Time to Detection of *Mycobacterium tuberculosis* Using the MGIT 320 System Correlates with Colony Counting in Preclinical Testing of New Vaccines

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Clinical studies have suggested that the enumeration of mycobacteria by using automated liquid systems is a faster and simpler alternative to quantitative cultures. Here, we show that the time to detection of *M. tuberculosis* growth as measured with the MGIT 320 liquid culture system inversely correlates with CFU determinations from culture on solid media and that mycobacterial quantification using the MGIT system is faster and easier to perform than CFU plating.

The limited effectiveness of the *Mycobacterium bovis* BCG vaccine in preventing adult pulmonary tuberculosis (TB) and the increasing incidence of deadly multidrug-resistant *M. tuberculosis* strains emphasize the need to develop improved vaccination strategies against TB (1–3). However, the recent disappointing results from the MVA85A TB vaccine trial in South Africa suggested that the search for new approaches to TB immunization is just beginning (4). Currently, most new vaccines against TB are evaluated in a multistep preclinical process that often includes testing in two or more animal models (5). Although established models are available for assessing novel TB vaccine preparations in mice, guinea pigs, rabbits, and nonhuman primates, testing new vaccines in these models is slow and often not reproducible in different laboratories. A time-consuming component of the vaccine evaluation protocols is the enumeration of postchallenge organ mycobacterial burdens by colony counting on solid media. Standard CFU determinations are labor-intensive, hampered by the tendency of mycobacteria to clump, and sometimes fail due to contamination (6). In many TB vaccine testing labs, CFU determinations can vary within different experiments because of the absence of standardized reagents for solid media preparation. Most importantly, CFU determinations are slow; usually about 3 weeks of incubation are required to detect colonies on mycobacterial growth plates (7).

For more than a decade, the MGIT liquid media system has been increasingly employed to assess whether *M. tuberculosis* bacilli are present in clinical specimens (8–11). The MGIT system uses an oxygen-quenching fluorescent sensor and appropriate software algorithms to determine whether significant mycobacterial growth has occurred (12). The instrument reports this bacterial growth as the time to detection (TTD). Overall, the MGIT system has been shown to be highly sensitive and to shorten the time needed to detect *M. tuberculosis* in clinical samples by 1 to 3 weeks (relative to growth on solid media). Several multicenter studies have demonstrated that MGIT-based protocols for detecting drug-resistant *M. tuberculosis* strains are relatively rapid and highly reproducible between different laboratories (13–15). Interestingly, the TTD measurements obtained using the automated liquid systems have been shown to closely compare with the results of CFU counting on solid media, and therefore the MGIT system represents a viable alternative to CFU determinations in evaluating responses to TB chemotherapy (9).

To potentially improve the reproducibility of preclinical vaccine assessments and to accelerate the testing process, we evaluated how the MGIT system compared to CFU counting in the quantification of mycobacteria during both in vivo and in vitro preclinical TB vaccine effectiveness assessments. For the in vivo testing, mice were vaccinated and challenged by the aerosol route with the Erdman strain of *M. tuberculosis* as described previously (16). Then, mycobacterial burdens were determined in lung homogenates from naive and immunized mice by using Middlebrook’s 7H11 solid agar plates (supplemented with oleic acid–albumin–dextrose–catalse and containing the antibiotics trimethoprim, cycloheximide, ampicillin, and thiophen 2-carboxylic acid hydrazide) and the MGIT liquid system (as described by the manufacturer [Becton, Dickinson]). For the in vitro analysis, mycobacterial growth inhibition assays using murine cocultures of *M. tuberculosis*-infected bone marrow macrophages and immune or naive spleen cells were established using protocols described earlier (17). Mycobacteria were quantified at day 7 for the cocultures on Middlebrook’s 7H11 plates and the MGIT system.

In our initial studies, we assessed the relationship between inoculum size and TTD for a standard culture of the *M. tuberculosis* Erdman strain, where the inoculum ranged from 10⁶ to 10⁷ CFU by comparing CFU plating results with MGIT data. In this experiment, a highly significant (*P* < 0.0001) linear inverse correlation was detected when the TTD of increasing CFU was evaluated over a 7-log₂⁰ range (Pearson *r* value, −0.99540). After establishing that TTD values from preclinical experiments inversely correlated with inoculum size, we compared MGIT and CFU results from 6 ongoing experiments in which vaccinated mice had been challenged with *M. tuberculosis* Erdman. For these studies,

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the infected lungs were homogenized in 5 ml of phosphate-buffered saline–Tween 80 and then serial dilutions of lung homogenates were plated for CFU determinations, while 0.5-ml aliquots of the 10^{-2} dilutions were added to MGIT tubes. These six experiments included assessing the long-term efficacy of BCG when administered by the subcutaneous and intranasal routes (Table 1, studies 1, 2, 4, and 5), the long-term postvaccination and postchallenge effectiveness of BCG vaccine and the secA2 gene-deleted M. tuberculosis mutant (study 6), and the effectiveness of the ESA6 antigen 85B (E6-85) M. tuberculosis fusion antigen formulated in trehalose dibehenate/dimethyldioctadecylammonium bromide (TDB/DDA) adjuvant or expressed from a DNA vaccine (study 3) (16, 17). As seen in Table 1, when significant reductions in lung CFU were detected for vaccinated groups relative to naive controls based on t test analysis, an inverse correlation was observed between the M. tuberculosis CFU reductions in immune cell cultures (relative to naive controls) and the corresponding TTD values. A correlation analysis (Table 2) again showed a highly significant inverse relationship (P = 0.0012; r = −0.9710) between CFU and TTD results.

