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Medium matters: modeling the impact of solid medium performance on tuberculosis trial sample size requirements

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SUMMARY

SETTING: Two-month solid medium culture conversion is a commonly used, if suboptimal, endpoint for phase 2 anti-tuberculosis treatment trials.

OBJECTIVE AND DESIGN: To model the effect of the performance characteristics (sensitivity and contamination rate) of solid medium 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 the 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 the efficiency of tuberculosis clinical trials.

KEY WORDS: *Mycobacterium tuberculosis*; clinical trials; mathematical modeling; microbiology

DESPITE RECENT ADVANCES in new molecular technologies, the diagnosis and management of tuberculosis (TB) remains heavily dependent on classical microbiological techniques such as smear microscopy for the detection of acid-fast bacilli and growth of *Mycobacterium tuberculosis* in culture. Cultivation of *M. tuberculosis* on culture medium is more sensitive than microscopy and is a necessary step in the TB diagnostic algorithm, as it allows for species identification, drug susceptibility testing, and genotyping.[1] Different types of culture medium are used, including both liquid and solid media. Growth in liquid media, which includes commercial broth detection systems such as BACTEC™ MGIT™ 460 (BD, Sparks, MD, USA) that use Middlebrook 7H12 media, is faster than in solid media; however, liquid medium does not allow for the examination of colony morphology or detection of mixed cultures.[1–3] The use of slower growing solid media, including Löwenstein-Jensen (LJ) or Middlebrook 7H10 or 7H11, is thus an essential diagnostic tool.

Reliable growth of *M. tuberculosis* on solid medium is also an important bacteriological endpoint for phase 2 clinical TB trials.[4,5] However, the performance of solid medium, notably culture sensitivity and contamination rate, varies.[2–6] Egg-based LJ medium is used more frequently as it does not require carbon dioxide incubation and is less expensive to prepare in local laboratories.[5] Middlebrook medium was designed to recover more fastidious *M. tuberculosis* strains and detect *M. tuberculosis* growth more quickly; 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.[5] LJ has historically been used as the solid medium of choice for clinical trials, but a recent prospective cohort study in patients on standard short-course treatment comparing five different solid media to a reference standard constructed using latent class analysis demonstrated that selective Middlebrook medium, in particular 7H11S, was a more reliable standard with lower rates of contamination than LJ medium.[2,4,5] Limited additional data exist on which solid medium has better performance characteristics.

Although there may not be a consensus regarding optimal solid medium selection, performance characteristics of solid media have important implications in the efficiency of conducting clinical TB trials and the reproducibility of results. To streamline phase 2 clinical trials of new anti-tuberculosis treatment regimens, we developed a mathematical model to examine the influence of solid medium characteristics, specifically sensitivity and contamination rate, on the sample size required for phase 2 clinical trials.

