

Annex 3

Recommendations to assure the quality, safety and efficacy of BCG vaccines

Replacement of Annex 2 of WHO Technical Report Series, No. 745, and Amendment to Annex 12 of WHO Technical Report Series, No. 771

Introduction	139
General considerations	139
Special considerations	140
Scope of the Recommendations	141
BCG vaccine strains	141
Potency-related tests	142
Part A. Manufacturing recommendations	143
A.1 Definitions	143
A.2 General manufacturing recommendations	145
A.3 Control of source materials	147
A.4 Control of vaccine production	149
A.5 Filling and containers	152
A.6 Control tests on final lot	152
A.7 Records	156
A.8 Retained samples	156
A.9 Labelling	156
A.10 Distribution and transport	157
A.11 Stability, storage and expiry date	157
Part B. Nonclinical evaluation of BCG vaccines	159
Part C. Clinical evaluation of BCG vaccines	160
C.1 General considerations	160
C.2 Special considerations	162
C.3 Post-marketing surveillance	164
Part D. Guidelines for NRAs	164
D.1 General	164
D.2 Release and certification	166
Authors and acknowledgements	166
References	170

Appendix 1	
History and genealogy of BCG substrains	174
Appendix 2	
Summary protocol for manufacturing and control of BCG vaccine	175
Appendix 3	
Model certificate for the release of BCG vaccine by NRAs	184

Recommendations published by the WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Recommendations be made only on condition that such modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the recommendations set out below. The parts of each section printed in small type are comments for additional guidance, intended for the benefit of manufacturers and NRAs.

Introduction

The last revision of the Requirements for dried bacille Calmette–Guérin (BCG) vaccine for human use was in 1985, and an amendment which updated the section on the expiry date was published in 1988 (1, 2). Recent WHO consultation meetings (3–6) have addressed issues concerning the improvement of vaccine characterization and quality control assays of BCG vaccine to reflect current state-of-the-art technology. In addition, a recommendation to replace the International Reference Preparation for BCG vaccine by substrain-specific Reference Reagents evaluated by collaborative studies has been proposed. This document provides: recommendations for the production and control of BCG vaccines (Part A); guidelines for nonclinical evaluation (Part B); guidelines for the content of the clinical development programme applicable to BCG vaccines (Part C); and recommendations for NRAs (Part D). The guidelines for nonclinical evaluation apply to classic BCG vaccine products that are still in need of such evaluation, including newly manufactured products requiring clinical trial studies or those produced following changes in the manufacturing process. The clinical part of this document aims to provide a basis for assessment of efficacy and safety of BCG vaccines in pre-licensing clinical trials as well as in post-marketing surveillance, monitoring consistency of production and clinical testing of new classic BCG vaccine products. If important changes have been introduced to an authorized production process, the need for preclinical and clinical testing should be considered on a case-by-case basis in consultation with the NRA(s) concerned.

General considerations

Tuberculosis (TB) was declared a global emergency by WHO in 1993, and *Mycobacterium tuberculosis* (*M. tuberculosis*) is now considered to be responsible for more adult deaths than any other pathogen. Vaccination with BCG still remains the standard for TB prevention in most countries because of its efficacy in preventing life-threatening forms of TB in infants and young children. It is inexpensive and usually requires only one administration in either newborns or adolescents (7, 8). As there is currently no suitable alternative, BCG will remain in use for the foreseeable future and may continue to be used as a prime vaccine in a prime-boost immunization schedule in conjunction with new TB vaccines (4).

BCG vaccine contains a live, attenuated strain of *M. bovis* that was originally isolated from cattle with tuberculosis and cultured for a period of 13 years and a total of 231 passages (7). The BCG vaccine was first used to immunize humans in 1921. Following its introduction into the WHO Expanded Programme on Immunization (EPI) in 1974, the vaccine soon reached global coverage rates exceeding 80% in countries endemic for TB (9).

Over the years, different BCG vaccine seed strains have evolved from the original vaccine strain for production. A number of BCG vaccine strains that are used worldwide differ in terms of their genetic and phenotypic properties, and their reactogenicity and immunogenicity profile when given to infants and children. With this background of a diversity of substrains, manufacturing processes, immunization schedules and levels of exposure to environmental mycobacteria and virulent *M. tuberculosis* infection, different levels of protective efficacy of BCG vaccines in adult populations have been reported (10). However, the data are insufficient to make recommendations on whether one strain should be preferred over the other (11). The United Nations agencies are the largest supplier of BCG vaccines, distributing more than 120 million doses each year to more than 100 countries. Worldwide, the most commonly used vaccine strains are currently Danish 1331, Tokyo 172-1 and Russian BCG-I because they are supplied by the United Nations Children's Fund (UNICEF) which purchases the vaccines through a published prequalification process which determines their eligibility for use in national immunization programmes (12).

There has been particular concern over the safety of BCG vaccination in subjects infected with the human immunodeficiency virus (HIV) (8). WHO previously recommended that in countries with a high burden of TB, a single administration of BCG vaccine should be given to all healthy infants as soon as possible after birth, unless the child presented a symptomatic HIV infection (9). However, recent evidence shows that children who were HIV-infected when vaccinated with BCG at birth, and who later developed acquired immunodeficiency syndrome (AIDS), were at increased risk of developing disseminated BCG disease. Among these children, the benefits of potential prevention of severe TB are outweighed by the risks associated with the use of BCG vaccine; thus the use of BCG vaccines at birth in relation to HIV-infected infants should follow the recommendations of the Global Advisory Committee on Vaccine Safety (GACVS) (13, 14).

Special considerations

The formulation of international requirements for freeze-dried BCG vaccine is complicated by the following: (a) a number of different substrains derived from the original strain of BCG are used in vaccine manufacture; (b) a number of different manufacturing and testing procedures are employed; (c) it is difficult to identify a link between significant differences *in vitro* and *in vivo* between different BCG vaccine strains and any possible differences in protective efficacy against TB in humans; (d) vaccines are produced with different total bacterial content and numbers of culturable particles; and (e) vaccines intended for administration by different routes are prepared. Therefore, the following considerations should be borne in mind regarding the scope of these recommendations, BCG vaccine strains, and potency-related tests.

Scope of the Recommendations

These revised Recommendations refer to freeze-dried BCG vaccines prepared from substrains derived from original BCG for use in the prevention of TB. Where BCG vaccine is issued in liquid form, the application of these Recommendations is entirely the responsibility of the NRA. In that case, only the relevant parts of this document apply since the limited stability of liquid BCG limits the possibility of completing the full recommended control test schedule. Although many of the principles expressed in this document (e.g. manufacturing, quality control) are expected to apply also to new recombinant BCG and other live, attenuated mycobacterial vaccines modified by molecular biology techniques, these novel vaccines are outside the scope of these Recommendations. The same pertains to the use of BCG for immunotherapy (e.g. treatment of bladder cancer). However, applicability of issues on nonclinical and clinical evaluations should be considered on a case-by-case basis. These Recommendations have been formulated primarily to cover vaccines intended for intradermal and percutaneous administration. Although WHO recommends intradermal administration of the vaccine, preferably in the deltoid region of the arm using syringe and needle, other administration methods such as percutaneous application by the multiple puncture technique are practised in some countries (9, 15–17).

BCG vaccine strains

The original BCG vaccine strain was formerly distributed by the Pasteur Institute of Paris and subcultured in different countries using different culture conditions that were not standardized. 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 (see Appendix 1). Recently, the various substrains have been studied by comparative genomics (18, 19). 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 mutations in the genome of the bacilli than the “late” strains. “Early” strains are represented by BCG Russia BCG-I, BCG Moreau-RJ, BCG Tokyo 172-1, BCG Sweden, and BCG Birkhaug; and the “late” strains include BCG Pasteur 1173P2, BCG Danish 1331, BCG Glaxo (Copenhagen 1077) and BCG Prague. The genomic sequences of BCG Pasteur 1173P2 as a “late” strain, and BCG Tokyo 172-1 and BCG Moreau as “early” strains were determined (18–20). There is insufficient direct evidence to suggest that various BCG substrains differ significantly in their efficacy to protect against TB in humans. However, evidence from animal and human studies indicates differences in the immune responses induced by different BCG vaccine strains (12, 21). Although the “early” strains may confer better protection against

TB in some animal studies (18, 22), commonly administered BCG vaccine strains including both evolutionary “early” and “late” strains induce comparable protective immunity against TB (23).

Only master seed lots that have been shown to be acceptable by laboratory and clinical tests on batches derived from them should be used for the production of working seed lots and/or final product. A suitable seed lot of BCG should yield vaccines that give protection in experimental animals, produce a relatively high level of immunological responses to *M. tuberculosis* antigens including tuberculin sensitivity in humans, and have an acceptably low frequency of adverse reactions (see section A.3.1).

Some manufacturers of freeze-dried BCG vaccine have modified their master seed lot strain to make it more suitable for their particular production procedure. The seed lots prepared in this way may not retain the same immunogenic properties, and should be used only with the approval of the NRA.

In practice, a product prepared from BCG seed lots may generally be investigated in humans only for the properties of producing tuberculin sensitivity and vaccination lesions. The former should be measured by the distribution of tuberculin reactions according to size in persons vaccinated with a given dose of BCG vaccine. A low dose of tuberculin should be employed (e.g. equivalent to 5 IU of the First WHO International Standard for purified protein derivative (PPD) of *M. tuberculosis*, or 2 tuberculin units (TU) of a batch of PPD RT23 with Tween 80).

Currently three substrain-specific Reference Reagents for BCG vaccines are available: BCG Danish 1331, Tokyo 172-1 and Russian BCG-I.

Potency-related tests

There is some evidence that BCG seed lots that have been shown to produce vaccines with protective potency in laboratory animals and tuberculin sensitivity in humans will give effective protection against TB in humans. It should be noted that tuberculin sensitivity is a marker for cell-mediated immune responses to mycobacteria and not a direct indicator of protective immunity. A number of alternative laboratory tests have been developed primarily for research purposes but, to date, none have been proved reliable indicators of protective immune conversion following administration of different vaccines.

Studies in animals should include protection tests, tests of vaccination lesions, and tests for tuberculin conversion. Immunizing efficacy should be measured in terms of degree of protection afforded to the test animals against a challenge with virulent *M. tuberculosis*. Sensitizing efficacy should be measured by the average dose of vaccine that will convert a negative tuberculin reaction in guinea-pigs to a positive one, as well as by the reaction time during which

the conversion takes place. In these animal tests, the inclusion for comparative purposes of an in-house reference BCG vaccine prepared from a seed lot known to be effective in animals and humans is recommended.

Currently there is no biomarker which directly correlates to clinical efficacy of BCG vaccine. These Recommendations are intended to be used for ensuring the manufacture of consistent lots. This means that new lots should not significantly differ from those that have already been shown to be safe and effective in humans.

At present, for batch control purposes, much reliance is placed on tests for the estimation of the total bacterial content and for the number of culturable particles. It is not possible to specify single requirements for the total bacterial content and for the number of culturable particles for all vaccines (24), since different substrains and methods of manufacture may yield different specifications for these parameters. For example, although the number of culturable bacteria in a single human dose may differ for different vaccines, these vaccines may show satisfactory properties as regards their ability to induce adequate sensitivity to tuberculin and their safety in humans. It is therefore essential that clinical studies for dose optimization in humans be carried out to estimate suitable total bacterial contents and the number of culturable particles for a particular manufacturer's product. For a particular vaccine, the difference between the lower and upper specification for the number of culturable particles should not be larger than fourfold. In addition, it is necessary to perform animal experiments that give an indication of the safety and efficacy of the vaccines to the satisfaction of the NRA.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name should be "freeze-dried BCG vaccine". The proper name should be the equivalent of the international name in the language of the country of origin. The use of the international name should be limited to vaccines that satisfy the recommendations formulated below.

