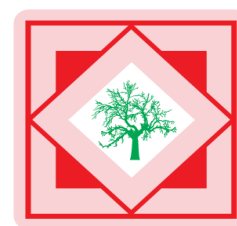




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GMP regulation of BCG vaccine

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ABSTRACT

Mycobacterium bovis, Bacille Calmette-Guérin (BCG) represents the most widely used viable vaccine for tuberculosis, with over 3 billion doses administered. General agreement exists that BCG can protect against severe forms of systemic tuberculosis in children. GMP covers all aspects of the manufacturing process: defined manufacturing process, validated critical manufacturing steps, suitable premises, storage, transport; qualified and trained production and quality control personnel, adequate laboratory facilities, approved written procedures and instructions, records to show all steps of defined procedures have been taken. The guiding principle of GMP of BCG vaccine is that quality is built in to a product and not just tested in to a product. Therefore, the assurance is that the product not only meets the final specifications, but that it has been made by the same procedures under the same conditions each and every time it is made. There are many ways this is controlled – validation is that part of GMP that ensures that facility systems, equipment, processes and tests procedures are in control and therefore consistently produce quality product.

Keywords- BCG Vaccine, GMP regulation, Advancement & Future Policy of BCG Vaccine

INTRODUCTION

Vaccine

Vaccines are a group of products that includes some of the oldest biological pharmaceutical entities. Disease resistance of some strains of organisms, such as tuberculosis, difficulties in providing vaccines suitable for immunosuppressed patients, as well as the need to provide vaccines to greater numbers of people at lower cost, are all challenges facing manufacturers and governments alike.¹

The theory of vaccination

When BCG is injected intradermally into an individual who is not infected with tubercle bacilli, and who, presumably therefore, does not possess specific resistance against tuberculosis, the bacilli will start multiplying rapidly at the site of vaccination.

Some of the invading bacilli will be destroyed and the rest surrounded by fibrous tissues. During this process, immunity develops and the body tissues will be sensitized to a state of hypersensitivity. The tissues at site of vaccination, where the largest number of the bacilli is concentrated, are destroyed and the destroyed tissues as well as dead bacilli come out as pus.¹

BCG immunization policies²

BCG vaccination policies differ greatly between countries. The various policies may be broken down into four groups:

1. BCG only at birth (or first contact with health services):

This is the current recommendation of the EPI and the Global Tuberculosis Programme (GTP), and is the policy in most of the world today, in particular in developing countries.

WHO has emphasized this policy in recent years, because of consistent evidence that BCG protects against serious childhood forms of tuberculosis, even where it may not protect to a high degree against adult pulmonary forms of the disease.

2. BCG once in childhood:

Some European nations have this policy, for example the United Kingdom, where BCG has been given routinely to tuberculin negative Adolescents (12-13 year olds). This particular policy was initiated in 1957 as an appropriate way to deliver the vaccine at an age of low disease incidence, prior to school leaving and just before individuals move into the higher incidence period associated with young adulthood. This policy is now being discontinued in some health authorities of the UK, which have moved to selective vaccination of high risk populations (e.g. immigrants, contacts).

3. Repeated/booster BCG:

Many countries have a tradition of repeated BCG vaccination³¹. For several countries (e.g. Switzerland, Portugal), this means BCG in infancy and then at school entry or leaving, but for others, in particular in Eastern Europe, BCG has been recommended up to five times, e.g. from birth to 30 years of age (as in Hungary and Russia). The criteria for revaccination differ between countries, some of which emphasize routine revaccination of everyone, and others restrict revaccination to individuals who lack a scar or who remain tuberculin “negative”. Criteria for negativity differ according to the tuberculin used, the method of administration and reading, and the interpretation of the indurations.

4. No routine BCG use:

Two countries (USA, the Netherlands) have never recommended routine universal BCG, and others have now moved to this policy (e.g. Sweden in 1975, parts of Czechoslovakia in 1986). All these countries license BCG for selective use among groups considered to be at particularly high risk

The mode of infection³

- Coughs
- Sneezes.
- Inhalations into the lungs
- Skin.

Tuberculin reactions not only vary in size but in internal consistency too and hence different persons measure them differently.

Table 1: Types of tuberculin reaction³

Type I A	Type II A	Type III A	Type IV
Sputum dense, hard and elevated with sharp borders.	Dense, hard and elevated with sharp borders but hardness is lower than Type I.	Mild density	Soft
Surrounded by oedema (swelling).	May not be surrounded by oedema.	Distinguishable by the eye.	Easily missed unless the reader is careful.

