

NIB/BVL/BCG/01

# GUIDANCE DOCUMENT

ON

QUALITY CONTROL

OF

**Bacillus Calmette-Guerin (BCG)  
Vaccine**



**National Institute of Biologicals,**  
(Ministry of Health & Family Welfare, Government of India)  
A-32, Sector 62, NOIDA  
U.P. 201309  
**Telephone:** +91-120-2400022, 2400072  
**Fax:** +91-120-2403014, 2400074  
**E- mail:** [info@nib.gov.in](mailto:info@nib.gov.in)  
**Website:** [www.nib.gov.in](http://www.nib.gov.in)

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine

**Document ID No. :** NIB/BVL/BCG/GD/01

**Effective Date:** 01.12.2014

**Validity:** till further revision

**Page** 1 of 18

### Amendment sheet

S. No.	Date	Page No.	Revision No.	Nature of Amendment Section/details	Authorization

## ACKNOWLEDGEMENT

I would take this opportunity to express my gratitude to all the staff members of bacterial vaccine laboratory Mr. Neeraj Malik, Mr. Harit Kasana, Ms Suchitra,

Ms. Gunjan Sikarwar for providing the valuable inputs in the preparation of the Guidance Manual on Quality Control of BCG Vaccine without which the endeavor would not have been possible. I also wish them good luck in their future scientific work.

The encouragement provided by Dr. Surinder Singh Director (i/c) to prepare this document is deeply acknowledged.

I hope the Guidance Manual on Quality Control of BCG vaccine will go a long way in Quality Control of BCG vaccine

Dr. G.R Soni  
Scientist-I & Head  
Vaccine Division

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine			
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 3 of 18

## FOREWORD

This document has been prepared for the purpose of inviting comments and suggestions from stakeholders of Bacillus Calmette-Guerin (BCG) Vaccine , by the Central Drugs Testing Laboratories (CDTL), Indian Pharmacopoeia Commission (IPC), Central Drugs Standard Control Organization (CDSCO), State Drugs Controller, and Academic Institutions which are involved in activities relating to testing of Bacillus Calmette-Guerin (BCG) Vaccine

This draft has been prepared in consultation with different pharmacopoeias, WHO TRS and experience gained by the team during testing of the vaccine. The comments will be considered by the team and will be incorporated accordingly.

This document will be useful to manufacturers, suppliers, NRA and testing laboratories which are involved in assurance of the Quality, Safety and Efficacy of freeze dried BCG vaccine.

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine			
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 4 of 18

## **ABBREVIATIONS:**

BP	British Pharmacopoeia
EP	European Pharmacopoeia
CDL	Central Drugs Laboratory
CDSCO	Central Drugs Standard Control Organization
CDTL	Central Drugs Testing Laboratory
CMC	Chemistry Manufacturing and Control
DCG (I)	Drugs Controller General of India
IP	Indian Pharmacopoeia
IPC	Indian Pharmacopoeia Commission
IPRS	Indian Pharmacopoeia Reference Standard
NCA	National Control Authority
NCL	National Control Laboratory
NIBSC	National Institute of Biological Standards & Control, U.K
SOP	Standard Operating Procedures
USP	United States Pharmacopoeia

## **CONTRIBUTORS:**

### **Bacterial Vaccine Laboratory -NIB**

1. Dr. G. R. Soni  
Scientist oI & Head  
sonibpl@msn.com
2. Late Sh. A.K. Sahu  
Scientist Gr-II
3. Mr. Neeraj Malik  
Scientist Gr-III  
neerajmalik13@rediffmail.com
4. Mr. Harit Kasana  
Jr. Scientist  
haritkasana.bt@gmail.com

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine			
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 6 of 18

**CONTENTS:**

<b>Content</b>	<b>Page No.</b>
Introduction	08
Objective and Background	09
Assuring Quality of Test Results	10
Definitions	11
Test Method and Test Validation	12
Reporting the Results	14
References	16
Annexure A	17

## 1. INTRODUCTION

WHO and various Pharmacopoeias regularly review and publish the amendment and address the issues relating to improvement in Quality Control test parameters of BCG vaccine. This document will help the stakeholders involved in the quality assurance of BCG vaccine.

Tuberculosis (TB) was declared a global emergency by WHO in 1993, and *Mycobacterium tuberculosis* is now considered to be responsible for more adult deaths than any other pathogens. Rates of infection and disease are highest throughout Asian and African region. A third of world's population is infected with tubercle bacilli. Every year between 8-9 million new cases appear & 3 million persons die from the disease. India is one of the worst affected countries. More than 40% of the population is infected & about 15 million suffer with the disease .Half a million die from the disease every year. India is the highest TB burden country with World Health Organisation (WHO) statistics for 2011 giving an estimated incidence figure of 2.2 million cases of TB for India out of a global incidence of 8.7 million cases. The estimated TB prevalence figure for 2011 is given as 3.1 million.

