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Report

Consultation on the characterization of BCG strains

**Imperial College, London, UK
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**WORLD HEALTH ORGANIZATION
Quality Assurance and Safety of Biologicals
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The meeting was opened by Dr U. Fruth (Initiative for Vaccine Research, BAC ; WHO, Geneva). Dr E. Griffiths (Health Canada, Ottawa, Canada) and Dr M. J. Corbel (NIBSC, Potters Bar, UK) were appointed Chairman and Rapporteur respectively. Dr Fruth stated that a key objective of the meeting was to facilitate the implementation of new vaccines against tuberculosis. It was clear that BCG would continue to play an important role in immunization against tuberculosis and would be included in forthcoming clinical trials, either as a primer to be boosted by new components or as an integral component of new vaccines. In this context it was important to consider the molecular characterization of BCG in the light of new knowledge. This had been strongly recommended by the TB Vaccines Working Group of the Global Partnership to Stop TB. This initiative would be implemented by the WHO Quality Assurance and Safety of Biologicals Unit (QSB).

Dr I. Knezevic (QSB, WHO, Geneva) noted that it was difficult to get support for work on 'old' vaccines such as BCG as it was not generally realized that these still had an important role to play and were subject to issues that required updated information. QSB was responsible for setting standards for biologicals, including vaccines through the Expert Committee on Biological Standardisation (ECBS) of which it formed the secretariat. The establishment of standards was a complex process involving identification of candidate material, performance of an international collaborative study, statistical analysis of the results, submission of a report to the ECBS which decided to recommend approval or discontinuation of a proposed preparation. Recommendations were usually published in the WHO Technical Report Series as Requirements, Recommendations or Guidelines. QSB interacted with National Regulatory Authorities which were concerned with the different stages of vaccine development, production and evaluation. In the case of BCG, the vaccine was well-established but the Requirements had been written many years ago (WHO Expert Committee on Biological Standardization, 1987, 1988) and it was time to consider if these needed to be updated and brought into line with those for other products. For example, there were several outstanding questions. It had been claimed that the large number of daughter strains of BCG had similar protective power but it was not clear if this view still prevailed. The importance of product characterization needed to be strongly emphasized as well defined vaccines offer the greatest chance of success. Poorly defined products make it difficult to analyze factors that can contribute to vaccine performance. It would seem that there is a need to improve the characterization of BCG vaccine. In relation to the current Requirements, methods for determining the identity of sub-strains appeared inadequate and do not allow specific identification.

The potential benefits of a consultation on improved characterization could include:

- improved vaccine characterization: molecular genetic studies could be used to differentiate sub-strains used by different manufacturers
- it may help to ensure consistency of production in terms of genetic stability
- this may help in the future clinical evaluation of BCG vaccines

The objectives of the present meeting were:

- to provide scientific advice to WHO in respect of future characterization of BCG strains for vaccine production,
- to provide WHO with advice on clinical data regarding the immunogenicity, efficacy and safety of current BCG vaccines
- to discuss the need for additional studies (genetic analysis, animal studies, clinical trials)
- to determine the need for a collaborative study on methodology
- if needed, to identify the questions to be answered by the study
- to progress towards a global consultation on the Requirements for production and control of BCG vaccines.

Dr E. Griffiths re-affirmed the objectives of this meeting and introduced the invited participants.

Dr M. Roumiantzeff (Consultant, Lyon, France) summarized the history of dissemination of BCG strains, starting from the original strain which emerged after 230 sub-cultures on glycerol-potato-bile medium between 1908 and 1921 of a *Mycobacterium bovis* strain isolated by Nocard in 1904. From this, 49 production sub-strains that have been in use at one time or another in various parts of the world could be traced to the original source on the basis of his own or WHO inquiries. However, there are still many other producers for local markets, using a variety of sub-strains, although others in recent years have stopped production. The total number of current producers was not known. The WHO Requirements for BCG vaccines were last revised in 1985, although the European Pharmacopoeia (EP) and US Pharmacopoeia monographs were revised more recently. The procedure for assessing the acceptability of vaccines for supply to UN agencies also needed to be considered. The number of suppliers pre-qualified by WHO has been reduced from 6 to 4 manufacturers who use 3 different BCG sub-strains (Japan, Russia, Danish). The current First International Reference Preparation for BCG Vaccine was set up in 1960 using the Japan strain. This had received very limited use in BCG studies. The need for future standards should be considered.

