STATISTICAL ANALYSIS OF STANDARD PLATE COUNTS
OF A FOOD SAMPLE SPLIT AMONG LABORATORIES

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Summary

Homogeneous one-gram samples of kitchen-contaminated egg salad were analyzed in nine public health laboratories on three successive days. Analysis of variance using a nested design was applied to estimate independently the major sources of variation both between and within laboratories. The variation between laboratories was found to be very great, but the variation within each laboratory was also substantial.

This study used a split-sample technique to study the variation in standard plate counts on a food specimen between and within laboratories. Each of nine laboratories (eight county and one state) in the San Francisco Bay Area analyzed portions of a "naturally contaminated" homogenized egg salad. It was not expected that the laboratories would agree closely with one another; rather, the goal was to assess the magnitude of the variation and compare it with variations in the results within each laboratory. The statistical technique used was analysis of variance of basically the same kind as that used by Donnelly et al. (2) in their study on split milk samples. The present analysis differs technically in using a nested design, as discussed by Scheffé (3), in order to obtain the within-laboratory components. The goal was to explore some of the difficulties involved in determining the bacteriological quality of foods rather than to establish any standards.

Materials and Methods

An egg salad was made in a kitchen with certain purposeful errors in technique: some salad was contaminated in the sink and on the floor, and the whole mixture was left to stand about four hours in a warm room. There was no deliberate introduction of bacteriological culture material. The resulting salad was homogenized in a Waring Blender. Three 1-g samples were dispatched by car to each of the participating laboratories with dry ice as a refrigerant.

Each laboratory was requested to analyze its samples on three successive days. Standard milk techniques (1) and a specified set of dilutions were used by all laboratories. Plate counts or estimates were reported for each of the two duplicate plates at the five dilutions (1:100 to 1:1,000,000). The microbiologists in these laboratories were all familiar with standard milk examination procedures and techniques for identifying the organisms commonly associated with food poisonings.

Analysis and Results

The two dilutions with plate counts running closest to the range of 30 to 300 colonies per plate were selected from each laboratory's results (Table 1). The results of one dilution (104 on second day) in laboratory E were rejected as probably reflecting a technical error; the next best dilution was substituted. The logarithm of the standard plate count (SPC) was used, rather than the actual count, to make the variance independent of the level of the count.

Variation in the log SPC was assumed attributable to four components: (a) variation among laboratories; (b) variation among days within laboratories; (c) variation among dilutions within days within laboratories; and (d) variations between plates within dilutions within days within laboratories. These variance components are set out in symbolic form in Table 2.

Estimates of the variance components are given by the formulae:

\[ \sigma^2 = \frac{\text{Observed MSPLATES} - \text{Observed MSDAYS}}{N} \]

Other symbols are explained in Table 2.

The analysis of variance table in numerical form is shown in Table 3. All of the F tests are significant.
## Table 1. Data From Participating Laboratories (SPC/10⁶)

<table>
<thead>
<tr>
<th>Day</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>10⁴</td>
<td>10⁵</td>
</tr>
<tr>
<td>Plate 1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Plate 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lab</td>
<td>38</td>
<td>6.0</td>
</tr>
<tr>
<td>Lab</td>
<td>4.7</td>
<td>6.8</td>
</tr>
<tr>
<td>Lab</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>Lab</td>
<td>4.6</td>
<td>4.7</td>
</tr>
<tr>
<td>Lab</td>
<td>120</td>
<td>98</td>
</tr>
<tr>
<td>Lab</td>
<td>370</td>
<td>410</td>
</tr>
<tr>
<td>Lab</td>
<td>200</td>
<td>180</td>
</tr>
<tr>
<td>Lab</td>
<td>310</td>
<td>320</td>
</tr>
</tbody>
</table>

**NOTE:** Laboratories are arranged in order of their average SPC/10⁶ values. Only the two dilutions yielding plate counts nearest to the 30-300 range are tabled.