It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB.

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine			
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 8 of 18



## 2. OBJECTIVE & BACKGROUND

The Objective of this document is to provide guidelines to manufactures to enable them to assure quality of the BCG vaccine for batch release.

Background: BCG vaccine is a live attenuated vaccine originated from culturing *Mycobacterium bovis* isolated from cattle and cultured for a period of 13 years and with a total number of 231 passages. It was first used to immunize humans in 1921. The vaccine was introduced in WHO expanded programme of immunization (EPI) in 1974. The vaccine is globally used and coverage rate is exceeding 80% in countries endemic for TB.

Development: The original strain of M.bovis used to make BCG was maintained by the serial passage at the Pasteur Institute. It was distributed to dozen of laboratories in many countries. Each laboratory produced its own BCG & maintained it by serial passage which resulted in the production of many daughter BCG strains that differ in terms of their genetic and phenotypic properties.

BCG vaccine strains:

Over the years, more than 14 substrains of BCG have evolved and have been used as BCG vaccine strains in different parts of the world. (Reference 10.7, 10.8) Recently, the various substrains have been studied by comparative genomics. BCG vaccine strains were thus divided into the 'early' strains, in which the original characteristics of 'authentic Pasteur' were conserved with fewer deletions, insertions and mutation in the genome of the bacilli than the 'late' strains. 'Early' strains are represented by BCGs Russia BCG-I, Moreau-RJ, Tokyo 172-1, Sweden, and Birkhaug; and the 'late' strains include BCGs Pasteur 1173P2, Danish 1331, Glaxo (Copenhagen 1077) and Prague.

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine			
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 9 of 18

Worldwide, the most commonly used vaccine strains are currently Danish 1331, Tokyo 172-1 & Russian BCG-1 (Reference 12.9)

## **2.1 Selection of methods**

The laboratory uses test methods, which are appropriate for the tests it undertakes and meet the needs of the manufacturer and NRA. Specifications of the test methods are in accordance of Indian pharmacopoeia 2014 and WHO TRS 979, 2013. The laboratory shall ensure that it uses the latest valid edition of a standard unless it is not appropriate or possible to do so. Laboratory has a well defined test plan with assignment of each test to be conducted by the Analysts.

## **2.2 Assuring the Quality of test methods**

The laboratory has quality control procedures for monitoring the validity of tests. The resulting data is recorded in a way that the trends are detectable and where practicable, statistical techniques are applied in reviewing of results. This monitoring is planned and reviewed which includes regular use of working reference standards provided by the manufacturer, retesting of test items. Equipment are validated/calibrated to ensure its proper functioning and to maintain the integrity of test data.

# **3. ASSURING THE QUALITY OF TEST RESULTS:**

## **3.1 POLICY ON HANDLING OF TEST SAMPLES**

3.1.1 The test plan is prepared and approved which describes the sequence of test from the receipt of samples to release of report by Lab Head for each batch of vaccine sample forwarded for purpose of test and analysis in the laboratory by SRRDU

3.1.2 The vaccine sample on receipt in the laboratory will be assigned by the Laboratory Head/In-charge to the concerned Analysts. The work assignment for functions and responsibilities are

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine			
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 10 of 18

issued to an individual scientific and technical staff for carrying out quality control test and other related laboratory activities.

3.1.3 Testing of samples to confirm compliance with the requirements and agreed specifications. The partial testing applies to Final Lots which have complied consistency under GMP conditions. It is assured that the minimum tests done are for Identification/ Counts of viable units and safety.

3.1.4 Protocols of production, summary protocol and Certificate of analysis of the concerned batch are provided.

3.1.5 There are instances that new products are received for the first time and require to meet the demand for critical care patients, demand of short supply in the market and supplying through tender process to government hospitals. Such cases are dealt on a case-by-case basis in an impartial manner to establish the quality. The samples are either released on the basis of minimal tests carried out for Identification, Potency, Safety, other possible tests for which the laboratory has the capability to assure the quality or completely on the basis of manufacturer's Protocol review. This shall apply to all reports released by NIB. The report will indicate that the product tested is of expected standard quality and the consistency of the product of the manufacturer is established. The check list prepared for the purpose to review manufacturer's documents/protocols is given in Annexure A

4. The definitions given below apply to the terms as used in these recommendations:

**4.1 Final bulk:** The homogeneous finished liquid vaccine present in a single container from which the final containers are filled, either directly or through one or more intermediate containers derived from the initial single container.