Dr F. Leguellec (Aventis, France) described the procedures used for production of BCG vaccine in France.. At one time both Pasteur and Glaxo sub-strains were used but these required different growth conditions and in recent years production had focused on the Glaxo strain. In both cases a seed lot system was used with strict limitation of the number of passages from the original seed. The final dosage forms differed, with lower dosage for the more aggressive Pasteur strain. The dosage had been decided on the basis of both clinical and laboratory studies.

Dr K. Haslov (Statens Serum Institut, Copenhagen, Denmark) reported on the revision of the E P monograph for BCG vaccine. This had been driven largely by the vaccines for cancer therapy rather than tuberculosis prophylaxis. Significant changes included the acceptance of molecular methods for identification and of biochemical assays for in-process monitoring of viability in place of colony counts.

Dr M. Behr (Montreal General Hospital, Canada) described the genetic evolution of BCG strains. This had been approached by examining strains with a defined history both by piecemeal analysis of variation in individual genes and by analysis of the whole genome. Examination of historical records showed that the Birkhaug, Brazil, Japan, Sweden and Russia sub-strains emerged soon after distribution of cultures derived from the 1921 parent strain. The Danish strain emerged in 1931, with the Glaxo and Prague strains diverging from this much later. The Frappier, Phipps, Connaught strains form another cluster derived from the Pasteur strain, with Tice developing from this independently. This pattern was confirmed by piecemeal analysis using the IS 6110 to detect polymorphisms and by the presence/absence of the *mpt 64* gene. The older sub-strains showed retention of *mpt 64* and 2 copies of IS 6110. Use of micro-chips and arrays showed that multiple deletions had occurred during the evolution of BCG sub-strains from *M.bovis* (Behr et al., 1999; Mostowy et al., 2003). Deletions in the RD1 region were observed in all BCG sub-strains, and in the vole and Dassie bacilli. All BCG sub-strains contained the same deletion RD1^{bcg} whereas RD1^{mic} and RD1^{das} were distinct (Mostowy et al., 2004). The older sub-strains such as the Japan, Moreau, Russia cluster, contained fewer chromosomal deletions than the sub-strains of the other clusters. Each sub-strain has its own signature molecular profile and differs from other sub-strains and the BCG of 1921. These differences can be used to characterize any sample of BCG and had shown some culture collection strains to have been incorrectly identified. BCG was undergoing genomic decay at a faster rate than wild type *M.bovis*. Regulatory genes were over-represented in this process although their role in relation to efficacy and adverse events remains to be determined. However, BCG had lost a number of antigens including ESAT-6, MPB64 and CFP-10. The genes for MPB70 and MPB83 were still present but were not expressed in post-1927 sub-strains. This may have implications for protective efficacy. The only vaccine candidates shown to be more effective than BCG in animal models were rBCG-30kDa and BCG::RD1. Since all current sub-strains differ from the 1921 BCG and from those used in clinical trials, it followed that no current BCG vaccine had been shown to be efficacious in a clinical trial. This raised the issue of whether a valid meta-analysis could be performed. It was proposed that in future human and animal studies, the vaccine strains should be characterized beforehand. It would be desirable to restrict the number of sub-strains used for vaccine production to a few. Molecular characterization should be performed as part of the quality control process. The clinical performance of difference sub-strains could be examined by alternating vaccines used in areas with reliable notification data.

Dr S. Cole (Institut Pasteur, Paris) reported on genomic studies performed together with Dr R. Brosch. The *M. tuberculosis* complex included *M.africanum*, *M.canetti*, *M.microti* and *M.bovis* with BCG forming a sub-group of the latter (Brosch et al., 2002). *M. bovis* had a smaller genome size than *M.tuberculosis* (4.32 Mb v. 4.41 Mb). The genome maps of the various species were almost identical but there were at least 14 regions of difference (RD1-14) between members of the complex (Gordon et al., 1999). Relative to virulent *M.tuberculosis* H37RV and *M.bovis*, the BCG genome contained 3 regions of difference, RD1-3, that were not shared and a large body of evidence has now been obtained that RD1 is the primary lesion responsible for attenuation of BCG. Relative to