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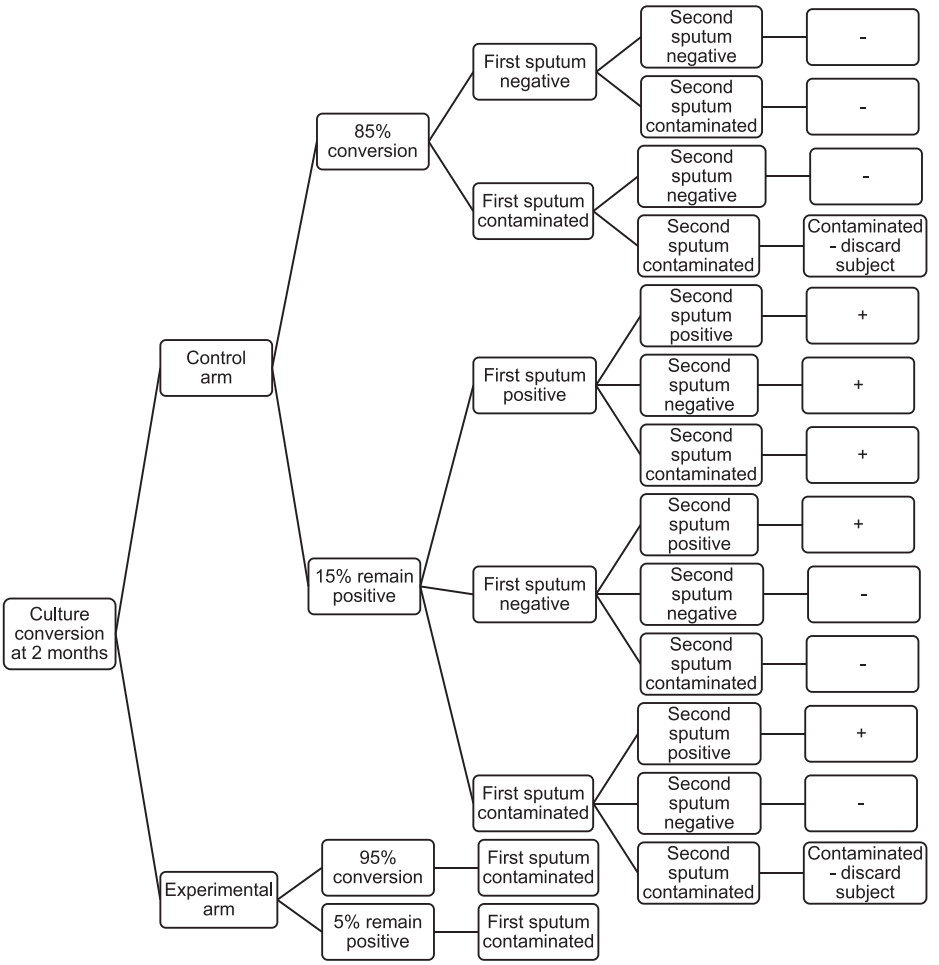

Figure 1 Hypothetical two-arm study design comparing the performance of solid medium for 2-month culture conversion with two sputum specimens between a control arm with 85% 'true' culture conversion to an experimental arm with 95% 'true' culture conversion. Probabilities in all nodes are conditional 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 'first sputum contaminated', $(0.7 * (1 - 0.2)) = 0.56$ for 'first sputum positive' if the sensitivity of the medium were 70%, and $((1 - 0.7) * (1 - 0.2)) = 0.24$ for 'first 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.

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 2 months of treatment. Similar to published trials, the assumed study procedure was to collect two sputum specimens after 2 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:

1 80% power to detect a significant difference with a two-sided alpha of 0.05;
2 85% culture conversion at 8 weeks detected by a 'perfect' (gold standard) solid medium in the control arm;[7]
3 95% culture conversion at 8 weeks detected by a 'perfect' (gold standard) solid medium in the experimental arm;[7]
4 Each sputum specimen from a given patient is an independent event (i.e., within-patient correlation was ignored);
5 No false-positive cultures.

'Perfect' solid medium is defined as a medium that

Table Estimated sample sizes required to detect observed differences between clinical trial arms with selected medium characteristics, given 'true' culture conversion of 85% in the control arm and 95% in the experimental arm, assuming 80% power at a significance of 0.05. The top five rows demonstrate the effect of increasing medium sensitivity while holding contamination rate constant, while the bottom five rows demonstrate the effect of increasing contamination rate while holding sensitivity constant

Sensitivity %	Contamination rate %	Calculated proportion of subjects with >1 positive culture at 8 weeks in control arm %	Calculated proportion of subjects with >1 positive culture at 8 weeks in experimental arm %	Subjects required per arm *n*
60	1	12.5	4.2	169
70	1	13.5	4.5	155
80	1	14.3	4.8	145
90	1	14.8	4.9	140
95	1	14.9	5.0	138
70	5	13.5	4.5	156
70	10	13.4	4.5	159
70	15	13.3	4.4	165
70	20	13.1	4.4	175
70	30	12.3	4.1	210

would grow *M. tuberculosis* with no contamination (0% contamination rate), and that would grow *M. tuberculosis* if any solid medium (e.g., LJ, 7H10, etc.) demonstrated growth. Note that a 'perfect' solid medium does not necessarily have the same sensitivity as other media types, such as liquid media, which are generally more sensitive than solid media and are able to grow populations of bacteria that are not cultivable on currently used solid media.