A.1.2 Descriptive definition

Freeze-dried BCG vaccine is a freeze-dried preparation containing live bacteria derived from a culture of the bacillus of Calmette and Guérin, known as BCG, intended for intradermal injection. The name of the freeze-dried vaccine intended for percutaneous vaccination, should be "freeze-dried BCG vaccine, percutaneous". The preparation should satisfy all the recommendations formulated below.

A.1.3 International Reference Preparations and Reference Reagents

The First WHO International Reference Preparation for BCG vaccine was established in 1965 and the First WHO International Standard for PPD of *M. tuberculosis* was established in 1951. Because of the age of these preparations, the need for replacements has been recognized, especially for the First WHO International Reference Preparation for BCG vaccine which is a live bacterial preparation. WHO has initiated the development of replacements. These were presented to the WHO Expert Committee on Biological Standardization in 2009 and 2010 as candidates for the First WHO International Reference Reagents for BCG vaccines of substrain Danish 1331, Tokyo 172-1 and Russian BCG-I (25, 26). These materials are available through the WHO web site (<http://www.who.int/entity/bloodproducts/catalogue/BlooFeb2013.pdf>). These International Reference Reagents cover the major proportion of BCG vaccine strains currently used in production. The establishment of substrain Moreau-RJ as the WHO International Reference Reagent for BCG vaccine is currently in progress and is scheduled for submission to the Committee in 2012 for adoption. These preparations are intended as International Reference Reagents, if required, for:

- periodical consistency monitoring of quantitative assays such as viability estimates (such as culturable particle count and modified ATP assays);
- residual virulence/local reactogenicity assays and protection assays in animal models for nonclinical evaluation.

They are also intended:

- as reference BCG substrains;
- for identity tests using multiplex polymerase chain reaction (PCR) as included in the collaborative study or in other molecular biology techniques.

The National Institute for Biological Standards and Control (NIBSC), England, distributes the WHO International Reference Reagents for BCG vaccines.

A.1.4 Terminology

The definitions given below apply to the terms as used in these Recommendations. They may have different meanings in other contexts.

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.

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.

In-house reference: a batch of vaccine prepared from the same BCG strain as the tested vaccine and used in parallel to the vaccine tested in:

- quantitative assays such as viability estimates (such as culturable particle count and modified ATP assays);
- residual virulence assays.

Master seed lot: a bacterial suspension of a single substrain originated from the bacillus of Calmette and Guérin that has been processed as a single lot and is of uniform composition. A seed lot should be maintained in the freeze-dried form stored at -20°C or below (in the liquid form it is stored at -80°C or below) in order to maintain viability. In each manufacturing establishment, a master seed lot is that from which material is drawn for inoculating media for the preparation of working seed lots or single harvests.

Single harvest: the material obtained from one batch of cultures that have been inoculated with the working seed lot (or with the inoculum derived from it), harvested and processed together.

Working seed lot: a quantity of bacterial organisms of a single substrain derived from the master seed lot by growing the organisms and maintaining them in aliquots in the freeze-dried form stored at -20°C or below (in the liquid form stored at -80°C or below). The working seed lot should be prepared from the master seed lot by as few cultural passages as possible (e.g. 3–6 passages from the master seed lot), having the same characteristics as the master seed lot and intended for inoculating media for the preparation of single harvests.

A.2 General manufacturing recommendations

The general manufacturing recommendations for manufacturing establishments contained in WHO's Good manufacturing practices: main principles for pharmaceutical products (27) and Good manufacturing practices for biological products (28) should apply to establishments manufacturing BCG vaccine. In addition, the compliance with current good manufacturing practices should apply with the addition of the following.

Details of standard operating procedures for the preparation and testing of BCG vaccines adopted by the manufacturer, together with evidence of appropriate validation of each production step, should be submitted for the approval of the NRA. As required, proposals for the modification of manufacturing and control methods should also be submitted for approval to the NRA before they are implemented.

The NRA should satisfy itself that adequate control of the manufacturing, shipping and storage of the BCG vaccine has been achieved. NRAs may consider

that a formal clinical lot-to-lot consistency study is not necessary if there are adequate and satisfactory data provided to support consistency of manufacture. However, several different lots of the product should be used in randomized studies and should elicit comparable immune responses in similar populations.

The degree of consistency in producing satisfactory final lots is an important factor in judging the efficacy and safety of a particular manufacturer's product.

The data that should be considered in determining the consistency of production should include the results obtained with consecutive vaccine lots when tested as described in Part A, section 6 (e.g. the test for viability in Part A, section 6.7, and the thermal stability test in Part A, section 6.8).

More than two consecutive vaccine lots should have been satisfactorily prepared before any vaccine from a given manufacturer, or resulting from a new method of manufacture, is released. In subsequent routine production, if a specified proportion of vaccine lots or a specified number of consecutive vaccine lots fails to meet the requirements, the manufacture of BCG vaccine should be discontinued and should not be resumed until a thorough investigation has been made and the cause or causes of the failures determined to the satisfaction of the NRA.

Conventionally, production of BCG vaccine should take place in a dedicated area, completely separate from areas used for production of other medicines or vaccines, and using dedicated separate equipment. Such areas should be so situated and ventilated that the hazard of contamination is reduced to a minimum. No animals should be permitted in the vaccine production areas. Tests for the control of vaccine that require cultures to be made of contaminating microorganisms should be carried out in a completely separate area. Tests in which animals are used should also be carried out in a completely separate area.

For the purposes of these requirements, the processes of vaccine production that should take place in dedicated facilities are all operations up to and including the sealing of the vaccine in the final containers.

In some countries, the production of BCG vaccine – although isolated – is carried out in a building in which other work takes place. This should be done only after consultation with, and with the approval of, the NRA. If production takes place in part of a building, the work carried out in other parts of the building should be of such a nature that there is no possibility of cross-contamination with the BCG vaccine.

No cultures of microorganisms other than the BCG vaccine strain approved by the NRA for vaccine production should be introduced into the manufacturing areas. In particular, no strains of other mycobacterial species, whether pathogenic or not, should be permitted in the BCG vaccine production area.

BCG is susceptible to sunlight. Therefore, the procedures for the preparation of the vaccine should be so designed that all cultures and vaccines are protected from direct sunlight and ultraviolet light at all stages of manufacture, testing and storage, until the vaccine is issued.

BCG vaccine should be produced by a staff consisting of healthy persons who do not work with other infectious agents; in particular, they should not work with virulent strains of *M. tuberculosis*, nor should they be exposed to a known risk of tuberculosis infection. Precautions should also be taken to ensure that no worker should be employed in the preparation of BCG vaccine unless he or she has been shown by medical examination to be free from TB. The scope and nature of the medical examination should be at the discretion of the NRA. It may include a radiological examination and/or a validated immunological blood assay that should be repeated at intervals or when there is reason to suspect illness. The frequency of radiological examination should be at the discretion of the NRA, taking into consideration the incidence of TB in the country.

It is advisable to keep radiation exposure to a minimum, but the examination should be of sufficient frequency to detect the appearance of early active TB. It is estimated that, if workers in BCG vaccine laboratories were given one or two conventional X-ray examinations of the chest each year, not using fluoroscopic methods, and if the best available techniques were employed to minimize the radiation dose, the doses received would be considerably lower than the maximum permissible doses for workers occupationally exposed to radiation that have been set by the International Commission on Radiological Protection (29, 30).

Should an examination reveal signs of TB or suspected TB in a worker, he or she should no longer be allowed to work in the production areas and the rest of the staff should be examined for possible TB infection. In addition, all cultures should be discarded and the production areas decontaminated. If it is confirmed that the worker has TB, all vaccine made while he or she was in the production areas should be discarded, and all distributed batches should be recalled.

Persons not normally employed in the production areas should be excluded from them unless, after a medical examination, including radiological examination, they are shown to be free from TB. In particular, persons working with mycobacteria other than the BCG seed strain should be excluded at all times.

A.3 Control of source materials

A.3.1 Seed lot system

The production of vaccine should be based on the seed lot system. A seed lot prepared from a strain approved by the NRA (see Part D, section 1.1) should be prepared under conditions satisfying the requirements of Part A, sections 2, 3 and 4.

The BCG vaccine strain used should be identified by historical records that include information on its origin and subsequent manipulation. It would be preferable for the master seed lot to have protection proven clinically through clinical studies on a batch derived from it by a production process that is representative of the commercial process. It is also recommended to use a batch derived from such a clinically “validated” seed lot as an in-house reference in the laboratory to help ensure consistency in production.

If a working seed lot is being used, the total number of passages for a single production harvest should not exceed 12, including the passages necessary for preparing the working seed lot.

A.3.2 Tests on seed lot

A.3.2.1 Antimicrobial sensitivity test

An antimicrobial sensitivity test should be carried out as part of the ongoing characterization of BCG vaccine strains. It would be appropriate to test this property at the level of master or working seed lot.

A.3.2.2 Delayed hypersensitivity test

When a new working seed lot is established, a suitable test for delayed hypersensitivity in guinea-pigs is carried out; the vaccine is shown to be not significantly different in activity from the in-house reference.

A.3.2.3 Identity test

The bacteria in the master and working seed lots are identified as *M. bovis* BCG using microbiological techniques (e.g. morphological appearance of the bacilli in stained smears and the characteristic appearance of the colonies grown on solid media). Manufacturers are encouraged to carry out the test using molecular biology techniques (e.g. PCR test) to identify the specific substrain of BCG. The techniques will also provide relevant information to ensure genetic consistency in production, from master seed through working seed and to final product (4).

A.3.2.4 Test for bacterial and fungal contamination

Each master and working seed lot should be tested for bacterial and fungal contamination by appropriate tests, as specified in Part A, section 5.2 of General requirements for the sterility of biological substances (31), or by the validated methods approved by the NRA.

A.3.2.5 Test for absence of virulent mycobacteria

The test for absence of virulent mycobacteria, described in Part A, section 4.2.3, should be made in at least 10 healthy guinea-pigs injected with a quantity of

vaccine not less than 50 single human doses and should be observed for at least six weeks. If none of the animals shows signs of progressive TB and at least 90% survive the observation period (i.e. should one of the 10 animals die), the seed lot should be considered to be free from virulent mycobacteria.

If more than 10% of the guinea-pigs die during the observation period (i.e. should two out of 10 animals die) and freedom from progressive TB disease is verified, the test should be repeated on at least 10 more guinea-pigs. On the second occasion, the seed lot passes the test if not more than 10% of the animals die during the observation period (i.e. should one of the 10 animals die) and the autopsy does not reveal any sign of TB.

A.3.2.6 Test for excessive dermal reactivity

The test for excessive dermal reactivity, described in Part A, section 6.4.2, should be made in six healthy guinea-pigs, each weighing not less than 250 g and having received no treatment likely to interfere with the test. Each guinea-pig should be injected intradermally, according to a randomized plan, with 0.1 ml of the reconstituted vaccine and of vaccine dilutions 1:10 and 1:100. The same dilutions of the appropriate international Reference Reagent or in-house reference should be injected into the same guinea-pigs at randomly selected sites. The guinea-pigs should be observed for at least four weeks. The vaccine complies with the test if the reactions it produces at the sites of injection are not markedly different from those produced by the appropriate international Reference Reagent or in-house reference.