M. bovis and *M. tuberculosis*, BCG contained 69 and 241 single nucleotide polymorphisms (SNPs) respectively, in a 400 kb segment of the genome. In contrast, *M. bovis* and *M. tuberculosis* contained 14 and 37 SNPs respectively, not present in BCG. In addition, pulsed field gel electrophoresis had identified tandem duplications of the *oriC* region, DU1, in BCG Pasteur but not in other sub-strains (Brosch et al., 2000). A second duplication was also detected at the DU2 region which contains a large number of regulatory genes that could influence expression of genes elsewhere in the genome. Unlike the DU1 duplication, this was not confined to the Pasteur sub-strain and in some cases (Danish sub-strain) was triplicated and showed further signs of instability. The evolution of these replications could be used to track the lineage of BCG sub-strains. Primers had been devised to differentiate the sub-strain clusters and individual sub-strains. Furthermore, the pattern of replications had been shown to change during the course of serial propagation of an individual sub-strain during vaccine production. It was clear that BCG strains were continuing to evolve, even during vaccine production. The phenotypic consequences of this were unclear but gave cause for concern. Molecular tools were now available and could be applied to the quality control and quality assurance of BCG vaccines.

Dr M. Gheorghiu (Institut Pasteur, Paris) summarised laboratory studies on BCG daughter strains. She pointed out that phenotypic changes occurred very readily in BCG strains during sub-culture (e.g. spreading and non-spreading colonial forms) and emphasized the importance of minimizing such changes during the course of vaccine production. Strict quality control was essential at all stages of production. The reduction in emergence of variants could be achieved by minimizing the number of passages from the master seed. The original specification of 12 sub-cultures was too high. Seed cultures should be established from single colonies and kept under stable conditions e.g. freeze-dried.

Sub-strains had been compared in a collaborative study involving 13 laboratories, by *in vivo* studies in mice and *in vitro* tests. The results indicated great variability between sub-strains. A correlation had been observed between residual virulence as indicated by persistence in murine tissues, and protective capacity. The sub-strains which persisted most strongly in mouse tissues and induced the largest local granulomas and strongest delayed type hypersensitivity (DTH) reactions, tended to produce the strongest protection e.g. Pasteur, Danish, Montreal. The less persistent and less reactogenic strains e.g. Glaxo, Japan, induced the least protection. In a comparison of 16 vaccines by 4 laboratories, no clear difference was seen in relation to sub-strain. There was some correlation between colony form and residual virulence but this was influenced by the culture medium. The French (Pasteur) strain was the only one to show no variation. Other studies had also indicated a correlation between protection and *in vivo* multiplication. In a more recent study, these and additional parameters such as cytokine production, lymphocyte proliferation, and CD 8 cytotoxicity, had also been examined (Lagranderie et al., 1996). The ranking of sub-strains for residual virulence was Pasteur>Russia>Glaxo but the order of protection was Pasteur>Glaxo>Russia. The Russia sub-strain also showed the highest cytotoxicity. Similar results were obtained if BCG was given by the oral route. Case control studies had suggested that Moreau, Glaxo

and Pasteur strains were more effective than the Japan strain but there was variation between individual studies. The Pasteur 1173P2 strain gave 80% protection in France and Hungary. Japanese studies had suggested that mouse and human responses to BCG were very different.

Dr Y. Lopez Vidal (Universidad Nacional de Autonoma, Mexico) summarised studies on the immune response of Balb/c mice to 10 sub-strains of BCG. It was to be expected that the complex genomic rearrangements, deletions and mutations present in BCG sub-strains would be reflected in phenotypic and immunological differences that could influence vaccine efficacy. The response of mice vaccinated subcutaneously with 10^4 cfu of the Pasteur, Phipps, Frappier, Connaught, Tice, Danish, Birkhaug, Sweden, Moreau or Mexico sub-strains, to intratracheal challenge with 10^6 *M.tuberculosis* H37RV was assessed by monitoring the development of DTH, bacterial loads in the lung, histopathological changes (pneumonitis and granuloma formation) and the immunological response (CD4, CD8 and CD69 lymphocyte type and cytokine profile). The patterns of response were complex and for each parameter, varied between sub-strains. All induced DTH compared with controls. More difference was apparent between sub-strains at 4 months compared with 2 months after vaccination but with no clear pattern. Bacterial loads varied widely, with the lowest loads seen in mice vaccinated with the Phipps, Frappier and Connaught sub-strains and the highest in the Tice group. Severity of pneumonitis was least at 2 months after vaccination with Connaught or Mexico, greatest with Danish, Pasteur and Tice. At 4 months, severity was least after vaccination with Phipps strain. Granuloma formation after 4 months was least severe in the Phipps and Mexico groups and worst in the Tice group. CD4 counts were highest in the Sweden, Birkhaug, Connaught, Danish and Mexico groups, CD8 counts highest in the Connaught and Frappier groups and CD69 counts highest in the Mexico and Danish groups. The Mexico group had the highest CD4:CD8 ratio. In other groups all counts were comparable with controls. IL-2 levels were comparable with controls except for the Mexico, Tice and Sweden groups which showed increases. The latter also showed the only significant increase in IL-10. γ -interferon responses were low in all groups.

