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Optional analyses of crossover trials having two treatments and a placebo

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The assumption of carryover effects is unavoidable due to the very nature of crossover designs. Even in case of crossover design with washout period, the hypothesis of no carryover effect should be tested and established. On the other hand, this assumption makes the analysis difficult and potentially biased or inefficient in case of two treatment two period crossover design. For a reasonable estimation, experimenters are advocated to employ a two period three treatment crossover design, or a three period two treatment crossover design. In this article, we present optional analyses of a uniform three period three treatment crossover design, consisting of a placebo and two active treatments. We develop a test for detecting presence of carryover effects which directs experimenter for a proper analysis of his crossover trial. We present ANOVA for each of the three possible carryover models, that both, single, or none of the active treatments has carryover effect, and illustrate through an example.

keywords: Repeated measurement design, Carryover effects, Active treatment, Placebo treatment, Test of carryover effects, Analysis of variance.

1 Introduction

Designs with a new therapy, standard therapy and a placebo are sometimes referred as 'gold standard' trials. There are two kinds of objectives associated with these trials, first, compare two active treatments and second, compare active treatments with placebo.

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These research objectives are mostly carried out using crossover designs (CODs) because within subject treatment comparisons are more efficient than between subject treatment comparisons. For example, Tsoy et al. (1990) used three-period crossover design in three treatments F12, F100, P as double-blind gold standard trial on patients suffering from exercise-induced asthma. They used data values of forced expiratory volume (FEV1) obtained after an exercise challenge for comparing the protective effect of a single dose of a study therapy formetorol solution aerosol 12 mg (F12), with a single dose of standard therapy salbutamol suspension aerosol 100 mg (F100) and with a placebo (P).

Since in crossover, each subject is measured according to a treatment sequence over successive periods of time, the carryover effects are natural and may sustain for different amounts of time. While Senn (1992) advocated use of adequate washout period, number of articles have discussed analysis of two and three treatment CODs conducted in two to four periods under various carryover models. Grizzle (1965) analyzed COD1 {AB, BA} and advised to use only first period data in presence of carryover. To deal with carryover COD1 has been modified to COD2 {AA, AB, BA, BB}, COD3 {ABB, BBA}, COD4 {ABA, BAB}, COD5 {ABB, ABA, AAB, BAA, BAB, BBA}, COD6 {ABAB, BABA}, COD7 {AABB, ABBA, BBAA, BAAB} and COD8 {AABA, ABAA, ABBA, AAAA, BBAB, BABB, BAAB, BBBB} having self and mixed carryover. A treatment followed by self is self carryover and a treatment followed by another treatment is a mixed carryover.

Senn and Lambrou (1998) investigated designs of COD5 type, under simple and steady state carryover models and concluded that, statisticians cannot provide a general design and estimation strategy that can be guaranteed to deal with carryover. The simple carryover model considers self carryover effects equal to mixed carryover, and, a steady state model considers self carryover as vanished to zero. Afsarinejad and Hedayat (2002) studied suitability of various two period CODs in more than two treatments assuming self and mixed carryover effects to be different. Kempton et al. (2001) showed that COD1 is $\overline{A}$-optimal and COD3 is MA-optimal when carryover effects are proportional to treatment effects. As per Hedayat and Stufken (2003), the best design choices for optimal treatment comparisons are, COD1 under no carryover model, COD2 and COD3 under mixed carryover model, COD4 and COD8 under self and mixed carryover model while, COD6 and COD7 under all three models being robust to changes in model. Yang and Stufken (2008) studied CODs having $(t+1)$ treatments for comparing t treatments with a control and identified them as efficient/robust under number of models including three period CODs having three treatments. Some discussion on carryover model with correlated errors is available in literature; it is for two treatment designs in more than two periods, for example, Kunert (1991) result showed that, COD7 is optimal for both treatment and carryover effects under correlated observations.

Koch et al. (1989) proposed a two period COD in ten sequences, for comparison of two active treatments in the presence of placebo, so that treatment and carryover effects are partially orthogonal. This resulted in variance for active treatment contrast remaining the same under no carryover and carryover models. Jones and Donev (1996) improved their design into three period design to optimize the active treatments comparison. In one of their designs, the treatment effects are orthogonal to the carryover effects.