**4.2 Final lot:** A number of sealed, final containers that are equivalent with respect to the risk of contamination during filling and, when it is performed, freeze-drying. A final lot should therefore have been filled from a single container and freeze-dried in one continuous working session.

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine			
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 11 of 18

**4.3 Working Reference Standard:** Manufacturer is to provide working reference standard of vaccine prepared from the same BCG strain as the tested vaccine and used in parallel to the vaccine tested in:

- i. Quantitative assays such as viability estimates (such as culturable particle count); and
- ii. Excessive dermal reactivity test.

#### **4.4 Control test on final lot**

As given in Table No.1 manufacturer will provide total quantity of sample to be forwarded to NIB. Samples are properly labeled and retained quantities are kept for future reference. As per the test parameter indicated in Table 1, each vial is allocated identification by giving T1-T57 before the test is initiated.

Tests are performed after reconstitution, except for appearance (before reconstitution) & residual moisture tests. The diluent supplied or recommended for reconstitution is used.

If the diluent would interfere with any of the tests in which case some other diluent is used. The vaccine is reconstituted to the concentrations at which it is to be used for injection into humans, (except in case of the test for absence of virulent mycobacteria) when a higher concentration of reconstituted vaccine may be necessary.

## **5. TEST METHOD & TEST VALIDATION:**

### **5.1 QUALITY EVALUATION**

The test methods given in concerned monographs are verified in the laboratory for its repeatability & reproducibility in quantitative test.

**5.1.1 Labelling:** The product container bears the number of colony forming units (C.F.U) as  $1 \times 10^6$  to  $33 \times 10^6$  CFU/ml. On the basis of formulations existing in Indian pharmacopoeia 2014 the

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine			
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 12 of 18

tests for Quality are carried out, calculations are performed and samples are reported to be of Standard Quality / Not of Standard Quality.

**5.1.2 Testing of BCG vaccine samples:** The product container bears claim of Pharmacopoeia mentioned, on the basis of which the sample is evaluated as per the procedure developed in NIB and in accordance with the specifications and method used by manufacturer. The standard operating procedures used in the laboratory for all QC tests are in accordance with the IP/EP and or WHO TRS.

**Table 1: Test parameters & Distribution of Quantity of Bacillus Calmette-Guerin (BCG) Vaccine required (Both 10 & 20 dose vials)**

S.No.	QC test	No. of vials required		
		Test	Retained	Reference
1.	Test for Identity	T1	RN1	REF1
2.	Test for Counts of viable units	T2 ó T6	RN2 ó RN6	REF2-REF6
3.	Temperature stability	T7 ó T11	RN7 ó RN11	
4.	Test for Sterility	T12 -T15	RN12 ó RN15	
5.	Residual moisture content	T16 ó T20	RN16 ó RN20	
6.	Excessive dermal reactivity	T 21	RN21	REF7
7.	* Test for Virulence/Safety	T 22-T57	RN22 ó RN57	
<b>Total vials required</b>		<b>: 57 + 57 = 114</b>		
<b>Total vials required ( working reference ) :</b>		<b>07 + 07(retained) = 14</b>		

\* May be omitted if test for virulent mycobacteria has been carried out with satisfactory results on the final bulk vaccine by the manufacturer as given in IP2014.

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine			
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 13 of 18

## REPORTING THE RESULTS

### 6. CRITERIA FOR ACCEPTANCE OF SAMPLES:

#### 6.1 Types of biologicals used:

Bacillus Calmette-Guerin vaccine (freeze dried) as per manufacturer's specifications is:

6.1.1 Ten dose vial (1 ml reconstituted vaccine; 10 doses & 20 doses).

#### 6.2 Condition of packing:

Vaccine should be in

6.2.1 Sealed containers

6.2.2 With proper labels

6.2.3 Supplied in cold chain at 2-8°C.

6.2.4 With minimum 12 months expiry date

#### 6.3 Quantity of samples essential:

Total vials required (Complete testing): 114 vials

#### 6.4 Documents required

6.4.1 Documents shall be checked as per the check list of documents given in Annexure -A

6.4.2 Document on description of samples shall be done by physical examination of sample container, diluent, labels and product insert.