A.3.3 Production culture medium

The production culture medium should contain no substances known to cause toxic or allergic reactions in humans. The use of material originating from animals should be discouraged. However, if constituents derived from animals are necessary, approval of the NRA should be sought and the materials should comply with current policy on TSEs (32–37). A risk assessment for TSE would need to be included for the materials of the culture medium. WHO's revised *Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (32) provide guidance on risk assessments for master and working seeds and should be consulted. Substances used in that medium should meet such specifications as the NRA may prescribe.

A.4 Control of vaccine production

A.4.1 Control of single harvests

All cultures should be examined visually, and any that have grown in an uncharacteristic manner should not be used for vaccine production.

A.4.2 Control of final bulk

A.4.2.1 Final bulk

The final bulk should be prepared from a single harvest or by pooling a number of single harvests.

A.4.2.2 Test for bacterial and fungal contamination

The final bulk should be tested for bacterial and fungal contamination by appropriate tests as specified in Part A, section 5.2 of the General requirements for the sterility of biological substances (31), or by the validated methods approved by the NRA. No vaccine lot should be passed for use unless the final bulk has been shown to be free from such contamination.

A.4.2.3 Test for absence of virulent mycobacteria

The test for absence of virulent mycobacteria should be carried out on each final bulk or final lot.

At least six healthy guinea-pigs, all of the same sex, each weighing 250–400 g should be used. They should not have received any treatment or diet, such as antibiotics, that is likely to interfere with the test. A sample of the final bulk intended for this test should be stored at 4 °C for not more than 72 hours after harvest.

A dose of BCG organisms corresponding to at least 50 single human doses of vaccine intended for intradermal injection should be injected into each guinea-pig by the subcutaneous or intramuscular route.¹ The guinea-pigs should be observed for at least six weeks. If, during that time, they remain healthy, gain weight, show no signs of progressive TB and not more than one dies, the final bulk should be considered to be free from virulent mycobacteria.

At the end of the observation period, the animals should be killed and examined postmortem for macroscopic evidence of progressive TB disease. Similarly, any animals that die before the end of the observation period should be subjected to a postmortem examination.

Should one third of the guinea-pigs die (i.e. should two out of six animals die) during the observation period (and freedom from progressive TB disease is verified), the test should be repeated on at least six more guinea-pigs. On the second occasion, the vaccine lot passes the test if not more than one

¹ When a more concentrated vaccine, intended for administration by the percutaneous route, is tested, a dilution factor approved by the NRA should be applied so that the mass of BCG injected corresponds to at least 50 human doses of intradermal vaccine.

animal dies during the observation period and the autopsy does not reveal any sign of TB.

Should a vaccine lot fail to satisfy the requirements of this test because animals die from causes other than TB, the procedure to be followed by the manufacturer should be determined with the approval of the NRA.

If signs of TB disease are seen, the vaccine lot should be rejected, all subsequent vaccine lots should be withheld, and all current vaccine stocks should be held pending further investigation. The manufacture of BCG vaccine should be discontinued and it should not be resumed until a thorough investigation has been made and the cause or causes of the failure determined and appropriate actions have been taken. Production should be allowed to resume only upon the approval of the NRA.

A.4.2.4 **Test for bacterial concentration**

The bacterial concentration of the final bulk should be estimated by a validated method approved by the NRA and should have a value within a range approved by the NRA (see Part D, section 1.2).

Based on manufacturers' experience, the opacity method is the method of choice. The International Reference Preparation of Opacity,² or an equivalent Reference Preparation approved by the NRA, may be employed in comparative tests.

Clumping issues should be considered during validation of the assay.

A.4.2.5 **Test for number of culturable particles**

The number of culturable particles on a solid medium of each final bulk should be determined by an appropriate method approved by the NRA. Alternatively, a bioluminescence or other biochemical method can be used (38, 39), provided that the method is properly validated against the culturable particle test for the production step in question. If properly validated, such tests can be used as equivalent methods. Regular calibration with the reference method as agreed with the NRA would be relevant.

The medium used in this test should be such that the number of culturable particles may be determined at an optimal time point (usually 3–5 weeks) after the medium has been inoculated with dilutions of the vaccine.

² The International Reference Preparation of Opacity is in the custody of the NIBSC, Potters Bar, England, which supplies samples on request.

There are various methods of determining the number of culturable particles in BCG vaccine, and it is essential that only one culture method be used for all the vaccine lots produced by a manufacturer (5). It is also desirable for assay validation that the clumping issue should be considered and that tests should be carried out in parallel with the appropriate international Reference Reagent or in-house reference, e.g. the same vaccine production that has been used in clinical trials and has assured safety (including immunogenicity) and efficacy.

A.4.2.6 Substances added to the final bulk

Substances used in preparing the final bulk should meet such specifications as the NRA may prescribe. In particular, the NRA should approve the source(s) of any animal-derived raw materials which should comply with the *WHO Guidelines on tissue infectivity distribution in transmissible spongiform encephalopathies* (34).

Substances added to improve the efficiency of the freeze-drying process or to aid the stability of the freeze-dried product should be sterile and of high and consistent quality, and should be used at suitable concentrations in the vaccine.

A.5 Filling and containers

The general requirements concerning filling and containers given in Good manufacturing practices for biological products (28) should apply to vaccine filled in the final form.

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

A.6 Control tests on final lot

Tests on the final lot should be performed after reconstitution, except for appearance and residual moisture tests. The diluent supplied or recommended for reconstitution should be used, unless such diluent would interfere with any of the tests, in which case some other suitable fluid should be used. The vaccine should be reconstituted to the concentration at which it is to be used for injection into humans; however, an exception may be made in the case of the test for absence of virulent mycobacteria (Part A, section 6.4.1), when a higher concentration of reconstituted vaccine may be necessary. It would be appropriate to monitor periodically the antimicrobial sensitivity in final lots.

A.6.1 Inspection of final containers

Every container in each final lot should be inspected visually, and those showing abnormalities should be discarded.

The appearance of the freeze-dried vaccine and the reconstituted vaccine should be described with respect to form and colour. If reconstitution with the product diluent does not allow for the detection of particulates, an alternative diluent may be used.

A.6.2 Identity test

An identity test should be performed on samples of the vaccine from each final lot. The identity test for final lots should be used to identify the product as BCG as approved by the NRA. The identity of each final lot of vaccine should be verified by the morphological appearance of the bacilli in stained smears and by the characteristic appearance of the colonies grown on solid media. A validated nucleic acid amplification technique (such as PCR) should preferably be used.

A.6.3 Test for bacterial and fungal contamination

Samples from each final lot should be tested for bacterial and fungal contamination by appropriate tests as specified in Part A, section 5.2 of the General requirements for the sterility of biological substances (31), or by the validated methods approved by the NRA.

A.6.4 Safety tests

A.6.4.1 Test for absence of virulent mycobacteria

Provided the test for virulent mycobacteria has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

If the test for the absence of virulent mycobacteria, applied to the final bulk, is unsatisfactory (and freedom from progressive TB disease is verified), it should be repeated with a sample of a final lot (see Part A, section 4.2.3).

A.6.4.2 Test for excessive dermal reactivity

Provided the test has been carried out with satisfactory results on the working seed lot and on at least three consecutive final lots produced from it, the test may be omitted on the final lot.

Historically the omission of the test with satisfactory results on five consecutive final lots has been accepted by the authorities (40).

A.6.5 Test for bacterial concentration

The total bacterial content of the reconstituted vaccine should be estimated for each vaccine lot by a validated method approved by the NRA, and should have a value within a range approved by the NRA (see Part D, section 1.2).

The estimation of total bacterial content may be made either directly, by determining the dry weight of organisms, or indirectly by an opacity method that has been calibrated in relation to the dry weight of the organisms.

The clumping issue should be considered during validation of the assay.

A.6.6 **Test for residual moisture**

The average moisture content of a freeze-dried vaccine should be determined by a validated method accepted by the NRA. Values should be within limits of the preparations shown to be adequately stable in the stability studies of the vaccine.

A.6.7 **Tests for viability**

A.6.7.1 **Test for number of culturable particles**

The number of culturable particles of each final lot should be determined by an appropriate method approved by the NRA (see Part A, section 4.2.5). The viable count should have a value within a range approved by the NRA that should not be wider than a fourfold difference between the lower and upper levels of the specification for numbers of culturable particles (see Part D, section 1.2). By comparison with the results of the test for number of culturable particles carried out on final bulk, as described in Part A, section 4.2.5, the percentage survival on freeze-drying may be calculated and this value should be not less than one approved by the NRA. The appropriate international Reference Reagent or in-house reference should be used for every test in order to validate the assay.

The purpose of including the appropriate international Reference Reagent or in-house reference is to have a check on the quality and consistency of the culture medium and the accuracy of the technique used for the determination of the number of culturable particles. It is not intended to adjust the count of the vaccine by comparison with the Reference Preparation.

The clumping issue should be considered during validation of the assay.

The survival rate after freeze-drying is usually not less than 20%.

A.6.7.2 **Rapid test for viability**

As an alternative to the colony counting method, a bioluminescence or other biochemical method can be used provided that the method is properly validated against the culturable particle test for the production step in question. If properly validated, such tests may be considered by the NRA to replace the culturable particle test.

The bioluminescence reaction occurring in fireflies depends on the presence of adenosine triphosphate (ATP), luciferin luciferase, oxygen and magnesium ions. This reaction can be reproduced *in vitro* by mixing these components. If all components except ATP are present in excess, the amount of light emitted is proportional to the amount of ATP coming from the vaccine.

Since ATP is present in all living cells and is immediately destroyed when the cell dies, ATP is a reliable marker for living cells.

Studies have shown that, if properly validated, measurement of ATP using the bioluminescence reaction can be used to estimate the viable count of freeze-dried BCG vaccine within 1–2 days as accurately as other, more time-consuming methods, once the mean content of ATP per culturable particle has been estimated for a given vaccine production.

A.6.8 Thermal stability test

The thermal stability test is part of the characterization and consistency demonstration of vaccine production. The requirement for this test should be at the discretion of the NRA and, if required, each final lot should be tested for thermal stability by a validated method approved by the NRA. If the production consistency is demonstrated, this test may be omitted on the final lot subject to NRA approval (6).

If performed, the test should involve the determination of the number of culturable particles before and after the samples have been held at appropriate temperatures and for appropriate periods.

For example, the thermal stability test may be carried out by taking samples of the vaccine and incubating them at 37 °C for 28 days.

The percentage decrease in the number of culturable particles is then compared with that of samples of the same vaccine lot stored at 2–8 °C. The number of culturable particles in the vaccine after heating should be not less than 20% of that stored at 2–8 °C (41). The absolute value should be approved by the NRA. The viability test should also be performed with the appropriate international Reference Reagent or in-house reference for checking validity of the assay. One method of determining the number of culturable particles should be adhered to, as suggested in Part A, section 4.2.5.

The purpose of including the appropriate international Reference Reagent or in-house reference is to check the quality and consistency of the medium used for determination of the number of culturable particles. It is not intended to adjust the count of the vaccine by comparison with the Reference Preparation.

All manufacturers should keep their product for the approved storage period and should determine the number of culturable particles from time to time to demonstrate that the number is being maintained at an adequate level.

In some countries, the thermal stability test is carried out only after the vaccine has been stored for 3–4 weeks after freeze-drying, since it is considered that the degree of stability during the first three weeks may not be related to the long-term stability of the product.

