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Media matters: Modeling the impact of solid media performance on tuberculosis trial sample size requirements

Matthew G. Johnson, MD,
Division of Infectious Diseases and International Health, Duke University Medical Center, Durham, NC, USA, matthew.g.johnson@duke.edu

Jason E. Stout, MD, MHS*,
Division of Infectious Diseases and International Health, Duke University Medical Center, Durham, NC, USA, jason.stout@dm.duke.edu

Debra A. Benator, MD,
Infectious Diseases Section, Veterans Affairs Medical Center and the George Washington University Medical Center, Washington, DC, USA, debra.benator@va.gov

William C. Whitworth, MPH, and
Division of Tuberculosis Elimination, Clinical Research Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA, wcw2@cdc.gov

David P. Holland, MD, MHS**
Division of Infectious Diseases and International Health, Duke University Medical Center, Durham, NC, USA, david.holland@emory.edu

SUMMARY

Setting—Two-month solid media culture conversion is a commonly used (if suboptimal) endpoint for phase 2 tuberculosis treatment trials.

Objective and Design—To model the effect of solid media performance characteristics (sensitivity and contamination rate) on required sample size for a two-arm clinical trial with 85% true (gold standard) culture conversion in the control and 95% in the experimental arm.

Results—Increasing sensitivity and decreasing contamination reduced sample size from 239 subjects/arm (60% sensitivity, 30% contamination) to 138 subjects/arm (95% sensitivity, 1% contamination).

Conclusion—Optimizing solid medium has significant potential to reduce sample size and increase tuberculosis clinical trial efficiency.

Keywords

Mycobacterium tuberculosis; clinical trials; mathematical modeling; microbiology

* Corresponding author.
** Current affiliation: Division of Infectious Diseases, Emory University, Atlanta, GA

Reliable growth of *Mycobacterium tuberculosis* (Mtb) on solid media is an important bacteriological endpoint for phase 2 clinical tuberculosis (TB) trials.[1,2] While growth in liquid media is faster than solid media and allows for automated growth detection, it does not allow for examination of colony morphology or isolation of mixed cultures.[3–5] Thus utilization of solid media (i.e., Löwenstein-Jensen (LJ), Middlebrook) is an essential diagnostic tool for clinical TB trials. But solid media performance, notably culture sensitivity and contamination rate, varies.[1,2,4–6]

Egg-based LJ medium is used more frequently because it does not require CO_2 incubation and is less expensive to prepare in local laboratories.[2] Middlebrook medium was designed to recover more fastidious Mtb strains and detect Mtb growth quicker; it is agar-based and requires a variety of supplements including oleic acid, albumin, dextrose, and catalase which add to the cost of the medium.[2] LJ has historically been used as the solid medium of choice for clinical trials, but a recent prospective cohort study comparing five different solid media demonstrated that selective Middlebrook medium was a more reliable standard with lower rates of contamination than LJ medium.[1,2,4] Limited additional data exist on which solid medium has better performance characteristics.

Although consensus may not exist regarding optimal solid medium selection, performance characteristics of solid media have important implications on the efficiency of conducting clinical TB trials and the reproducibility of results. Therefore, we developed a mathematical model to examine the influence of solid medium characteristics on the sample size required for a phase 2 clinical trial.

STUDY POPULATION AND METHODS

We modeled a theoretical, two-arm, phase 2 clinical TB trial with a primary endpoint of culture conversion on solid medium after two months of treatment. Similar to published trials, the assumed study procedure was to collect two sputum specimens after two months of treatment. Culture conversion was defined as a negative culture for both specimens, or a negative culture for one specimen and a contaminated result for the other. Results from subjects with two contaminated specimens were considered uninterpretable and were not included in the required sample size; in other words, these subjects would need to be replaced with subjects with interpretable results (Figure 1).