6.4.3 Documents on Results of various tests shall be done on records of tests in form specified

### 7. POLICY ON TESTING

#### 7.1 Opening of samples for laboratory test:

Fifty seven (57; 10 & 20 doses) shall be kept in the laboratory and will be opened just before use for testing in the laboratory and 57 (10 & 20 doses) vials shall be kept as retained (In case of complete testing).

### 8. QUALITY CONTROL EVALUATION:

Quality control evaluation shall be based on various Quality Control Tests, their acceptance criteria and interpretation of results as mentioned in S.No 13.

#### 8.1 Initial Test

8.1.1. Appearance (reconstituted vaccine).

#### 8.2 Final Tests

8.2.1 Test for Identity

8.2.2 Test for Counts of Viable Units

8.2.3 Test for Temperature Stability

8.2.4 Test for Sterility

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine

Document ID No. : NIB/BVL/BCG/GD/01

Effective Date: 01.12.2014

Validity: till further revision

Page 14 of 18

- 8.2.5 Test for Moisture Content
- 8.2.6 Test for Excessive Dermal Reactivity
- 8.2.7 Test for Virulence/Safety

**8.3 Policy on use of control sample:**

- 8.3.1 **Reference vials:** 14 vials of internal reference preparation used by the manufacturer.
- 8.3.2 **Positive control:** One batch of vaccine with specified Quality control parameters (from manufacturer) shall be obtained and validated against International Reference Standard. This shall be used as an Internal Reference Standard.
- 8.3.3 **Negative control:** Shall be used as per the individual test.
- 8.3.4 **Repeat testing (if essential):** In case of any deviation in the test or objective/ observation of Head, repeat test shall be done with approved competent authority.
- 8.3.5 **Validation of tests:** Records of validation of individual tests shall be maintained in the laboratory.
- 8.3.6 **Presentation of Results:** Individual test results shall be mentioned in the test report.

**8.4 Verification of Results and Signature:**

- 8.4.1 Test report of each test shall be signed by analyst
- 8.4.2 The results shall be verified by Lab. In-charge/ Head

**8.5 Policy on calibration of equipment:**

- 8.5.1 Only calibrated equipments of the lab shall be used.
- 8.5.2 If any fault occurs, it shall be notified to the engineering division for corrective measures as per SOP/QA/14.

**8.6 Policy on preparation of report:**

- 8.6.1 **Signature on reports:** Certificate of Analysis shall be signed by analyst and Head of Division.
- 8.6.2 **Release of Report:** Shall be done by Director, NIB.
- 8.6.3 **Turn around time:** Shall be within 90 days from the date of receipt of sample in SRRDU.

**9. POLICY ON RETENTION OF SAMPLES AND REPORT :( Ref. Table 1)**

- 9.1 **No. of samples to be retained:** 57 vials (1 ml, 10 & 20 doses)
- 9.2 **Copies of report to be retained:** One copy shall be retained by Sample Receipt & Report Dispatch Unit in Archives.
- 9.3 Policy of Emergency/Exigency testing: Exigency testing done in public interest.

**10. POLICY ON ADMINISTRATIVE REVIEW:**

- 10.1 **Reports to be submitted for Administrative Review:** The reports shall be submitted as per NIB/QMU/SOP/26/R1.
- 10.2 **Authority for Administrative Review:** Expert

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine		
<b>Document ID No. :</b> NIB/BVL/BCG/GD/01	<b>Effective Date:</b> 01.12.2014	<b>Validity:</b> till further revision
		<b>Page</b> 15 of 18

**11.0 POLICY ON DESTRUCTION OF REPORT ON BIOLOGICAL SAMPLES:**

11.1 **Duration of retention of samples:** Samples shall be retained under specified condition for one year after expiry, in sample receipt and report dispatch unit.

11.2 **Retention of documents:** The documents shall be retained in Archives as per NIB/QMU/SOP/26/R1

**12. REFERENCES:**

12.1 Indian Pharmacopoeia (2014)

12.2 European Pharmacopoeia 7.0

12.3 WHO TRS 979 (2013)

12.4 Drugs and Cosmetics Act: 1940

12.5 WHO/1V/B/05.23 (2005). Guidelines on the International Packing and Shipping of Vaccines.

12.6 Brosch R et al. Genome plasticity of BCG and impact on vaccine efficacy. *Proceedings of the National Academy of Sciences*, 2007, 104 (13):5596-5601.

12.7 Seki M et al. Whole genome sequence analysis of *Mycobacterium bovis* Bacillus Calmette Guerin (BCG) Tokyo 172: a comparative study of BCG vaccine substrains. *Vaccine*, 2009, 27:1710-1716.