As a guide to stability, some manufacturers of freeze-dried BCG vaccine determine the residual moisture content of the final vaccine, since failure to achieve a certain degree of desiccation results in an unstable product. However, such a test cannot be regarded as an alternative to tests involving the determination of the number of culturable particles.

A.7 Records

The recommendations in section 8 of Good manufacturing practices for biological products (28) should apply.

Written records should be kept of all seed lots, all cultures intended for vaccine production, all single harvests, all final bulk vaccines, and all vaccine in the final containers produced by the manufacturing establishments, including all tests irrespective of their results.

The records should be of a type approved by the NRA. An example of a suitable protocol is given in Appendix 2.

A.8 Retained samples

The recommendations in section 9.5 of Good manufacturing practices for biological products (28) should apply.

It is desirable that samples should be retained for at least one year after the expiry date of the final lot.

A.9 Labelling

The recommendations in section 7 of Good manufacturing practices for biological products (28) should apply, including the following guidance.

The label, and/or the packaging insert in some countries, printed on or affixed to each container should show the volume and nature of the diluent. Also, this label, or the label on the carton holding several final containers, or the leaflet accompanying the containers, should carry the following additional information:

- the fact that the vaccine fulfils the requirements of this document;
- instructions for use of the vaccine and information concerning contraindications and the reactions that may follow vaccination;

- the volume and nature of the diluent to be added to reconstitute the vaccine, specifying that only the diluent supplied by the manufacturer should be used;
- the conditions recommended during storage and transport, with information on the reduced stability of the vaccine if exposed to temperatures higher than that stated on the label;
- warnings that the vaccine should be protected from direct sunlight;
- a statement that the reconstituted vaccine should be used as soon as possible, or should be stored at 2–8 °C, protected from direct sunlight and used within six hours (42);
- information on antimicrobial sensitivity.

The label for the diluent should state “Reconstituting fluid for BCG vaccine [proprietary name]”.

A.10 **Distribution and transport**

The recommendations given in section 8 of Good manufacturing practices for biological products (28) should be followed, along with the guidance provided in *Safe vaccine handling, cold chain and immunizations* (43). Further guidance is provided in Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (44).

It is desirable that samples should be retained for at least one year after the expiry date of the final lot.

A.11 **Stability, storage and expiry date**

A.11.1 **Stability testing**

Adequate stability studies form an essential part of vaccine development. The recommendations provided in WHO’s Guidelines for stability evaluation of vaccines should be followed (46). Stability testing should be performed at different stages of production if stored for a given time period, namely as appropriate for single harvests or pool of single harvests, final bulk or final lot. Stability-indicating parameters should be defined or selected appropriately according to the stage of production. It is advisable to assign a storage period to all in-process materials during vaccine production, particularly intermediates such as single harvests and final bulk, and a shelf-life period to the final lots.

BCG vaccines require special precautions to ensure sufficient stability. In this connection the most important measures are lyophilization, the use of an effective stabilizer, and proper sealing of vaccine containers.

Historically the use of ampoules sealed under vacuum was the most common practice for increasing stability. However, vacuum-sealing is difficult compared to sealing in the presence of inert gas. There were no significant differences between BCG vaccines sealed under vacuum and under nitrogen or carbon dioxide at either 4 °C or 37 °C (41). Manufacturers now prepare BCG vaccines in vials/ampoules and, under well-validated conditions, the product is adequately stable.

A.11.2 Storage conditions

The *Guideline for establishing or improving primary and intermediate vaccine stores* (47) should apply.

Storage conditions should be based on stability studies and approved by the NRA. Before being distributed by the manufacturing establishment, or before being issued from a depot for the storage of vaccine, all vaccines in their final containers should be stored constantly at 2–8 °C (41, 48) and vaccine diluents should be stored as recommended by the manufacturer. Freeze-dried BCG vaccines, regardless of their substrain, are sensitive to ultraviolet and fluorescent light. They should be protected from direct sunlight (41).

BCG vaccines are sensitive to light as well as to heat. Normally, these vaccines are supplied in vials/ampoules made from dark brown glass, which gives them some protection against light damage, but care should still be taken to keep them covered and protected from strong light at all times (48).

Freeze-dried BCG vaccines may be kept frozen at –15 °C to –25 °C if cold chain space permits, but this is neither essential nor recommended (41).

Precautions should also be taken to maintain the vaccine during transport and up to the time of use at the temperature and under the storage conditions recommended by the manufacturer.

A.11.3 Expiry date

The expiry date should be approved by the NRA and should be based on the stability of the final product, as well as on the results of the stability tests referred to in section A.11.1 above. It is established for each batch by adding the shelf-life period to the date of manufacture. Most freeze-dried BCG vaccines are stable at temperatures of 2–8 °C for at least two years (41) from the date of manufacture. The storage of final product at –20 °C to extend the shelf-life should be validated.

A.11.4 Expiry of reconstituted vaccine

Stability studies should be undertaken on reconstituted vaccine. Freeze-dried BCG vaccines become much more heat-sensitive after they have been

reconstituted with diluent (41). After multidose containers of freeze-dried BCG have been reconstituted, the vaccine should be used as soon as possible. Any reconstituted vaccine remaining should be stored at 2–8 °C until used, and the expiry time should be defined by stability studies (4, 42, 45).

Part B. Nonclinical evaluation of BCG vaccines

Details on the design, conduct, analysis and evaluation of nonclinical studies are available in the WHO Guidelines on nonclinical evaluation of vaccines (49).

Nonclinical testing of a new strain (i.e. a strain derived by selection from existing BCG strains in Appendix 1) or of a strain from a new manufacturer of a BCG vaccine is a prerequisite for initiation of clinical studies in humans. Nonclinical testing includes immunogenicity, protection studies (proof of concept) and safety testing in animals. The vaccine lots used in nonclinical studies should be adequately representative of the formulation intended for clinical investigation and, ideally, should be the same lots, manufactured according to current good manufacturing practice (cGMP), as those used in clinical studies. If this is not feasible, the lots used clinically should be comparable to those used in the nonclinical studies with respect to potency, stability and other characteristics of quality. The technical manufacturing consistency lots may often be used for these purposes.

New manufacturers of BCG vaccine for human use will need to refer to the range of nonclinical safety and characterization tests that are recommended for existing licensed BCG vaccines. Although there is currently no requirement for additional nonclinical testing beyond that already described for licensed BCG vaccines, the development of new variants of BCG, the potential for new fermentation technologies and the possibility of novel live vaccines against TB have shown that additional nonclinical studies beyond that required for licensed BCG vaccine can be helpful in demonstrating that a new BCG product has satisfactory nonclinical efficacy, safety and stability.

Guideline example on protective potency testing: Hartley guinea-pigs are used for potency testing. The guinea-pigs are vaccinated with a small amount of BCG (~10³ colony-forming units (CFU)). Eight weeks after the vaccination, the guinea-pigs are challenged with virulent *M. tuberculosis* H37Rv (ATCC 27294) by the pulmonary route with a low dose (10–15 CFU) per animal. Five weeks after the infection, the guinea-pigs are killed and the spleen and the lung lobes are removed. These organs are homogenized separately. Appropriate dilutions are inoculated on to duplicate solid medium and incubated at 37 °C for three weeks. The number of *M. tuberculosis* H37Rv colonies is counted, and is expressed as mean log₁₀ CFU per tissue. The CFU results are compared between the vaccinated and non-vaccinated groups (50).

If there are two pharmacologically relevant species for the clinical candidate (one rodent and one non-rodent), both species should be used for short-term (up to one month duration) toxicology studies. If the toxicological findings from these studies are similar in both species, longer-term studies in one species are usually considered sufficient; the rodent species should be considered unless there is a rationale for using non-rodents. Studies in two non-rodent species are not appropriate. Other *in vivo* studies should address both potency (such as tuberculin sensitivity and immunological tests) and safety issues (such as tests for excessive dermal reactivity and absence of virulent mycobacteria) of the classical BCG vaccines.

It may be of benefit for new BCG vaccine developers to consider the points raised in recent meetings establishing recommendations for new live vaccines against TB (51, 52).

Part C. Clinical evaluation of BCG vaccines

Clinical trials should adhere to the principles described in the Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (53) and the general principles described in WHO's Guidelines on clinical evaluation of vaccines: regulatory expectations (54). All clinical trials should be approved by the relevant NRAs and local ethics committees. Continued licence of BCG vaccines should be viewed in the light of ongoing post-marketing data on the safety, immunogenicity and effectiveness of BCG vaccines in the target population.

Part C considers the provision of clinical data required (a) when a new candidate "classical" BCG vaccine derived from (the same master seed of) one of the recognized strains (see Appendix 1) is developed; (b) when there have been major changes to the manufacturing process of an established vaccine, including preparation of a new master seed lot of an established strain; (c) when technology transfer of existing vaccine is planned to a new manufacturer; and (d) when revalidation of existing vaccines used in national immunization programmes is considered.

Vaccines manufactured using a "new strain" (i.e. a strain derived by selection from existing BCG strains in Appendix 1) should require a full clinical development programme that provides evidence of safety, efficacy and the reactogenicity profile in all target age-groups.

Other vaccines against *M. tuberculosis* derived from *M. bovis* or other mycobacterial strains cannot be considered as BCG. They would require a full clinical development programme and are not included here.

C.1 General considerations

C.1.1 Comparative or placebo-controlled clinical trials

It would not be considered ethical to conduct a placebo-controlled trial of protective efficacy of a BCG vaccine in a TB-endemic area, particularly in

infants. A comparative trial with a licensed, or internationally accepted (WHO prequalified), BCG vaccine could be accepted.

C.1.2 Value of PPD response

It is recognized that the response to tuberculin PPD is not an indicator of a protective immune response. Nonetheless this has been used for more than 50 years to indicate a cellular immune response to an infection with *M. tuberculosis* or as evidence of “successful” BCG vaccination. At best, a PPD reaction is an indicator of exposure to antigens of TB, and the generation of a cellular immune response. Thus, it can be used in a PPD-naive population as an indicator of an immune response to the BCG vaccine (55). Other immunological measures may be more closely related to *M. tuberculosis* infection or vaccination, but currently none has been agreed as a correlate of protection from infection or disease.

C.1.3 BCG in HIV-infected infants

A very important safety consideration with regard to vaccination policy is to establish, during clinical trials, the potential for disseminated BCG disease in immunocompromised children. In this regard, the use of BCG vaccines at birth should follow the recommendations from the GACVS (13, 14). The GACVS recommendations consider the policies for immunization exclusion of infants known to be infected with HIV, infants symptomatic for HIV infection, and those infants born to mothers known to be HIV-infected and who therefore may be infected.

C.1.4 Post-vaccination reactions and complications

Vaccines intended for intradermal or percutaneous injection should be given strictly intradermally or percutaneously, and vaccinators should be trained accordingly. Incorrect vaccination technique can result in adverse reactions, including discharging ulcers, abscesses and keloid scars.

Current BCG vaccines have a known reactogenicity profile after intradermal inoculation (56). Local reaction at the vaccination site is normal after a BCG vaccination. It may take the form of a nodule that, in many cases, will break down and suppurate. The reaction developing at the vaccination site usually subsides within 2–5 months and in practically all children leaves a superficial scar of 2–10 mm in diameter. The nodule may persist and ulcerate. Swelling of regional lymph nodes may also be seen, and this may be regarded as a normal reaction, but the size should be limited.

Keloid and lupoid reactions may occur at the site of the vaccination. Children with such reactions should not be revaccinated. Inadvertent subcutaneous injections produce abscess formations and may lead to ugly retracted scars. Among the major complications, suppurative lymphadenitis has been observed.