The model was created by specifying a decision tree (Figure 1) representing all potential outcomes of the two 8-week sputum specimens obtained from subjects in the theoretical phase 2 clinical trial described above. 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 sensitivity of solid medium were 70% and contamination rate 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%. Similarly, using the same assumptions, the proportion of subjects with at least one observed positive culture in the experimental arm ('true' rate 5%) would be $0.05 * (0.7 * (1–0.2) + (0.7 * (1–0.2) * (1 – (0.7 * (1 – 0.2)))))) = 4.0\%$. 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] For the example mentioned above, a sensitivity of 70% with solid medium and a contamination rate of 20%, the per-arm sample size needed would be based on 80% power at an alpha level of 0.05 to detect a difference between proportions of 12.1% and 4.0%, with an inflation factor of 4% (20% x 20%) for subjects with two contaminated sputum specimens. The calculated per-arm sample size (substituting these values into the normal approximation for the binomial) would therefore be as follows: $(((1.96 + 0.84)^2 * (0.121 * (1 – 0.121) + 0.04*(1 – 0.04))) / (0.121 – 0.04)^2) / (1 – 0.2^2) = 182.1$, or, rounding up, 183 subjects per arm.

All calculations were performed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA).

RESULTS

As shown in the Table, increasing the sensitivity of the medium reduces the number of subjects required for the hypothetical phase 2 study. Conversely, increasing contamination rates increases the number of subjects required, both because subjects with two contaminated cultures are discarded (they do not contribute to the sample size because they have no interpretable endpoint) and due to a smaller effect related to subjects with one negative and one contaminated 8-week culture (i.e., only a single valid result instead of two results in effective attenuation of medium sensitivity). Figure 2 gives the number of subjects required per arm across the range of 1–30% contamination and 60–95% sensitivity, both plausible ranges based on previous studies.[9,10] Reducing the sensitivity of solid medium attenuates the 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 2-month culture results, also reducing effective statistical power due to the lower effective sample size. For example, this model predicts that employing solid medium with a sensitivity of 85% and a 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 required sample size per arm of 141 subjects. A total of 40 fewer subjects (20 subjects/arm) would thus be required to perform a clinical TB trial if