BCG vaccine**History**³

The BCG vaccine (Bacillus Calmette-Guerin) was introduced into the UK in 1953. Robert Koch first distinguished *Mycobacterium bovis* from *Mycobacterium tuberculosis*. Albert Calmette, a French bacteriologist, and his assistant and later colleague, Camille Guérin, a veterinarian, were working at the Institute Pasteur de Lille in 1908.⁽²³⁾ Their work included subculturing virulent strains of the tubercule bacillus and testing different culture media. The injection is usually given into the left upper arm. As the vaccine is only effective in about 8 in 10 people.

Description³

BCG Vaccine is a live freeze-dried vaccine derived from attenuated strain of *mycobacterium bovis*. (Bacillus Calmette Guerin) used for the prevention of tuberculosis. BCG is **Conjugate vaccines** also hold promise for emergent problems with old diseases. For example, the BCG vaccine for tuberculosis cannot be used in those immunosuppressed, for such as through HIV infection, because it is a whole, live, attenuated bacterial vaccine. However, a conjugate of suitable toxoids with a tuberculosis cell wall saccharide could provide a relatively simple vaccine that would be stable, well characterized and able to be made in quantity and which could be effective in this target population.

Composition⁴

- Live, attenuated BCG Vaccine (Bacillus Calmette Guerin strain)
- Each 0.1 ml contains between: 1×10^5 and 33×10^5 C.F.U.
- Reconstitute with Sodium Chloride Injection. Dose: 0.05 ml, intradermally for infants under one year old.

Reconstitution⁴

- BCG Vaccine vial of 10 doses (0.05 ml) for infants under one year old, to be reconstituted with 0.5 ml of sodium chloride injection.
- BCG Vaccine vial of 20 doses (0.05 ml) for infants under one year old, to be reconstituted with 1 ml of sodium chloride injection.
- Use immediately after reconstitution. If the vaccine is not used immediately then it should be stored in the dark at 2° to 8° C for no longer 6 hours (1 immunization session).

Dosage and administration⁴

- Intradermal route, avoiding the subcutaneous route.
- The vaccination dose is 0.05 ml for children under one year of age including the new born.

- The skin should not be cleaned with antiseptic.
- The vaccine should be preferably given with a tuberculin syringe or sterile needle.

Intradermal injection technique⁴

The skin is stretched between thumb and forefinger and sterile needle (25 G/26 G) inserted bevel upwards for about 2mm into superficial layers of the dermis (almost parallel with the surface).

Storage⁵

- BCG (Freeze-dried) should be stored in dark between 2°C to 8°C.
- It is stable if stored in temperatures as low as -20°C. Protect from light.
- The diluents should not be frozen, but should be kept cool.

Shelf Life⁵

24 months from the date of last satisfactory potency test if stored in a dark place at recommended temperature.

Various effect of BCG vaccine^{6, 11}

Adverse Effects

- Lymphadenitis (Swelling in lymph node).
- Lymphangitis (can occur if the vaccine is administered close to the shoulder injection)
- Lymphadenopathy (lymph nodes, in young children).

Side Effects

- Papule develops at site of vaccination
- Abscess formation.
- Shallow ulcer covered with a crust.

Contraindication

- Leukaemia
- Generalized malignancy
- HIV Patient.

Role of BCG in tuberculosis control^{7, 8}

The WHO Expert Committee on tuberculosis (1964) gave its considered opinion that “BCG vaccination should have an important place in and form an integral part of the tuberculosis programmed in most countries.” The Expert Committee (1974) noted with satisfaction that during the last decade the scale of BCG vaccination had increased. The most important arguments and doubts expressed against BCG vaccination are:

- 1) BCG vaccinated persons are rendered sensitive to tuberculin and this will interfere with the diagnostic and epidemiological value of the tuberculin test.
- 2) It is well known that there now exists several daughter strains of BCG which vary widely. Attempts at a rationalization about the relative potency of different daughter strains are not easy because of the involved and long drawn nature of assays.
- 3) Excepting long drawn trials in man it cannot be stated with certainty which of the BCG vaccines (i.e. daughter strains) affords sustained immunity in man, to what degree and for how long. It is in this important aspect that different controlled trials have varied widely.

GMP regulations of BCG vaccine

General provision finished product^{9, 10}

This Laboratory through National Productivity Council of Govt. of India made necessary action towards ISO-9002 certification for BCG manufacture. The preliminary discussions,

documentation of Standard Operating Protocols were completed. By March, 2001 this Laboratory will get the ISO-9002 certification.