Sample size calculations were based upon the following assumptions:

- **a.** 80% power to detect a significant difference with a two-sided alpha of 0.05
- **b.** 85% culture conversion at 8 weeks detected by a “perfect” (gold standard) solid medium in the control arm[7]
- **c.** 95% culture conversion at 8 weeks detected by a “perfect” (gold standard) solid medium in the experimental arm[7]
- **d.** Each sputum specimen from a given patient is an independent event (i.e., within-patient correlation was ignored)

e. No false positive cultures

The observed proportion of patients in each arm was derived from the "true" proportion by rolling back the tree in Figure 1. For example, if the solid medium sensitivity were 70% and contamination rate was 20%, the proportion of subjects with at least one observed positive culture in the control arm ("true" rate 15%) would be 0.15×(0.7×(1−0.2) + (0.7×(1−0.2)×(1−(0.7×(1−0.2))))), or about 12.1%. Per-arm sample size estimates were derived from standard formulas that use the normal approximation to the binomial distribution, dividing the calculated number in each arm by [1−(proportion of subjects with two contaminated specimens)] to simulate discarding data from patients with two contaminated specimens as described above.[8] All calculations were performed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA).

RESULTS

Figure 2 displays the number of subjects required per arm with 1–30% contamination and 60–95% sensitivity, both plausible ranges based on prior studies.[9,10] Decreasing solid medium sensitivity attenuates observed differences between arms, resulting in less actual statistical power for a given sample size. Similarly, increasing contamination rates were associated with a higher number of participants with uninterpretable two-month culture results, also reducing effective statistical power due to lower effective sample size. For example, this model predicts that employing solid medium with a sensitivity of 85% and contamination rate of 20% would be associated with a sample size of 161 subjects required per arm. Alternatively, employing solid medium with a sensitivity of 95% and contamination rate of 10% would be associated with a sample size required per arm of 141 subjects. Thus a total of 40 less subjects (20 subjects/arm) would be required to perform a clinical TB trial if employing solid medium with the latter performance characteristics. Varying the sensitivity and contamination rate resulted in sample size requirements of between 138–239 subjects per arm.

DISCUSSION

Phase 2 clinical trials of new TB treatment regimens often use two-month solid medium culture conversion as a surrogate marker, although imperfect, for an appropriate response to TB therapy. Liquid media have distinct performance characteristics from solid media, and may be more advantageous to use in many settings, but we focused on solid medium in this analysis.[2,4–6,10] This hypothetical modeling study illustrates that utilizing solid medium with higher sensitivity and lower contamination rates can result in smaller sample sizes required to perform clinical trials, thus reducing the time and effort required to conduct phase 2 clinical TB trials. Preliminary studies suggest that selective Middlebrook medium may have higher sensitivity and lower contamination rates than LJ medium.[1,2,4] Confirmatory studies will be important to verify these performance differences.

However, the model used for this analysis does not address the effect of within-patient correlation of sputum culture results. The magnitude of within-patient correlation is not known, but we did two separate simulations that introduced within-patient correlation using constants (e.g., if the first specimen was contaminated, we increased the likelihood that the

second specimen would also be contaminated (or negative in the second simulation)). Introducing an arbitrary level of within-patient correlation changed the specific numerical results but not the overall trend (data not shown).

CONCLUSIONS

Solid media play an important role in clinical TB trials, and optimizing solid media performance has significant potential to increase TB clinical trial efficiency by reducing the cost, time, and resources needed to conduct phase 2 clinical trials of new TB treatment regimens.

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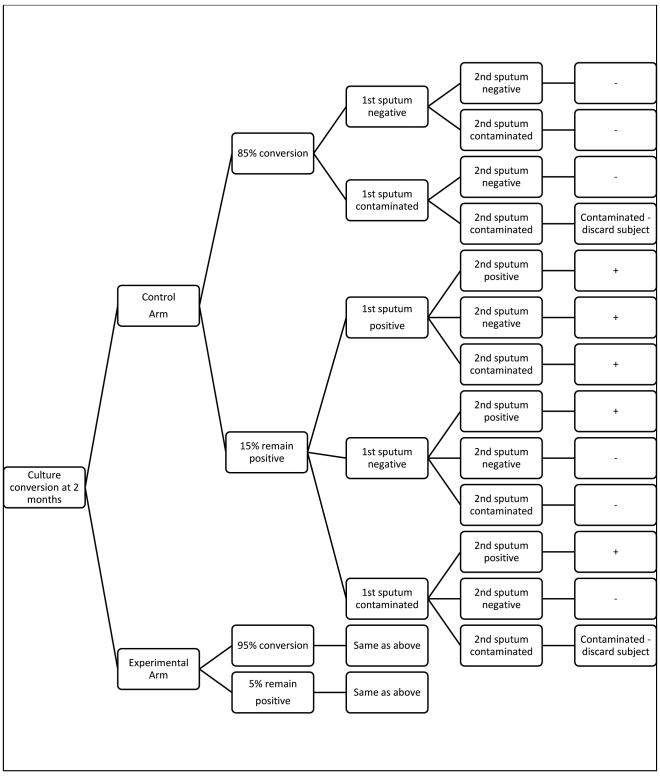

