



QUALITY CONTROL OF BCG VACCINES BY THE WORLD HEALTH ORGANIZATION:  
A REVIEW OF FACTORS THAT MAY INFLUENCE VACCINE EFFECTIVENESS AND SAFETY

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## 1. INTRODUCTION

The World Health Organization oversees the quality control of BCG vaccine via a system which includes regular testing of products by *in vitro* tests and clinical trials. Three parent strains of BCG (Glaxo-1077, Tokyo-172, and Pasteur-1173P2) account for over 90% of the vaccines currently in use world wide. Characteristics of the vaccine preparations are summarized, along with known physical-chemical properties of preparations. In cases where diagnostic criteria of tuberculosis are stringent, there is no evidence of differing efficacy among preparations when vaccine is administered to newborns; the incidence of BCG-associated adverse reactions does correlate with preparation, however. Other factors including dose, administration technique, and recipient characteristics are also important in vaccine-associated reactions.

## 2. HISTORY OF WHO-SPONSORED INTERNATIONAL QUALITY CONTROL OF BCG VACCINE

The involvement of WHO in quality control of BCG vaccine began after the Second World War when WHO took over responsibility for the large-scale international BCG vaccination programmes using liquid vaccine. Since that time, the system for quality control of BCG vaccine has undergone changes, as has the vaccine's production and testing. The purpose of this paper is to summarize the information gained from studies on efficacy, adverse reactions, and quality control, in order to provide a reference for future activities to maintain and improve the quality of the vaccine.

In 1974 the twenty-seventh World Health Assembly (via Resolution WHA 27.54) reaffirmed the importance of quality control of BCG vaccines and recommended that all member countries producing or importing BCG vaccine use the international quality control system set up by WHO "until they have established a competent national control service."<sup>a</sup> All producers of freeze-dried vaccine supplied by or through UNICEF were already using this system, which consisted of evaluation in international reference laboratories and centres both by laboratory experiments and by clinical testing. A description of this system can be found in WHO/TB/Technical Guide/77.8, "WHO-Sponsored International Quality Control of BCG Vaccine."

The system was based on keeping the number of BCG production and control centres to a minimum. Quality control testing is coordinated by the BCG Section of the Quality Control Department of the Statens Seruminstitut in Copenhagen; training of staff in the production and control of BCG vaccine also takes place in Copenhagen.

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<sup>a</sup>In the future, it is expected that responsibility for quality of individual vaccines will be shifted almost exclusively to National Control Authorities.

This international system was originally set up by the Tuberculosis Unit in WHO, but by 1976 responsibility for in vitro assays had been transferred to the Biologicals Unit, and, in December of 1982, all responsibility for international quality control of BCG was transferred to Biologicals. The Statens Seruminstitut has continued to coordinate quality control of BCG with assistance of three to four collaborating laboratories in Europe and Asia, and of the Pan American Zoonoses Center (CEPANZO) in Buenos Aires, which serves as a reference centre for the Americas Region and performs the coordinating, testing, and training functions for this region with advice from Copenhagen.

The coordinating laboratory does the following: coordinates the International Quality Control System; provides technical advice to national BCG laboratories at the request of WHO; provides training in BCG production and quality control in consultation with the Tuberculosis Unit; distributes references and seed lots of BCG vaccine; tests candidate BCG vaccines from producers; and tests samples from vaccine lots supplied to national immunization programmes through or by UNICEF. The International Quality Control System at present consists of for each lot of BCG vaccine supplied to UNICEF review of summary protocols, testing of random samples, and clinical testing of products. The WHO procedure for evaluating the acceptability of lyophilized BCG vaccines offered for sale to UNICEF for use in the Expanded Programme on Immunization (EPI) has been described (1).

### 3. QUALITY CONTROL TESTING

There is consistent evidence from multiple clinical trials that BCG seed lots used to produce vaccines having protective efficacy in laboratory animals and inducing tuberculin sensitivity in humans are effective against disseminated disease and meningitis in childhood tuberculosis, and probably to a lesser degree against other forms of tuberculosis in children as well (2-7). At present, however, there is no laboratory test that correlates with protective efficacy of any BCG vaccine preparation.

For this reason, the strategy used has been to evaluate the protective efficacy of several different preparations of BCG through careful clinical trials, using vaccines whose safety and in vitro characteristics have already been verified. Once vaccine efficacy in humans is demonstrated, repeated measurement of tuberculin sensitivity and lesion size, and various in vitro tests on cultured BCG bacteria, are used to verify that later lots of vaccine grown from these preparations are being reproduced satisfactorily. The laboratory tests are thus designed to verify that successive batches are indeed uniform or vary within only narrow limits. In addition, the WHO Requirements for Dried BCG Vaccine (8) suggest to the national control authority the possible need to conduct trials in tuberculin-negative human subjects to determine the optimum content of BCG organisms whenever there is a change in manufacturing procedure. It is required, in any case, that manufacturers conduct such studies in children on at least one batch each year.

### In vitro tests

The in vitro tests are described in WHO/TB/Technical Guide/77.9, "In-Vitro Assays of BCG Products," and in BLG/UNDP/82.1 Rev.1, "Manual of Tests on Vaccines in the Expanded Programme on Immunization." One of these tests is for the number of culturable particles, which is the most informative viability test. The test is subject to considerable variability depending on the type of media used, the components of the media, and the test procedures. A laboratory therefore needs to establish consistency in the test performance and to be able to relate the numbers of BCG particles determined for a particular vaccine to clinical effects such as scar size, post-vaccination tuberculin hypersensitivity, and incidence of common toxic effects such as regional lymphadenitis. A rapid test for viability is based on bioluminescence, which, when conditions are adjusted so that it is proportional to the adenosine triphosphate content, is a reliable marker for living cells. This test is useful once the mean content of adenosine triphosphate per culturable particle has been estimated for a given vaccine strain. Colony morphology is another useful test.

### Viability

The viability of a vaccine (proportion of living and dead bacilli) is an important determinant of its characteristics. The final product is filled in containers according to a standard bacterial mass, estimated by weight and/or opacity. The percentage of total bacterial particles which is culturable is then determined. This percentage is subject to further decrease after freeze-drying.

It is known that the size of the local reaction is proportional to the total bacterial mass, while the level of tuberculin sensitivity is related to the number of culturable particles (WHO/TB/Technical Guide/77.8). The Tokyo strain of BCG generally has a high viability and a high resistance to freeze drying (9). Gheorghiu et al. (10) found a Pasteur strain vaccine prepared in dispersed culture had a high viability compared to the classically grown strain.