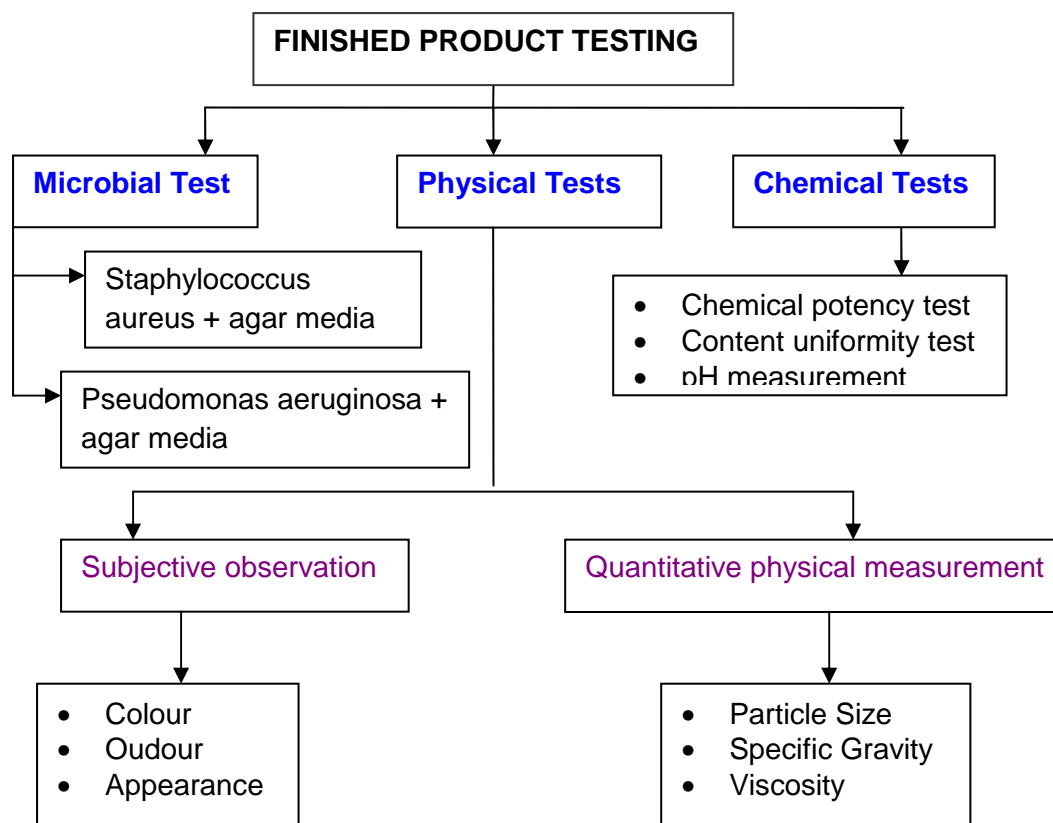


Fig: 1: Outlines of Finish Product Testing

1. Personnel⁹

- All personnel (including those concerned with cleaning, maintenance or quality control) employed in areas where biological medicinal products are manufactured should receive additional training specific to the products manufactured and to their work. Personnel should be given relevant information and training in hygiene and microbiology.
- Persons responsible for production and quality control should have an adequate background in relevant scientific disciplines, such as bacteriology, biology, biometry, chemistry, medicine, pharmacy, pharmacology, virology, immunology and veterinary medicine.
- The immunological status of personnel may have to be taken into consideration for product safety. All personnel engaged in production, maintenance, testing and animal care (and inspectors) should be vaccinated where necessary with appropriate specific vaccines and have regular health checks.
- Production of BCG vaccine and tuberculin products should be restricted to staff who are carefully monitored by regular checks of immunological status or chest X-ray.

2. Building & Premises¹⁰

- The degree of environmental control of particulate and microbial contamination of the production premises should be adapted to the product and the production step, bearing in mind the level of contamination of the starting materials and the risk to the finished product.

- The risk of cross-contamination between biological medicinal products, especially during those stages of the manufacturing process in which live organisms are used. The nature of the product as well as the equipment used will determine the level of segregation needed to avoid cross-contamination.
- In principle, dedicated facilities should be used for the production of BCG vaccine and for the handling of live organisms used in production of tuberculin products.
- Air filtration units should be specific to the processing area concerned and recirculation of air should not occur from areas handling live pathogenic organisms.
- The layout and design of production areas and equipment should permit effective cleaning and decontamination (e.g. by fumigation). The adequacy of cleaning and decontamination procedures should be validated.

