Incomplete block designs for slope ratio assays

Aloke Dey[a], K. Balasubramanian[a], Sudhir Gupta[b,*]

[a] *Indian Statistical Institute, New Delhi 110 016, India*

[b] *Division of Statistics, Northern Illinois University, DeKalb, IL 60115, USA*

Abstract

Incomplete block designs for slope ratio assays are investigated. These designs leave the two important contrasts, viz., 'blank' and 'intersection' unconfounded. Several families of such designs are reported.

AMS classifications: 62K10

Keywords: Bioassay; Slope ratio assays; Block designs; Blank and intersection contrasts; Switching

1. Introduction and preliminaries

Incomplete block designs have been used extensively in many areas of investigation. In bioassays also, incomplete block designs may be used profitably. Groups of experimental units, such as litters of animals, adjacent tubes in an incubator, animals kept in the same cage, cultures on a single plate or paper discs on an agar plate are generally taken for providing homogeneous blocks of experimental units in bioassays. If blocks of homogeneous experimental units of right size are available, one can adopt a randomized complete block design for the assay. Due to experimental limitations however, it is not always possible to find homogeneous blocks that can accommodate all the doses; for example, litters may be too small, cage accommodation may be limited or the agar plate may be incapable of accommodating all the doses. The problem becomes more acute with increasing number of doses. In such a situation, adoption of a suitable incomplete block design becomes necessary.

Incomplete block designs for bioassays (in particular for parallel line assays) with some desirable properties have been investigated by several authors; see e.g., Das and Kulkarni (1966), Kyi Win and Dey (1980) and Gupta and Mukerjee (1990) and the

references given therein. For an account of the recent developments on designs for parallel line assays, a reference may be made to Gupta and Mukerjee (1996). The literature on incomplete block designs for another important class of assays, namely the slope ratio assays is rather scanty. Das and Kulkarni (1966) obtained some designs for slope ratio assays by augmenting each block of a design for parallel line assays with the 'blank' dose. A similar approach was followed by Kulshreshtha (1972). However, this method is not very efficient in the sense that these designs do not always permit the estimation of the major contrasts of slope ratio assays free from block effects.

The purpose of this paper is to present general techniques of construction of incomplete block designs for slope ratio assays. All these designs permit the estimation of the two important contrasts viz., 'intersection' and 'blank' free from block effects, i.e., these designs allow the estimation of these two major contrasts as if the block effects were absent from the model. Several families of designs are reported.

To begin with we briefly describe some important aspects of bioassays and in particular, of slope ratio assays. In bioassays, two materials are compared by utilizing the response that they produce in living organisms. The comparison is made on the basis of two sets of doses, one from *standard* preparation (material of known strength) and the other from *test* preparation (material of unknown strength) such that they produce the same response. If z_s and z_t denote the doses of standard and test preparations producing the same response, then the parameter of interest, called *relative potency*, is $\rho = z_s/z_t$. If the two preparations involved in an assay contain the same effective ingredient and all other substances that may be present in the preparations are totally inert, the assay is called analytical dilution assay. We consider only analytical dilution assays; it is also assumed that the response is quantitative.

In most assays the dose producing a specific response is not directly measurable and, recourse has to be taken to indirect methods to estimate the dose corresponding to a given response via a dose response relationship. If the dose-response relationship is not linear, often a transformation of the dose is made so that the relationship between the transformed dose and response is nearly linear. If z represents the dose in the original scale, then the two important transformations that have been found useful in bioassay work are (i) $x = \log_e z$ and (ii) $x = z^{\lambda}$, where $\lambda > 0$ is a known constant. The first of these gives rise to parallel line assays and assays based on the second transformation are called slope ratio assays.

In symmetrical slope ratio assays, there are m doses of each preparation and the doses are in arithmetic progression, i.e., if s_i and t_i denote respectively the ith dose of standard and test preparations, then for $i = 1, 2, \ldots, m$, $s_i = t_i = i/m$. In slope ratio assays, it is hypothesised that the regression lines of response on dose transform intersect on the response axis, and therefore, it is necessary to include a *blank* (control) dose in the assay to test the validity of this assumption. Thus a symmetrical slope ratio assay consists of $(2m + 1)$ doses.

In slope ratio assays, the *blank* (L_B), the *intersection* (L_I) (defined below) and the two regression contrasts are of major importance, as these contrasts are used for making

validity tests and for the estimation of relative potency. For further details on these aspects, a reference may be made to Ch. 7 of Finney (1978).

Throughout this paper we denote an m-component column vector of all ones by 1_m and an m-component null column vector by 0_m. A prime over a matrix or a vector denotes its transpose.

Let τ_i and τ_{m+i}, $i=1,2,\ldots,m$ denote the effect of s_i and t_i, respectively, and τ_c, that of the blank dose. Then the blank and intersection contrasts are given by

$$L_B = g\tau_c + \sum_{i=1}^{m} \{3(i-1) - h\}(\tau_i + \tau_{m+i}), \tag{1.1}$$

$$L_I = \sum_{i=1}^{m} \{h - 3(i-1)\}(\tau_i - \tau_{m+i}), \tag{1.2}$$

where $g = m(m-1)$, $h = 2(m-1)$.