### Thermal stability

The degree of thermal stability of each final lot is another important characteristic of a vaccine, and one of the few tests for which the WHO Requirements specify a numerical requirement: that is, the number of culturable particles in a vaccine after incubation for 28 days at 37° shall be not less than 20% of that in samples of the same vaccine stored at 4°.

There have been several studies on heat stability of freeze-dried vaccines (c.f. for examples, references 9, 11). Some of the difference in thermal stability can be attributed to the growth characteristics of the vaccine (9); other differences are clearly related to the preparation and packaging of the freeze-dried vaccine (11). Ladefoged (personal communication, 1989) has recently compared the characteristics of the freeze-dried Copenhagen vaccine in ampoules and vials. The vaccines were prepared, dispensed from the same apparatus, and freeze-dried, the only difference between the products being that the ampoules were sealed under vacuum, while the vials were sealed after filling with nitrogen. She found no difference in viability determined immediately after freeze drying, nor in heat stability. After three months' storage at 4°C, there was slightly better stability of vaccine in ampoules than in vials for only a few lots. The observations have now been extended to 12 months with the conclusion that for this period of time, freeze-dried BCG vaccine in vials has satisfactory stability. This stability is strongly dependent on the quality and treatment of vials and stoppers.

#### Delayed type hypersensitivity in animals

An analysis of BCG potency in animals is beyond the scope of this paper. Several studies have been done comparing a number of preparations by in vivo methods (12, 13). Animal experiments show that cellular immune responses induced under experimentally controlled conditions are higher for the traditionally "stronger" preparations, i.e. Pasteur, Copenhagen (9). These are also the vaccine preparations which give a higher incidence of adverse reactions such as suppurative lymphadenitis.

#### 4. CHARACTERISTICS OF PREPARATIONS OF BCG IN USE

In order to learn which preparations of BCG are presently in use and determine their characteristics, information was requested from 15 manufacturers who had been included in the WHO 1984 List of Availability of Vaccines and Sera, and who had responded with information for the 1989 edition. Responses have been received from all of these. Some of the results are summarized in Table 1.

It should be noted in Table 1 that the range of number of culturable particles per dose is a compilation of numbers reported by all producers of a vaccine prepared from a particular parent strain. Therefore, a wide range in number of culturable particles per dose does not necessarily imply a wide variability of the product. However, given the dose dependence of both induced tuberculin sensitivity and reactogenicity, it is clear that an important characteristic of a vaccine is its homogeneity in terms of range of number of culturable particles. The WHO Requirements reserve the setting of the limit of inhomogeneity allowed to the National Control Authority. Those vaccines in Table 1 having relatively low numbers of culturable particles per dose (Pasteur, Copenhagen) are more reactogenic than those having higher numbers (Tokyo, for example).

The stabilizer is important as it will contribute to ease of reconstitution as well as to stability properties. Vaccine stabilized with monosodium glutamate may be more difficult to reconstitute, while the presence of albumin, though it is easily soluble, may lead to foaming of the product during reconstitution.

It is clear that the vast majority of the BCG vaccine use in the world is limited to three preparations, Pasteur, Glaxo, and Tokyo. Accordingly, this report will concentrate on the characteristics of these preparations, although others will be considered as well. A summary of the history of four of the most widely used strains, Pasteur-1173P2, Tokyo-172, Copenhagen-1331, and Glaxo-1077, can be found in Osborn (14), and an extensive summary of the history of the Pasteur strain is reported by Gheorghiu et al. (15).

#### Physical-chemical characteristics of BCG preparations

A number of different studies comparing various preparations of BCG vaccine have been performed, including biochemical and immunological analyses of secreted proteins, and chromatographic analyses of lipid production, as shown in Table 2. It should be noted that vaccine preparations, though referred to as being of one or another strain, are in fact complex mixtures of a variety of different BCG Mycobacteria. Methods of production may influence the composition of these mixtures; thus "strains" of BCG may differ among production facilities.

MPB70 is a unique BCG-specific antigen which elicits a delayed skin reaction in guinea pigs sensitized with viable cells of BCG. Guinea pigs sensitized with heat-killed BCG do not show delayed-type hypersensitivity to MPB70 (16). MPB70 has been found to have a relative molecular mass of 15100, to contain no sugar, and to comprise up to 10% of the total protein content of the culture medium of the Tokyo strain of BCG, while being present in only trace quantities in other strains (17). It will be seen from Table 2 that strains of BCG fall into two groups with respect to content of this protein, with the Glaxo strain being distinctive in containing an intermediate amount. This grouping is also reflected by antigen analysis of 46000 dimer (18) or antigen 15 (19), and by secretion of methoxymycolate (20-22). It is not clear whether all of these phenomena are related to the expression of one protein, which is present in different forms due to break-down. It is interesting to note that the Swedish strain produces methoxymycolate whether prepared in Copenhagen or in Gothenberg (20), suggesting that this characteristic is present under at least two different production conditions.

On the other hand, production of mycoside B (glycosylphenolphthiocerol dimycoerates) appears to be associated with colony morphology (21), both characteristics having been observed, based on studies on four preparations, to vary with production method. It is not known to what extent these laboratory characteristics correlate with protective efficacy and adverse reactions of BCG vaccine.

Efficacy of BCG preparations in producing tuberculin sensitivity when given to newborn children

One measure of potency of a BCG vaccine is the post-vaccination delayed sensitivity to tuberculin (PVS) induced by that vaccine in children who are tuberculin-negative before vaccination. Vallishayee et al. (23) performed a study with 11 different BCG preparations prepared under uniform conditions and tested for PVS using 2 units of tuberculin 9-11 weeks after vaccination. With the exception of the Prague strain, very little difference was found among the preparations in the mean size of PVS induced.

Tuberculin PVS will of course depend on the method of measurement; both the dose of tuberculin used and the interval between vaccination and testing will affect the sensitivity of the test. A number of studies have been performed in newborn infants to measure the ability of different vaccines to produce PVS. Strains that do produce a high proportion of conversions in neonates include Glaxo (24, 25), Tokyo (25), Pasteur (26), and Copenhagen (26, 27). One study using the Glaxo strain in Nigeria found that satisfactory tuberculin sensitivity was induced even in preterm infants (28). However, some studies have shown a less than optimal result in Asian children (29-31), though these populations may still be protected against tuberculosis. A trial by Fillastre et al. (32) showed the same Pasteur freeze-dried vaccine gave a lower tuberculin hypersensitivity among Indonesian children (64%) than among French children (88%), supporting the possible importance of living conditions, previous atypical mycobacterial infections, and genetic characteristics in BCG immunogenicity.