Approximately 23,000 sqft of manufacturing areas including:

- Master Cell Bank (MCB)
- Cell Culture Expansion / Inoculation
- Cell Culture Harvest Filtration
- Chemical Inactivation
- Ultrafiltration
- Buffer Formulation
- Downstream Processing

Design Features

- 6,000 sqft BSL-3 Cell Culture Suite
 - Batch culture process to 800 liters (4 x 200 liters)
 - 5 major processing areas with Biosafety Cabinets
 - 100 & 200 liter mobile culture tanks (>50 vessels)
 - Culture vessel sparging systems
 - 4 walk-in incubators
 - Cold storage (2-8 °C) and decontamination autoclave
- 5,000sqft BSL 3 Downstream Processing Suite
 - 6 fixed inactivation process tanks (1,000 liter each)
 - Chromatography room (K-Prime)
 - Multiple mobile process tanks (UF & Chromatography)
 - 2 fixed process buffer tanks (1,500 liters each)
 - Centrifugation room
 - Multiple Biosafety Cabinets
 - Steam Stations for mobile tanks
- 3,100 sqft Aseptic Processing Area
 - Aseptic Formulation / Filling of syringes
 - Multiple Class 100 areas
 - 2 HVAC Systems
 - Container / closure system

3. List of equipments¹⁰

Equipment used during handling of live organisms should be designed to maintain cultures in a pure state and uncontaminated by external sources during processing. Effluents which may contain pathogenic micro-organisms should be effectively decontaminated. Due to the variability of biological products or processes, some additives or ingredients have to be measured or weighed during the production process (e.g. buffers). In these cases, small stocks of these substances may be kept in the production area.

Laboratory has following equipments: ¹⁰

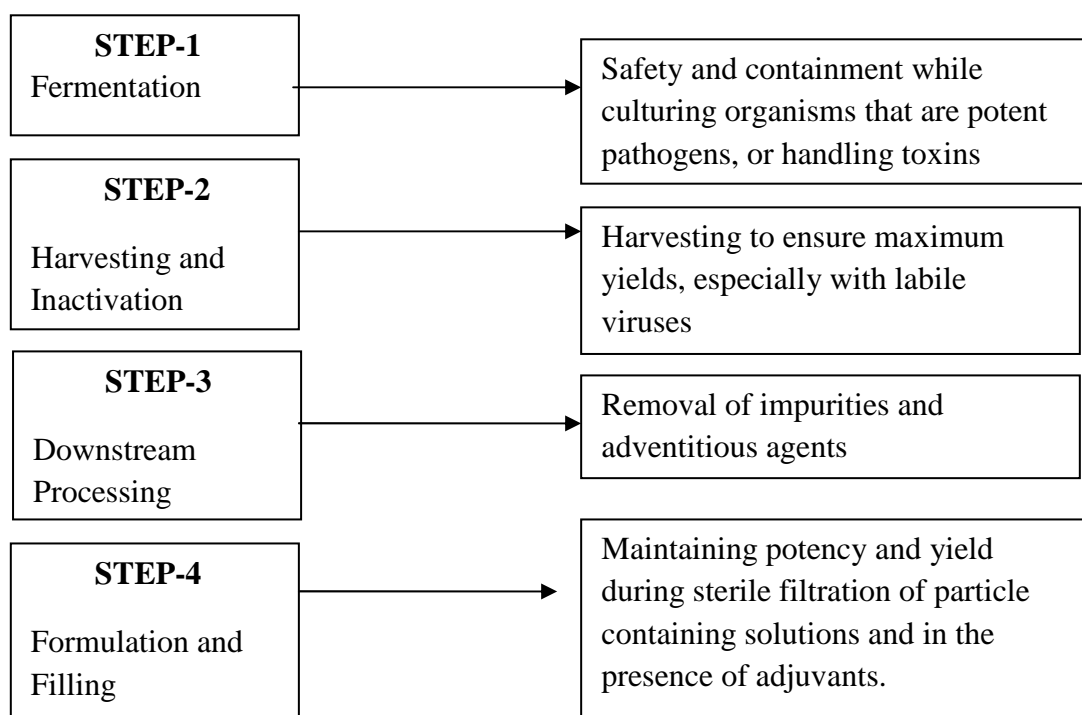
- Freeze Driers
- Autoclaves
- Clean room facility at Class 10,000 level.
- Laminar Flow Benches at Class 100 level.
- Vial filling & vial capping machines.
- Blister forming machineries.