## Table 2. Analysis of Variance in Symbolic Form

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Expected mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratorizes</td>
<td>K-1</td>
<td>(\sigma^2 + N \sigma^2_M + MN \sigma^2_L + LMN \sigma^2_K)</td>
</tr>
<tr>
<td>Days</td>
<td>(L-1)K</td>
<td>(\sigma^2 + N \sigma^2_M + MN \sigma^2_L)</td>
</tr>
<tr>
<td>Dilutions</td>
<td>(M-1)LK</td>
<td>(\sigma^2 + N \sigma^2_M)</td>
</tr>
<tr>
<td>Plates</td>
<td>(N-1)MLK</td>
<td>(\sigma^2)</td>
</tr>
<tr>
<td>Total</td>
<td>NMLK-1</td>
<td></td>
</tr>
</tbody>
</table>

**Symbols:**
- \(K\) = number of laboratories
- \(L\) = number of days
- \(M\) = number of dilutions
- \(N\) = number of plates
- \(\sigma^2_K\) = variance component due to laboratories
- \(\sigma^2_L\) = variance component due to days (within laboratories)
- \(\sigma^2_M\) = variance component due to dilutions (within days within laboratories)
- \(\sigma^2\) = variance component due to plates (within dilutions within days within laboratories)
As a specific example, the data for Laboratory G are given in Table 4. The standard error of the log of the geometric mean plate count is (from the above) 0.754. A 95\% confidence interval would be obtained from the laboratory mean (2.296) as follows:

\[
2.296 \pm 1.96 \times 0.754 = (0.818, 3.774)
\]

Converting back from logarithms, the laboratory means and the 95\% confidence interval are as follows:

Laboratory mean = 1.98 x 10^8
95\% confidence interval = 6.58 x 10^6, 5.94 x 10^8

It is important to note that this rather large confidence interval is almost entirely due to consistent differences between laboratories. If Laboratory G had run several more days’ worth of samples, they could at best have reduced the standard error from 0.754 to 0.724 (the square root of 0.5239). By the same token, if the laboratory had run only two days’ samples and had counted only one plate at one dilution each day, the lab standard error would have been:

\[
\sqrt{\frac{0.5239}{2}} + \frac{0.1034}{2} + \frac{0.0587}{2} + \frac{0.0056}{2} = 0.779
\]

If two laboratories did this simple routine, the standard errors would be reduced to:

\[
\sqrt{\frac{0.5239}{2}} + \frac{0.1034}{2} + \frac{0.0587}{2} + \frac{0.0056}{2} = 0.587
\]

Three laboratories participating would drive the standard error down to 0.508 and four to 0.463.

To the extent that this limited study permits generalizations, it seems clear that standardization of techniques among laboratories is the most important problem to tackle. Simply replicating results within laboratories will not overcome this barrier to establishment of food standards based on plate counts. Sending food samples to laboratories in several counties is hardly practical for everyday use.

Some of the suggested sources of variation between laboratories could have been: refrigeration failure in transporting specimens, differences in personnel, differences in water bath and incubator temperatures, differences in counting techniques (one lab used a dissecting scope), use of glass versus plastic petri dishes, and differences in media. Within laboratories, some sources of variation between days could have been: water bath and incubator temperatures, personnel, food samples, interactions between bacterial populations, and chance (often only part of the plate was counted). The smaller dilution and plate errors are probably purely technical. The predominance of pin-point colonies and the presence of tiny food particles in the lower dilutions were additional factors. The pin-point colonies were often hazy at 24 hr; in one laboratory, they were examined at 48 hr and were more distinct. Counting at 48 hr might
have improved the results. As to the petri dishes, the plastic ones had an inside area of about 57-58 cm² while the glass ones were 62-67 cm². All the laboratories assumed an area of 65 cm². Standard serological pipettes may not be the best for making dilutions of food materials. There may be a serious error due to deposition of food particles on the walls.

In a split sample study when the objective is to measure the major sources of variability within as well as among laboratories, a nested design may be appropriate. Such knowledge from samples processed in different laboratories would be helpful in setting up uniform procedures for standard plate counts on foods. When one is examining results within laboratories, the first day’s results for all laboratories are not expected to be consistently different from the second or third day’s results. The nested design is appropriate because it compares each day’s results within a given laboratory with the average for the three days in that laboratory; similarly, dilutions are studied within days and plates within dilutions.

Summary and Conclusions

A split sample study on a kitchen-contaminated egg salad was done in nine public health laboratories. Each laboratory received three homogeneous 1-g samples to be analyzed on three successive days using a specified routine. All of the plate counts were reported. For statistical analysis, the best two of ten dilutions were selected on the basis of the 30 to 300 colonies per plate criterion. Analysis of variance (nested design) was used to estimate independently the major sources of variation. While the variation between labs was enormous, the variation within labs was also substantial. Much development work remains to be done before laboratories can reproduce each other’s results to the extent that arbitrary SPC’s can be established for different foods.

Acknowledgement

The consulting time for the analytical work on this study was paid for by the Contra Costa County Health Department. The project was developed by the Laboratory Methods Committee of the California Association of Public Health Laboratory Directors, Kenneth Jernigan, Chairman.

References