A clear vaccine dose-response relationship in the size of the PVS has been demonstrated (33, 34); however, increasing doses yield only small increases in size of induration for doses above the lowest that produces PVS in most vaccinees. Stated in another way, the slope of the dose-response curve is small, and, in fact, may vary with vaccine preparation.

While it is generally accepted that tuberculin delayed hypersensitivity after natural infection is associated with partial protection against reinfection tuberculosis, the relation of BCG PVS to protective efficacy of BCG has not been well studied.

Efficacy of different BCG preparations in preventing tuberculosis when given to newborn children

The least ambiguous way to assess the protective efficacy of a vaccine is through a randomized, double-blind, placebo-controlled trial. Eight randomized community trials were reviewed by Clemens et al. (35). The vaccine efficacy observed ranged from 0 to 80%; explanations for this variability have included prevalence of infection with non-tuberculosis mycobacteria, variability in intensity of exposure to tuberculosis, questionable potency of some of the BCG preparations tested, and variations in intrinsic host resistance to tuberculosis. However, only three prospective community trials have evaluated

efficacy of BCG given at birth (see reference 36 for a review), though it is the time recommended by the standard immunization schedule of the Expanded Programme on Immunization. A randomized trial carried out in newborns in Hong Kong<sup>a</sup> compared efficacy of the Glaxo and Pasteur strains, using 0.3 mg/ml of Glaxo vaccine and 0.1 mg/ml of Pasteur-1173P2 both produced by Japan BCG Laboratory. In 162953 newborns receiving vaccine by the intradermal route, 3.43/10000 of those receiving the Pasteur vaccine developed tuberculosis within a four year period, compared to 4.55/10000 of those receiving the Glaxo vaccine, a small but statistically significant difference. Absolute efficacy could not be measured in this case, as there was no unvaccinated group. The report did not provide details of case ascertainment.

Some retrospective studies (for example, by Curtis et al., 6) have reported good efficacy of BCG vaccination. Recently, WHO has sponsored studies to evaluate the protective efficacy of BCG immunization in infants and/or children by two low-cost methods: case-control studies (3, 37) and contact studies (38).

In evaluating such studies it is important to consider the rigour with which the case definition was formulated and applied, the vaccination coverage, the assessment of vaccination status, and the comparability of vaccinated and nonvaccinated groups with respect to tuberculosis exposure and infection, ability to mount a cell-mediated immune response, and access to accurate diagnosis of tuberculosis disease. Cases and controls should therefore be comparable in age, sex, race or ethnic background, and socioeconomic status. In only a handful of published studies have all these features been considered in evaluating the efficacy of BCG immunization in newborn infants. Two of these are the studies reported by Tidjani et al. (39) in Lomé, Togo, and by Miceli et al. (40, also discussed in Smith, 37), in Buenos Aires, Argentina, both of which show a high protective effect of BCG (60-70%), and an even higher efficacy against more severe forms of tuberculosis for which diagnostic criteria are more stringent.

Unfortunately, only one of these two studies gives information on the type of vaccine in use during the study period. A few other studies do give this information, so that an idea of strain-specific efficacy may be inferred. Table 3 summarizes the data from such studies (41-44). Two other studies, referred to by Smith (37), give a protective efficacy of 74% and 89% against tuberculous meningitis in Brazil, where the Moreau strain has been used for many years; another study also mentioned by Smith (37) showed an efficacy of 40% against all forms of the disease and 75% protective efficacy against tuberculous meningitis in Indonesia, where locally produced Pasteur-1173P2 vaccine is used.

A recent re-analysis has been done of two case-control BCG efficacy studies in children from Indonesia and Colombia (69). The re-analysis controlled for selection bias in each trial by comparing efficacies of two different BCG's used sequentially, to allow a judgement of the relative efficacy between the pairs of vaccines used in each trial. The Japanese strain appeared more effective than preparations derived from the Pasteur or Danish (Copenhagen) strains in each case. Significance was not tested.

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<sup>a</sup>Chan, S.L. et al., XXXVI Conf. IUAT, Singapore, 4-7 November, 1986.

The general conclusion from these studies is that when vaccine is administered to newborns, there is no evidence of efficacy difference between preparations for which data are available in cases where the diagnostic criteria are stringent.

Adverse reactions related to newborn immunization using different preparations of BCG

It is well established that the use of BCG vaccine may be associated with a significant number of adverse reactions. However, the incidence of these reactions is generally low compared with the risk of contracting tubercular infection in the developing world, and the most frequent reaction, suppurative lymphadenitis, is usually self-limiting and requires no treatment. The risk-benefit equation for BCG vaccination is thus heavily weighted to the benefit side in developing countries. However, use of BCG in newborns probably increases the risk of adverse reactions. A number of other factors also affect the frequency of these reactions. One potentially serious consequence of these side effects, non-completion of the immunization series in children whose mothers had seen or heard of such reactions, has been very poorly assessed.

Lotte et al. (45) have developed a classification system for BCG complications, along with an estimate of their frequency. Two of these, regional suppurative adenitis (Category 1.2) and osteitis (Category 2.6) will be further considered, both because they are relatively more frequent than other complications (0.1-38 per thousand and 0.01-330 per million, respectively) and because cases from a number of countries are fairly well documented.

Regional suppurative adenitis. The incidence of this complication depends on many factors, including the type and concentration of vaccine, the age of the vaccinated subjects, and the use of proper technique in performing the intradermal injection (46). For example, a report from Egypt (47) shows a 10% incidence of adenitis needing treatment for patients vaccinated in public health welfare clinics compared with 0.02% for those receiving BCG in a chest clinic, where it was administered under strict medical supervision to an older population. Lotte et al. (45) cite an incidence of 0.1 per thousand for this complication in Hong Kong when a low dose of Glaxo vaccine was given to newborn infants compared with 38 per thousand in a mass BCG campaign in Algeria, where infants received a high dose (0.1 ml) of Glaxo vaccine. At the same time, use of what was reported to be the same amount of Pasteur-1173P2 strain vaccine in maternity wards in Algeria gave an incidence of only 5 per thousand.

In a retrospective study on BCG complications in six countries in Europe, Lotte et al. (48) concluded that the number of viable units injected had an important influence on the risk of regional complications. Quast et al. (49) found a dose-response relationship between number of culturable particles and incidence of suppurative lymphadenitis with the Copenhagen-1331 strain used in West Germany. A similar result was observed with the Pasteur strain in Hungary (50). On the other hand, studies in India using the Madras BCG Laboratory vaccine made from the Copenhagen-1331 strain (34) showed the incidence of complications to differ little between newborn infants given half doses and those given full doses.

