Essential statistics for the pharmaceutical sciences

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Additional topics as supplements to the book:

Paired t-tests based on percentage changes – a better way?

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A scenario – hormone production in batches of cultured cells and the effect of changing the culture medium

We determine the rate of production of the hormone erythropoietin in a cell culture system. Several batches of cultured cells are used. Each batch is studied using an established culture medium (Referred to as the controls) and a possible new medium.

The results with seven batches of cells are shown in Table 1.

Table 1 Production of erythropoietin (nMol/h) with existing and new growth medium.

Batch no.	Control medium	New medium	Change	% Change
1	1.10	1.22	+0.12	+10.9
2	4.65	5.50	+0.85	+18.3
3	7.94	9.11	+1.17	+14.7
4	1.56	1.73	+0.17	+10.9
5	0.83	0.86	+0.03	+ 3.6
6	27.27	29.59	+2.32	+ 8.5
7	2.88	3.26	+0.38	+13.2

With every batch, the production of erythropoietin is increased when the new medium is used (See penultimate column). Common sense would suggest that when all seven batches show an increase, there is almost certainly a real effect.

Testing by a standard paired t-test based on simple changes in the end-point

As each batch of cells is studied with both media, our first thoughts for an analysis would be a paired t-test. The standard approach to such a test would be to calculate the change seen with each batch and then produce a 95% C.I. for the change in yield, based on our sample of seven batches. However, the figures in the penultimate column smell strongly of positive skew with a mean of 0.72 nMol/h but a maximum value more than three times as great. If we were to go ahead anyway and calculate a confidence interval based on these figures it would be -0.038 to +1.478 nMol/h. The interval would include zero and the outcome would be non-significant.

A modified form of paired t-test using percentage changes

We already know that non-significant results based on non-normal data should not be accepted. We could adopt the approach used in Chapters 5 and 17, where log transforms were used to convert positively skewed data to a more normal distribution. However, in this case, there is a more natural and transparent solution. The two very large increases in hormone production (1.17 and 2.32 nMol/h) are seen with the cultures that produced the highest amounts of hormone under control conditions and the lowest producer gave the smallest increase. The new growth medium seems to increase hormone production by a fairly constant percentage rather than by a fixed amount.

In the final column of Table 1, the changes caused by the use of the new medium are expressed as a percentage of the control figures. These have a mean of 11.4% and the individual values are distributed reasonably symmetrically about that figure (Range 3.6 to 18.3%). We can now set up a null hypothesis worded in terms of the percentage rather than the actual changes:

> *Null hypothesis: The mean change brought about by changing to the new medium is zero percent.*

Then all we have to do is calculate a 95% C.I. for the mean change using the figures in the final column. The result is an interval of +7.11 to +15.77%. The outcome is now very clearly statistically significant.

General applicability of paired t-tests based on percentage change.

Many biological and chemical systems respond in the way we have just seen – a change in a relevant factor alters the outcome by a certain percentage rather than increasing or decreasing it by a set amount. Whenever a paired t-test is contemplated, it is probably a good idea to look for correlation between the values under control conditions and the amount by which they change. If there is clear positive correlation, a paired t-test based on percentage changes should be considered.