Apart from design and model modifications, different procedures have been suggested to handle carryover effects at the analysis stage. Since, a COD is highly suitable for treatment comparison beyond elimination of carryover effects; the first step in the analysis of CODs has been the test of equality of carryover effects. In the context of COD1, Grizzle (1965) developed F-test for testing the equality of carryover effects based on between sequence sum of squares and within sequence sum of squares, then treatment comparison is made as per COD only if carryover effects are not significant. Lehmacher (1991) discussed an alternative approach, consisting of testing multiple tests of hypothesis about equality of treatment effects and /or equality of carryover effects using Hoteling T^2 test and t-test. Their test statistics were defined in terms of sequence total differences and within sequence treatment differences. Laird et al. (1992) considered the data resulting from a COD as representing longitudinal data from number of subjects, and developed GLS and REML estimators considering two period observations as bivariate normal observations. They advocated to employ COD2 or COD9 instead of COD1 to ensure availability of adequate information on carryover effects.

From the above discussion it is clear that, reasonable analysis of COD for carryover models is discussed for CODs in three / four periods, or CODs in two periods having at least one subject receiving no active treatments. In this paper, we consider analyzing a three treatment, three period, uniform, minimum balanced COD in six sequences, COD (3, 6, 3) as a COD in two treatments and a placebo, under carryover model assuming no carryover from placebo. As per medical knowledge, when placebo is given to patients, in patients' mind the psychology is that drug is given to them, but in reality it is placebo, so whatsoever psychological effects the patients' has, get measured as direct effects of the placebo, and in the next period there are no carryover of the psychological effects. Therefore it is logical to assume no psychological carryover and obviously, there is no pharmacological carryover from a placebo treatment.

There are two advantages of using one treatment as placebo. Firstly, carryover effects are estimated and tested independently prior to embarking upon the least squares analysis. Secondly, the efficiency of separability of treatment and carryover effects increases. An individual test for testing the significance of carryover effects prior to analysis is developed which directs for optional analysis of crossover experiment. For analytical convenience, we consider subject effects as random during development of the individual tests and, as fixed while making treatment comparisons with a reasonable restriction that sum of subject effects across all sequences is nil. We present an estimation of model terms and ANOVAs for three cases, (i) both active treatments have significant carryover, (ii) only one of the active treatments has carryover and, (iii) none of the active treatments has significant carryover. The paper is divided into three sections, Section 2 provides model and characterization of the COD, Section 3 develops analysis under three models, and Section 4 illustrates analyses by an example. MATLAB code for the individual test of the carryover effects and analysis under one of the three models is available upon request to authors.

2 The model and characterization of COD(2+1,6,3)

The COD (3, 6, 3) belongs to the series of uniform, minimum balanced COD$(t, t(t-1), t)$; $t > 2$; prime power which has been considered by many authors (Martin and Eccleston (1998), Hedayat and Yang (2004), Yang and Stufken (2008), Divecha and Gondaliya (2015)). We consider this design as COD(2+1,6,3), specially to represent a clinical trial design in two active treatments, A and B and the third treatment P as a placebo, because in execution, this design is close to the design consisting of three replications of COD1 and in analysis it is possible to obtain a test for testing significance of carryover effects. The carryover effect tests have an immediate generalized form for the corresponding series. The treatment sequences of COD(2+1,6,3) are {ABP,BPA,PAB,APB,BAP,PBA}.

2.1 The model and notations

We consider that data from COD(2+1,6,3) follows fixed effect simple carryover model given by

$$y_{ijk} = \mu + \tau_{d(k,j)} + \gamma_{d(k-1,j)} + \pi_k + \xi_{ij} + e_{ijk}, \tag{1}$$

$$i = 1, \ldots, 6; j = 1, \ldots, n; k = 1, 2, 3,$$

where, y_{ijk} denotes the response from sequence i, subject j, in period k to which treatment $d(k, j)$ was assigned, μ is the general mean, $\tau_{d(k,j)}$ is the effect of treatment $d(k, j)$, $\gamma_{d(k-1,j)}$ is the carryover effect of treatment $d(k-1, j)$ on the response observed on subject j in period k, π_k is the effect due to period k, ξ_{ij} is the effect due to subject j having sequence i, every sequence replicated n times ($n \geq 1$) and the e_{ijk} are independently normally distributed error terms with mean 0 and variance σ^2. It is obvious that there is no carryover effect in the first period, i.e. $\gamma_{d(0,j)} = 0$. We further assume that placebo does not produce carryover effect, i.e. $\gamma_{d(k-1,j)} = 0$ if $d(k-1, j) = P$.

We use the notation G, T_A, T_B, T_P, R_A, R_B, P_k and U_{ij} to denote in order, the grand total, treatment total for A, B, P, residual total for A, B, period total for k^{th} period, $k = 1, 2, 3$ and total for j^{th} subject receiving i^{th} sequence, $j = 1, \ldots, n,\ i = 1, \ldots, 6$.