Assuming consistency of immunization technique and a standard vaccine formulation in use in a country, a number of reports show a change in incidence of suppurative lymphadenitis when the preparation was changed. In Saudi Arabia (51), the incidence of regional lymphadenitis following BCG vaccination of newborns increased when the only change was the introduction of the Tokyo strain of BCG. Likewise, when the Gothenberg strain was replaced by the Copenhagen-1331 strain in the Federal Republic of Germany in 1975 (52), there was an immediate rise in the incidence of suppurative inguinal lymphadenitis after immunization of newborns, to 1.5% in one area. This fell to 0.02% after investigations into the dose-response relationship and subsequent lowering of the dose (53). A more recent study in Zaire (54) of an outbreak of axillary and epicondylar abscesses ruled out underlying Human Immunodeficiency Virus (HIV) infection as a cause, and concluded that it was due to the change of the BCG vaccine to the Pasteur-1173P2 product.

Comparative studies in which different vaccines were used simultaneously in the same population also show that the number of complications varies with the manufacturer and preparation of the vaccine. Bleiker (55) in a study in Cakovec, Yugoslavia, found the incidence of enlarged axillary nodes to be 5.05% in 1601 newborns vaccinated with Dutch BCG vaccine (Pasteur-1173P2 strain), 0.3% in 1871 vaccinated with the Copenhagen-1331 vaccine, and 3.15% in 1594 vaccinated with the Yugoslav product, prepared from the Pasteur-1173P2 strain.

Gheorghiu et al. (56) cite results in Togo in which the incidence of non-suppurative adenitis was 4.3% in newborns receiving the Pasteur-1173P2 vaccine, while this complication was rare in infants receiving the London F10 strain. With good injection technique in Togo, the incidence of adenitis with the Pasteur strain fell to 0.44% (M. Gheorghiu, personal communication, 1989). In Turkey (45), the Tokyo strain gave a risk of 0.3 cases of regional suppurative adenitis per 1000 infants compared with the risk of 1.3 per thousand with the local vaccine prepared from the Pasteur strain.

In Hong Kong the risk of complications, including peripheral lymph node enlargement and abscess formation, was 0.514/10000 infants receiving the Pasteur strain compared with none of 81304 infants receiving the Glaxo strain.

It is generally accepted that some preparations are associated with lymphadenitis less closely than others. The Tokyo strain (45) and the Moreau strain in Brazil (57) are rarely associated with this complication, while the Pasteur strain gives rise to a higher incidence (45, 56). The Copenhagen strain is also said to cause a relatively higher frequency of lymphadenitis. It is of unknown significance that this ranking of BCG preparations by reactogenicity shows a roughly inverse relationship with protein and methoxymycolate secretion, as shown in Table 2

A recent report by Stuckey (Stuckey, J. Prevalence of BCG Related Adenitis in Healthy Children, Maputo City, Mozambique, UNICEF Report, October, 1988) of a recent outbreak of BCG-associated lymphadenitis in an area with good access to relatively high-quality health services clearly demonstrates the chief determinants of the occurrence of adenitis. Infants were immunized at birth, or in the first two weeks of life if their birth weight was below 2500 g, with 0.05 ml of either Pasteur-1173P2 strain vaccine (in a new presentation aimed at minimizing the possibility of confusion in reconstitution) or vaccine of the Glaxo or Tokyo strain, depending on the month in 1988 in which they received immunization. Cases of adenitis were sought by active surveillance, although most were diagnosed retrospectively. A statistically significant difference was found between the incidence of lymphadenitis after Pasteur vaccine (9.9% of 531 children) and that after Glaxo/Tokyo strain vaccine (0% of 221 children). Other factors seemed comparable, since the same immunization centres used each of the three vaccines sequentially during the period of 1988 when each was available.

There was a striking difference in adenitis rates between different centres (range 0% to 17.6%), which was only partly explained by the fact that the centre with the lowest incidence did not give BCG to any children under two weeks of age. Birth weight data were available for 500 of the children and had no effect on the incidence of adenitis (Chi-square 0.02). Vaccination technique may have been important, however.

The most complete review, by Lotte et al. (45), attempted to collect, evaluate, and tabulate data from all reported cases through 1983, showing the incidence of regional suppurative lymphadenitis to vary widely by country and preparation of vaccine used. A study in six European countries (48) again showed a wide difference in risk of complications by country, ranging from very slight in Romania, to 0.6% in Hungary and 1.7% in Yugoslavia, all of which use their respective national preparations of the Pasteur-1173P2 strain. High incidence of regional suppurative adenitis have been found with Pasteur-1173P2 preparations from France (58); especially high incidences have been reported following use of this vaccine in Africa (56).

Although it is clear that improvement of intradermal injection technique and appropriate adjustment of dose will minimize this reaction, which is noted to occur especially with the Pasteur strain, recent data collected in Paris after administration of dispersed grown Pasteur strain BCG to newborn infants under conditions where technique was strictly controlled, showed an incidence of fistulated adenitis of from 0 to 10% depending on the lot of vaccine used (N. Guerin, personal communication, 1989). In one lot of vaccine, when the concentration ( $8.8 \times 10^6$  particles/ml) was halved by giving a 0.05 mg dose instead of 0.1 mg, the incidence of this complication dropped from 10% to 0. There was a fairly good correlation between concentration in number of culturable particles and incidence in this investigation; moreover, the more reactogenic lots showed a lower thermal stability.

In evaluating most of the above-mentioned reports of BCG-associated lymphadenitis, it should be remembered that careful case definitions of BCG adenitis were rarely made, or applied to the cases discussed, and that in most cases were sought through passive surveillance only. Small axillary lymph nodes, pea-sized or less, are commonly palpable after BCG immunization, and are rarely noted by the mother unless searched for specifically.

In summary, the reasons for the differences in incidence of BCG-associated lymphadenitis include dose for a given preparation of vaccine, age of the recipient, and technical quality of the intradermal injection of vaccine. Additional determinants, including the method of preparing the vaccine and the characteristics of the recipient population, may also be important.

