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Serial dilution with a confirmation step

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Abstract

A serial dilution test estimates the concentration of a microbe in a broth by inoculating several tubes with portions of the broth. The test may include both presumptive and confirmation steps. For the confirmation step considered here a portion of the contents of each tube indicating a change in the presumptive step is streaked on a plate. Several colonies are selected and examined to determine if the tube contained the target microbe. A statistical model accounts for the possibility of not selecting the target microbe from a tube that contains it and a similar appearing microbe. Simulations show that this model sometimes gives estimates similar to those currently used, but at other times can make large corrections. Also, a commonness measure helps check the validity of the assumptions of this statistical model.
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1. Introduction

A serial dilution test measures the concentration of a target microbe in a broth. At each dilution several tubes are inoculated with a portion of the broth. After a growth step, the tubes containing the target microbe show a change. From the number of tubes at each dilution that show a change, the concentration of the target microbe in the original broth can be estimated. This estimate is called the most probable number (MPN). Many authors (Cochran, 1950; Eisenhart and Wilson, 1943; Halvorson and Ziegler, 1933; McCrady, 1915) have discussed a statistical model to make this estimate. Hurley and Roscoe (1983) and Best (1990) present an overview of serial dilution tests. This model will be called the BAM model because it is in the FDA Bacteriological Analytical Manual (2001) with further explanation in Garthright and Blodgett (2003).

Although the tubes may contain substances to favor the target microbes, sometimes other microbes can cause a change in a tube. Particularly when a distinction between similar microbes is important, a confirmation step may be necessary. For the confirmation step discussed here, a small amount of the contents of each tube that showed a change is streaked on a medium in a separate plate. After another growth step, colonies form. Some colonies that look like the target microbe are selected and tested. A streaked tube is confirmed when at least one of its selected colonies is confirmed. Even in streaked tubes containing the target microbes, the colonies selected may all be ones that appear like the target microbes, but are different. If only similar appearing colonies are selected for a tube, then it may be wrongly classified as negative. The statistical model presented below accounts for selections of colonies that miss the target microbe. The appendices should help implement this model.

2. The model

The model allows for both the target microbe and another similar appearing microbe in the original broth.

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It estimates the concentration of each and the probability that a colony selected from a streaked tube containing both is the target microbe. The estimates of these quantities are the value of each that makes the observed outcome most likely. This confirmation model accounts for the possibility that the selection of colonies missed the target microbe in a streaked tube containing it.

If some dilution does not require a confirmation step, then the investigator is assuring that change in its tubes is adequate evidence of the target microbe. The contribution of these dilutions to the likelihood of an outcome is similar to the BAM model and will be left for the appendix. Appendix A develops the likelihood. Appendix B derives equations to estimate the parameters from the maximum likelihood principle.

The confirmation model uses more data than the BAM model does for dilutions requiring a confirmation step. For these dilutions the confirmation model needs (1) the number of tubes, (2) the amount of broth inoculated into each tube, (3) the number of tubes with a colony that appears like the target microbe after a plate is streaked, (4) the number of colonies selected from each plate, and (5) the number of selected colonies that confirmed as the target microbe for each plate. If the appearance of selected colonies differ between plates, that should be noted.

The BAM model selects three dilutions, but this step can be eliminated. The BAM model both for three dilutions and using all dilutions differ from the confirmation model by how unconfirmed, streaked tubes affect them. The BAM models treat unconfirmed, streaked tubes as negatives. If all dilutions were used, the resulting MPN would be a lower bound for the confirmation model. If all streaked tubes are confirmed, then the BAM model using all dilutions and the confirmation model give the same estimate. If there is at least one positive tube, then treating each of the unconfirmed, streaked tubes as though it did not occur gives an upper bound for the confirmation model.

A measure of an outcome's commonness can indicate whether the model's assumptions hold. Blodgett (2002) discusses several measures of how rare an outcome appears. The measure used here divides the likelihood of the observed outcome by the likelihood of the most probable outcome at the estimated values of the parameters. Consequently, this commonness measure is positive and at most one. It is near zero for very unusual outcomes and is larger for more common outcomes. Appendix C discusses a method of finding the most probable outcome.

3. Simulations

This section compares the confirmation model with the BAM model with three dilutions using randomly generated simulations. The basic simulation contains 1000 outcomes. It has five tubes at each of five dilutions with 1, .1, .01, .001, or .0001 g of the original broth. The concentration of the target microbe is 10 colony forming units (cfu)/g; the similar microbe, 6 cfu/g. Also, the probability that a colony, selected from a streaked tube containing both types of microbes, will be the target microbe is .4. Finally, the confirmation step selects three colonies from each plate.

For this basic simulation, 74 outcomes had all streaked tubes confirmed. The geometric mean for the MPN from the BAM model equals 3.70. For the confirmation model, it equals 10.07 which is closer to the population value of 10. For the simulations used here, the number of streaked tubes with no colonies confirmed in the lowest dilution plays an important role. For simulations with other values of the parameters, this quantity may not be as important. The four tables for the first simulation give the number of outcomes and have rows for the number of unconfirmed tubes in the lowest dilution.

The columns in Table 1 give the MPN from the BAM model. The rows for one or more tubes show most outcomes with an MPN below the population value of 10. A few outcomes have higher MPN-values with a large gap between the two groups. The BAM model picks three dilutions. If a higher dilution has all tubes positive, then the lowest dilution is ignored which could account for the outcomes with high MPN. Also, the outcomes in rows for one or more unconfirmed tubes tend to have lower MPN-values as the number of unconfirmed tubes in the lowest dilution increases.

The columns in Table 2 give results on unusual outcomes for the BAM model. Since unusual outcomes may have probabilities many times less than the most probable outcome, the negative log of the commonness measure is used. The outcomes with higher values for this negative log are less common. Table 2 shows that as the number of unconfirmed tubes in the lowest dilution increases, the outcomes become more unusual.

The columns in Table 3 give the ratio of the estimated concentration from the confirmation model over the

Table 1
Unconfirmed tubes by MPN from the BAM model showing that unconfirmed tubes in the lowest dilution can decrease MPN

Unconfirmed tubes in lowest dilution	MPN						
	<1	1–2	2–4	4–8	8–16	16–32	32<
3+	23	56	0	0	0	3	2
2	0	125	89	0	0	6	3
1	0	25	341	9	0	16	8
0	0	0	15	134	92	47	6