2.2 Characterization of COD(3,6,3) as COD(2+1,6,3)

It is known that, in general in a COD, the treatment effects and carryover effects are not orthogonal. Every COD must be measured for its ability to separate the treatment and carryover effects for model 1 because, a design that is poor in separating these effects may cause treatment effects to be declared significant when it may be due to positive carryover effects. The ability of separating the effects depends upon the number of observations free from carryover and the number of ordered pair of treatments occurrences in two consecutive periods. A measure of separability can be calculated for a *COD* on the basis of observed frequencies of carryover and the expected frequencies from an independent model (Hanford (2005)). The efficiency of separability of COD(2+1,6,3) is 50% greater than that of COD(3,6,3), the efficiency of separability of former is 63.5% and that of latter is 42.3%.

The COD(2+1,6,3) retains all the characteristics of COD(3,6,3) such as uniformity, minimal balanced and so on. Also, this design is optimal and efficient for five carryover models (Gondaliya and Divecha (2018, 2019)). An additional characteristic is that there are 10 observations free from carryover effects in the former design instead of only 6 in the latter. This facilitates to divide the total 18 observations into three equal size sets of 6 observations, each complete in occurrence of treatment, period and subject factor levels. Exploiting this feature, we develop tests of carryover effects based on means of sets and discuss analysis in the following Section.

3 Analysis

In practice, presence of carryover effects leads to lengthy clinical trials. It has a large impact on the estimation and testing of treatment effects. Few CODs, including COD$(t, t(t-1), t)$; t prime power with last period repeated, are universally optimal for estimation of treatment effects, as well as carryover effects of treatments (Martin and Eccleston (1998)). However, this period addition does not carry any clinical significance. Undoubtedly, a COD that allows test of significance of carryover effects prior to its analysis for treatment effects should be useful in practice.

3.1 Test of carryover effects

To develop test statistics for testing significance of carryover effect of active treatments of COD(2+1, 6n, 3), we divide the $6n$ subjects from the above COD in three sets say, f_1, f_2 and f_3, consisting of specific observations respectively as $\{U_{11}, \ldots, U_{1n}, U_{51}, \ldots, U_{5n}\}$, $\{U_{21}, \ldots, U_{2n}, U_{61}, \ldots, U_{6n}\}$ and $\{U_{31}, \ldots, U_{3n}, U_{41}, \ldots, U_{4n}\}$. We define,

$$\begin{aligned} \overline{U}_m &= \frac{1}{2n} \sum_{l \in f_m} U_l;\ m = 1, 2, 3 \\ s_m^2 &= \frac{1}{2n-1} \sum_{l \in f_m} (U_l - \overline{U}_m)^2;\ m = 1, 2, 3 \end{aligned}$$

then, under the random effect consideration, and the assumption that subject effects are independently normally distributed with mean 0 and variance σ_ξ^2, the sampling distributions of group means $(\overline{U}_m)$ and variances (s_m^2) are:

$$\begin{aligned} \overline{U}_m &\sim N(\mu_m, \sigma_1^2/2n);\ m = 1, 2, 3; \\ &\quad \text{Where } \sigma_1^2 = 3\sigma^2 + 3\sigma_\xi^2 \\ \overline{U}_1 - \overline{U}_2 &\sim N(\gamma_A, \sigma_1^2/n) \\ \overline{U}_1 - \overline{U}_3 &\sim N(\gamma_B, \sigma_1^2/n) \\ \frac{(2n-1)s_m^2}{\sigma_1^2} &\sim \chi^2_{(2n-1)};\ m = 1, 2, 3 \end{aligned}$$

Here, μ_m indicates expectation of group mean $\overline{U}_m$. Consequently, independent two sample t-tests with null hypothesis $H_0 : \gamma_A = 0$ against alternative $H_1 : \gamma_A \neq 0$ and with hypothesis $H_0 : \gamma_B = 0$ against $H_1 : \gamma_B \neq 0$ provide tests for testing significance of carryover effects of A and carryover effects of B defined respectively by 2 and 3.

$$\frac{(\overline{U}_1 - \overline{U}_2) - \gamma_A}{\sqrt{(s_1^2 + s_2^2)/2n}} \sim t_{(4n-2)} \tag{2}$$

$$\frac{(\overline{U}_1 - \overline{U}_3) - \gamma_B}{\sqrt{(s_1^2 + s_3^2)/2n}} \sim t_{(4n-2)} \tag{3}$$

Although this test is developed using a heuristic approach, and carries less power, it is useful as a confirmatory test with say 10 or more % level of significance.

In practice, clinical trial data from a COD may result in, both carryover effects of A as well as B are significant, or carryover from either A or B is significant, or carryover from neither A nor B is significant. Accordingly three cases of analysis of above COD arise. We present analyses under fixed effects model with varying assumptions of presence or absence of carryover effects.

3.2 Estimation in fixed effect carryover models

We present estimation of parameters given by model 1 in three cases about the two active treatments as: both treatments, only one treatment and none of the treatments possess significant carryover effects.

