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To triplicate or not to triplicate?

Julio M. Singer [a,*], Antonio C. Pedroso-de-Lima [a], Nelson I. Tanaka [a], Verónica A. González-López [b]

[a] *Departamento de Estatística, Universidade de São Paulo, Brazil*
[b] *Departamento de Estatística, Universidade Estadual de Campinas, Brazil*

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Abstract

A common practice in scientific experimentation in areas such as Medicine, Pharmacy, Nutrition, among others, is to measure each sample unit three times (in triplicate) or more generally, m times (in m–plicate) and take the average of such measurements as the response variable. This is generally done to improve the precision of model parameter estimates. When the objective is to estimate the population mean, we use a random effects model to show that the efficiency of working with m-plicates is related to the magnitude of the intraclass correlation coefficient, which essentially measures the contribution of the variance between sample units to the total variance. We show that above certain values of this parameter, the use of m-plicates does not bring significant improvement (say, of 10% or more) to the precision of the estimates. Additionally, taking the costs of sampling units and making measurements into account, we compare sampling schemes with and without m-plicates designed to obtain fixed width confidence intervals for the mean. We illustrate the results through a practical example.

Keywords: Cost based optimization; Intraclass correlation; Precision of estimates

1. Introduction

In many experimental studies, observations are obtained in triplicate (or more generally, in m-plicate) and their average is taken as the response variable. This is a common practice in areas like Medicine, Pharmacy, Nutrition etc., as evidenced in Vaughan and Oram [1], Paquet et al. [2] or Viyoch et al. [3], among others. In many cases, like in Nutter et al. [4] or Thuresson et al. [5], the objective is to evaluate intraobserver variability. Also, there are instances where the procedure is adopted simply by tradition. We focus on situations where the use of m-plicates is intended to improve the precision of model parameter estimates, in particular, the mean.

A practical example involves the estimation of the average amount of oil contained in lemon juice, an important feature for the decision about the destination of this commodity (plain consumption, as a cosmetic ingredient etc) and hence, about its trade price. Each of 60 samples was obtained from a batch of lemon juice and divided into three portions (haphazardly labeled A, B and C) each of which was analyzed with respect to the amount of oil (kg/ton). The data for the 60 triplicates are displayed in Table 1.

We are interested in evaluating the effect of using triplicates in the precision of the estimate of the mean amount of oil per ton of lemon juice.

Assuming a Gaussian model we obtained four 95% confidence intervals for the mean: the first three ones are based on the observations (A, B or C) considered separately; the fourth is based on the average of the three within sample units observations. The results are presented in Table 2 and show that the precision of the four intervals is practically the same, suggesting that the use of triplicates is unnecessary, in the sense that, a single observation per sample unit would generate confidence intervals with similar widths and consequently reduce costs.

A similar problem was considered in Fagan et al. [6] to evaluate the need for triplicate blood pressure measurements. Considering Analysis of Variance for repeated measurements and correlation methods, the authors conclude that averaging the triplicate within sample units observations or simply using the first of the three observations produces similar results. Shapiro et al. [7], on the other hand, argue that more replicates are better,

* Corresponding author.
E-mail address: jmsinger@ime.usp.br (J.M. Singer).

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Table 1
Amount of oil in lemon juice (kg of oil/ton of juice)

Sample	A	B	C	Sample	A	B	C	Sample	A	B	C
1	5.29	5.10	5.13	21	5.66	5.64	5.46	41	4.90	4.75	4.84
2	5.34	5.34	5.27	22	5.62	5.49	5.73	42	4.88	4.57	4.54
3	5.20	5.07	5.08	23	5.36	5.33	5.46	43	4.80	4.82	4.94
4	5.43	5.38	5.36	24	4.91	5.01	4.86	44	5.29	5.29	5.10
5	5.18	5.03	5.02	25	5.28	5.35	5.14	45	4.53	4.66	4.63
6	5.33	5.07	5.07	26	5.02	4.80	4.64	46	4.39	4.49	4.39
7	5.16	5.40	5.23	27	5.57	5.54	5.29	47	4.50	4.51	4.52
8	4.91	5.10	4.84	28	5.09	5.22	4.95	48	4.82	4.80	4.66
9	5.07	5.01	4.87	29	5.58	5.45	5.32	49	5.06	4.96	4.94
10	4.85	4.76	4.54	30	5.04	4.90	4.94	50	5.20	4.97	5.11
11	5.31	5.42	5.52	31	5.79	5.65	5.58	51	5.63	5.75	5.63
12	5.12	5.40	5.27	32	5.46	5.38	5.36	52	5.38	5.51	5.14
13	5.29	5.47	5.13	33	5.21	5.20	5.07	53	5.37	5.06	5.13
14	5.04	5.09	4.98	34	4.84	4.98	4.91	54	5.06	5.20	5.07
15	5.11	5.11	5.11	35	5.27	5.11	5.25	55	5.15	5.32	4.99
16	4.96	5.07	4.94	36	5.06	5.08	4.89	56	4.74	4.74	4.64
17	5.36	5.06	5.10	37	5.10	5.24	5.05	57	4.48	4.4	4.37
18	5.36	5.40	5.33	38	5.32	5.51	5.22	58	4.26	4.12	4.37
19	5.39	5.13	5.34	39	4.80	4.70	4.58	59	4.46	4.37	4.62
20	5.49	5.60	5.28	40	5.18	4.83	4.80	60	5.20	4.93	5.07