Osteitis. The mean risk of BCG osteitis varies greatly from country to country, with some countries reporting extremely low incidence (e.g. 0.01 per million in Japan) compared with a high incidence elsewhere (e.g. more than 300 per million in newborns in Finland) (45). An increased incidence of osteitis has been seen in countries where a change in vaccine or vaccine manufacture has been introduced. Thus the increase in the incidence of osteitis in Czechoslovakia coincided with the substitution of the Prague strain by the Russian strain of BCG (59), and that in Finland and Sweden with a change in the manufacture of the Gothenberg strain (60).

Use of the Prague strain for universal vaccination of the newborn in Czechoslovakia since 1951 had coincided with the disappearance of tuberculous meningitis in children and the almost complete disappearance of tuberculosis in children in general by 1982 (61). In 1980, however, the Russian strain was substituted. This strain had been thought to be more immunogenic (23), though Sula et al. (61) could demonstrate no substantial differences in the immunogenicity of the Czechoslovak and Russian BCG strains in guinea pigs. The risk of osteitis in Czechoslovakia in the period 1982-1985 rose to 35 per million (48), with many cases bacteriologically confirmed (62). In contrast, no cases were reported in the USSR, where the same strain was used (48).

The dramatic rise in the incidence of osteitis in Sweden and Finland coincided with the replacement in 1971 of BCG produced by the Swedish BCG laboratory in favor of a vaccine produced by the Statens Seruminstitut in Copenhagen which was also based on the Gothenberg strain. Continuous checking of the virulence in animals during manufacture and comparison in guinea pigs of the two products manufactured in Sweden and Denmark provided no support for the hypothesis that the process of manufacture was responsible for the observed reactions (63).

A retrospective study beginning in 1948 (60) shows that cases of osteitis were present in Sweden from 1949 onward. The reported incidence was 1/40000 for children born between 1960 and 1969 and rose to between 1/3000 and 1/4000 for neonatally vaccinated children in the period 1972 to 1975. It should be noted that compulsory notification of BCG reactions to the Swedish Adverse Drug Reaction Committee was instituted during this period, and there was increasing

publicity about this reaction. However, a recent retrospective study in Europe (48) failed to find any reports of BCG osteitis in six European countries, while cases are still being reported in Finland (though at a lower rate) where the Glaxo vaccine is in use. Osteitis cases have never been notified in Great Britain where the Glaxo vaccine is also in use (48).

The osteitis story remains unsolved. The impact of several factors - active case-finding, vaccine strain, manufacturing technique, body site of vaccination - is undoubtedly important, but the available evidence give no consistent explanation.

Relationship of biologic characteristics of the BCG preparation to vaccine efficacy and reactogenicity

Although vaccine efficacy as measured by induced tuberculin sensitivity and vaccine safety as measured by incidence of adverse reactions such as BCG-lymphadenitis both show a dose-response relationship, the slopes of these dose-response curves differ for different preparations. The acceptability of a BCG vaccine preparation will depend on the relative slopes of these two curves. For the more reactogenic strains, the dose at which good efficacy and low reactogenicity is found may be more difficult to determine.

Although there has been substantial speculation about the clinical effects of changing the method of production of BCG vaccine, very few reliable data exist. Osborn (14) has shown that while the Pasteur-1173P2 strain appears to be homogeneous with respect to colony morphology, both the Tokyo and Copenhagen strains carry a minority population that will become a majority under certain changed production techniques, causing them to exhibit non-spreading colony morphology like that of the Glaxo strain. Abou-Zeid et al. (21) confirmed this observation when these four different strains were prepared in four different production laboratories, and correlated the colony morphology with the presence or absence of the lipid mycoside B.

The work of Osborn and colleagues (14, 21, 64) suggests that even in those studies where a number of strains are compared after preparation in a single laboratory, their characteristics will not necessarily be maintained nor will they be comparable. Thus the Japanese BCG vaccine generally exhibits spreading morphology, but when prepared by either the Glaxo or the Pasteur method, it showed non-spreading colony morphology (21). The Glaxo strain prepared in Copenhagen (12, 13) or in Paris (9) grew poorly, making comparison of its in vitro characteristics with other preparations difficult.

Although there is an observed correlation between colony morphology and excretion of mycoside B (21), several other properties do not correlate well with colony morphology. Gheorghiu and Lagrange (9) report that the Glaxo and Tokyo strains grown in their laboratory, both with non-spreading colony morphology, are at opposite ends of the spectrum with respect to viability and stability. Moreover, though measures of immunopotency such as tuberculin sensitivity in guinea pigs seem to be higher in the spreading strains (9), these workers found that the non-spreading Glaxo strain preparation, along with the spreading Pasteur strain preparation, gave better protection in mice.

In contrast to these mixed results, however, a recent publication by Gheorghiu et al. (10) describes the preparation of a dispersed-grown BCG vaccine with better immunogenicity, viability, and heat stability than the classical surface-grown Pasteur BCG, and at least 10% non-spreading colony morphology compared with the 100% spreading colony morphology of the classical strain. In the population studied, the higher immunogenicity and dose were not accompanied by increased side effects. Further studies on this product are in progress.

Preliminary data from a trial of this new dispersed-grown Pasteur vaccine in 1588 newborn children in Togo, using different intradermal doses, revealed suppurative lymphadenitis incidences ranging from 0.8% for the lowest dose to 8.8%. When a slightly higher dose of the same vaccine was given to school-age children in Europe, the incidence of lymphadenitis was 0% of 528 (Report to the Tuberculosis Unit, WHO, May, 1987).

The presence or absence of certain antigens and lipids, shown in Table 2, appears to divide BCG strains into different categories. Again, there is no obvious correlation with either immunogenicity or efficacy, although some of the more attenuated strains (Tokyo, Moreau) show production of the MPB70 antigen and of methoxymycolates, while those known to be more potent (Copenhagen, Pasteur) do not. It is of interest that the Swedish strain contains methoxymycolate whether cultivated in Copenhagen or in Gothenberg (20), suggesting that this marker did not change with the production laboratory. Furthermore, this characteristic lipid production did not correlate with a change in colony morphology (21).

A recent study using DNA restriction mapping analysis of BCG preparations (70) shows a division of the BCG strains tested into the same categories as shown in Table 2 for MPB70 expression, with the Swedish, Russian, Tokyo, and Moreau strains in one category, and the Copenhagen and Pasteur in another. These authors suggest that the former group is most like the original BCG strain established as an avirulent vaccination strain in 1921.

Recent work (65) on *Mycobacterium leprae* has suggested that cell wall protein is a major contributor to cell-mediated immune reactivity, whereas removal of mycolates did not affect this activity. It is clear that further work needs to be done to define those biochemical correlates that are predictive of efficacy and reactogenicity in humans.