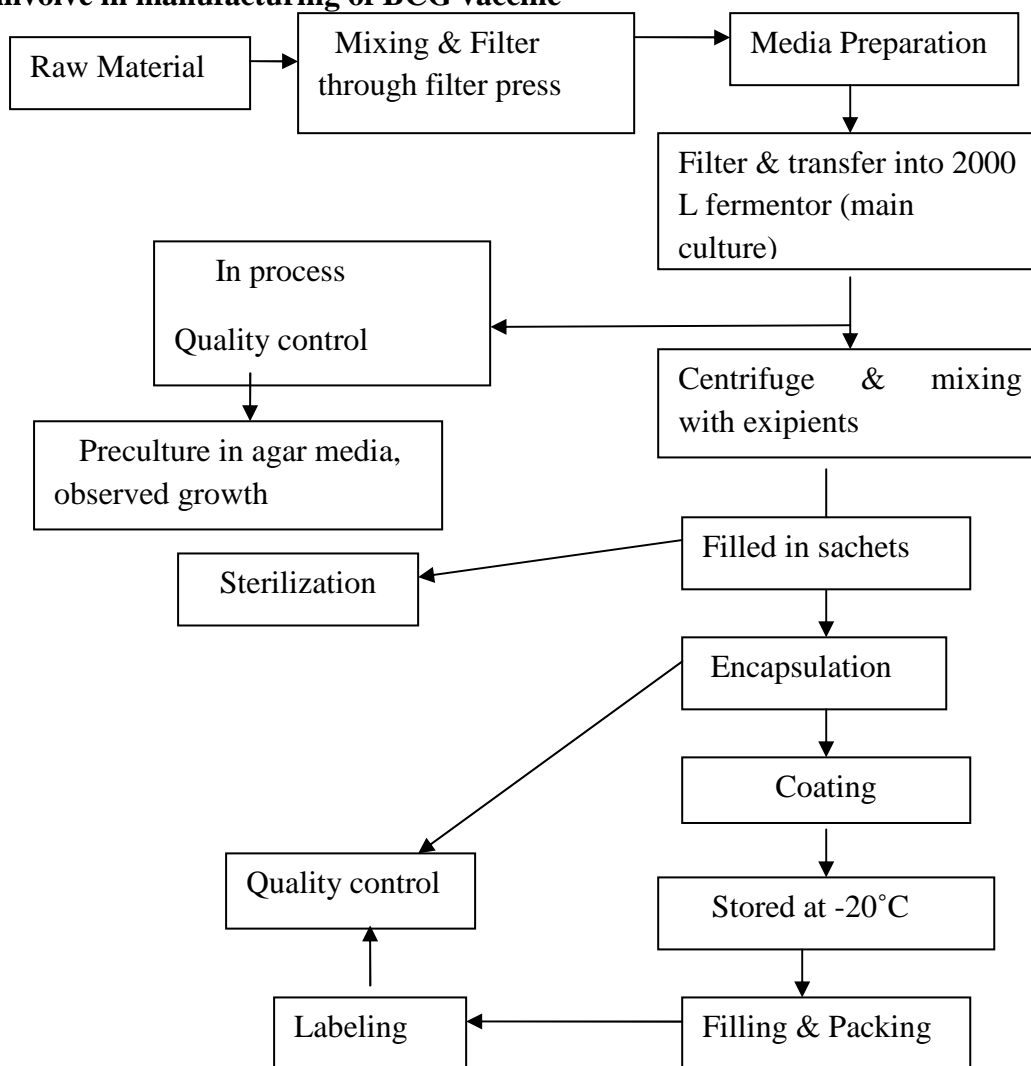
4. List of raw material ¹¹

- BCG Vaccine (Freeze-Dried) is a culture preparation of Bacillus Calmette-Guérin, Connaught sub strain, an attenuated strain of *Mycobacterium bovis* suspended in monosodium glutamate.
- BCG cultures are propagated on Sauton growth medium, then harvested, mixed with monosodium glutamate and lyophilized (freeze-dried).
- BCG cultures are viable upon reconstitution. BCG vaccine is supplied in multi-dose vials with accompanying diluents, which consists of sterile phosphate-buffered saline containing 0.025% polysorbate 80.
- The concentration of the reconstituted vaccine is 8 x 10⁵ to 32 x 10⁵ colony forming units (CFU) per adult dose of 0.1 mL (= 0.1 mg BCG), equivalent to 4 x 10⁵ to 16 x 10⁵ CFU per infant dose of 0.05 mL (= 0.05 mg BCG).

Table2: Required raw materials

Medium	Bile-potato medium, barren potato, liquid Sauton medium.
Strain	Bovine tubercle bacillus
Ingredient	Water,Auxin, Biotin

Stepwise production process of BCG vaccine

Steps involve in manufacturing of BCG vaccine¹¹**Fig 2: General steps involve in manufacturing of BCG vaccine****4. Production & control of bcg vaccine**

The original B.C.G. strain was bovine tubercle bacillus attenuated by repeated subculture for 230 generations in three years in bile-potato medium. The strain had become eugenic and a virulent for animals. Synthetic liquid medium (Sauton medium) subculturing every 2 weeks. More frequent transfers enhance the virulence and longer transfers cause more attenuation. With suitable virulence the growth should completely cover the surface of medium in an Erlenmeyer flask in 12 to 14 days. At Gothenberg laboratory in Sweden the B.C.G. are grown successively on barren potato for 14 days; on glycerine potatoes for 11 days; 11 days in Sauton I and 11 days in Sauton II and then they are prepared as vaccine.¹¹

