Analysis of Variance and Design of Experiments-II

MODULE VII

LECTURE - 29

CROSS-OVER DESIGNS

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The reduction of error to improve the statistical inference from experiments is an important objective in any experimental design.

Such experimental variability is higher in certain types of experiments, e.g., those experiments involving biological entities, animals, humans etc. One approach to reduce such natural variability is to use each animal or human as a block rather than experimental units. In general, such animals or humans, are termed as **subjects.**

Different treatments are then applied successively, i.e., in different time periods and, to each subject, so that the combination of subject and time now represents an experimental unit. Then the comparisons between the treatments can be made within subjects rather than between subjects. This is referred to as **each subject acting as its own control**.

Some conditions are to be fulfilled in such procedures.

These conditions are as follows:

1. A subject reacts to the treatment soon after the treatment has been applied.
2. The treatment effects last only for a limited time.
3. After this time, the subject is restored to its original state.
4. The treatment effects are the same in each period.

If these conditions are satisfied, then some form of the block design can be used in which the subjects are the blocks and the treatments to be administered are applied at random to the subjects.

If the subjects change systematically over the time of trial, then period effect can be suspected. In such cases, some sort of row-column design can be used in which rows represent the periods and columns represent the subjects. The designs suitable for these situations are called **cross-over designs, change-over designs** or **repeated measurements designs.**

Consider an example from clinical trial. Whenever a new medicine is developed, it is experimented on human beings before it is finally declared to be used or discarded. There is always a risk involved with the health of those human beings to whom the medicine is administered. In order to minimize such risks, an option is to choose the minimum number of persons who can provide the relevant size of data needed for the statistical tool required for further analysis to judge the efficacy of the medicine. The cross-over designs help in such situations.

In cross-over designs, each patient is successively treated with two or more treatments. To do so, the persons are randomly divided into two groups and the treatments are given in certain orders.

For example, in a 2 x 2 cross-over design, each person is given two treatments. These treatments are labeled, say, as *A* and *B.* Then the total number of persons are divided into two groups at random. Then half of the persons are given the treatment *A* first and then they are crossed over to treatment *B*. The remaining half persons are given the treatment *A* first and then they are crossed over to treatment *B*.

The treatments *A* and *B* are applied at some difference of time interval. So a suitable time period is chosen between which the two treatments are administered. This is called as **washout period** and is chosen to avoid the persistence of a treatment applied in one period to a subsequent period of time.

If the washout period is chosen to be not long enough, then the effect of a treatment may persist in the subsequent period of treatment effect. This is called as **carry-over effect.** Such carry-over effect makes the estimation of **direct treatment effects** more difficult or nearly impossible.

The treatments are applied in a double blinded manner so that the patients and the doctors don't know that which of the treatment (*A* or *B*) is being applied. This avoids the presence of psychological effects in the observations.

Generally, the variation is more between the subjects than within the subject which leads to more powerful tests in comparison to the simple comparison of two independent groups using between-subject information. The variation between the subjects is eliminated as a source of error because each subject acts as per his own control. The main aim of cross-over designs is to estimate most of the main effects using the differences within the subject.

Residual and direct effects

One of the advantages of cross – over designs is that certain treatment contrasts may be estimated more precisely on a within–subject basis as compared to designs where only between–subject information is available.

- There are some disadvantages like the length of time required for a trial.

- Another major disadvantage is related to the treatments that exhibit effects beyond the period in which they are applied.

These lingering effects are termed as **residual effects** or **carry-over effects**.

If these effects cannot be accounted for, then they may introduce bias in the estimates of contrasts among treatment effects and they are termed as **direct effects**.

The washout periods (with either no treatment or a standard treatment) have been used to eliminate this problem, but they may in some cases be unethical and it also prolongs the duration of the trial even more.

There may be situations in which one is interested in estimating the residual effects but generally we are interested only in the direct effects. It is, therefore, important to construct designs that allow us to estimate the direct effects separately from the residual effects.

Linear model and notations

Suppose the total available subjects are divided into *s* groups. Each group receives the *M* treatments. The administration of these treatments is done in a different order. There are total *M*! possible orders to administer *M* treatments. It is good to use all of the *M*! orderings of treatments. For example, when *M* = 2, then there are two orders – *AB* and *BA.* When *M* = 3, then there are 3! = 6 orders as *ABC, BCA, CAB, ACB, CBA, BAC.* In general, with *M* treatments, the total numbers of orders are *s* = *M*!.