## 5. RELATIONSHIP OF HOST CHARACTERISTICS TO ADVERSE REACTIONS

In the review by Lotte et al. (45), and in decades of programme experience, the main characteristic of immunologically normal hosts which is associated with increased risk of lymphadenitis is age at immunization of less than one month. While the incidence of lymphadenitis in these children is approximately twice that in children over three months of age, there is no evidence for an increased risk of life-threatening reactions in neonates. Thus the EPI recommends BCG vaccination at birth.

In spite of the appearance of birth weight below 2500 g as a contraindication on some package circulars, there is no good evidence that otherwise normal children who are moderately premature or have low birth weights, but are able to leave the maternity institution, are at any increased risk of toxicity from vaccination at the time of going home. The experience in Mozambique showed no evidence of a higher incidence of complications in infants below 2500 g (Stuckey, J. Prevalence of BCG Related Adenitis in Healthy Children, Maputo City, Mozambique, UNICEF Report, October, 1988).

The presence of major congenital immune deficiency has been found to be associated with risk of both local and disseminated BCG infection after immunization on a number of occasions, but this subject is reviewed well in other places and will not be further discussed here. However, an important unresolved issue is the effect of symptomatic and asymptomatic infection with HIV on BCG complications.

The present WHO recommendation is not to give BCG to infants with symptomatic AIDS (66). This recommendation is based on the existence of five cases of disseminated BCG infection after immunization in such children; while the incidence of such complications could not be calculated, it seems reasonable to assume a significantly increased risk in children with symptomatic AIDS. The situation is even less clear for children with asymptomatic HIV infection, which will be the condition for almost all infected neonates considered for BCG immunization in HIV-endemic countries.

One carefully followed cohort of HIV-positive infants, with controls, has been reported from Zaire (Dr Medi Mvula, presentation at EPI Research and Development Meeting, Abidjan, Ivory Coast, October 1988). A cohort of 470 children of HIV-positive mothers and 600 children of HIV-negative age-parity matched mothers was followed for at least one year, after immunization with all EPI vaccines including BCG in the neonatal period. No cases of dissemination were seen in either group, and minor complications (adenitis, fistulization) were not significantly different. The two groups also did not differ in their rate of tuberculin sensitivity when tested at the age of 12 months. In a second study of cases of BCG adenitis in Rwanda, though incidence of adenitis could not be calculated directly, there appeared to be no evidence of a higher risk of BCG-adenitis in HIV-positive infants by comparison with percent HIV-positivity of women of child-bearing age from the same population (Mercier, personal communication, 1989).

Determinants of occurrence of BCG-osteitis have been discussed above. Taking into account the limitations in the observational data available, it appears that persons of Swedish and Finnish national origin may have a substantially higher risk of developing osteitis even after differences in use of BCG vaccine preparations are taken into account. No other patient variable appeared to be associated with occurrence of osteitis.

There is convincing observational evidence for a substantial association of risk of local cutaneous reactions (i.e. keloids) with racial group (45).

To summarize, the major host characteristics that may affect adverse reactions to BCG in EPI programmes are the approximately doubled incidence of adenitis in neonates compared to older infants and children, and the increased risk of disseminated (and possibly of local as well) reactions in infants with serious immune deficiency involving the T-cell-mediated system. In practical terms, the major risk of concern is abnormal T-cell function secondary to HIV infection, which is rarely present until a period of several months after birth in perinatally infected infants. Thus the existence of endemic HIV infection should provide EPI programme managers with another strong incentive to provide BCG at or near birth, when available evidence does not suggest increased risk of adverse reactions.

## 6. RECENT REPORTS OF INCREASED INCIDENCE OF BCG-ASSOCIATED LYMPHADENITIS

As pointed out above, the varying incidence of regional suppurative adenitis (0.1-38/1000) reported under different conditions depends on the technique for administering the vaccine (and thus the training of staff), the dose and preparation of vaccine used, and the characteristics of the recipient population. In general, an immunization programme will be damaged if reported incidence of regional lymphadenitis following vaccination rises above approximately 1%.

### Mozambique

Mozambique reported a suspected increase in neonatal lymphadenitis in Maputo in March 1987. An investigation of cases revealed enlarged suppurative axillary nodes (67). The incidence of regional lymphadenitis was 1.3% without active case finding, but rose to 7.4% when active case finding was utilized. During the preceding year, the type of BCG vaccine used in Maputo was changed five times, and included products from Japan BCG Laboratory, Connaught, and Pasteur. An outbreak of lymphadenitis reported in the province of Imbahane in mid-1987 when only Pasteur vaccine was in use was associated with administration of twice the recommended dose (F. Cutts, personal communication, 1988).

Subsequently there has been another outbreak of lymphadenitis in Mozambique (Stuckey, J. Prevalence of BCG Related Adenitis in Healthy Children, Maputo City, Mozambique, UNICEF Report, October, 1988). Since this outbreak was also positively associated with the use of the Pasteur BCG vaccine, it is unlikely that the cause can be attributed to errors in dosage.

### Zimbabwe

One of the best documented reports comes from Zimbabwe, which has produced a "Report on the Investigation of Complications of BCG Immunization, Zimbabwe, 1987," a joint report of the Ministry of Health and WHO with consultants from Pasteur Vaccins, Paris. Beginning in the second half of 1986, sporadic reports indicated an increasing number of children developing regional lymphadenitis after BCG vaccination. A team was formed to investigate the extent of the problem and its possible causes. Available data showed that around 5% of those vaccinated in Harare developed regional BCG lymphadenitis, more than half of which was suppurative. A case definition was developed to characterize abnormal reactions, data were compiled on reported cases, case records were examined, and vaccination techniques were analysed.

It was noted that the BCG vaccine in use at vaccination centres was changed from the Mérieux product (derived from the Glaxo strain) to the Pasteur (Paris) vaccine during the second and third quarters of 1986. A similar increase in complications had been observed in 1983 when Pasteur Vaccins products were also used for a brief period. It was planned to start using the Mérieux vaccine again in January, 1988, and recent communication from Zimbabwe indicates that the incidence of lymphadenitis has declined since autumn 1988.

A survey of vaccination technique indicated a number of errors in administration. Only 4 of 17 (24%) reconstitutions were correct, only 4 of 28 (14%) injections were done correctly, and in only 1 of 14 (7%) cases was both reconstitution and intradermal injection performed in the prescribed manner. The investigators concluded that the outbreak of BCG complications might have been caused by the faulty techniques combined with a more reactogenic type of vaccine than had previously been used.