Table 1: Estimates of model parameters.

Parameter	model 1	model 4
μ	$G/18n$	$G/18n$
γ_A	$(3(R_A - R_B) + (T_A - T_B) + \sum U_{2j} + \sum U_{6j} - \sum U_{3j} - \sum U_{4j})/16n$	-
γ_B	$(3(R_B - R_A) + (T_B - T_A) + \sum U_{3j} + \sum U_{4j} - \sum U_{2j} - \sum U_{6j})/16n$	-
τ_A	$T_A/6n - G/18n - \widehat{\gamma}_B/3$	$T_A/6n - G/18n$
τ_B	$T_B/6n - G/18n - \widehat{\gamma}_A/3$	$T_B/6n - G/18n$
τ_P	$T_P/6n - G/18n$	$T_P/6n - G/18n$
π_1	$P_1/6n - G/18n$	$P_1/6n - G/18n$
π_2	$P_2/6n - G/18n$	$P_2/6n - G/18n$
π_3	$P_3/6n - G/18n$	$P_3/6n - G/18n$
ξ_{1j}	$U_{1j}/3 - G/18n$	$U_{1j}/3 - G/18n$
ξ_{2j}	$U_{2j}/3 - G/18n - \widehat{\gamma}_B/3$	$U_{2j}/3 - G/18n$
ξ_{3j}	$U_{3j}/3 - G/18n - \widehat{\gamma}_A/3$	$U_{3j}/3 - G/18n$
ξ_{4j}	$U_{4j}/3 - G/18n - \widehat{\gamma}_A/3$	$U_{4j}/3 - G/18n$
ξ_{5j}	$U_{5j}/3 - G/18n$	$U_{5j}/3 - G/18n$
ξ_{6j}	$U_{6j}/3 - G/18n - \widehat{\gamma}_B/3$	$U_{6j}/3 - G/18n$

3.2.1 Estimation when both active treatments have significant carryover effects

The normal equations are:

$$
\begin{aligned}
18n\mu + 6n(\tau_A + \tau_B + \tau_P) + 4n(\gamma_A + \gamma_B) + 6n(\pi_1 + \pi_2 + \pi_3) + 3\sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= G \\
6n\mu + 6n\tau_A + 2n\gamma_B + 2n(\pi_1 + \pi_2 + \pi_3) + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= T_A \\
6n\mu + 6n\tau_B + 2n\gamma_A + 2n(\pi_1 + \pi_2 + \pi_3) + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= T_B \\
6n\mu + 6n\tau_P + 2n(\gamma_A + \gamma_B) + 2n(\pi_1 + \pi_2 + \pi_3) + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= T_P
\end{aligned}
$$

$$
\begin{aligned}
4n\mu + 2n(\tau_B + \tau_P) + 4n\gamma_A + 2n(\pi_2 + \pi_3) + \sum_{j=1}^{n}\xi_{1j} + \sum_{j=1}^{n}\xi_{3j} + \sum_{j=1}^{n}\xi_{4j} + \sum_{j=1}^{n}\xi_{5j} &= R_A \\
4n\mu + 2n(\tau_A + \tau_P) + 4n\gamma_B + 2n(\pi_2 + \pi_3) + \sum_{j=1}^{n}\xi_{1j} + \sum_{j=1}^{n}\xi_{2j} + \sum_{j=1}^{n}\xi_{5j} + \sum_{j=1}^{n}\xi_{6j} &= R_B \\
6n\mu + 2n(\tau_A + \tau_B + \tau_P) + 6n\pi_1 + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= P_1 \\
6n\mu + 2n(\tau_A + \tau_B + \tau_P) + 2n(\gamma_A + \gamma_B) + 6n\pi_2 + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= P_2 \\
6n\mu + 2n(\tau_A + \tau_B + \tau_P) + 2n(\gamma_A + \gamma_B) + 6n\pi_3 + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= P_3 \\
3\mu + (\tau_A + \tau_B + \tau_P) + (\gamma_A + \gamma_B) + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{1j} &= U_{1j} \\
3\mu + (\tau_A + \tau_B + \tau_P) + \gamma_B + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{2j} &= U_{2j} \\
3\mu + (\tau_A + \tau_B + \tau_P) + \gamma_A + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{3j} &= U_{3j} \\
3\mu + (\tau_A + \tau_B + \tau_P) + \gamma_A + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{4j} &= U_{4j} \\
3\mu + (\tau_A + \tau_B + \tau_P) + (\gamma_A + \gamma_B) + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{5j} &= U_{5j} \\
3\mu + (\tau_A + \tau_B + \tau_P) + \gamma_B + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{6j} &= U_{6j}
\end{aligned}
$$

The solutions of the normal equations with restrictions $\sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} = 0$, $\tau_A + \tau_B + \tau_P = 0$, $\gamma_A + \gamma_B = 0$ and $\pi_1 + \pi_2 + \pi_3 = 0$ are shown in Table 1.