Dr M. M. Ho (NIBSC, Potters Bar, UK) summarized attempts to develop improved methods for control testing of BCG vaccines. Emphasis had been placed on the development of improved viable counting methods, a more specific identity test and a reproducible aerosol challenge assay for protection. Experience with the ATP chemiluminescence assay suggested that this did not consistently reflect viability in freeze-dried BCG. An assay dependent on active metabolism of the tetrazolium substrate XTT was developed (Kairo et al., 1999). This was much more rapid than colony counting (2-4 days rather than weeks) but lacked sensitivity at very low count levels and required a stable reference preparation. Problems with the latter drove the search for alternatives. A commercial procedure that monitored uptake and metabolism of a chromogenic substrate by individual cells (Chemscan^R) was evaluated. This was shown to be rapid (2 hrs) and reliable and to correlate fairly well with colony counts. It can also differentiate individual cells from clumps. It is now undergoing validation for routine use.

A multiplex PCR test that can differentiate individual sub-strains was developed to replace the acid fast stain identity test that lacked specificity (Bedwell et al., 2001). It has

proved effective in routine use for batch release testing of BCG vaccines for immunization and for cancer therapy at NIBSC.

A murine challenge model that allows delivery of a consistent challenge as an aerosol has been used by Dr K B Walker to assess immunogenicity of tuberculosis vaccines. Although primarily a research tool, it could be used for BCG sub-strain characterization.

Dr T. Brewer (Harvard University, Cambridge MA, USA) reviewed the clinical and epidemiological evidence for BCG strain variability. Although Calmette and Guérin had believed that BCG was a 'fixed' strain, by 1929 there was evidence of colonial variation on egg media. It rapidly became apparent that cultural and production methods can affect the phenotype of BCG sub-strains. For clinical trial purposes, the strain is defined by the location where a vaccine lot was maintained or the individual responsible for introducing it to a particular locale. The strain is not defined by specific phenotypic or genotypic characteristics. The ancestry of BCG strains used in various clinical trials between 1921 and 1995 has been summarized (Brewer and Colditz, 1995). The number of these trials showed that BCG is a well-studied vaccine, with 13 placebo-controlled efficacy trials, 10 case-control studies and at least five trials examining revaccination and the same number assessing protection against leprosy. The results of these showed wide variation in efficacy against both clinical tuberculosis and fatal outcome. Since 1996 there have been at least 14 additional case-control studies. These indicated a protective efficacy of 60-64% against pulmonary tuberculosis and 47-87% protective efficacy against tubercular meningitis. Determining the effect of strain on efficacy was complex but it was clear that BCG sub-strains differed in genetic composition, growth requirements, viability, induction of tuberculin sensitivity in people and animals, side effects, and virulence and protective efficacy in animals. Evidence for strain differences in efficacy was provided by a comparative trial of the Glaxo and Paris (Pasteur) strains in Hong Kong children from 1978-82. This showed a relative risk of contracting tuberculosis of 0.63 for recipients of the Paris strain compared with those receiving the Glaxo strain. Follow-up of this cohort showed that re-vaccination increased the risk of disease but no data on strain effectiveness were reported.

