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Statistical Analysis of the Recovery of Coliform Organisms on Gelman and Millipore Membrane Filters

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The recovery of coliform organisms on Gelman and Millipore membranes was analyzed by using both a model I (which assumes no error in the x variable) and model II (which allows errors in both the variables) regression analysis. The two models afford estimates of the slope which agree within their 95% confidence limits. Using equations derived in this paper, the model II confidence limits on the intercept are obtained. This range does not include the model I intercept limits, thereby demonstrating the differences between results from an incorrect (model I) and correct (model II) approach. In addition, fecal coliform show no differences in response to the two membranes, whereas total coliform exhibit higher recoveries on Gelman membranes.

Recently, Presswood and Brown (4) have reported the results of a comparison of the recovery of coliform organisms on Gelman and Millipore membranes. They concluded that the Gelman filters were more efficient at recovering these organisms than were Millipore filters. The statistical procedure used by these authors was a model I (least squares) regression analysis (5). The assumptions underlying this model are: (i) Normally and randomly distributed observations of the dependent variable, y, for any given value of the independent variable, x. (ii) The independent variable, x, is measured without error. (iii) The expected value for the variable y (for a selected x) has a mean, μ, and a constant variance σ^2, and is described by the linear function, $\mu = \alpha + \beta x$. The mathematical model is specified by equation (1)

$$(1)\ y = \alpha + \beta x + v$$

where α = y intercept, β = slope or regression coefficient, and v is a normally distributed error term with a mean of zero and standard deviation $\sigma_{y \cdot x}$.

In the case of coliform data, conditions (i) and (iii) are met by employing log-transformed raw data (3) in the analysis. Because the errors in both x and y are substantial and approximately equal when comparing membrane filters, a model II regression analysis, which has separate error terms for the x and y variables, is more appropriate. As used here, a model II linear regression is one in which each variate (x and y) includes a random component. In this model, only assumption (ii) of the least squares or model I analysis is changed. The new condition is: (ü') the independent variable, x, is measured with an error, u, which is random, normally distributed with constant variance σ^2, and is given by Bartlett (2) as relation (2):

$$(2)\ y = \alpha + \beta x + v + \beta u$$

The intentions of this note are to present a comparison of the results obtained from each model on paired coliform samples cultured on Gelman and Millipore membrane filters, and to indicate a need for further microbiological assays comparing the two membranes.

MATERIALS AND METHODS

The data recorded in Table 2 were obtained by using the methods described in Standard Methods (1) for natural samples.

RESULTS

From the information presented in Table 1, it can be seen that results by the two models agree with each other to within the tabulated error limits. Even though these models seem to afford similar statistical estimates, the large inherent error in data of this type, however, makes a model I analysis inappropriate because of the requirement made by assumption (ii) of the model.

Although the difference in the location of the origin between the fecal and total coliform results (model II) probably has a real microbiological basis, it is possible that it arises from the small amount of fecal coliform data used in the computations. A t test on the means of the

TABLE 1. *Statistical summary*[a]

Determination	Model I		Model II	
	Fecal	Total	Fecal	Total
n[b]	19	33	19	33
a	0.2611	0.3727	0.2191	0.5148
b	0.8384	0.8305	0.8659	0.7339
r	0.9433	0.7096	0.8861	0.6270
Lower limit on b[c]	0.6102	0.5284	0.6291	0.3824
Upper limit on b	1.0666	1.1327	1.1323	1.0625
Lower limit on a	−0.0885	−0.0746	0.1824	0.4648
Upper limit on a	0.6106	0.8201	0.2558	0.5548

[a] Log transformed data (x = Millipore, y = Gelman).
[b] Raw data given in Table 2.
[c] Confidence limits calculated at $P = 0.95$.

log-transformed data of the paired samples in Table 2 was not significant for the fecal coliform ($t = -0.7692$) but was highly significant for the total coliform results [$d_{(\text{Gelman - Millipore})} = -0.1235$, $t = -4.9470$ ($t_{0.01} = 2.74$)]. This suggests that microbiological differences are significant factors in explaining the differences in the performance of the two membranes.

To compare our results more directly with those of Presswood and Brown (4), a least squares line through the origin was fitted to the data. Thus, by assuming in equation 1 that $\alpha = 0$ (the null hypothesis), we get equation (3):

$$(3)\quad y = \beta x + v$$

For the total coliform data, use of equation (3) gives a least squares interval estimate of β of $1.0807 \pm 0.1481\ t$, and a t test of the null hypothesis that the line, assumed straight, goes through the origin, was not significant. For the fecal coliform data, the corresponding limits are $1.007 \pm 0.1082\ t$ and the t test of the null hypothesis was not significant. Although these results are in accord with the confidence limits reported in Table 1 for the intercepts determined by the model I analysis, the assumption of a zero intercept (equation 3) is different than computing an intercept (equation 1) and testing whether or not it differs to a statistically significant extent from zero. If the two types of membrane exhibit the same characteristics of organism growth, the inhibition (or growth) of certain organisms on one membrane would exactly parallel those observed on the other. Under these circumstances a zero intercept, as assumed in equation 3, might be a reasonable, if untested, assumption. Since we do not know in advance, however, that growth characteristics on the two membranes are the same, the assumption of a zero intercept can lead to erroneous conclusions. Since the models described by equations 1 and 3 have the same restrictions, even if the assumption of a zero intercept were justified, this model would be inappropriate since assumption ii of the model is not met.