3.2.2 Estimation when only one active treatment has significant carryover effect

When only one active treatment has carryover effect, the normality restriction on carryover effects is unavailable and hence, the normal equations containing the non-zero carryover effects parameter cannot be solved. The only way to analyze the model 1 is to substitute the non-zero carryover effect terms by the empirical estimates. Define, four sets h_1, h_2, h_3 and h_4 consisting of model observations $\{y_{1j2}, y_{2j1}, y_{3j3}, y_{4j2}, y_{5j3}, y_{6j1}\}$, $\{y_{1j3}, y_{2j2}, y_{3j1}, y_{4j3}, y_{5j1}, y_{6j2}\}$, $\{y_{1j3}, y_{2j2}, y_{3j1}, y_{4j1}, y_{5j2}, y_{6j3}\}$ and $\{y_{1j1}, y_{2j3}, y_{3j2}, y_{4j2}, y_{5j3}, y_{6j1}\}$ and having means $\overline{y}_1$, $\overline{y}_2$, $\overline{y}_3$ and $\overline{y}_4$ respectively. When 2 results in γ_A to be non-zero, use sets h_1 and h_2 to estimate γ_A, $\widehat{\gamma}_A = 1.5(\overline{y}_1 - \overline{y}_2)$. Similarly, when 3 results in γ_B to be non-zero, use sets h_3 and h_4, and obtain $\widehat{\gamma}_B = 1.5(\overline{y}_3 - \overline{y}_4)$. Without loss of generality

let us assume, γ_A is significant. Then model 1 can be transformed into a no carryover effects model given by,

$$y'_{ijk} = \begin{cases} y_{ijk} - \widehat{\gamma}_A, & \text{if } d(k-1,j) = A; \\ y_{ijk}, & \text{otherwise.} \end{cases}$$

where,

$$y'_{ijk} = \mu + \tau_{d(k,j)} + \pi_k + \xi_{ij} + e_{ijk}, \quad i = 1,\ldots,6; j = 1,\ldots,n; k = 1,2,3, \tag{4}$$

Then estimates of model 4 parameters are as shown in Table 1. Note that G, $\tau_{d(k,j)}$, p_k, and U_{ij} are calculated using observation y'_{ijk} in place of y_{ijk}.

3.2.3 Estimation when none of the active treatments have significant carryover effect

When carryover effects are present, they are confounded with the treatment period interaction effects, therefore the treatment period interaction terms was ignored in above two cases. Now, in the absence of carryover effects, there is an opportunity to analyze the following model,

$$y_{ijk} = \mu + \tau_{d(k,j)} + \pi_k + \xi_{ij} + (\tau\pi)_{d(k,j)k} + e_{ijk}, \tag{5}$$

$$i = 1,\ldots,6; j = 1,\ldots,n; k = 1,2,3,$$

where, $(\tau\pi)_{d(k,j)k}$ is treatment period interaction effects due to treatment $d(k,j)$ and period k and the remaining terms are as defined in 1. Under the side conditions, $\sum_{d(k,j)}(\tau\pi)_{d(k,j)k} = 0$ and $\sum_k(\tau\pi)_{d(k,j)k} = 0$, estimate of $(\tau\pi)_{d(k,j)k}$ is $[3(TP)_{d(k,j)k} - T_{d(k,j)} - P_k - V_{d(k,j)k} + 2G/3]/6n$, where $(TP)_{d(k,j)k}$ is sum of observations receiving treatment $d(k,j)$ in period k and $V_{d(k,j)k}$ is the sum of subjects who receive treatment $d(k,j)$ in period k. Estimates of the remaining model terms are same as model 4 shown in Table 1.

3.3 Testing

3.3.1 Testing when both active treatments have significant carryover effects

Analysis of COD1 cannot test hypothesis about equality of treatment effects eliminating carryover. Grizzle (1965) has suggested to use only first period data to test treatment effects under carryover model. Lehmacher (1991) made a detailed study of all possible hypotheses about treatment and carryover effects and recommended that COD1 can be useful for crossover trial, if no or if only positive carryover is assumed. He suggested to test following set of hypothesis to conclude about treatment effects when carryover effects are present, $H_0 : (\tau_A - \tau_B) - (\lambda_A - \lambda_B) = 0$, $H_1 : \tau_A - \tau_B = 0$ (no treatment effects in first period), $H_2 : (\tau_A - \tau_B) - (\lambda_A - \lambda_B)/2 = 0$, $H_3 : \lambda_A - \lambda_B = 0$ and $H_4 : (\tau_A - \tau_B) - (\lambda_A - \lambda_B) = 0$ (no second period difference). Then, subject to rejection of H_0, if H_1 is rejected, treatment effects are concluded as significant, however if it fails to reject H_1 but H_3 and

Table 2: Analysis of variance of model 1 for treatment effects.