12.8 Ritz N et al. Influence of BCG vaccine strain on the immune response and protection against tuberculosis. *FEMS Microbiology Review*, 2008, 32:821-841.

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine		
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision
		Page 16 of 18



**ANNEXURE –A**  
**CHECK LIST OF DOCUMENTS**

**1. DOCUMENTS REQUIRED TO BE SUBMITTED ALONG WITH SAMPLE OF BATCH FOR QUALITY CONTROL TESTING:**

**1.1 Indigenous manufacturer:**

- 1.1.1 Forwarding letter from DCG (I) or Port Office/ as per the QC manual of the Institute.
- 1.1.2 A copy of Manufacturing License.
- 1.1.3 Batch Release Certificate of Manufacturer for three consecutive batches (for new manufacturer).
- 1.1.4 Certificate of Analysis of Manufacturer for three consecutive batches (for new manufacturer).
- 1.1.5 Summary Protocol for Production and Q.C Testing.
- 1.1.6 Manufacturing Protocols for three consecutive batches (for new manufacturer).

**1.2 Additional documents from indigenous manufacturer processing imported bulk**

- 1.2.1 A copy of Import License.
- 1.2.2 Bulk release certificate from National Control Authority.
- 1.2.3 Certificate of Analysis of bulk.
- 1.2.4 Manufacturing Protocols
- 1.2.5 QC test done on final lot.

**1. 3 Imported manufacturer:**

- 1. 3.1 Forwarding letter from DCG (I) or port office/ as per the QC manual of the NIB.
- 1. 3.2 A copy of Import License.
- 1. 3.3 Batch Release Certificate from the National Control Authority/ country of origin
- 1. 3.4 Certificate of Analysis of National Control Laboratory
- 1. 3.5 Summary Protocol for Production and Testing
- 1. 3.6 List of three countries where the product is sold/marketed.

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine			
Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 17 of 18



### 13. Template Certificate of Analysis:

NATIONAL INSTITUTE OF BIOLOGICALS  
(Ministry of Health & Family Welfare)  
An ISO 17025:2005, Accredited Institute



Certificate No. T-2010 & T-2011

Dated:

**CERTIFICATE OF ANALYSIS**

Date of sample receipt :  
Start date of analysis :  
CDR No :  
Analytical Report No. :  
Product & Dosage Form :  
Name of the Manufacturer :  
Batch/Lot No. :  
Manufacturing date :  
Expiry date :  
Whether legal/Regulatory/Non regulatory sample :

S No.	Test Parameters (Method)	Specifications	Result
1	Test for Identity (Microscopic examination of the bacilli in stained smear).	Demonstration of Acid fast property (IP2014).	Complies
2	Counts of Viable Units. (By culture on solid medium).	Colony count $1-33 \times 10^6$ CFU/ml at $4 \pm 1^\circ\text{C}$ (IP2014).	Complies
3	Temperature Stability (by incubation/heating of vaccine at $37^\circ\text{C}$ for 4 weeks)	The number of viable units in the incubated/heated vaccine should not be <20% of that in unheated vaccine (IP2014).	Complies
4	Test for Sterility (Direct inoculation)	No evidence of bacterial and mycotic growth after 14 days of incubation, of both content of final container and diluent(s) (IP2014).	Complies
5	Residual moisture content (Karl Fischer-coulometric)	The average residual moisture content should not be > 2.5%; and of individual vial should not be 3% or greater (IP2014).	Complies
6	Test for Excessive dermal reactivity (In-vivo).	The vaccine complies with the test if the reaction it produced is not markedly different from that produced by the comparison vaccine (IP2014).	Complies
7	Test for virulence/safety (In-vivo).	Observe the animal for atleast 42 days. If two animals die during this period and autopsy does not reveal signs of tuberculosis, repeat the test on six other Guinea pigs. The vaccine complies with the test if not more than one animal dies during the 42 days following the injection and autopsy does not reveal any sign of tuberculosis (IP2014).	Complies

**CONCLUSION:** The Batch /Lot No. of BCG vaccine (Freeze dried) **complies/do not complies** the requirement(s) as per IP2014. The product is of **standard quality/not of Standard quality**.

Signature of the Analyst  
Name & Designation:  
Date:

Signature of the Lab.i/c/Head  
Name & Designation:  
Date :

Guidance Document on QC of Bacillus Calmette-Guerin (BCG) Vaccine

Document ID No. : NIB/BVL/BCG/GD/01	Effective Date: 01.12.2014	Validity: till further revision	Page 18 of 18
-------------------------------------	----------------------------	---------------------------------	---------------