Taken together, our results demonstrate that the MGIT system mycobacterial quantification results are highly correlated with CFU data during preclinical in vivo and in vitro evaluations of TB vaccines tested against the M. tuberculosis Erdman strain. Importantly, the MGIT results were usually available 7 to 10 days before the CFU data. Interestingly, similar highly significant correlations were also recently seen between CFU plating results and MGIT data for in vivo and in vitro TB vaccine studies when using the Beijing-type M. tuberculosis HN878 strain (M. Parra, unpublished data). In summary, the MGIT data are comparable to CFU results, the MGIT analysis shortens the assay period by at least 1 week and is easy to perform, and the MGIT system is better standardized than CFU determinations done on solid media by most laboratories. Thus, the MGIT analysis should be strongly considered as an alternative for CFU testing during preclinical assessments of TB vaccines.

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### Table 1

| Study group no. and timing of regimen | Treatment group | log_{10} CFU | TTD (h) |
|--------------------------------------|----------------|-------------|---------|
| 1: 2 mos PV, 1 mos PC                | Naive          | 5.82 ± 0.05 | 200 ± 2 |
|                                      | BCG            | 4.64 ± 0.04* (1.16) | 245 ± 4* (45) |
| 2: 2 mos PV, 5 mos PC                | Naive          | 6.19 ± 0.07 | 205 ± 4 |
|                                      | BCG            | 4.71 ± 0.09* (1.48) | 269 ± 5* (64) |
| 3: 2 mos PV, 1 mos PC                | Naive          | 5.64 ± 0.05 | 187 ± 3 |
|                                      | E6-85 DNA      | 5.24 ± 0.04* (0.40) | 222 ± 7* (35) |
|                                      | E6-85/DDA/TDB  | 4.77 ± 0.17* (0.87) | 228 ± 7* (41) |
|                                      | BCG            | 4.74 ± 0.14* (0.90) | 232 ± 7* (45) |
| 4: 8 mos PV, 1 mos PC                | Naive          | 5.90 ± 0.01 | 193 ± 1 |
|                                      | BCG i.n.       | 5.40 ± 0.07* (0.50) | 209 ± 1* (16) |
|                                      | BCG s.c.       | 5.15 ± 0.12* (0.75) | 220 ± 7* (27) |
| 5: 8 mos PV, 3 mos PC                | Naive          | 6.45 ± 0.08 | 166 ± 11 |
|                                      | BCG i.n.       | 5.77 ± 0.16* (0.68) | 203 ± 8* (37) |
|                                      | BCG s.c.       | 5.68 ± 0.15* (0.77) | 200 ± 6* (34) |
| 6: 8 mos PV, 1 mos PC                | Naive          | 5.47 ± 0.10 | 209 ± 4 |
|                                      | BCG            | 4.72 ± 0.09* (0.73) | 258 ± 10* (49) |
|                                      | secA2 mutant   | 4.67 ± 0.15* (0.80) | 264 ± 1* (55) |

**Abbreviations:** PV, postvaccination; PC, postchallenge with M. tuberculosis\| via aerosol route; i.n., intranasal; s.c., subcutaneous; *, P < 0.05 compared to naive controls based on t test analysis.

**Values are means ± standard errors of the means of the log_{10} CFU. Values in parentheses represent the naive minus experimental CFU. a, P < 0.05 relative to naive controls based on t test analysis.**

### Table 2

**References of CFU culture compared to MGIT TTD**

| Treatment          | Log_{10} CFU | TTD (h) |
|--------------------|--------------|---------|
| Naive              | 6.45 ± 0.02  | 101 ± 4 |
| E6-85 Ad5          | 5.65 ± 0.06* (0.80) | 121 ± 4* (20) |
| E6-85 DNA          | 5.63 ± 0.06* (0.82) | 127 ± 6* (26) |
| E6-85/DDA/TDB      | 5.63 ± 0.13* (0.82) | 134 ± 6* (33) |
| BCG                | 5.25 ± 0.15* (1.20) | 154 ± 1* (53) |
| BCGA4/DDA/TDB      | 4.63 ± 0.07* (1.82) | 182 ± 3* (81) |

**Values are means ± standard errors of the means of the log_{10} CFU. Values in parentheses represent experimental minus naive TTD result. a, P < 0.05 relative to naive controls based on t test analysis.**

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