Source of Variation	Sum of Squares Expression	Degrees of Freedom
Treatments	$\widehat{\tau}_A Q_A + \widehat{\tau}_B Q_B + \widehat{\tau}_P Q_P$	2
(eliminating carryover)		
Active Treatments	$(5(T_A - T_B) + 3(R_A - R_B) + \sum_j U_{2j} + \sum_j U_{6j}$	1
(eliminating carryover)	$- \sum_j U_{3j} - \sum_j U_{4j})^2/240n$	
Carryover of treatments	$3[(R_A + P_1/3 + \sum U_{2j}/3 + \sum U_{6j}/3 - 4G/9)^2$	1
(ignoring treatments)	$+(R_B + P_1/3 + \sum U_{3j}/3 + \sum U_{4j}/3 - 4G/9)^2]/10n$	
(eliminating subjects)		
Periods	$\sum_{i=1}^{3} P_i^2/6n - G^2/18n$	2
Subjects	$\sum_{i=1}^{6} \sum_{j=1}^{n} U_{ij}^2/3 - G^2/18n$	$6n - 1$
(ignoring carryover)		
Error	By Subtraction	$12n - 5$
Total	$\sum y_{ijk}^2 - G^2/18n$	$18n - 1$
Where $Q_A = T_A - G/3 - 2n\widehat{\gamma}_B$; $Q_B = T_B - G/3 - 2n\widehat{\gamma}_A$; $Q_P = T_P - G/3$		

H_4 are rejected, it implies either treatment effects are significantly positive or carryover effects are significantly negative. These set of hypothesis give proper conclusion when $\lambda_A = \lambda_B$ and are erroneous otherwise. It is desirable to obtain test of treatment effects based on treatment effect estimates that have been adjusted for carryover effects. Such estimates of treatment effects for two treatment crossover designs have been discussed by Lucas (1957) for COD3, Ebbutt (1984) for COD{ABB,ABA}, Senn and Lambrou (1998) for COD5 type, etc., but all have been limited to estimation. We provide analysis of variance for COD(2+1,6n,3) to test treatment effects eliminating carryover effects as shown in Table 2. Here, primary interest is to compare active treatments. Under $H_0 : \tau_A = \tau_B$, the sum of squares due to active treatments $(5(T_A - T_B) + 3(R_A - R_B) + \sum_j U_{2j} + \sum_j U_{6j} - \sum_j U_{3j} - \sum_j U_{4j})^2/240n$ normed by σ^2 follows chi-square distribution with one degree of freedom. As a result, active treatment effects adjusted for carryover effects are also tested by analysis of variance shown in Table 2.

Subject effects may be significantly different because different sequences of treatments are received or due to biological variation. In the second analysis of variance shown in Table 3, we test hypothesis about subject effects to get some idea about the cause of variation when they are significant. Here, G_1 and G_2 are the grand total of first and second Latin squares which include sequences {ABP,BPA,PAB} and {APB,BAP,PBA} respectively.

3.3.2 Testing when any one active treatment has significant carryover effect

When any one active treatment has significant carryover effect, the test procedures suggested in literature, specifically Grizzle (1965), Lehmacher (1991), etc. are not useful because of the assumption that carryover of all treatments should be equal. Here it is better to transform model 1 into the form 4 according to the procedure suggested in Section 3.2.2. Model 4 is free from carryover and hence the hypothesis $H_0 : \tau_A = \tau_B = \tau_P$ gets tested using analysis of variance shown in Table 4. Note that, here primary objective is to compare active treatments. Under null hypothesis $H_0 : \tau_A = \tau_B$, the test

Table 3: Analysis of variance of model 1 for subject effects.