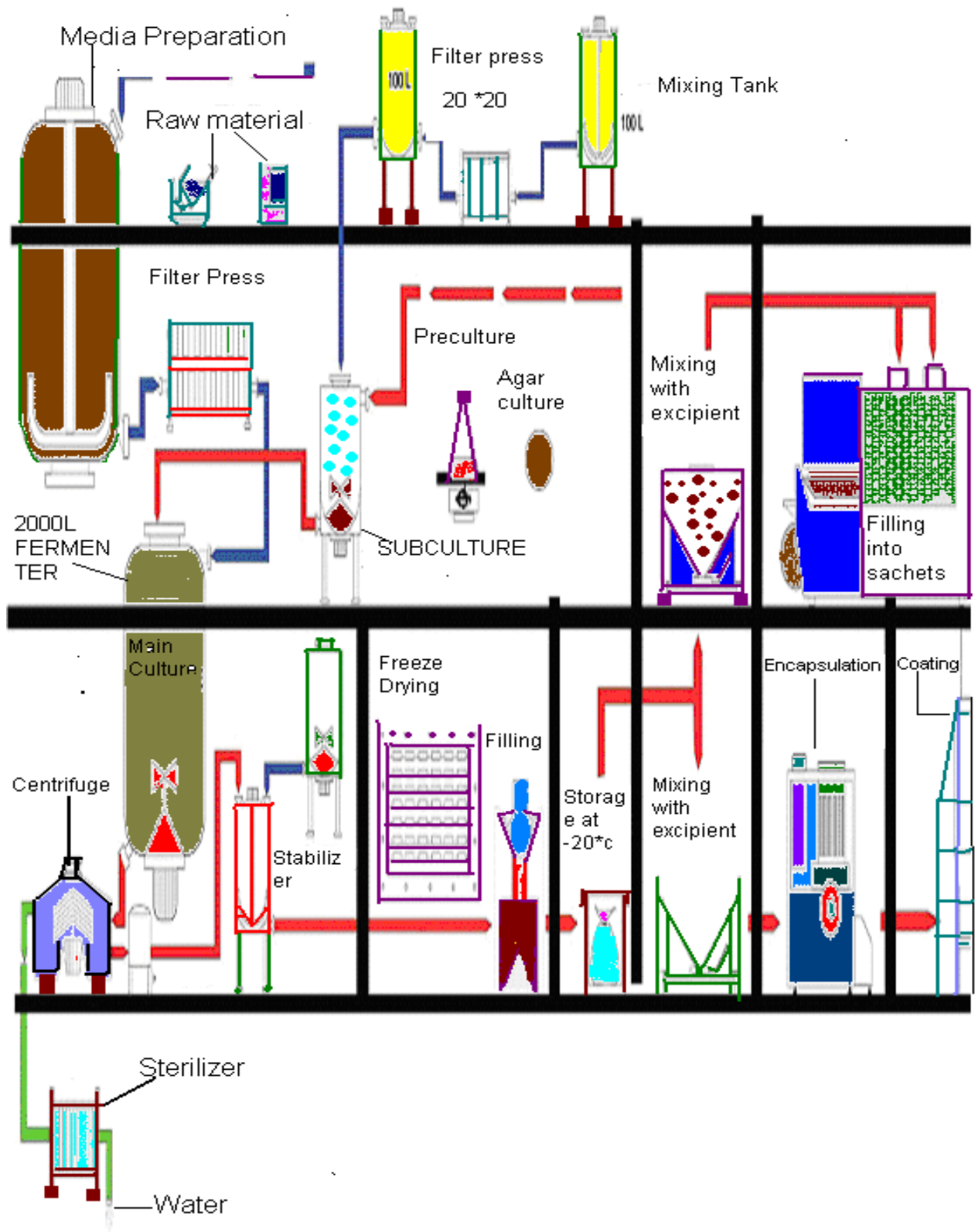


Fig 3: Schematic representation of Industrial production of BCG vaccine^{11,21}

Sampling of BCG vaccine¹¹

The following sample should be supplied to the official Medicine Control Laboratory performing batch release:

<i>IN VITRO</i>	<i>IN VIVO</i>
Least 50 single & multiple dose containers each final lots.	At least 320 single dose of each new final bulk.