Evidence against variation in efficacy between strains was provided by the UK MRC Trial in the 1950s where similar protection was provided by the Copenhagen strain and vole bacillus (*Mycobacterium microti*) vaccines (Medical Research Council, 1972). A similar conclusion could be drawn from the Madras trial where both the Copenhagen and Paris (Pasteur) strains failed to protect. These trials showed that efficacy varied by population rather than vaccine strain. Thus, the Copenhagen strain produced 78% efficacy after 20 years in the UK and no protection after 15 years in India. Similarly, the Glaxo strain produced 93% protection in the UK and no protection in Malawi (although it was effective against leprosy). In a Haitian trial, both Montreal and isoniazid-resistant Montreal BCG strains produced significant protection compared with a placebo. In the UK, similar levels of protection were produced by a liquid Copenhagen BCG (89%) and a freeze-dried Glaxo BCG (93%) vaccine. Studies on Canadian indigenous people between 1933 and 1983, showed that different BCG preparations achieved comparable

efficacy over time (range 57-80%). Examination of protection data between 1930 and 1990 showed a similar scatter of efficacy values over this period.

If there was no clear evidence for strain variation as a cause of variation in efficacy, other explanations needed to be sought. Both 'biological' and 'methodological' reasons have been identified and contribute substantially to the observed variations.

Dr L. Barker (Aeras Global Tuberculosis Vaccine Foundation, Rockville MD, USA) reviewed the evidence for BCG strain variation and adverse reactions. It has been widely recognised that certain sub-strains such as Pasteur and Danish were more reactogenic than others such as Tokyo, Glaxo or Moreau and were more likely to produce large ulcers at the inoculation site, local lymphadenopathy and suppurative adenitis. Reports of 'outbreaks' of adenitis in vaccination programmes were always associated with a change in vaccine strain, almost invariably to the Pasteur (Paris) sub-strain (Milstien and Gibson, 1990). Review of the complications reported in 55 countries, involving vaccine from 44 manufacturers prepared from 14 sub-strains, implicated the Pasteur (Paris), Danish and Sweden (Goteborg) sub-strains most frequently in all types of complications, although all vaccines and sub-strains could produce these. Risk factors other than the sub-strain included methods of vaccination, modalities of administration, strength of the vaccine and individual dose given, and the accuracy and vigilance of surveillance (Lotte et al., 1984).

Reported rates ranged from 0.01/1000 or less for local complications and adenitis in the former GDR and Rumania, to 17.2/1000 in Croatia. The different sub-strains in use were not identified. In another study, a complication rate of 36.61/1000 with the Pasteur (Paris) strain fell to 6.25/1000 vaccinations after switching to the Danish sub-strain. In Brazil, a reaction rate of 0.17/1000 in primary vaccinates and 0.39/1000 in re-vaccinates was reported with the Moreau strain.

Disseminated BCG disease was rare in individuals without underlying disease (0.59/1,000,000 neonates in France). Most cases were associated with HIV/AIDS or other cause of immunodeficiency.

Representatives of the manufacturers pre-qualified by WHO to supply BCG vaccines to UN programmes summarized the backgrounds of their products.

Dr M. Chouchkova (BCG Laboratory, Sofia, Bulgaria) reported that production of liquid BCG vaccine began in Bulgaria in 1949. They initially used the Pasteur strain for production but changed to the Russia sub-strain because they were encountering an incidence of about 1% cervical adenitis. The effect of implementation of BCG vaccination was established in 1951 and was to reduce the incidence of tuberculosis in vaccinated neonates fourfold in comparison with non-vaccinates. The current incidence was 54/100,000 population. Regular production of freeze-dried vaccine began in 1963. Its biological activity has been studied under laboratory and field conditions. In 1972 a seed lot system was introduced. The properties of lots produced from the seed lot 222 Sofia (derived from the original Russian sub-strain BCGI) have been studied. The vaccine is

produced by conventional methods to comply with WHO Requirements. The variations in viable count between lots were within acceptable limits (1.5-6.0 million /ml) and were not associated with any significant differences in effect when investigated in the field. The vaccine is also monitored for adequate heat stability and for oxygen uptake rate using Warburg manometry. Surveys are performed to check local reactogenicity and tuberculin conversion rate in school children. Neither factor varied significantly between batches or between ampoules within a batch. At the request of WHO, a randomized double blind clinical trial was performed and showed that the Bulgarian vaccine elicited the highest tuberculin conversion rate. No lymphadenopathy was observed in any of the vaccinated groups. The vaccine was approved for UN supply in 1991.