In the case of certain vaccines, it has been revealed that there is a strong correlation between the incidence of these complications in newborns and the number of culturable particles in the vaccine.

The concentration of the vaccine should be shown to be effective and tolerated in the age groups for which the vaccine is intended.

A reduction of the dose for newborns may be based on the evidence and approved by the NRA (57).

The NRA should issue guidelines for the treatment of complications.

C.2 Special considerations

This section is limited to the clinical development of new “classical” BCG vaccines manufactured following these Recommendations and using strains of BCG that are derived from (the same master seed of) one of the strains recognized in Appendix 1.

The use of comparative studies with a licensed BCG vaccine can provide evidence of the similarity of safety and immune responses to a new classical BCG vaccine product.

The target population for the vaccine would be newborns or infants according to current recommendations on the use of BCG vaccines.

The nonclinical expectations for a new classical BCG vaccine are outlined in Part B.

For such a new classical BCG vaccine, these nonclinical studies should be conducted in comparison to an existing licensed BCG vaccine, preferably derived from the same BCG substrain. It would be expected that the results of preclinical studies would be similar for the new vaccine product and for the comparator.

The clinical development programme should ideally be designed to show the safety and protective efficacy of the vaccine. However, for such a new classical BCG vaccine product, comparative studies with an existing licensed BCG vaccine, using immunological responses as a marker for efficacy, may be acceptable to the responsible NRA.

Comparable PPD response (proportion of PPD converters, intensity of response) may be acceptable.

Clinical studies should provide evidence of safety in all the potential target populations, including those with a high incidence of diseases that may affect the safety or efficacy of the new vaccine product. In phase I and phase II studies this should include evaluation of:

- safety and reactogenicity in healthy adults (comparative);

- end-points;
- safety and reactogenicity – can include healthy HIV-infected adults;
- immune responses – non-inferior PPD response, and may include other immunological markers.

These studies are difficult to interpret as adults will most likely have received BCG vaccination at birth. Dose-finding studies may be considered unnecessary for these vaccines. The safety in HIV-infected individuals and in infants needs to be considered.

Dose-finding and age de-escalation can be included in these studies, but review at each step by a suitable independent safety committee should be considered.

In phase III studies evaluation should be made of:

- safety and reactogenicity in infants (comparative)
- end-points
- safety and reactogenicity
- non-inferior PPD immune response.

Post-marketing risk management

As it may not be practically possible to evaluate protective efficacy for a new classical BCG vaccine, the responsible NRA in the country of manufacture should require post-marketing surveillance activities for safety and effectiveness in a suitable environment. Sentinel surveillance sites in an endemic country may be considered.

In the past, the responsible NRA of a country of manufacture required a demonstration that adequate control of BCG vaccine had been achieved, by arranging for studies of some of the final lots to be performed at regular intervals in children.

Studies of immunological responses to *M. tuberculosis* antigens should be made, such as sensitivity to tuberculin. In at least 100 tuberculin-negative persons per year, records of tuberculin-induced reaction (distribution of tuberculin reactions by size) by a defined dose of tuberculin³ in vaccines, local skin lesions (nature and size of reaction at injection site), and the occurrence of untoward vaccination reactions should be obtained. Such tests should be performed in parallel on two or more vaccine lots in the same population group, one of the vaccine lots being preferably a reference vaccine.

³ An intradermal test with a dose of tuberculin equivalent to 5 IU of tuberculin PPD is suitable. A description of an appropriate method and a design for a study to assess BCG vaccines in humans are available on application to the World Health Organization, 1211 Geneva 27, Switzerland.

C.3 Post-marketing surveillance

The NRA in the country of manufacture may require periodic updates of reports on BCG safety and immunogenicity.

C.3.1 BCG vaccine used in a national immunization programme

As in all immunization programmes, the adverse events following immunization with BCG vaccines should be monitored and recorded.

The following events are important after BCG vaccination (58):

- injection site abscesses
- BCG lymphadenitis
- disseminated BCG diseases
- osteitis/osteomyelitis.

Appropriate training of health-care workers is important as some medical incidents can be related to immunization even if they have a delayed onset.

C.3.2 WHO prequalified BCG vaccines

Prequalified vaccines may be used in a wide range of countries worldwide. Periodic safety update reports supplied to WHO should include specific analysis of countries where the vaccine has been used.

Part D. Guidelines for NRAs

D.1 General

The general recommendations for NRAs provided in the Guidelines for national authorities on quality assurance for biological products (59) should apply. These specify that no new biological substance should be released until consistency of manufacturing and quality as demonstrated by a consistent release of batches has been established. The detailed production and control procedures, as well as any significant change in them that may affect the quality, safety or efficacy of BCG vaccine, should be discussed with and approved by the NRA. For control purposes, the NRA should obtain the WHO International Reference Reagents as comparators for potency-related testing and, where necessary, should establish national working Reference Preparation(s) calibrated against the international reference.

In addition, the NRA should provide a reference vaccine or approve one used by a manufacturer, and should give directions concerning the use of the reference vaccine in specified tests. The NRA should also give directions to

manufacturers concerning the BCG substrain to be used in vaccine production, the total content of bacteria, the number of culturable particles, and the stability required of the vaccine, and should specify the requirements to be fulfilled by the manufacturer in accordance with the provisions of Part A of this document, including those for consistency of quality in respect of the points referred to in Part A, section 2.

D.1.1 BCG vaccine strain

The substrain of BCG (maintained in the form of a seed lot) used in the production of vaccine should be derived from the original strain maintained by Calmette and Guérin and should be identified by historical records that include information on its origin and subsequent manipulation. On the basis of cultures and biochemical and animal tests, the BCG seed lot should show characteristics that conform to those of BCG and generally differ from those of other mycobacteria. The identity test should be supplemented by molecular biology techniques to identify the specific BCG substrain used. The seed lot should show consistency in the morphological appearance of colonies and genetic stability on serial subculture. It should also have been shown to yield vaccines that, upon administration by intradermal injection to children and adults, induce relevant immunological responses to *M. tuberculosis* antigens, including sensitivity to tuberculin, and with a low frequency of untoward effects. In addition, the seed lot should have been shown to give adequate protection against TB in experimental animals in tests for protective potency.

D.1.2 Concentration of BCG vaccine

The concentration of BCG vaccine varies with different vaccine products and is dependent on a number of factors, such as the substrain of BCG used and the method of manufacture. It is therefore essential for each manufacturer, as well as for each method of manufacture, for the optimum potency of vaccine to be ascertained by trials in tuberculin-negative subjects (newborns, older children, and adults) to determine the response to vaccination in respect of the induction of relevant immunological responses to *M. tuberculosis* antigens – including sensitivity to tuberculin, the production of acceptable local skin lesions, and the occurrence of a low frequency of untoward reactions. As a result of such trials, the NRA should give directions to the manufacturer concerning the total bacterial content and the number of culturable particles required for the vaccine.

If a manufacturer changes its procedure for preparing BCG vaccine, and if the NRA considers that the change might affect the final product, it may be necessary to conduct further clinical trials in order to determine the optimum content of BCG organisms in the new product.

D.2 Release and certification

A vaccine lot should be released only if it fulfils the national requirements and/or Part A of these Recommendations. Before any vaccine lot is released from a manufacturing establishment, the recommendations for consistency of production provided in the Guidelines for national authorities on quality assurance for biological products (59) should be met. In addition, the general recommendations for NRAs provided in the Guidelines for independent lot release of vaccines by regulatory authorities (60) should be followed. A protocol based on the model given in Appendix 2, signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for release of a vaccine for use.

A statement signed by the appropriate official of the NRA (or authority as appropriate) should be provided if requested by a manufacturing establishment and should certify whether or not the lot of vaccine in question meets all national requirements, as well as Part A of these Recommendations. The certificate should also state the date of manufacture, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, the date of the last satisfactory potency test, as well as the expiry date assigned on the basis of shelf-life, should be stated. A copy of the official national release document should be attached. The certificate should be based on the model given in Appendix 3. The purpose of the certificate is to facilitate the exchange of vaccines between countries.

Authors and acknowledgements

The scientific basis for the revision of the Requirements published in WHO Technical Report Series, Nos. 745 and 771 was developed at meetings from 2003 to 2007 attended by:

Dr L. Barker, Aeras Global Tuberculosis Vaccine Foundation, Rockville, MD, USA; Professor M. Behr, Montreal General Hospital, Quebec, Canada; Dr T. Bektimirov, Tarashevich State Research Institute for Standardization and Control of Medical Biological Preparations, Moscow, Russian Federation; Dr T. Brewer, Brigham and Women's Hospital, Boston, MA, USA; Dr R. Brosch, Institut Pasteur, Paris, France; Dr L.R. Castello-Branco, Fundacao Atauilpho de Paiva, Brazilian League Against Tuberculosis, Rio de Janeiro, Brazil; Dr M. Chouchkova, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria; Dr S. Cole, Institut Pasteur, Paris, France; Dr M. Corbel, National Institute for Biological Standards and Control, Potters Bar, England; Dr J. Darbyshire, MRC Clinical Trials Unit, London, England; Dr J. Daviaud, World Health Organization, Geneva, Switzerland; Dr H. Dockrell, London School of Hygiene and Tropical Medicine, London, England; Dr U. Fruth, World Health Organization, Geneva, Switzerland; Mr I. Fujita, Japan BCG Laboratory, Tokyo, Japan; Dr M. Gheorghiu,

Institut Pasteur, Paris, France; Dr E. Griffiths, Health Canada, Ottawa, Canada; Dr C. Gutierrez Pèrez, Institut Pasteur, Paris, France; Dr K. Haslov, Statens Serum Institut, Copenhagen, Denmark; Dr M.M. Ho, National Institute for Biological Standards and Control, Potters Bar, England; Dr S. Jadhav, Serum Institute of India Ltd, Pune, India; Dr S.E. Jensen, Statens Serum Institut, Copenhagen, Denmark; Dr I. Knezevic, World Health Organization, Geneva, Switzerland; Dr M. Lagranderie, Institut Pasteur, Paris, France; Mr F. Leguellec, Aventis Pasteur, Val de Reuil, France; Dr Y. Lopez Vidal, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico; Dr G. Marchal, Institut Pasteur, Paris, France; Dr H. Mayanja-Kizza, Makarere Medical School, Kampala, Uganda; Dr S. Mostowy, McGill University Health Centre, Montreal General Hospital, Montreal, Quebec, Canada; Dr M. Roumiantzeff, Lyons, France; Dr M. Seki, BCG Laboratory, Tokyo, Japan; Dr V. Vincent, Institut Pasteur, Paris, France; Dr F. Weichold, Aeras Global Tuberculosis Vaccine Foundation, Rockville, MD, USA; Dr D. Wood, World Health Organization, Geneva, Switzerland; Dr S. Yamamoto, National Institute of Infectious Diseases, Tokyo, Japan; Dr I. Yano, Japan BCG Laboratory, Tokyo, Japan; Dr D. Young, Imperial College of Science, Technology & Medicine, London, England; Dr T. Zhou, World Health Organization, Geneva, Switzerland.

