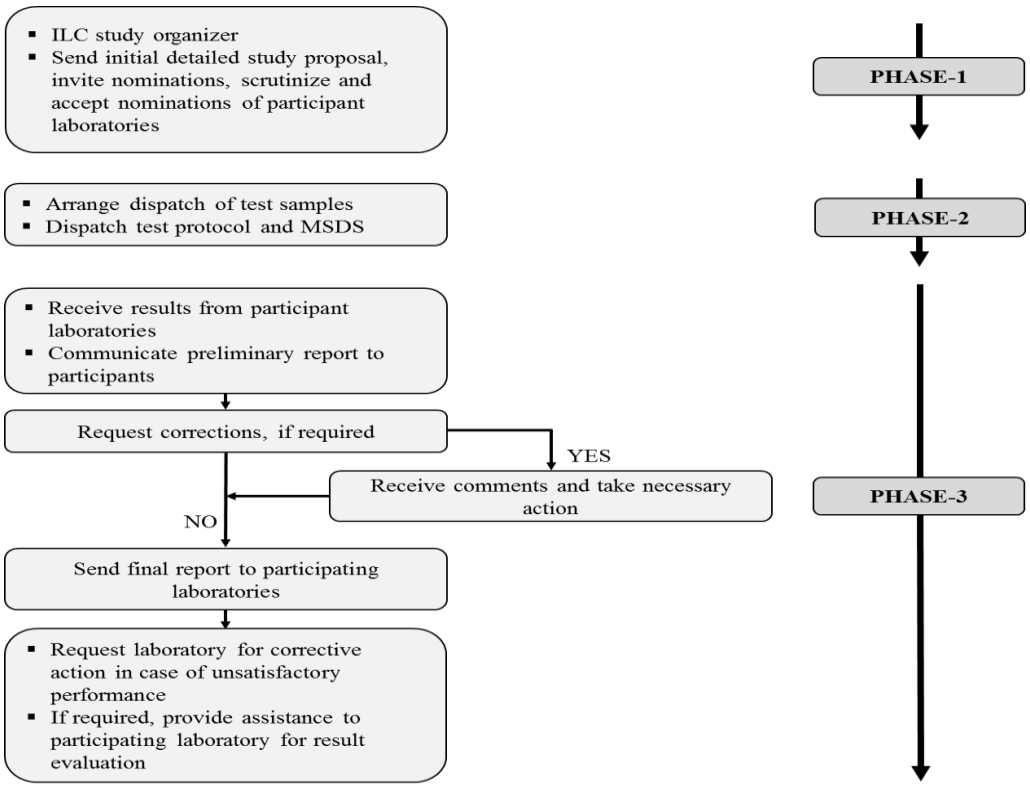

Figure 1: Process flow showing stages for inter-laboratory collaborative study

Participants

Eight laboratories agreed to participate in the collaborative study for ILC. Participants are listed in (**Table 1**) and in this article participants are referred to by a code number which is unrelated to their order of listing in the table. The participants included two pharmacopoeia laboratories and six laboratories of biotech manufacturers in India. QMU of NIB served as the coordinating laboratory for this collaborative study.

Table 1: Participants of the ILC study from India#

S. No.	Participant Name and Organization
1.	Sriram Akundi and Harshad Joshi, Biocon Limited, Bangalore
2.	Raman Mohan Singh, Indian Pharmacopoeia Commission, Ghaziabad
3.	Krithika Balasubramanian and Sridevi Khambhampati, Dr. Reddy's Laboratories, RR District, Hyderabad
4.	Susobhan Das and Kinnary Vyas, Intas Pharmaceuticals Limited, Ahmedabad
5.	Sunil Gairola, Serum Institute of India Limited, Pune
6.	Dhaivat Desai and Nilesh Trivedi, Torrent Pharmaceuticals Limited, Mehsana
7.	Ranjan Chakrabarti and G. Pradeep, USP India Private Limited, RR District, Hyderabad
8.	P. S. Maruthi Sai and Nimesh Thaker, Zydus Biologics, Ahmedabad

Test Samples

Four preparations of test samples were provided to the participants with details given in (**Table 2**). Test samples selected for the study were finished recombinant biological products received for quality control testing at NIB and identity of these samples is not disclosed in this report. Test samples were coded and dispatched under cold chain conditions in March 2014. The consignment was accompanied with test protocol and material safety data sheet.