Figure 1.

Hypothetical 2-arm study design comparing solid media performance for two-month culture conversion with two sputum specimens between a control arm with 85% “true” culture conversion using solid media to an experimental arm with 95% “true” culture conversion. Probabilities in all nodes are conditional probabilities that sum to 1 given the condition in the attached node to the left. For example, the conditional probabilities of the three nodes to the right of the “15% remain positive” node in the control arm, assuming 20% contamination would equal 0.2 for “1st sputum contaminated”, (0.7 × (1−0.2))=0.56 for “1st sputum positive” if the sensitivity of the medium were 70%, and ((1−0.7) × (1−0.2))=0.24 for “1st sputum negative.” The conditional probabilities (0.2 + 0.56 + 0.24) sum to 1, and the actual probabilities of observing each of these outcomes would be 0.15 multiplied by the conditional probabilities.

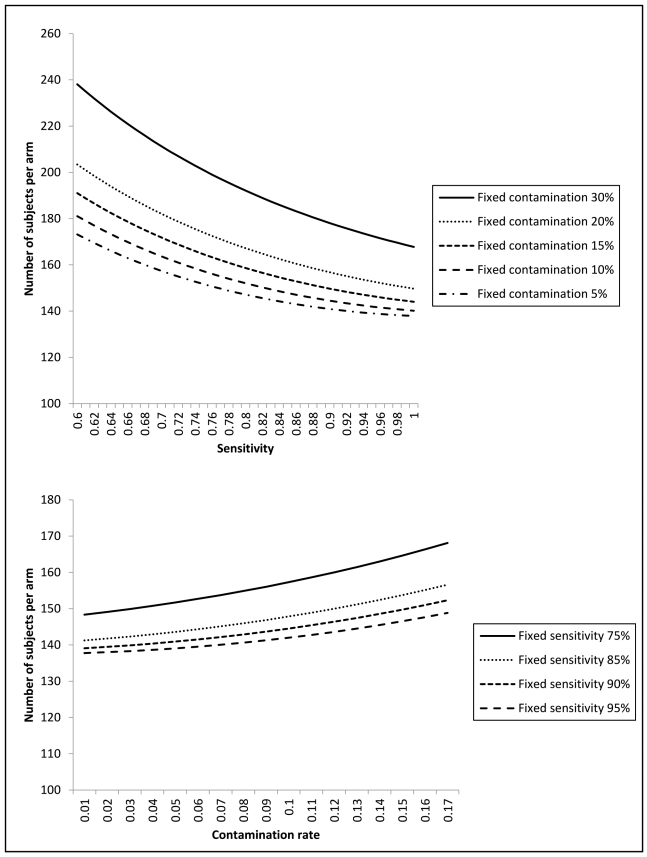

Figure 2.

Number of subjects/arm required for hypothetical study design with varying fixed sensitivities and contamination rates for solid media (varying the contamination rate between 1–30% and sensitivity between 60–95%). The top chart displays the number of subjects/arm using solid media with a sensitivity of 60–95% and fixed contamination rates of 5%, 10%, 15%, 20%, and 30%, while the bottom chart displays the number of subjects/arm using solid media with a contamination rate of 1–17% and fixed sensitivities of 75%, 85%, 90%, and 95%.