The first draft was prepared by Dr H-N. Kang, World Health Organization, Geneva, Switzerland, taking into account comments received from:

Dr M. Andre, Agence Française de Sécurité Sanitaire des Produits de Santé, Lyons, France; Dr I.S. Budiharto, Bio Farma, Bandung, Indonesia; Dr H. Chun, Korea Food and Drug Administration, Seoul, Republic of Korea; Dr M. Corbel, National Institute for Biological Standards and Control, Potters Bar, England; Dr R. Dobbelaer, Lokeren, Belgium; Dr S. Gairola, Serum Institute of India, Hadapsar, India; Dr L. Grode, Vakzine Projekt Management GmbH, Hannover, Germany; Dr M.M. Ho, National Institute for Biological Standards and Control, Potters Bar, England; Dr S. Jadhav, Serum Institute of India Ltd, Pune, India; Professor D. Levi, Tarashevich State Research Institute for Standardization and Control of Medical Biological Preparations, Moscow, Russian Federation; Dr S. Morris, United States Food and Drug Administration, Silver Spring, MD, USA; Dr V. Öppling, Paul-Ehrlich-Institute, Langen, Germany; Dr M. Roumiantzeff, Lyons, France; Dr K. Shibayama, National Institute of Infectious Diseases, Tokyo, Japan; Dr J. Southern, Cape Town, South Africa; Mr A. Supasansatorn, Ministry of Public Health, Nonthaburi, Thailand; Dr S. Wahyuningsih, National Agency of Drug and Food Control, Jakarta Pusat, Indonesia; Dr K.B. Walker, National Institute for Biological Standards and Control, Potters Bar, England; Dr S. Yamamoto, National Institute of Infectious Diseases, Tokyo, Japan; Mrs A. Zhao, National Institute for the Control of Pharmaceutical & Biological Products, Beijing, China.

Following the informal consultation meeting on standardization and evaluation of BCG vaccines in September 2009, held in Geneva, Switzerland, draft

Recommendations were revised by Dr H.-N. Kang, World Health Organization, Geneva, Switzerland, taking into account information on current manufacturing and regulatory practice provided at the meeting attended by:

Dr M. Andre, Agence Française de Sécurité Sanitaire des Produits de Santé, Lyons, France; Dr L.F. Barker, Aeras Global Tuberculosis Vaccine Foundation, Rockville, MD, USA; Dr A. Bhardwaj, Central Research Institute, Kasuli, Himachal Pradesh, India; Dr M. Brennan, Aeras Global TB Vaccine Foundation, Rockville, MD, USA; Dr I.S. Budiharto, Bio Farma, Bandung, Indonesia; Dr L.R. Castello-Branco, Fundacao Atauolpho de Paiva, Brazilian League Against Tuberculosis, Rio de Janeiro, Brazil; Dr M. Chouchkova, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria; Dr H. Chun, Korea Food and Drug Administration, Seoul, Republic of Korea; Dr M. Corbel, National Institute for Biological Standards and Control, Potters Bar, England; Dr B. Eisele, Vakzine Projekt Management GmbH, Hannover, Germany; Dr S. Gairola, Serum Institute of India, Hadapsar, India; Dr L. Grode, Vakzine Projekt Management GmbH, Hannover, Germany; Dr K. Haslov, Statens Serum Institut, Copenhagen, Denmark; Dr M.M. Ho, National Institute for Biological Standards and Control, Potters Bar, England; Dr P. Hubrechts, Statens Serum Institut, Copenhagen, Denmark; Dr G. Hussey, South African TB Vaccine Initiative, Cape Town, South Africa; Dr H.-N. Kang, World Health Organization, Geneva, Switzerland; Dr I. Knezevic, World Health Organization, Geneva, Switzerland; Professor D. Levi, Tarassevich State Research Institute for Standardization and Control of Medical Biological Preparations, Moscow, Russian Federation; Dr Y. Lopez Vidal, Universidad Nacional Autonoma de Mexico, Copilco-Universidad, Mexico City, Mexico; Dr K. Markey, National Institute for Biological Standards and Control, Potters Bar, England; Dr C. Martin, Universidad de Zaragoza, Zaragoza, Spain; Dr S. Morris, FDA/CBER, Bethesda, MD, USA; Dr V. Öppling, Paul-Ehrlich-Institute, Langen, Germany; Dr M. Roumiantzeff, Lyons, France; Dr. M. Seki, Japan BCG Laboratory, Tokyo, Japan; Dr K. Shibayama, National Institute of Infectious Diseases, Tokyo, Japan; Dr J. Southern, Advisor to Medicines Control Council in South Africa, Cape Town, South Africa; Mr A. Supasansatorn, Ministry of Public Health, Nonthaburi, Thailand; Dr S. Wahyuningsih, National Agency of Drug and Food Control, Jakarta Pusat, Indonesia; Dr K.B. Walker, National Institute for Biological Standards and Control, Potters Bar, England; Dr S. Yamamoto, Japan BCG Laboratory, Tokyo, Japan; Dr Z. Yan, Chengdu Institute of Biological Products, Chengdu, China; Dr L. Zhang, Chengdu Institute of Biological Products, Chengdu, China; Mrs A. Zhao, National Institute for the Control of Pharmaceutical & Biological Products, Beijing, China.

Several draft Recommendations were subsequently prepared by Dr M.M. Ho, National Institute for Biological Standards and Control, Potters Bar, England, with support from the drafting group of Dr M. Corbel, Milton Keynes, England; Dr R. Dobbelaer, Lokeren, Belgium; Dr H.-N. Kang, World Health

Organization, Geneva, Switzerland; Dr J. Southern, Cape Town, South Africa; and Dr K.B. Walker, National Institute for Biological Standards and Control, Potters Bar, England.

Following the meeting of the drafting group in March 2011, held in Potters Bar, England, draft Recommendations were updated taking into account comments received from:

Dr M. Andre, Agence Française de Sécurité Sanitaire des Produits de Santé, Lyons, France; Dr M. Chouchkova, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria; Dr H. Dockrell, London School of Hygiene and Tropical Medicine, London, England; Dr S. Gairola, Serum Institute of India, Hadapsar, India; Dr P. Hubrechts, Statens Serum Institut, Copenhagen, Denmark; Dr J. Joung, Korea Food and Drug Administration, Chungchengbuk-do, Republic of Korea; Dr M. Lagranderie, Institut Pasteur, Paris, France; Professor D. Levi, Tarashevich State Research Institute for Standardization and Control of Medical Biological Preparations, Moscow, Russian Federation; Dr Y. Lopez Vidal, Universidad Nacional Autonoma de Mexico, Facultad de Medicina, Mexico City, Mexico; Dr V. Öppling, Paul-Ehrlich-Institute, Langen, Germany; Mrs G. Trisnasari, Bio Farma, Bandung, Indonesia; Dr V. Vincent, Institut Pasteur, Paris, France; Dr S. Wahyuningsih, National Agency of Drug and Food Control, Jakarta Pusat, Indonesia; Dr T. Ying, China National Biotec Group, Beijing, China; Mrs A. Zhao, National Institute for the Control of Pharmaceutical & Biological Products, Beijing, China.

The draft Recommendations were posted on the WHO Biologicals web site for public consultation from 24 May to 23 June 2011. The WHO/BS/2011.2157 document was then prepared by Dr M.M. Ho, National Institute for Biological Standards and Control, Potters Bar, England; Dr H.-N. Kang, World Health Organization, Geneva, Switzerland; Dr J. Southern, Cape Town, South Africa; Dr K.B. Walker, National Institute for Biological Standards and Control, Potters Bar, England, taking into account comments received from the following reviewers:

Dr C. Ahn, Korea Food and Drug Administration, Chungchengbuk-do, Republic of Korea; Dr M. Andre, Agence Française de Sécurité Sanitaire des Produits de Santé, Lyons, France; Dr M. Brennan, Aeras Global TB Vaccine Foundation, Rockville, MD, USA; Dr I.S. Budiharto, Bio Farma, Bandung, Indonesia; Ms Y. Choi, Korea Food and Drug Administration, Chungchengbuk-do, Republic of Korea; Dr M. Chouchkova, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria; Dr S. Gairola, Serum Institute of India, Hadapsar, India; Dr K. Haslov, Statens Serum Institut, Copenhagen, Denmark; Dr H. Hozouri, Pasteur Institute of Iran, Tehran, Islamic Republic of Iran; Dr P. Hubrechts, Statens Serum Institut, Copenhagen, Denmark; Dr J. Joung, Korea Food and Drug Administration, Chungchengbuk-do, Republic of Korea; Mr H. Kim, Korea Food and Drug Administration, Chungchengbuk-do, Republic of Korea; Mr J.-G. Kim, Korea Food and Drug Administration, Chungchengbuk-do, Republic of Korea;

Ms Y.L. Kim, Korea Food and Drug Administration, Chungchengbuk-do, Republic of Korea; Dr K. Lee, Korea Food and Drug Administration, Chungchengbuk-do, Republic of Korea; Dr Y. Lopez Vidal, Universidad Nacional Autonoma de Mexico, Copilco-Universidad, Mexico City, Mexico; Dr V. Öppling, Paul-Ehrlich-Institute, Langen, Germany; Dr M. Roumiantzeff, Lyons, France; Dr M. Seki, Japan BCG Laboratory, Tokyo, Japan; Dr J. Shin, World Health Organization, Geneva, Switzerland; Mr S.-C. Shin, Green Cross, Yongin, Republic of Korea; Dr V. Vincent, Institut Pasteur, Paris, France; Dr S. Yamamoto, Japan BCG Laboratory, Tokyo, Japan.

Further changes were made to WHO/BS/2011.2157 by the Expert Committee on Biological Standardization, resulting in the current document.

References

1. Requirements for dried BCG vaccines. In: *WHO Expert Committee on Biological Standardization. Thirty-sixth report*. Geneva, World Health Organization, 1987 (WHO Technical Report Series, No. 745), Annex 2.
2. Requirements for dried BCG vaccines. In: *WHO Expert Committee on Biological Standardization. Thirty-eighth report*. Geneva, World Health Organization, 1988 (WHO Technical Report Series, No. 771), Annex 12.
3. Corbel MJ et al. Report on a WHO consultation on the characterization of BCG strains, Imperial College, London 15–16 December 2003. *Vaccine*, 2004, 22:2675–2680.
4. Ho MM et al. Report on a WHO consultation on the characterization of BCG vaccines held at WHO Headquarters, Geneva, 8–9 December 2004. *Vaccine*, 2005, 23:5700–5704.
5. Knezevic I, Corbel MJ. WHO discussion on the improvement of the quality control of BCG vaccines held at Pasteur Institute, Paris, France, 7 June 2005. *Vaccine*, 2006, 24:3874–3877.
6. Ho MM et al. Report on a WHO informal consultation on standardization and evaluation on BCG vaccines, 22–23 September 2009, WHO, Geneva, Switzerland. *Vaccine*, 2010, 28:6945–6950.
7. *State of the art of new vaccine research and development*. Geneva, World Health Organization, 2006 (WHO/IVB/06.01; http://whqlibdoc.who.int/hq/2006/WHO_IVB_06.01_eng.pdf, accessed 6 February 2013).
8. Fine PEM et al. *Issues relating to the use of BCG in immunization programmes: a discussion document*. Geneva, World Health Organization, 1999 (WHO/V&B/99.23; http://whqlibdoc.who.int/hq/1999/WHO_V&B_99.23.pdf, accessed 6 February 2013).
9. BCG vaccine: WHO position paper. *Weekly Epidemiological Record*, 2004, 79:27–38.
10. Colditz GA et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *Journal of the American Medical Association*, 1994, 271:698–702.
11. Global Advisory Committee on Vaccine Safety, 12–13 June 2007. *Weekly Epidemiological Record*, 2007, 82:252–259.
12. Ritz N et al. Influence of BCG vaccine strain on the immune response and protection against tuberculosis. *FEMS Microbiology Review*, 2008, 32:821–841.
13. Revised BCG vaccination guidelines for infants at risk for HIV infection. *Weekly Epidemiological Record*, 2007, 82:193–196.