We generally assume that the trial lasts *p* periods (i.e., *p* = *M* periods if all possible ordering are used). Let y_{ijk} denotes the response observed on the k^{th} subject ($k = 1, 2\ldots, n_i$) of group *i* (*i* = 1, 2…, *s*) in period *j* (*j* = 1, 2…, *p*). The model in such a case is expected to incorporate the effect of a subject in the group, effect of period, direct effect, and cross-over effect besides the general mean effect. We first consider the following linear model or the **classical approach model.**

$$y_{ijk} = \mu + s_{ik} + \pi_j + \tau_{[i,j]} + \lambda_{[i,j-1]} + \varepsilon_{ijk},$$

where

y_{ijk} is the response of the k^{th} subject of group *i* in period *j;*

μ is the overall mean;

s_{ik} is the effect of subject *k* in group $i (i = 1, 2, ..., s, k = 1, 2, ..., n_i)$;

π_j is the effect of period *j* ($j = 1, 2, .., p$);

$\tau_{[i,j]}$ is the direct effect of the treatment administered in period *j* of group *i* (treatment effect);

$\lambda_{[i,j-1]}$ is the carry-over effect (effect of the treatment administered in period *j* -1 of group *i*) that still persists in period *j;* and where $\lambda_{[i,0]} = 0$ and

ε_{ijk} is random error.

The subject effect s_{ik}'s are taken to be random. Sample totals will be denoted by capital letters, sample means by small letters. A small letter "*o*" will replace a subscript to indicate that the data has been summed over that subscript. For example,

total response : $Y_{ijo} = \sum_{k=1}^{n_i} Y_{ijk}$, $Y_{ioo} = \sum_{j=1}^{p}\sum_{k=1}^{n_i} Y_{ijk}$, $Y_{ooo} = \sum_{i=1}^{s}\sum_{j=1}^{p}\sum_{k=1}^{n_i} Y_{ijk}$,

means: $\bar{y}_{ij} = Y_{ijo} / n_i$, $\bar{y}_{ioo} = Y_{ioo} / pn_i$, $\bar{y}_{ooo} = Y_{ooo} /(p\sum_{i=1}^{s} n_i)$.

To begin with, we assume that the response has been recorded on a continuous scale.

The problem of **sequence effect** becomes apparent in classical model particularly when higher order designs are used. This shows some inconsistencies concerning the effect caused by the order in which the treatments are given. For example, consider the following plan in a cross-over design trial:

Sequence	Period			
	1	2	3	4
	A	*B*	*C*	*D*
	B	*D*	*A*	*C*
	C	*A*	*D*	*B*
	D	*C*	*B*	*A*

Then the actual sequence (group) might have a fixed effect on the response. Then the between–subject effect s_{ik} would also be stratified by sequences (groups). This effect is to be considered as an additional parameter in the classical model. Applying the classical approach model without this sequence effect will result as the sequence effect that gets confounded with the other effects.

2 x 2 Cross-Over (Classical approach)

We now consider the common comparison of $M = 2$ treatments A and B using a 2 x 2 cross-over trial with $p = 2$ periods as follows:

	Period 1	Period 2
Group 1	*A*	*B*
Group 2	*B*	*A*

There will be four sets of observations available. So there are four sample means $\bar{y}_{11o}$, $\bar{y}_{12o}$, $\bar{y}_{21o}$ and $\bar{y}_{22o}$ available from the 2 x 2 cross-over design. Thus there are three degrees of freedom which are used to estimate the period effect, treatment effect and carry-over effect. Thus, we have to omit the direct *treatment* x *period* interaction effect. This effect now has to be estimated as an aliased effect confounded with the carry-over effect.

Therefore, special parameterization is needed in a 2 x 2 cross-over as

$$\tau_1 = \tau_A \text{ and } \tau_2 = \tau_B.$$

The carry-over effects are simplified as

$$\left.\begin{aligned} \lambda_1 &= \lambda_{[1,1]} = \lambda_{[A,1]} \\ \lambda_2 &= \lambda_{[2,1]} = \lambda_{[B,1]}. \end{aligned}\right\}$$

Then λ_1 and λ_2 denote the carry-out effect of treatments A and B respectively, applied in the first period . The effects in the full model are shown in the table. The subject effects s_{ik} are regarded as random.

Table: Effects of 2 X 2 cross-over model

Group	Period 1	Period 2
1 (*AB*)	$\mu+\pi_1+\tau_1+s_{1k}+\varepsilon_{11k}$	$\mu+\pi_2+\tau_2+\lambda_1+s_{1k}+\varepsilon_{12k}$
2 (*BA*)	$\mu+\pi_1+\tau_2+s_{2k}+\varepsilon_{21k}$	$\mu+\pi_2+\tau_1+\lambda_2+s_{2k}+\varepsilon_{22k}$

The random effects are assumed to be distributed as follows:

The effect s_{ik} are assumed to be identically and independently distributed following $N(0,\sigma_s^2)$ and the effect ε_{ik} are assumed to be identically and independently distributed following $N(0,\sigma^2)$. Further ε_{ijk} and s_{ik} are also assumed to be uncorrelated for all *i, j* and *k.*

Analysis using *t*-tests

The analysis of data from a 2 x 2 cross-over trial using *t*-tests and this analysis remains valid irrespective of the covariance structure of the two measurements y_A and y_B taken on each subject during the active treatment periods.