since blood pressure varies considerably from beat to beat. They seem to misunderstand the role of within sample unit variability in the evaluation of total variability as pointed by Fagan et al., who show that the magnitude of intraunit short term measurements is not as large as to produce more precise measurements. We try to clarify such issues in a broader context by attacking the problem from two perspectives. The first consists of knowing under what circumstances the precision of the estimate of the mean of a Gaussian distribution based on a sample of n units is affected by considering m within sample units measurements; this is the object of Section 2. The second, refers to the choice between two experimental designs (with and without repeated within sample unit measurements) when the costs of obtaining sample units and performing measurements are taken into account; this is discussed in Section 3. The conclusion with a brief discussion is presented in Section 4.

2. Using *m*-plicates to reduce the width of confidence intervals

Assuming a Gaussian distribution, the data (collected in m-plicates) can be represented by the random effects model

$$y_{ij} = \mu + a_i + e_{ij}, \tag{1}$$

where $a_i \sim N(0,\sigma_a^2)$ and $e_{ij} \sim N\ (0,\ \sigma_e^2)$, are independent, $i=1,...,n$ and $j=1,...,m$. In the example shown in Table 1, we have $n=60$ and $m=3$. This model induces a dependence in the within sample units observations that may be quantified by the intraclass correlation coefficient,

$$\rho = \sigma_a^2/(\sigma_a^2 + \sigma_e^2). \tag{2}$$

When the within sample units observations are independent, it follows that $\rho=0$; otherwise, when the dependence between the within sample units observations is large, we have ρ close to 1.

Under Eq. (1), a 95% confidence interval for μ based on just one of the within sample units observations (the first one, for instance) is given by

$$\bar{y}_{+1} \pm 1.96\ \sigma/\sqrt{n}, \tag{3}$$

where $\bar{y}_{+1} = n^{-1}\sum_{i=1}^{n} y_{i1}$ and $\hat{\sigma}^2 = (n-1)^{-1}\sum_{i=1}^{n}(y_{i1}-\bar{y}_{+1})^2$ is an estimate of

$$\sigma^2 = \sigma_a^2 + \sigma_e^2. \tag{4}$$

Alternatively, under the same model (1), a 95% confidence interval for μ based on the average of the m within sample units observations is

$$\bar{y}_{++} \pm\ 1.96\sqrt{\frac{\hat{\sigma}_a^2 + \hat{\sigma}_e^2/m}{n}}, \tag{5}$$

where $\bar{y}_{++} = n^{-1}\sum_{i=1}^{n}\ \bar{y}_{i+}$, $\bar{y}_{i+} = m^{-1}\sum_{j=1}^{m} y_{ij}$, $\hat{\sigma}_e^2 = [n(m-1)]^{-1}$ $\sum_{i=1}^{n}\sum_{j=1}^{m}(y_{ij}-\bar{y}_{i+})^2$ and $\hat{\sigma}_a^2 + \hat{\sigma}_e^2/m = (n-1)^{-1}\sum_{i=1}^{n}(\bar{y}_{i+}-\bar{y}_{++})^2$.

Using Eqs. (2) and (4), we can show that the width of the confidence interval Eq. (5) is

$$2 \times \frac{1.96}{\sqrt{n}}\sqrt{\hat{\sigma}_a^2 + \hat{\sigma}_e^2/m} = 2 \times 1.96\frac{\hat{\sigma}}{\sqrt{n}}\sqrt{\hat{\rho} + (1-\hat{\rho})/m}. \tag{6}$$

Table 2
95% confidence intervals for the mean amount of oil in lemon juice

Data	Lower bound	Upper bound	Width
First observations (A)	5.04	5.21	0.17
Second observations (B)	5.00	5.18	0.18
Third observations (C)	4.94	5.11	0.17
Average	5.00	5.16	0.16