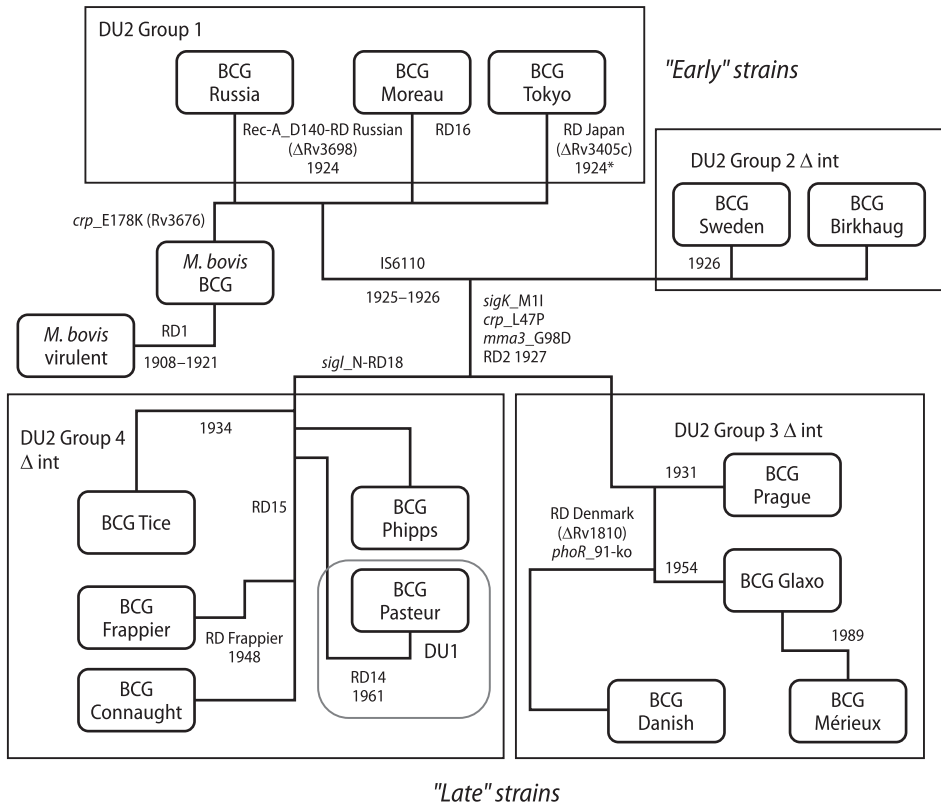
14. Use of BCG vaccine in HIV-infected infants. *Weekly Epidemiological Record*, 2010, 85:32–33.
15. Birkhaug K. Protective value of the intracutaneous and percutaneous methods of BCG vaccination. *Acta Medica Scandinavica*, 1944, CXVII, fasc. III–IV:274–312.
16. Mande R, Fillastre C, Pinelli L. A propos des complications de la vaccination B.C.G. *Annales de Pédiatrie*, 1976, 23:219–225.
17. Mori T, Yamauchi Y, Shiozawa K. Lymph node swelling due to bacilli Calmette-Guérin vaccination with multipuncture method. *Tubercle and Lung Disease*, 1996, 77:269–273.
18. Brosch R et al. Genome plasticity of BCG and impact on vaccine efficacy. *Proceedings of the National Academy of Sciences*, 2007, 104:5596–5601.
19. Seki M et al. Whole genome sequence analysis of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) Tokyo 172: a comparative study of BCG vaccine substrains. *Vaccine*, 2009, 27:1710–1716.
20. Gomes LHF et al. Genome sequence of *Mycobacterium bovis* BCG Moreau, the Brazilian vaccine strain against tuberculosis. *Journal of Bacteriology*, 2011, 193:5600–5601.
21. Castillo-Rodal AI et al. *Mycobacterium bovis* BCG substrains confer different levels of protection against *Mycobacterium tuberculosis* infection in a BALB/c model of progressive pulmonary tuberculosis. *Infection and Immunity*, 2006, 74:1718–1724.
22. Hayashi D et al. Comparable studies of immunostimulating activities in vitro among *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) substrains. *FEMS Immunology and Medical Microbiology*, 2009, 56:116–123.
23. Horwitz MA et al. Commonly administered BCG strains including an evolutionarily early strain and evolutionarily late strains of disparate genealogy induce comparable protective immunity against tuberculosis. *Vaccine*, 2009, 27:441–445.
24. *In vitro assays of BCG products*. Geneva, World Health Organization, 1977 (WHO working document WHO/TB/Technical guide/77.9).
25. Biological substances and reference reagents. In: *WHO Expert Committee on Biological Standardization. Sixtieth report*. Geneva, World Health Organization, 2013 (WHO Technical Report Series, No. 977), Annex 4.
26. Biological substances: International standards and reference reagents. In: *WHO Expert Committee on Biological Standardization. Sixty-first report*. Geneva, World Health Organization, 2013 (WHO Technical Report Series, No. 978), Annex 7.
27. Good manufacturing practices: main principles for pharmaceutical products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fifth report*. Geneva, World Health Organization, 2011 (WHO Technical Report Series, No. 961), Annex 3.
28. Good manufacturing practices for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second report*. Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 822), Annex 1.
29. International Commission on Radiological Protection. *Report of Committee II on permissible dose for internal radiation*. London, Pergamon Press, 1959 (ICRP Publication 2).
30. United Nations Scientific Committee on the Effect of Atomic Radiation. *Ionizing radiation: levels and effects. Report*. New York, NY, United Nations, 1972.
31. General requirements for the sterility of biological substances (Requirements for biological substances, No. 6, revised 1973). In: *WHO Expert Committee on Biological Standardization. Twenty-fifth report*. Geneva, World Health Organization, 1973 (WHO Technical Report Series, No. 530), Annex 4.
32. *Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products*. Geneva, World Health Organization, 2003 (WHO/BCT/QSD/2003.01).

33. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. In: *WHO Expert Committee on Biological Standardization. Sixty-first report*. Geneva, World Health Organization, 2013 (WHO Technical Report Series, No. 978), Annex 3.
34. *WHO Guidelines on tissue infectivity distribution in transmissible spongiform encephalopathies*. Geneva, World Health Organization, 2006 (<http://www.who.int/bloodproducts/tse/WHO%20TSE%20Guidelines%20FINAL-22%20JuneupdatedNL.pdf>, accessed 11 April 2011).
35. *WHO tables on guidelines on tissue infectivity distribution in transmissible spongiform encephalopathies*. Geneva, World Health Organization, 2010 (<http://www.who.int/bloodproducts/tablestissueinfectivity.pdf>, accessed 11 April 2011).
36. *Update of the opinion on TSE infectivity distribution in ruminant tissues*. Brussels, European Commission, 2002 (http://ec.europa.eu/food/fs/sc/ssc/out296_en.pdf, accessed 11 April 2011).
37. Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 Rev. 2 - October 2003) adopted by the Committee for Proprietary Medicinal Products (CPMP) and by the Committee for Veterinary Medicinal Products (CVMP). *Official Journal of the European Union, 2004* (http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003700.pdf, accessed 24 February 2013).
38. Ho MM et al. Report of an international collaborative study to establish the suitability of using modified ATP assay for viable count of BCG vaccine. *Vaccine*, 2008, 26(36):4754–4757.
39. Jensen SE et al. Development and validation of an ATP method for rapid estimation of viable units in lyophilised BCG Danish 1331 vaccine. *Biologicals*, 2008, 36(5):308–314.
40. BCG vaccine, freeze-dried. In: *European Pharmacopoeia*, 6th ed. Strasbourg, Directorate for the Quality of Medicines of the Council of Europe (EDQM), 2008 (01/2008:0163).
41. *Temperature sensitivity of vaccines*. Geneva, World Health Organization, 2006 (WHO/IVB/06.10).
42. *WHO policy statement: the use of opened multi-dose vials of vaccine in subsequent immunization sessions*. Geneva, World Health Organization, 2000 (WHO/V&B/00.09; <http://www.who.int/vaccines-documents/DocsPDF99/www9924.pdf>, accessed 11 April 2011).
43. *Safe vaccine handling, cold chain and immunizations*. Geneva, World Health Organization, 1998 (WHO/EPI/LHIS/98.02).
44. Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (jointly with the Expert Committee on Biological Standardization). In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fifth report*. Geneva, World Health Organization, 2011 (WHO Technical Report Series, No. 961), Annex 9.
45. Proper handling and reconstitution of vaccines avoids programme errors. *Vaccines and Biologicals Update*, 2000, 34 (<http://www.who.int/vaccines-documents/DoxNews/updates/updat34e.pdf>, accessed 11 April 2011).
46. Guidelines for stability evaluation of vaccines. In: *WHO Expert Committee on Biological Standardization. Fifty-seventh report*. Geneva, World Health Organization, 2011 (WHO Technical Report Series, No. 962), Annex 3.
47. *Guideline for establishing or improving primary and intermediate vaccine stores*. Geneva, World Health Organization, 2002 (WHO/V&B/02.34; <http://www.who.int/vaccines-documents/DocsPDF02/www715.pdf>, accessed 11 April 2011).
48. *Immunization in practice: a practical resource guide for health staffs, 2004 update*. Geneva, World Health Organization, 2004 (WHO/IVB/04.06).

49. WHO guidelines on nonclinical evaluation of vaccines. In: *WHO Expert Committee on Biological Standardization. Fifty-fourth report*. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 927), Annex 1.
50. Yamamoto T et al. *Mycobacterium bovis* BCG vaccination modulates TNF- α production after pulmonary challenge with virulent *Mycobacterium tuberculosis* in guinea pigs. *Tuberculosis*, 2007, 87:155–165.
51. Walker KB et al. The second Geneva consensus: recommendations for novel live TB vaccines. *Vaccine*, 2010, 28:2259–2270.
52. Kamath AT et al. New live mycobacterial vaccines: the Geneva consensus on essential steps towards clinical development. *Vaccine*, 2005, 23:3753–3761.
53. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products. In: *WHO Expert Committee on the Use of Essential Drugs. Sixth report*. Geneva, World Health Organization, 1995 (WHO Technical Report Series, No. 850), Annex 3.
54. Guidelines on clinical evaluation of vaccines: regulatory expectations. In: *WHO Expert Committee on Biological Standardization. Fifty-second report*. Geneva, World Health Organization, 2004 (WHO Technical Report Series, No. 924), Annex 1.
55. Roth A et al. Vaccination technique, PPD reaction and BCG scarring in a cohort of children born in Guinea-Bissau 2000–2002. *Vaccine*, 2005, 23:3991–3998.
56. Hoft DF et al. Clinical reactogenicity of intradermal bacille Calmette-Guérin vaccination. *Clinical Infectious Disease*, 1999, 28:785–790.
57. *WHO Expert Committee on Tuberculosis. Ninth report*. Geneva, World Health Organization, 1974 (WHO Technical Report Series, No. 552).
58. *Surveillance of adverse events following immunization*. Geneva, World Health Organization, 1997 (WHO/EPI/TRAM/93.02. Rev 1).
59. Guidelines for national authorities on quality assurance for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second report*. Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 822), Annex 2.
60. Guidelines for independent lot release of vaccines by regulatory authorities. In: *WHO Expert Committee on Biological Standardization. Sixty-first report*. Geneva, World Health Organization, 2013 (WHO Technical Report Series, No. 978), Annex 2.