TABLE 2. *Colonies counted*

Sample type	Colonies counted		Volume plated (ml)
	Millipore	Gelman	
Fecal	18	22	10
	18	20	10
	19	26	0.01
	51	43	0.1
	62	44	0.1
	38	41	0.1
	53	45	0.1
	36	46	0.1
	34	41	0.1
	46	53	0.1
	43	49	0.1
	39	34	0.1
	44	63	0.1
	27	24	10
	44	48	10
	23	18	10
	48	43	10
	18	21	10
	21	20	1
Total	77	79	0.0001
	58	76	0.0001
	50	70	0.0001
	60	81	0.0001
	23	41	10
	24	28	10
	22	23	10
	23	19	10
	24	25	10
	24	18	1
	20	24	1
	19	43	1
	20	57	1
	18	23	1
	21	29	1
	29	28	1
	22	74	1
	33	44	1
	32	44	1
	33	49	1
	28	57	1
	38	50	1
	48	65	1
	43	54	1
	28	60	1
	42	46	1
	48	44	1
	41	50	1
	19	22	1
	21	21	1
	27	27	1
	20	27	1
	23	34	1

DISCUSSION

The confidence interval for the model II intercept, α, and the estimate of the population correlation coefficient, r, are not discussed in the literature. Bartlett (2) does, however, discuss the joint confidence regions for both α and β. Using Bartlett's estimate of β, b', and following the conventional approach (5), we provide an estimate of the correlation coefficient as equation (4):

$$(4)\ r = b's_x/s_y$$

where s_x is the standard deviation of x, and s_y is the standard deviation of y.

If β is given, the best estimate of α, as suggested by Bartlett, is a', where equation (5):

$$(5)\ a' = \bar{y} - b'\bar{x}$$

where $\bar{x}$ and $\bar{y}$ are sample means of the variates x and y, respectively, and $b' = (\bar{y}_3 - \bar{y}_1)/(\bar{x}_3 - \bar{x}_1)$.

We have equation (6):

$$(6)\ a - \alpha = (\bar{y} - \overline{Y}) - \alpha(\bar{x} - \overline{X}) = v - \alpha u$$

where $x = \xi + u$ and $y = \eta + v$, when both the variables x and y have experimental errors, u and v, respectively, and ξ and η are the true values of x and y, and $\overline{X}$ and $\overline{Y}$ are the population means of x and y, respectively.

Hence, $a - \alpha$ is a normally distributed variable with zero mean and variance $(\sigma_v^2 + \alpha^2\sigma_u^2)/n$.

Bartlett has shown that $(s_y^2 - 2\beta s_{xy} + \beta^2 s_x^2)/(n - 3)$ is an estimate of the variance $(\sigma_v^2 + \alpha^2\sigma_u^2)$ with $(n - 3)$ degrees of freedom. Thus, the statistic equation (7):

$$(7)\ t = \sqrt{n}\ (a - \alpha)/\sqrt{s_{(\beta)}}$$

where $s_{(\beta)} = s_y^2 - 2\beta s_{xy} + \beta^2 s_x^2$, and s_x and s_y are the standard deviations of x and y, respectively, and s_{xy} is the covariance, has the Student's t distributions with $(n - 3)$ degrees of freedom.

Denoting the critical value of t by $t_{p/2}$, according to the chosen probability level p, the $(1 - p)\%$ confidence interval for α is given by the interval equation (8):

$$(8)\ a' \pm t_{p/2}\sqrt{s_b/n}$$

where $s_b = s_y^2 - 2b's_{xy} + b'^2 s_x^2$, and b' is Bartlett's estimate of β.

In sum, after log transformation, the model I analysis gave slopes which fell within the 95% confidence limits of the slopes computed on the basis of model II. The two models provide distinctly different estimates of the intercepts. Since the model I analysis is inappropriate for the reasons discussed previously, it is not surprising that differences between the estimates provided by the two models are obtained. Based on the model II results, it appears that coliform data cannot be assumed to provide regression estimates which pass through the origin. Also, whereas fecal coliform are recovered equally well by either membrane, Gelman membranes exhibit higher recoveries of total coliforms than do Millipore membranes.

The questions raised by the differences noted in this study, as well as the differences noted in this manuscript and in the published studies (4, 6, 7), suggests that additional work on the interaction dynamics of coliform organisms with membrane filters is necessary. Such work has the potential of ultimately providing a highly selective technique for distinguishing the various organisms by changing the composition of the membrane.

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