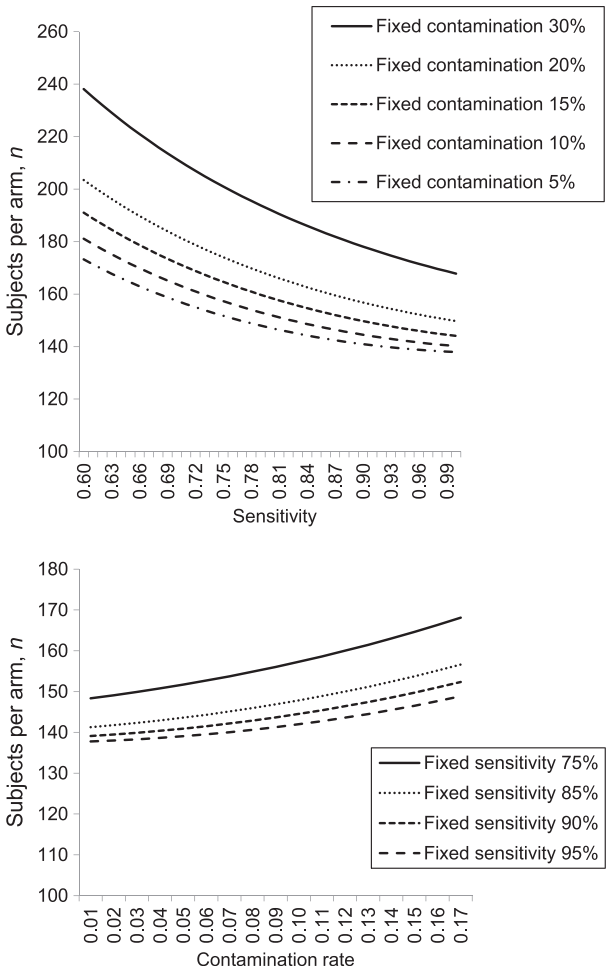

Figure 2 Number of subjects/arm required for hypothetical study design with varying fixed sensitivities and contamination rates for solid medium (varying the contamination rate between 1% and 30% and sensitivity between 60% and 95%). The top chart displays the number of subjects/arm using solid medium, 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 medium with a contamination rate of 1–17% and fixed sensitivities of 75%, 85%, 90%, and 95%.

employing solid medium with the latter performance characteristics. Varying the sensitivity and contamination rate resulted in sample size requirements of between 138 and 239 subjects per arm.

DISCUSSION

Phase 2 clinical trials of new anti-tuberculosis treatment regimens often use 2-month culture conversion on solid medium as a surrogate marker, although imperfect, for an appropriate response to TB therapy. Although the performance characteristics of liquid media are very distinct from those of solid media, and may be more advantageous in many settings, we focused on solid media in this analysis.[2,3,5,6,10] This hypothetical modeling study shows that using solid media with higher sensitivity and lower contamination rates can result in smaller sample sizes being 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.[2,4,5] Confirmatory studies will be important to verify these differences in performance.

The model used for this analysis does not, however, address the effect of within-patient correlation of sputum culture results. The magnitude of within-patient correlation is not known; however, we performed 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. Optimizing the performance of solid media 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 anti-tuberculosis treatment regimens.

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Conflicts of interest: none declared.

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RESUME

CONTEXTE : La conversion de culture sur milieu solide après 2 mois est un paramètre généralement utilisé, bien qu'il soit sous optimal, pour les essais de traitement de la tuberculose (TB) en phase 2.

OBJECTIF ET SCHÉMA : Modéliser les caractéristiques de l'effet de la performance de la culture sur milieu solide (sensibilité et taux de contamination) sur la taille d'échantillon requise pour un essai clinique à deux bras avec 85% de véritable conversion de culture (étalon or) dans le groupe témoin et 95% dans le groupe d'expérimentation.

RÉSULTATS : Une sensibilité accrue et une plus faible contamination ont réduit la taille de l'échantillon de 239 sujets par bras (60% de sensibilité, 30% de contamination) à 138 sujets par bras (95% de sensibilité, 1% de contamination).

CONCLUSION : Optimiser le milieu solide a un potentiel significatif de réduction de la taille de l'échantillon et d'accroissement de l'efficacité des essais cliniques de la TB.

RESUMEN

MARCO DE REFERENCIA: La conversión de los cultivos en medio sólido a los 2 meses de tratamiento se utiliza con frecuencia, aunque es imperfecto, como el criterio de valoración en los ensayos clínicos de segunda fase de los tratamientos antituberculosos.

OBJETIVO Y MÉTODOS: Modelar el efecto de las características del rendimiento de los medios de cultivo sólido (sensibilidad y tasa de contaminación) sobre el tamaño necesario de la muestra de un ensayo clínico con dos grupos, en el cual el grupo testigo presenta una conversión real de 85% (referencia) y el grupo experimental una tasa de 95%.

RESULTADOS: Al aumentar la sensibilidad y disminuir la tasa de contaminación se disminuyó el tamaño necesario de la muestra de 239 personas por grupo (sensibilidad de 60% y 30% de contaminación) a 138 personas por grupo (sensibilidad de 95% y 1% de contaminación).

CONCLUSIÓN: La optimización de la calidad de los medios sólidos puede disminuir de manera considerable el tamaño necesario de la muestra y aumentar la eficacia en los ensayos clínicos de los medicamentos antituberculosos.