Appendix 1

History and genealogy of BCG substrains



Note: This diagram provides only a historical overview of the use of different substrains derived from BCG vaccine strain. It does not indicate any WHO "qualification" or "approval" of the strains or vaccines in the context of this document.

* Yamamoto S, Yamamoto T. Historical review of BCG vaccine in Japan. *Japanese Journal of Infectious Disease*, 2007, 60:331–336.

Appendix 2

Summary protocol for manufacturing and control of BCG vaccine

The following protocol is intended for guidance, and indicates the information that should be provided as a minimum by the manufacturer to the NRA. Information and tests may be added or omitted as required by the NRA, if applicable.

It is thus possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO recommendations of a particular product should be given in the protocol submitted.

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must also be accompanied by a lot release certificate from the NRA or NCL of the country in which the vaccine was produced stating that the product meets national requirements as well as Part A of the recommendations of this document published by WHO.

Summary information on the finished product (final lot)

International name: _____

Trade name: _____

Product licence (marketing authorization) number: _____

Country: _____

Name and address of manufacturer: _____

Site of manufacture of final lot: _____

Name and address of licence-holder
(if different): _____

BCG substrain: _____

Authority that approved the BCG substrain: _____

Date approved: _____

Final bulk number: _____

Volume of final bulk: _____

Final product: _____

Type of vaccine: Intradermal/Percutaneous/Other

Final lot number: _____

Type of container: _____

Number of doses per container: _____
 Number of filled containers in this final lot: _____
 Date of manufacture of final lot: _____
 Date on which last determination of the bacterial count
 was started, or date of start of period of validity: _____
 Shelf-life approved (months): _____
 Expiry date: _____
 Diluent: _____
 Storage conditions: _____
 Volume of single human dose: _____
 Volume of vaccine per container: _____
 Number of doses per container: _____
 Summary of the composition (include a summary of the
 qualitative and quantitative composition of the
 vaccine per human dose): _____
 Release date: _____

Production information

A genealogy of the lot numbers of all vaccine components used in the formulation of the final product will be informative.

The following sections are intended for the reporting of the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production. Thus, if any test has to be repeated, this must be indicated. Any abnormal results should be recorded on a separate sheet.

Control of source materials (section A.3)

The information requested below is to be presented on each submission. Full details on master and working seed lots are required on first submission only and whenever a change has been introduced.

Master seed lot

Origin of seed lot: _____
 Master seed lot number: _____
 Name and address of manufacturer: _____
 Passage level: _____
 Date of preparation of seed lot: _____
 Date of receipt of seed lot (if applicable): _____
 Date of reconstitution of seed lot ampoule: _____
 Date approved by the NRA: _____

Working seed lot

Working seed lot number: _____

Name and address of manufacturer: _____

Passage level: _____

Date of reconstitution of seed lot ampoule: _____

Date approved by the NRA: _____

Tests on working seed lot production (section A.3.2)**Antimicrobial sensitivity test (section A.3.2.1)**

Method used: _____

Date test started: _____

Date test completed: _____

Results: _____

Delayed hypersensitivity test (section A.3.2.2)

	Vaccine	Reference vaccine
Method used:	_____	_____
Dilutions injected:	_____	_____
Inoculation route:	_____	_____
Number of guinea-pigs given injection:	_____	_____
Observation period (specification):	_____	_____
Date test started:	_____	_____
Date test completed:	_____	_____
Result:	_____	_____

Identity test (section A.3.2.3)

Method used: _____

Date test started: _____

Date test completed: _____

Results: _____

Test for bacterial and fungal contamination (section A.3.2.4)

Method used: _____

Number of containers tested: _____

Volume of inoculum per container: _____

Volume of medium per container: _____

Observation period (specification): _____

Incubation	Media used	Inoculum	Date test began	Date test ended	Results
20–25 °C					
30–36 °C					
Negative control					

Test for absence of virulent mycobacteria (section A.3.2.5)

Method used: _____
 No. of human doses injected per guinea-pig: _____
 Inoculation route: _____
 No. of guinea-pigs given injection: _____
 Weight range of guinea-pigs: _____
 Observation period (specification): _____
 Date test started: _____
 Date test completed: _____
 Health of animals during test: _____
 Weight gains (losses): _____
 Result: _____

Test for excessive dermal reactivity (section A.3.2.6)

	Vaccine	Reference vaccine
Method used:	_____	_____
Dilutions injected:	_____	_____
Inoculation route:	_____	_____
No. of guinea-pigs given injection:	_____	_____
Observation period (specification):	_____	_____
Date test started:	_____	_____
Date test completed:	_____	_____
Mean diameter of lesions (for each dilution):	_____	_____
Result:	_____	_____

Production of culture medium (section A.3.3)

Any components of animal origin: _____
 Certificate for BSE/TSE-free: _____

Control of vaccine production (section A.4)

Control of single harvests (section A.4.1)

Derived from master seed lot number: _____

Working seed lot number: _____

Passage level from master seed: _____

Culture medium: _____

Number and volume of containers inoculated: _____

Date of inoculation: _____

Temperature of incubation: _____

Date of harvest: _____

Results of visual inspection: _____

Control of final bulk (section A.4.2)

Tests for bacterial and fungal contamination (section A.4.2.2)

Method used: _____

Number of containers tested: _____

Volume of inoculum per container: _____

Volume of medium per container: _____

Observation period (specification): _____

Incubation	Media used	Inoculum	Date test began	Date test ended	Results
20–25 °C					
30–36 °C					
Negative control					

Test for absence of virulent mycobacteria (section A.4.2.3)

(if not performed on final lot)

Method used: _____

No. of human doses injected per guinea-pig: _____

Inoculation route: _____

No. of guinea-pigs given injection: _____

Weight range of guinea-pigs: _____

Observation period (specification): _____

Date test started: _____

Date test completed: _____

Health of animals during test: _____

Weight gains (losses): _____

Result: _____

Test for bacterial concentration (section A.4.2.4)

Method used: _____

Date test started: _____

Date test completed: _____

Specification: _____

Result: _____

Test for number of culturable particles (section A.4.2.5)

Method used: _____

Date test started: _____

Date test completed: _____

Specification: _____

Result: _____

Details of working Reference Preparation: _____

Substances added (section A.4.2.6)

Any components of animal origin: _____

Certificate for BSE/TSE-free: _____

Filling and containers (section A.5)

Lot number: _____

Date of filling: _____

Volume of final bulk filled: _____

Filling volume per container: _____

Number of containers filled (gross): _____

Date of freeze-drying: _____

Number of containers rejected during inspection: _____

Number of containers sampled: _____

Total number of containers (net): _____

Maximum period of storage approved: _____

Storage temperature and period: _____

Control tests on final lot (section A.6)

Inspection of final containers (section A.6.1)

Appearance: _____

Date of test: _____

Specification: _____

Result: _____
 Recommended reconstitution fluid: _____
 Volume of reconstitution fluid per final container: _____

Identity test (section A.6.2)

Method used: _____
 Date test started: _____
 Date test completed: _____
 Specification: _____
 Result: _____

Tests for bacterial and fungal contamination (section A.6.3)

Method used: _____
 Number of containers tested: _____
 Volume of inoculum per container: _____
 Volume of medium per container: _____
 Observation period (specification): _____
 Specification: _____

Incubation	Media used	Inoculum	Date test began	Date test ended	Results
20–25 °C					
30–36 °C					
Negative control					

Safety tests (section A.6.4)

Test for absence of virulent mycobacteria (section A.6.4.1) (if not performed on final bulk)

Method used: _____
 No. of human doses injected per guinea-pig: _____
 Inoculation route: _____
 No. of guinea-pigs given injection: _____
 Weight range of guinea-pigs: _____
 Observation period (specification): _____
 Date test started: _____
 Date test completed: _____
 Health of animals during test: _____
 Weight gains (losses): _____

Specification: _____

Result: _____

Test for excessive dermal reactivity (section A.6.4.2) if applicable

	Vaccine	Reference vaccine
Method used:	_____	_____
Dilutions injected:	_____	_____
Inoculation route:	_____	_____
No. of guinea-pigs given injection:	_____	_____
Observation period (specification):	_____	_____
Date test started:	_____	_____
Date test completed:	_____	_____
Mean diameter of lesions (for each dilution):	_____	_____
Specification:	_____	_____
Result:	_____	_____

Test for bacterial concentration (section A.6.5)

Method used: _____

Date test started: _____

Date test completed: _____

Specification: _____

Result: _____

Test for residual moisture (section A.6.6)

Method: _____

Date: _____

Specification: _____

Result: _____

Tests for viability (section A.6.7)

Test for number of culturable particles (section A.6.7.1)

Method used: _____

Medium: _____

Date test started: _____

Date test completed: _____

	Before lyophilization	After lyophilization
No. of containers tested:	_____	_____
Mean count of culturable particles per ml:	_____	_____
Mean survival rate (%):	_____	_____

Specification: _____

Result: _____

Details of working Reference Preparation: _____

Rapid test for viability (section A.6.7.2) if applicable

Method: _____

Mean survival rate (%): _____

Date: _____

Specification: _____

Result: _____

Thermal stability test (section A.6.8)

Method used: _____

Date test started: _____

Date test completed: _____

	Unheated containers	Heated containers
No. of containers tested:	_____	_____
Culturable particles in each container per ml:	_____	_____
Mean survival rate (%):	_____	_____
Specification:	_____	
Result:	_____	
Details of working Reference Preparation:	_____	

Submission addressed to NRA

Name of responsible person (typed) _____

Certification by the person from the control laboratory of the manufacturing company taking over responsibility for the production and control of the vaccine:

I certify that lot no. _____ of BCG vaccine, whose number appears on the label of the final container, meets all national requirements and/or satisfies Part A¹ of the WHO Recommendations to assure the quality, safety and efficacy of BCG vaccines (2013)²

Signature: _____

Name (typed): _____

Date: _____

¹ With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

² WHO Technical Report Series, No. 979, Annex 3.

Appendix 3

Model certificate for the release of BCG vaccine by NRAs

Lot release certificate

Certificate no. _____

The following lot(s) of BCG vaccine produced by _____¹ in _____,² whose numbers appear on the labels of the final containers, complies with the relevant specification in the marketing authorization and provisions for the release of biological products³ and Part A⁴ of the WHO Recommendations to assure the quality, safety and efficacy of BCG vaccines (2013)⁵ and comply with WHO good manufacturing practices: main principles for pharmaceutical products;⁶ Good manufacturing practices for biological products;⁷ and Guidelines for independent lot release of vaccines by regulatory authorities.⁸

The release decision is based on _____⁹

The certificate may include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers, packaging lot numbers if necessary);

¹ Name of manufacturer.

² Country of origin.

³ If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the NRA.

⁴ With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

⁵ WHO Technical Report Series, No. 979, Annex 3.

⁶ WHO Technical Report Series, No. 961, Annex 3.

⁷ WHO Technical Report Series, No. 822, Annex 1.

⁸ WHO Technical Report Series, No. 978, Annex 2.

⁹ Evaluation of summary protocol, independent laboratory testing, and/or specific procedures laid down in defined document etc., as appropriate.

- type of container;
- number of doses per container;
- number of containers/lot size;
- date of start of period of validity (e.g. manufacturing date) and/or expiry date;
- storage condition;
- signature and function of the authorized person and authorized agent to issue the certificate;
- date of issue of certificate;
- certificate number.

The Director of the NRA (or other authority as appropriate):

Name (typed) _____

Signature _____

Date _____