Dr S. S. Jadhav (Serum Institute of India Ltd, Pune, India (SIIL)) reported that the company had recently been pre-qualified by WHO to supply BCG vaccine to UN Agencies. They had originally intended to use the Danish sub-strain but the Indian Government had not authorised this. They now used the Russia sub-strain obtained from the Tarashevich State Research Institute for Standardization and Control of Medical and Biological Preparations, Moscow, Russia. The vaccine seed strain has been characterised by PCR-RFLP to establish its molecular profile. Protection tests in guinea pigs indicate that it is effective against a challenge of *M.tuberculosis* H37RV. It meets the standard pharmacopoeial and WHO requirements and an in-house specification of inducing a tuberculin reaction not less than 5 mm. The release limits are 1-33 million cfu/ml with a 2 year expiry date in accordance with the Indian Pharmacopoeia specifications. In a Phase 3 clinical trial, it produced results for tuberculin conversion and local reactogenicity rates very similar to those of the BCG vaccine manufactured by the BCG Laboratory, Guindy, India, that has been used in India for many years. Currently SIIL supplies BCG vaccine to about 50 countries and provides about 20% of the requirement for India. The Guindy laboratory provides the remainder.

Dr K. Haslov (Statens Serum Institute, Copenhagen, Denmark) commented that the SSI vaccine based on the Danish strain had been in production for many years and was supplied to numerous countries, both through the UNICEF programme and independently. It had a good safety and efficacy record. It was produced to pharmacopoeial and WHO requirements. Although not used routinely, molecular genetic methods had been explored for characterization.

Dr I Yano (BCG Laboratory, Tokyo, Japan) briefly summarized the history of the Japanese BCG vaccine. This had been produced by conventional procedures for many years using the original Japan (Tokyo) strain. The product met Japanese and WHO requirements and had a good safety record. It had been subject to extensive monitoring for reactogenicity and tuberculin conversion rate and had featured in many laboratory and clinical studies.

The final phase of the meeting was a discussion of the information presented and the future course of action. There was a consensus that the current WHO requirements for BCG vaccines were outdated and should be reviewed. The evidence for molecular genetic variation in BCG sub-strains was overwhelming. This was also reflected in laboratory

studies of their phenotypic and immunological properties. Clinical and epidemiological studies also indicated differences in vaccine performance in clinical trials but the presence of many confounding factors made it difficult to determine the influence of sub-strain. Nevertheless, for future use of BCG vaccine, both as conventional preparations for routine immunization or as part of a prime-boost strategy, and as genetically modified strains, it was essential to achieve much better characterization. It was agreed that the application of molecular characterization methods to the production and control of BCG merited further evaluation. The significance of known genetic variations and genomic decay in relation to the quality and performance of BCG vaccines needed to be determined. WHO was urged to promote further studies on these issues.

Recommendations

1. The current Requirements for BCG vaccines need to be reviewed in the light of current knowledge and the best available methods. The review should also consider the definition and nomenclature of BCG sub-strains in the light of new information. It should also take account of the position of the current standard, the replacement /development of appropriate standards and their application .
2. Currently, it was not feasible to conduct clinical trials to compare the efficacy and safety of currently used BCG. This could be re-considered in the light of the revised recommendations
3. The relevance of recent advances in the genomics and proteomics to the production and control of BCG vaccines needs to be assessed as part of this process.
4. Further information should be obtained by WHO on the number of BCG vaccine manufacturers currently in production and on the sub-strains used.
5. WHO should invite pre-qualified manufacturers to participate in the programme for improved characterization of seed strains.
6. More information is needed on the differences, if any, between the master seed, working seed and final lots of currently approved BCG vaccines.
7. The relevance of tandem replications in the genome to vaccine quality needs to be established
8. There is a need for a test that can identify BCG sub-strains specifically, exclude virulent mycobacteria and detect variation during production.
9. A BCG Working Group should be established to follow up on these recommendations. This would review progress early in 2005.
10. A sub-group (Dr M A Behr, Dr S T Cole, Dr Y Lopez Vidal, Dr I Knezevic and Dr M J Corbel -Coordinator) should pursue the development of a molecular procedure to fulfil the requirements of item 5. They will report progress by mid-2004.
11. When such an assay is available, a feasibility study should be performed to assess its applicability to the production and control of BCG vaccines.
12. WHO should identify funds to support this programme.

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ANNEX 1

List of Participants

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