In process quality control of BCG¹²

- In-process controls play an important role in ensuring the consistency of the quality of biological medicinal products. Those controls which are crucial for quality (e.g. virus removal) but which cannot be carried out on the finished product should be performed at an appropriate stage of production.
- It may be necessary to retain samples of intermediate products in sufficient quantities and under appropriate storage conditions to allow the repetition or confirmation of a batch control.
- Continuous monitoring of certain production processes is necessary, for example fermentation. Such data should form part of the batch record.
- Where continuous culture is used, special consideration should be given to the quality control requirements arising from this type of production method.

Quality control of BCG vaccine^{10, 12}

Major laboratory test systems generally used to control:

- The viability of the BCG products.
- The residual virulence of the BCG strains.
- The allergenic potency.
- The immunogenic potency of the BCG vaccines
- Intermediate and final product analyses of bacterial vaccines
- Stability tests of the products
- Handling of reference samples of bacterial vaccines (toxoids and final product vaccines), tuberculin and semi-finished BCG products.

<i>IN VITRO</i>	<i>IN VIVO</i>
Identity	Test for virulent mycobacteria (on every new working seed lot.)
Count of viable units (potency test)	Excessive dermal reactivity (on every new working seed lot.)

5. Packaging¹³

WHO prequalification scheme, vaccine manufacturers are expected to ensure that their packaging complies with the criteria specified below. Validation data should be produced in three consecutively successful runs. Any changes introduced in the packaging must be validated again.

Containers

The containers should be in a form that renders the process of reconstitution as simple as possible. Their packaging should be such that constituted vaccine is protected from daylight.

The containers may be used---

1. Single dose containers
2. Multiple dose containers

All containers sealed under vacuum shall be tested for leak not less than 2 months after sealing & all defective containers shall be discarded.

Packaging according to storage temperature

A. Class A packaging

Prior to – and at the time of packing – the vaccines must be kept within the storage temperature limits recommended by the manufacturer.

The vaccine must be packed to ensure that the warmest temperature inside the insulated package does not rise above +8 °C in continuous outside ambient temperatures of +43 °C for a period of at least 48 hours.

B. Class B packaging

Prior to and at the time of packing – the vaccines must be kept within the storage temperature limits recommended by the manufacturer.

The vaccines must be packed to ensure that the warmest temperature inside the insulated package does not rise above +30 °C in continuous outside ambient temperatures of +43 °C for a period of at least 48 hours.

C. Class C packaging

Prior to – and at the time of packing – the vaccines must be kept within the storage temperature limits recommended by the manufacturer.

Table-3: Types of packaging for BCG vaccine¹³

Primary packaging	Secondary packaging	Tertiary packaging
Constitutes the first level of container for vaccine: the vaccine vial or ampoule itself.	It must be clearly labeled for recipient, giving information on its contents.	Outer box, shipping container that contains secondary packages & clearly labeled.

6. Labeling^{13, 14}

• Labelling for secondary packaging

A label must be affixed either to the top and/or front surface of the secondary packages. It should indicate the type of vaccine, the name of the manufacturer, presentation, batch number and date of manufacture, date of expiry, quantity and storage conditions.

• Labelling for tertiary packaging

The external surface of insulated packages should be either white or in the natural colour of corrugated carton.

7. Validation^{15, 17}

A. PURPOSE

1 The **shake test** is designed to determine whether adsorbed vaccines have been affected by freezing.

2 After freezing, the lattice (bond between adsorbent and antigen) gets broken.

3 Separated adsorbent tends to form granules that get bigger in particle size and weight then gradually settle to the bottom after the vial has been shaken.

4 The size of the granules seems to increase after repeated freezing and thawing cycles.

5 Sedimentation occurs faster in a vaccine vial that has been frozen than in a vaccine vial (from the same manufacturer) that has never been frozen.

6

B. Test Procedure

To validate BCG vaccine various parameter are considered & evaluate in comparison with standard. The shake test should be conducted on a random sample of vaccines.

However, if there is more than one lot of vaccine in the shipment, the random sample must include a vial taken from each and every lot.

1 Take a vial of vaccine of the same type and batch number as the vaccine you want to test, and made by the same manufacturer. This is your **control** vial.

2 Clearly mark the control vial: “**FROZEN.**”

3 Freeze the **control** vial at -20°C overnight, until the contents are completely solid.

4 Let the **control** vial. Do **not** heat it!

5 Take a “**test**” vial from the batch that you suspect has been frozen.

6 Hold the **control** (“**frozen**”) vial and the “**test**” vial together in one hand.

7 Shake both vials vigorously for 10–15 seconds.

8 Place both vials on a flat surface side-by-side and start continuous observation of the vials until the test is finished. (If the vials have large labels that conceal their contents, turn both vials upside down and observe sedimentation in the neck of each vial.)