Source of Variation	Sum of Squares Expression	Degrees of Freedom
Subjects		$6n-1$
(ignoring carryover)		
Groups	$(G_1^2+G_2^2)/9n - G^2/18n$	1
Subjects within group	$\sum_{i=1}^{3}\sum_{j=1}^{n} U_{ij}^2/3 - G_1^2/9n + \sum_{i=3}^{6}\sum_{j=1}^{n} U_{ij}^2/3 - G_2^2/9n$	$6n-2$
Periods	$\sum_{i=1}^{3} P_i^2/6n - G^2/18n$	2
Treatments	$(T_A^2+T_B^2+T_P^2)/6n - G^2/18n$	2
(ignoring carryover)		
Carryover of treatments	$\widehat{\gamma}_A R_A^{'} + \widehat{\gamma}_B R_B^{'}$	1
(eliminating treatments)		
(eliminating subjects)		
Error	By Subtraction	$12-5$
Total	$\sum y_{ijk}^2 - G^2/18n$	$18n-1$
Where $R_A^{'} = 3(R_A - R_B) + (T_A - T_B) + \sum U_{2j} + \sum U_{6j} - \sum U_{3j} - \sum U_{4j}$;		
$R_B^{'} = 3(R_B - R_A) + (T_B - T_A) + \sum U_{3j} + \sum U_{4j} - \sum U_{2j} - \sum U_{6j}$		

Table 4: Analysis of variance of model 4.

Source of Variation(SS)	Sum of Squares Expression	DF
Treatments(SST)	$(T_A^2+T_B^2+T_P^2)/6n - G^2/18n$	2
Active Treatments (SSAT)	$(T_A - T_B)^2/8n$	1
Periods (SSP)	$\sum_{i=1}^{3} P_i^2/6n - G^2/18n$	2
Subjects (SSS)	$\sum_{i=1}^{6}\sum_{j=1}^{n} U_{ij}^2/3 - G^2/18n$	$6n-1$
Error	TSS-SST-SSP-SSS	$4(3n-1)$
Total (TSS)	$\sum y_{ijk}^2 - G^2/18n$	$18n-1$

statistics $(T_A - T_B)^2/8n$ normed by σ^2 follows chi-square distribution with one degree of freedom. Therefore, $H_0 : \tau_A = \tau_B$ gets tested by SSAT as shown in Table 4. Also one more advantage due to model transformation is that period and subject effects are now orthogonal with the treatment effects and hence, period and subject effects are also tested using analysis of variance shown in Table 4.

3.3.3 Testing when none of the active treatments has significant carryover effect

In absence of significant carryover of active treatments, sum of squares due to treatment period interaction can also be split from the total sum of squares. We split the total sum of squares of model into sum of squares due to treatments, periods, subjects and treatment period interaction. Also active treatments are tested by the test statistics $(T_A - T_B)^2/8n$. The analysis of variance of the model 5 for testing hypothesis about

Table 5: Analysis of variance of model 5.

Source of Variation(SS)	Sum of Squares Expression	Degrees of Freedom
Treatments (SST)	$(T_A^2 + T_B^2 + T_P^2)/6n - G^2/18n$	2
Active Treatments (SSAT)	$(T_A - T_B)^2/8n$	1
Periods (SSP)	$\sum_{i=1}^{3} P_i^2/6n - G^2/18n$	2
Treatments × Periods (SSTP)	$\sum_{d(k,j)} \sum_k (TP)^2_{d(k,j)k}/2n - G^2/18n - \text{SST} - \text{SSP}$	4
Subjects (SSS)	$\sum_{i=1}^{6} \sum_{j=1}^{n} U_{ij}^2/3 - G^2/18n$	$6n - 1$
Error	TSS-SST-SSP-SSTP-SSS	$3(4n - 3)$
Total (TSS)	$\sum y_{ijk}^2 - G^2/18n$	$18n - 2$

treatments, active treatments, periods, treatment period interaction and subjects is shown in Table 5.

4 Conclusion

A uniform three period three treatment crossover design, consisting of a placebo and two active treatments is presented. The COD is preferable in practice because, the design is not only more ethical, but also provides adequate flexibility to experimenters in analyzing their crossover experiment data. Experimenter can test the individual carryover effects prior to analysis of crossover design. This individual test lead the experimenter to choose proper analysis from all three possible cases as both, single and none of the two active treatments has carryover effect. We recommend that, when experimenter is not sure about the nature of carryover effect, this design should be selected because this design is not only uniform but also gives complete and proper analysis.

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Supplementary Material

We use simulated data shown in Table 6 to illustrate computations involved in estimating effects and performing analysis of variance for the case, both active treatments have significant carryover effects.

Table 6: Simulated Data from COD(3,12,3).