The Zimbabwe surveillance system recorded the following data in all cases of complications: date, date of birth, date of presentation, date and place of vaccination, site of vaccination, type of complications, and birth weight.

#### Zaire

In the second half of 1986, an increase in cases of BCG-adenitis was also observed in Kinshasa, Zaire (54). The outbreak was investigated for the following possible causes: poor injection technique, incorrect dose, immunodeficiency of recipients, preparation of vaccine used. Although poor technique and incorrect dose may have been used, the investigators felt that it was unlikely that all 158 administrators of BCG vaccine would have changed their technique simultaneously. Moreover, as mentioned above, 19 infants with BCG-adenitis were examined and found to be HIV-seronegative.

A survey of the Central Vaccine Stores indicated that the supplier of BCG vaccine was changed from Glaxo to Pasteur in the autumn of 1985. The Pasteur vaccine would have been distributed from the end of 1985 and largely used in 1986. The investigators thus concluded that the outbreak was caused by the preparation of vaccine used.

#### The Caribbean

In late 1982 and early 1983 cases of severe BCG lymphadenitis were noted in Saint Lucia. An investigation involving passive surveillance (68) uncovered 37 cases. Immunization policy in Saint Lucia was to give BCG at two to three months of age. The mean attack rate was 3.4%, although this was higher in some clinics; immunization techniques had been good, and variation among clinics was attributed largely to chance and perhaps to other factors such as inadequate mixing of freeze-dried vaccines. The outbreak occurred after a change to Pasteur vaccine.

In addition, outbreaks of BCG-associated lymphadenitis have been reported to WHO from Rwanda, Jamaica, Dominica, and Kampuchea. Because of the insensitivity of the WHO reporting system for such adverse reactions, it is likely that a number of other national programmes have also had such occurrences in the past three years.

### Conclusions regarding recent outbreaks

Every reported outbreak of BCG-adenitis has occurred after a change in vaccine strain, in almost every case to the Pasteur-Paris vaccine. According to a WHO publication (WHO/TB/86.147, "BCG Vaccination of the Newborn. Rationale and Guidelines for Country Programmes"), all outbreaks of suppurative adenitis in BCG country programmes in the last two decades have been connected with a change in vaccine.

Further investigation of these recent outbreaks is needed to clarify the role of possible confusion over the reconstitution and dosing instructions for Pasteur vaccine. Until January 1988, users of the Pasteur Vaccins product were instructed to reconstitute the vaccine to different concentration for use in newborns and in older children; it is possible that some health workers are confused and are still administering to newborns a full 0.1 ml of the vaccine reconstituted to normal strength according to the new instructions.

In addition, the investigations in Zimbabwe suggested that poor technique, both in reconstitution and in administration of the vaccine, could contribute to the problem. This hypothesis is supported by the repeated field observations in Africa of use of 1.0 cc tuberculin syringes for BCG injections to infants. Use of the same syringe for injections to multiple children, with replacement of the needle between doses, is also not a rare practice and contributes to dosage inaccuracy. Thus follow-up studies are needed to assess the impact of training and changes in vaccine packaging on these outbreaks.

However, evaluation of the above reports suggests that, notwithstanding the effect of poor technique of administration, the preparation of BCG has been an important independent contributory factor in the recent cluster of outbreaks of BCG lymphadenitis.

It should be stressed however that there is no debate concerning the compliance of all of these vaccines, including the Pasteur-Paris product, with WHO Requirements. At the same time it is apparent that WHO Requirements must be reviewed with the intent of addressing the problem of BCG vaccine reactogenicity.

### 7. RECOMMENDATIONS

Based on the information reviewed in this paper, a number of recommendations can be made to maximize the effectiveness of BCG immunization programmes and to prevent further outbreaks of BCG-associated lymphadenitis.

### Efficacy of BCG immunization

There is good evidence that the efficacy of modern BCG vaccines is in the range of 60-90% for disseminated tuberculosis or meningitis in young children, but somewhat lower for other forms of primary tuberculosis disease.

There is no evidence that any BCG preparation tested is more efficacious than any other under these conditions, and most of the preparations currently in commercial production have been tested at least once in careful clinical trial. There is therefore no evidence at present to support the choice of one preparation or manufacturer of BCG over another on the basis of protective efficacy. However, there is a need for the development of a single *in vitro* test capable of predicting the induction of immune resistance of humans to infection or dissemination of *Mycobacterium tuberculosis*.

### Prevention of BCG-associated lymphadenitis

- Because of the increasing number of outbreaks of BCG-adenitis in the last four years, changing the preparation of BCG vaccine supplied to a country where no problem has been encountered should be avoided.
- More research on exact dosage needed for optimal protection with fewer reactions is needed. Further, there is a need to devise tests which may correlate with higher reactogenicity of a given strain.
- Vaccine type and lot number in use in immunization programmes should be registered by EPI Programme Managers, and training to do this should be extended to the local level.
- Training in techniques of BCG immunization should be reemphasized, concentrating on reconstitution for proper homogenization; technique of intradermal administration; proper dosage; use of each syringe for a single child; proper vaccine storage; stock rotation to avoid use of vaccine after expiry.
- If there is a suspected increase in reactions reported with the use of BCG vaccine, active case finding in connection with a standard case definition should be initiated. Data should include age of immunization, sex, ethnic background, time to onset of symptoms, vaccine manufacturer and lot number, immunization site, and conditions of storage of vaccine.
- There is a need for studies of the effect of occurrence of BCG-lymphadenitis on immunization drop-out rates in infants who experienced adenitis, or who live in a community where this complication is reported.

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## FULL SUMMARY

For over forty years, when WHO took over responsibility for large scale international BCG vaccination programmes, it has overseen the quality control of BCG vaccines. This International Quality Control System includes provision of reference and seed lots of vaccines, coordination of clinical and laboratory testing of lots of vaccines used in immunization programmes, and training and technical advice in BCG production and control.

Laboratory tests are designed to insure consistency of production once a product has been clinically tested and found to have the appropriate characteristics. These tests include viability, thermal stability, and delayed type hypersensitivity in animals

The vast majority (>90%) of all BCG vaccines used in the world are produced from three strains: Pasteur-1173P2, Tokyo-172, and Glaxo-1077, though there are at least 15 major manufacturers who use different stabilizers and doses in their products. Available data on the physical-chemical characteristics of these strains have not been successful in predicting efficacy nor toxicity of BCG vaccines. Nor is post-vaccination delayed sensitivity to tuberculin in children a completely satisfactory correlate.