9 Use an adequate source of light to compare the sedimentation rates between vials.

8. Documentation ^{16, 18}

Copies of the documentation for the goods to be shipped must be sent at least seven days in advance of arrival of the shipment.

The documentation must include the following:

1. Airway bill (AWB)
2. Supplier’s invoice
3. Packing list
4. Lot release certificate (LRC) issued by the national regulatory authority (NRA) of the country of manufacture for each lot of vaccine supplied

The pre-advice must contain the following information:

1. Purchase order reference;
2. Consignee requisition reference;
3. Number of packages, gross weight (in kilograms) and volume (in cubic meters)
4. Type of vaccine, total number of vials and number of doses per vial/ampoule/tube
5. Date and time for place of departure, transit and arrival
6. Instructions for collection;
7. Any other information specified in the individual contract must also be included for the consignee.

Procedure for reporting vaccine arrival

1. Arrival of vaccines and customs clearance.
2. Inspection at airport or central cold stores. Vaccine Arrival Report (VAR) filled in and signed.
3. Copy of VAR sent to UNICEF Country Office.
4. Copy of VAR sent to UNICEF, Copenhagen (SD)

Clinical Trials for a Candidate Vaccine^{7, 19}**Table 4: Phases of clinical trials**

Phase I	Phase II	Phase III
First human tests of a candidate vaccine, conducted on small numbers (10-30) of healthy adult volunteers who are not at risk for the disease in question.	Involves a larger number of volunteers (50-500), usually a mixture of low-risk people and higher-risk individuals from the population.	Phase III trials are definitive test of whether a vaccine is effective in preventing disease using thousands of volunteers from high risk population.
The main goal is evaluation of safety & to a lesser extent, analysis of immune responses evoked by the vaccine.	Phase II trials generate additional safety data as well as information for refining the dosage and immunization schedule.	Successful demonstration of efficacy in Phase III trial can lead to application for licensure of vaccine.
Phase I trial usually takes 8-12 months to complete.	These trials generally take 18-24 months.	Required a minimum 3 years to enrollment, immunizations.

MARKET VALUE²⁰

BRAND NAME	OTHER NAME OF BCG
TheraCys [®] BCG	BCG live
TICE [®] BCG	BCG vaccine

- BCG 40mg vaccine in the local market by which the patient gets the vaccine through the stockiest.
- It is pertinent to mention here that by marketing of 40mg BCG Cancer Vaccine, this Laboratory will earn a revenue of approximately Rs.1.5 crore to 2 crore.

Advancement & future policy of BCG vaccine

There is much interest in the potential use of BCG as a live vector to deliver a variety of recombinant antigens and hence as a “super vaccine”²¹. Thus antigens from HIV, *Borrelia burgdorferi* and pneumococcus have been expressed in BCG in such a way as to induce immune responses in experimental animals²²⁻²⁴. The fact that BCG can be delivered at birth, that it has a good safety record (despite its local reactogenicity), and that it has general adjuvant activity, enhances the attractiveness of this approach. Among the implications of this research is the need to consider the possibility of broader uses of BCG in the future, and hence to maintain the acceptance of BCG in the immunization community.

B.C.G. vaccination in the bush is impracticable without a reliable freeze-dried vaccine; active steps must be taken to solve the problem of standardization. Intensive research into concomitant vaccination and the possible protective effect of B.C.G. against leprosy must also be carried out.

CONCLUSION

The BCG vaccine (Bacille Calmette Guérin) is a live virus vaccine prepared from attenuated strains of *Mycobacterium Bovis*. Use of BCG vaccine is recommended by the Expanded

Programme on Immunizations of the World Health Organization for administration at birth and is currently used in more than 100 countries, including Guatemala. BCG vaccine is used to prevent disseminated and other life threatening manifestations of Tuberculosis in infants and young children. However BCG vaccine does not prevent infection with Mycobacterium Tuberculosis. The various BCG vaccines used throughout the World differ in composition and efficacy. During the manufacturing we need a highly hygienic condition for patient welfare the number of problem related with production of pharmaceuticals are rectified by the skilled worker itself as they are having sufficient experience of manufacturing as well as sorting out the problem related with machine. FDA regulates various programmes to control and validate the production of BCG vaccine. FDA provides some standard to maintain GMP of industry for manufacturing of BCG vaccine. The (clinical) efficacy of a vaccine is measured in terms of the percentage reduction in disease among vaccinated individuals that is attributable to vaccination. Though the WHO now emphasizes BCG's utility in prevention of severe childhood disease (e.g. tuberculous meningitis), the main public health burden of tuberculosis is associated with adult pulmonary disease.¹⁸ It is therefore important to consider BCG vaccine efficacy against childhood tuberculosis, separately from that against adult tuberculosis, leprosy and other mycobacterial infections.

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