Table 2: Details of study samples along with their endotoxin limits

Sample No.	Sample Code	Potency	Endotoxin Limit
Sample-1	NIB/ILC/BET-01	100 U/ml	Less than 80 EU per 100 U
Sample-2	NIB/ILC/BET-02	100 U/ml	Less than 80 EU per 100 U
Sample-3	NIB/ILC/BET-03	100 U/ml	Less than 80 EU per 100 U
Sample-4	NIB/ILC/BET-04	5000 IU/Vial	Not more than 15 EU/500 IU

BET Testing

Test protocol provided to participants for BET by gel clot method was based on the pharmacopoeia monograph published in Indian pharmacopoeia-2014 [5,6]. Participants were requested to follow the method detailed in the protocol and carry out required testing and to submit the results on prescribed data recording form. Instructions were given on critical requirements on sample receipt, storage and handling, depyrogenation of glass tubes, essential equipment and calculation of maximum valid dilution (MVD).

Data Analysis and Preparation of Report

All the eight participant laboratories submitted their results in May 2014 on prescribed data recording forms. Information on material usage for Control Standard Endotoxin (CSE) and Limulus Amebocyte Lysate (LAL) reagent was compiled and results were analyzed. A preliminary report prepared along with the deficiencies observed and communicated to the participating laboratories with a request to comment upon. Clarifications received from the participants were considered to resolve the deficiencies and final report was prepared and communicated to participants in September 2014.

RESULTS AND DISCUSSION

In the preliminary report, results of four laboratories were found to be satisfactory while results for remaining four laboratories shown deviations with deficiencies observed in terms of calculation of MVD and expression of final results. After resolution of deficiencies, six laboratories qualified for ILC study with satisfactory results and two laboratories still reported unsatisfactory results. Final results of the ILC study along with the details of reagent used are summarized in (**Table 3**).

Table 3: Summary of the results of the ILC study after resolution of deficiencies along with details of reagents used

Laboratory Code	Reagent Source	CSE	LAL Reagent Sensitivity (EU/ml)	Sample Nos.	Calculated MVD	Test performed at	Reported Result	Deficiency Observed, if any
NIB/ILC-Lab-02	Endosafe Charles River	100 EU/vial	0.03	Sample-1, 2, 3	2560	MVD/4	<20 EU/ 100 IU	Error in expression of results. At MVD/4 dilution the results will be <40 EU/100 IU and <7.5 EU/500 IU respectively.
				Sample-4	480		<3.75 EU/500 IU	
NIB/ILC-Lab-03	Endosafe Charles River	40 EU/ml	0.125	Sample-1, 2, 3	640	MVD/2	<80 EU/ 100 IU	None observed
				Sample-4	120		<15 EU/ 500 IU	
NIB/ILC-Lab-04	Associates of Cape Cod	1000 EU/vial	0.06	Sample-1, 2, 3	1333.33	MVD/4	<40 IU/ 100 U	None observed
				Sample-4	2500		<75 EU/ 5000 IU	
NIB/ILC-Lab-05	Associates of Cape Cod	1000 EU/ml	0.06	Sample-1, 2, 3	1333	MVD/4	<40 EU/ 100 IU	None observed
				Sample-4	250		<7.5 EU/ 500 IU	
NIB/ILC-Lab-06	Endosafe Charles River	40 EU/ml	0.125	Sample-1, 2, 3	64000	MVD	<0.06 EU/ 100 IU	Error in MVD calculation. Error in expression of results due to wrong MVD calculation.
				Sample-4	60000		<0.06 EU/ 500 IU	
NIB/ILC-Lab-07	Associates of Cape Cod	1000 EU/ml	0.125	Sample-1, 2, 3	640	MVD/2	<80 EU/ 100 IU	None observed
				Sample-4	120		<15 EU/ 500 IU	
NIB/ILC-Lab-08	Endosafe Charles River	20 EU/ml	0.06	Sample-1, 2, 3	1333.33	1:250 dilution	<15 EU/ 100 IU	None observed
				Sample-4	250	1:50 dilution	<3 EU/ 500 IU	
NIB/ILC-Lab-09	Endosafe Charles River	20 EU/ml	0.03	Sample-1, 2, 3	2666.66	MVD/2	<80 EU/ 100 IU	None observed
				Sample-4	500		<15 EU/500 IU	