A number of studies have attempted to establish efficacy of BCG vaccines. In cases in which BCG has been given to newborn infants, it has been shown to have a high efficacy against severe forms of tuberculosis for which diagnostic

criteria are more stringent. The available data do not demonstrate a preparation dependent difference in efficacy.

Studies on BCG-related adverse reactions do show a clear preparation dependence, particularly for the reaction regional suppurative adenitis. Thus, the Pasteur-1173P2 strain is associated with an increased risk of BCG-adenitis relative to the Tokyo-172 and Glaxo-1077 strains. However, recent reports have suggested host factors including age of recipient and immunological status may be important in incidence and timing of these reactions, as are dose and technique of administration. BCG-osteitis also shows a preparation dependence, and, in addition, a strong dependence on the recipient population.

All reports of increased incidence of adverse reactions have been associated with a change of preparation of vaccine used in a country immunization programme. Because of the potential damage to immunization programmes when adverse reactions occur, a change in BCG vaccine product should be avoided, except in those cases when the preparation in use is causing problems.

Recommendations, including better training of BCG administrators, improved record keeping, and active case finding are given to help EPI programme managers prevent or quantitate problems associated with BCG-reactogenicity.

Table 2. Characteristics of BCG Strains<sup>a</sup>

Strain	MPB70 <sup>b</sup>	46kD dimer <sup>d</sup>	Methoxymycolate <sup>e</sup>	Mycoside B <sup>f</sup>	Colony <sup>f</sup> Morphology
Tokyo-172	++	+	+ <sup>f</sup>	+	Spreading
Moreau (Brazil)	++	+	+	ND	ND
Russian	++	+	+ <sup>g</sup>	ND	ND
Swedish	++	ND	+	ND	ND
Glaxo-1077	+	±	-	-	Nonspreading
Tice	-	-	ND	ND	ND
Copenhagen 1331	-	-	-	+	Spreading
Pasteur 1173P2	-	-	-	+	Spreading
Beijing	- <sup>c</sup>	ND	-	ND	ND
Prague	ND	-	-	-	ND
Dutch	ND	-	ND	ND	ND
Indonesian	ND	-	ND	ND	ND
Dakar	ND	-	ND	ND	ND

<sup>a</sup>ND = not determined

<sup>b</sup>++ means 50-100% of amount found in Tokyo strain

+ means 1-10% of amount found in Tokyo strain

- means <1% of the amount found in Tokyo strain

Data from reference 17

<sup>c</sup>Reference 16

<sup>d</sup>Reference 18

<sup>e</sup>Reference 20

<sup>f</sup>Reference 21

<sup>g</sup>Reference 22

Table 3. Summary of Some Studies on Protective Efficacy of BCG

Date and Place of Study	Study Population	Vaccine Used	Type of Study	Diagnostic Criteria	Recruitment of Controls	Ascertainment of Vaccination	Comments
Boksburg-Benoni Hospital, S.Afr. 1972-1976 <sup>a</sup>	Blacks 0-4 Yr.	Tokyo (after October 1972)	TB-Cohort	Hospital and clinic records, 538 cases	NA	Hospital records	19 cases vaccinated, 0/8 with meningitis. Efficacy >60% all forms, 100% TB meningitis
Rangoon Children's Hospital, Burma, July 1982 <sup>b</sup>	0-4 Yr.	Tokyo half-dose	Case-control	WHO scoring system, 311 cases	Hospital controls, 5/case, match on sex, age, residence	Scar, documents, parental recall	Efficacy 38% all forms, 52% TB meningitis, 80% disseminated TB
Israel 1956-1979 <sup>c</sup>	Jewish 0-12 Yr.	Glaxo (after late 1960s)	TB-Cohort	Notification by MCH, 299 cases	NA	MCH or hospital card, 86% coverage after 1962	Age and sex adjusted efficacy 24% for pulmonary TB, 64% for extrapulmonary TB
Bangkok Central Chest Clinic, Sept. 1981- June 1984 <sup>d</sup>	0-4 Yr.	Mérieux	Contact (Retrospective)	WHO scoring system, 218 cases	1506 TB cases followed, age, sex, residence, socioeconomic status found	Scar, documents	Efficacy 53% all TB, 72% bacteriologically confirmed cases
Manitoba, Canada, 1979-1983 <sup>e</sup>	Indians 0-14 Yr.	Connaught	Case-control	Clinical, lab eval., 71 cases	213 controls same age and community but not matched, stratified analysis	Records, 72% coverage confirmed cases	Efficacy >60% all TB, 73% bacteriologically confirmed cases

Table 3, continued

Lomé, Togo 1983-1985 <sup>f</sup>	0-6 Yr.	Glaxo	Contact	WHO scoring system	1421 child household contacts	Records, scars, 62% coverage	Efficacy 61.5%, higher for more severe disease and children under 6
Seoul, Korea 1984-1986 <sup>g</sup>	<5 Yr.	Paris seed lot 1173P2 produced by Japan BCG Lab	Contact	WHO scoring system	1293 child household contacts	Scars vaccination certificates	Efficacy 72%, unpublished study

- <sup>a</sup>Reference 25  
<sup>b</sup>Reference 41  
<sup>c</sup>Reference 42  
<sup>d</sup>Reference 43  
<sup>e</sup>Reference 44  
<sup>f</sup>Reference 39  
<sup>g</sup>Jin et al., personal communication, 1989

Table 1. Responses from Manufacturers of BCG Vaccine

Parent Strain	No. Mfrs.	Manufacturers' Report of No. Culturable Particles/Dose	Stabilizer	Total Production, Approx. No. Doses/Yr.
Pasteur 1173P2	6	37,500-500,000 <sup>a</sup>	sodium glutamate +dextran, glucose	59,000,000
Copenhagen 1331	3	150,000-300,000 <sup>b</sup>	Haemacel, glucose or monosodiumglutamate	3,000,000
Glaxo 1077	2	200,000-1,000,000	dextran, dextrose, or albumin	40,000,000
New York	1	525,000-1,125,000 <sup>c</sup>	7.5% lactose, salts	100,000
Tokyo-172	1	3,000,000	sodium glutamate	54,000,000
Montreal	2	200,000-3,200,000 <sup>b</sup>	dextran, sucrose, or monosodiumglutamate	9,000,000

<sup>a</sup>Four of six suppliers recommend a half or smaller dose for infants

<sup>b</sup>Half dose recommended for infants

<sup>c</sup>Three-quarters dose recommended for infants