Period	Treatment Sequence	Subject U_{11}	U_{12}	Treatment Sequence	Subject U_{21}	U_{22}	Treatment Sequence	Subject U_{31}	U_{32}
1	A	18	18	B	16	16	P	15	15
2	B	18	18	P	18	18	A	18	18
3	P	18	18	A	18	18	B	18	18
Period	Treatment Sequence	Subject U_{41}	U_{42}	Treatment Sequence	Subject U_{51}	U_{52}	Treatment Sequence	Subject U_{61}	U_{62}
1	A	18	18	B	16	16	P	15	15
2	P	17	17	A	21	21	B	16	16
3	B	16	16	P	17	18	A	21	21

Here $f_1 = \{54, 54, 54, 55\}$, $f_2 = \{52, 52, 52, 52\}$ and $f_3 = \{51, 51, 51, 51\}$. According to f_1, f_2 and f_3 the numerical values of the statistics $\bar{U}_1$, $\bar{U}_2$, $\bar{U}_3$, s_1^2, s_2^2 and s_3^2 are respectively as 54.25, 52, 51, 0.25, 0 and 0. Calculated values of test statistics 2 and 3 are, respectively 9 and 13. Both test statistics 2 and 3 show that carryover effect of active treatments are significant at 5% as well as 1% level of significance. Estimates of the model parameters in this case follows from Table 1 and are shown in Table 7. It shows that active treatment A has 2.0625 unit more effects than B (i.e., $\widehat{\tau}_A - \widehat{\tau}_B = 2.0625$). Similarly

Table 7: Estimates of model 1 for data in Table 6.

Parameter	Estimates
μ	17.4722
γ_A	-0.4062
γ_B	0.4062
τ_A	1.3924
τ_B	-0.6701
τ_P	-0.7222
π_1	-1.1389
π_2	0.5278
π_3	0.6111

analysis of variance in this case for treatments, active treatments and carryover effects follows from Table 2, is shown in Table 8. All three effects in this table are significant, specifically active treatment effects, as simulated in the data.

Further, when we perform computations involved in estimating effects and analysis of variance for the case in which one active treatment has significant carryover effect on simulated data we get results as shown in Table 9. Here, calculated values of test statistics 2 and 3 are respectively 1 and 13. Test statistics 3 shows that carryover effect of active treatment B is significant whereas, that of A is insignificant at 5% as well as 1% level of significance. The empirical estimate of $\hat{\gamma}_B$ from the sets $h_3 =$

Table 8: Analysis of variance of model 1 for data in Table 6.

Source of Variation	SS	DF	MS	F
Treatments (eliminating carryover)	34.9123	2	17.4562	22.9959
Active Treatments (eliminating carryover)	20.4185	1	20.4185	26.8987
Carryover of treatments (ignoring treatments) (eliminating subjects)	16.6093	1	16.6093	21.8803
Periods	23.3889	2	11.6944	
Subjects (ignoring carryover)	7.6389	11	0.6944	
Error	14.4229	19	0.7591	
Total	96.9722	35		

$\{18, 18, 15, 18, 21, 21, 18, 18, 15, 18, 21, 21\}$ and $h_4 = \{18, 18, 18, 15, 15, 15, 18, 18, 18, 15, 16, 15\}$ is $1.5 \times (18.5 - 16.583) = 2.875$. Now we transform the observations which are affected by carryover effects of treatment B. Transformed observations are shown in parenthesis beside original values in Table 9. The corresponding estimates of model parameters and analysis of variance for the model 4 are shown in Table 10 and Table 11 respectively. Active treatment A has 2.0417 unit more effects than B (i.e., $\widehat{\tau}_A - \widehat{\tau}_B = 2.0417$). Also analysis of variance shows that treatment effects and the active treatment effects are significant whereas periods and subject effects are insignificant.

Table 9: Simulated Data from COD(3,12,3) when active treatment B has carryover effect.

Period	Treatment Sequence	Subject U_{11}	U_{12}	Treatment Sequence	Subject U_{21}	U_{22}	Treatment Sequence	Subject U_{31}	U_{32}
1	A	18	18	B	16	16	P	15	15
2	B	16	16	P	18(15.125)	18(15.125)	A	18	18
3	P	18(15.125)	18(15.125)	A	18	18	B	16	16
Period	Treatment Sequence	Subject U_{41}	U_{42}	Treatment Sequence	Subject U_{51}	U_{52}	Treatment Sequence	Subject U_{61}	U_{62}
1	A	18	18	B	16	16	P	15	15
2	P	15	15	A	21(18.125)	21(18.125)	B	16	16
3	B	16	16	P	15	16	A	21(18.125)	21(18.125)

Table 10: Estimates of model 4 under $\gamma_B \neq 0$ for data in Table 9.

Parameter	Estimates
μ	16.3889
τ_A	1.6528
τ_B	-0.3839
τ_P	-1.2639
π_1	-0.0556
π_2	-0.0139
π_3	0.0694

Table 11: Analysis of variance under $\gamma_B \neq 0$ for data in Table 9.

Source of Variation	SS	DF	MS	F
Treatments	53.7639	2	26.8819	1138.5294
Active Treatments	37.5156	1	37.5156	1588.8971
Periods	0.0972	2	0.04861	2.0589
Subjects	0.3472	11	0.3157	1.3369
Error	0.4722	20	0.0236	
Total	54.6806	35		