As per the protocol communicated to the participants, the expected results at MVD/2 dilution were <80 EU/100 IU for sample-1, 2, 3 and <15 EU/500 IU for sample-4 (**Table 2**). Laboratories with code-03, 04, 05, 07, 08 and 09 produced valid test results as the positive and negative controls met the acceptance criteria and final results were reported as expected. Laboratories with code-03, 07 and 09 performed the test at MVD/2 dilution and reported results for test samples were as expected above. Laboratory with code-04 performed the test at MVD/4 dilution and reported result for sample-1, 2, 3 as <40 IU/100 U and for sample-4 as <75 EU/5000 IU. Laboratory with code-05 performed the test at MVD/4 dilution and reported results for sample-1, 2, 3 as <40 EU/100 IU and for sample-4 as <7.5 EU/500 IU. Laboratory with code-08 performed the test at 1:250, 1:500 and 1:1000 dilutions for sample-1, 2, 3 and reported result at 1:250

dilution as <15 EU/100 IU. Similarly, the test for sample-4 was performed at 1:50, 1:100 and 1: 200 dilutions and reported results at 1:50 dilution were <3 EU/500 IU.

Laboratories with code-02 and 06 produced errors in expression of their results due to errors made in MVD calculations. Therefore, overall performance of these laboratories was considered as unsatisfactory and these laboratories also agreed with this conclusion. Laboratory with code-02 reported results at MVD/4 dilution as <20 EU/100 IU for sample-1, 2, 3 and <3.75 EU/500 IU for sample-4. However, the expected results at the given dilution would be <40 EU/100 IU for sample-1, 2, 3 and <7.5 EU/500 IU for sample-4. Laboratory with code-06 made errors in MVD calculation and test samples were diluted beyond the MVD which would be considered as major noncompliance.

BET is a complex qualitative test procedure and numerous sources of errors are possible. These sources include but not limited to errors in handling of samples and solutions, dilution schemes, pipetting, incubation of reaction tubes, calibrations of temperature controlled equipment, MVD calculation, reporting of results and misinterpretation of results by coordinating laboratory. It is important to note that qualitative tests offer challenges in drawing conclusions based on the results obtained in ILC studies and comparison of results reported by different laboratories become difficult. In case of BET it is observed that though the test is qualitative, the calculations done during dilution of the test samples and reagents play an important role for assuring the quality of end result. Importantly, the errors made in the calculation may go unnoticed during internal audits and external assessments leading towards accreditation of a laboratory with incompetent personnel. Therefore, participation in an ILC provides a valuable opportunity to the testing laboratories to evaluate the competence of staff and overall performance of the laboratory.

An ILC is an external way of assuring the quality of test results reported by the participating laboratories [1]. It also allows the participants to detect unsuspected errors and deficiencies in their methodology. Results of the present ILC program provided valuable information allowing comparison of performance and in-consistency of test results among participant laboratories. It also provided early warning for systematic problems observed during technical operations, objective evidence of testing quality and indicated the areas that need improvement and identified training needs for personnel. Individual laboratories can use results of the ILC study to identify problems in their laboratory practices allowing for appropriate corrective action [2]. Whenever the laboratories find their results differ from the expected results as obtained by other participating laboratories then an investigation should be carried out to establish the reason for the problem. Appropriate corrective action shall be initiated together with measures to check that the corrective action was effective. Even if the performance of a laboratory is found satisfactory, a record must be made for audit purposes [2]. Successful participation in national and international ILC programs enhances the confidence in the competence of their analyst and laboratories.

CONCLUSION

To conclude, results of this study indicate that there is a scope for improvement in technical competence of the laboratory personnel. This would present opportunities to the participants to determine their necessities in terms of staff trainings and improving the quality of their test results. We expect that participation in such ILC programmes by more pharmaceutical testing laboratories would lead us towards assuring the quality of test results produced in pharmacopoeia laboratories, control laboratories and manufacturing facilities. This ILC study also provides insight for the risk based approach initiative as every product and every process has an associated risk and testing laboratories should have methodology for evaluating the risk and generating intervention for Risk Management Plan.

NIB would like to continue ILC studies on BET and other test parameters and expect collaborations from more participant laboratories in India and other neighbouring countries to strengthen the laboratory proficiency for assuring the

quality of their test results and clinical or public health scenario.

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Conflict of Interest

None to declare

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