We can rewrite the contrasts L_B and L_I as

$$L_B = (g, \boldsymbol{\alpha}', \boldsymbol{\alpha}')\,\boldsymbol{\tau}, \tag{1.3}$$

$$L_I = (0, -\boldsymbol{\alpha}', \boldsymbol{\alpha}')\,\boldsymbol{\tau}, \tag{1.4}$$

where

$$\boldsymbol{\alpha} = (2-2m, 5-2m, \ldots, m-1)',$$

$$\boldsymbol{\tau} = (\tau_c, \tau_1, \tau_2, \ldots, \tau_{2m})'.$$

Consider now an *equireplicate*, binary block design d with $(2m+1)$ treatments (doses) and b blocks, and let N_d be the incidence matrix of d. We may write N_d as

$$N_d = \begin{bmatrix} \boldsymbol{n}'_c \\ N_{d1} \\ N_{d2} \end{bmatrix}, \tag{1.5}$$

where $\boldsymbol{n}'_c = (n_{c1}, \ldots, n_{cb})$ is a row vector of b components, representing the incidence of the blank dose in d, N_{d1} is the $m \times b$ incidence matrix for the m doses of the standard preparation and N_{d2} is the corresponding matrix for the test preparation. Note that it is not necessary in general that each block of a design has doses from each of the two preparations, standard and test. Using Lemma 3.1 of Gupta and Mukerjee (1996), one can show that under a standard fixed effects model, the contrasts L_B and L_I can be estimated free of block effects through the design d if and only if

$$(g, \boldsymbol{\alpha}', \boldsymbol{\alpha}')N_d = 0'_b, \tag{1.6}$$

$$(0, -\boldsymbol{\alpha}', \boldsymbol{\alpha}')N_d = 0'_b. \tag{1.7}$$

Let $J \subset \{1,2,\ldots,b\}$ such that $n_{cj} = 1$ for $j \in J$ and $\bar{J} \subset \{1,2,\ldots,b\}$ such that $n_{cj} = 0$ for $j \in \bar{J}$. The cardinalities of J and $\bar{J}$ need not be the same. Clearly $J \cup \bar{J} = \{1,2,\ldots,b\}$.

Let β_{j1} (respectively, β_{j2}) be the jth column of N_{d1} (respectively, of N_{d2}) for $j \in J$. Then, from Eqs. (1.6) and (1.7), we must have

$$\alpha' \beta_{j1} = \alpha' \beta_{j2}, \tag{1.8}$$

$$\alpha' \beta_{j1} + \alpha' \beta_{j2} = -m(m-1). \tag{1.9}$$

Similarly, let γ_{j1} (respectively, γ_{j2}) be the jth column of N_{d1} (respectively, of N_{d2}) for $j \in \bar{J}$. Then, in order that the blank and intersection contrasts are estimated free from block effects, we must have

$$\alpha' \gamma_{j1} = \alpha' \gamma_{j2}, \tag{1.10}$$

$$\alpha' \gamma_{j1} + \alpha' \gamma_{j2} = 0. \tag{1.11}$$

Summarizing, we therefore have the following.

Lemma 1.1. *The blank and intersection contrasts can be estimated free from block effects through a binary equireplicate design d if and only if*

(i) $\alpha' \beta_{j1} = \alpha' \beta_{j2} = -m(m-1)/2, \quad \forall j \in J,$

(ii) $\alpha' \gamma_{j1} = \alpha' \gamma_{j2} = 0, \quad \forall j \in \bar{J}.$

In the next two sections we use Lemma 1.1 to arrive at equireplicate binary block designs for slope ratio assays which leave the blank and intersection contrasts free from block effects.

Remark 1.1. The designs constructed in this paper are necessarily equireplicate. It should be possible to construct non-equireplicate designs with the desired properties. A general procedure for constructing incomplete block designs for slope ratio assays that leave the blank and intersection contrasts free from block effects and are not equireplicate is not available at the present moment and further work is necessary to arrive at those designs.

2. A general method of construction of designs

In order to obtain designs satisfying the conditions of Lemma 1.1, we adopt the following strategy. First, for $n_{cj} = 1$, we obtain a *basic* incidence vector for one of the doses, say standard. Once the basic incidence vector is obtained, several other vectors, satisfying the conditions of Lemma 1.1 can be obtained by a procedure of *switching*, explained below.

Observe that the entries in the vector α are in arithmetic progression, with common difference 3. Consider any incidence vector, excluding its first entry (the one corresponding to the blank dose). Then, such a vector is a sequence of zeros and unities. Suppose there are two unities at positions i_1 and i_2 and two zeros at positions $i_1 + l$ and

$i_2 - l$. Then one can derive another vector with the same properties as the original one by bringing the unity at i_1th position to $(i_1 + l)$th position and that at i_2th position to $(i_2 - l)$th position. This is the procedure of switching. This procedure can be repeated any number of times, and we still call the procedure as switching. It is clear that all incidence vectors with the desired properties, mentioned earlier can be obtained from the basic incidence vectors by the process of switching.

Suppose $\boldsymbol{a}_i = (a_{i1}, a_{i2}, \ldots, a_{im})'$, $i = 1, 2, \ldots, t$, $a_{ij} = 0, 1$ is a collection of incidence vectors for the standard preparation doses, and similarly, let $\boldsymbol{c}_i = (c_{i1}, c_{i2}, \ldots, c_{im})'$, $i = 1, 2, \ldots, t$, $c_{ij} = 0, 1$ be a collection of incidence vectors for the test preparation doses, satisfying (i) of Lemma 1.1. Further, let

$$(\boldsymbol{a}_i', \boldsymbol{c}_i') 1_{2m} = k_1, \quad \forall i = 1, 2, \ldots, t, \tag{2.1}$$

where k_1 is a constant. The final incidence matrix of an equireplicate design for slope ratio assays permitting the estimation of blank and intersection contrasts free from block effects is then given by

$$N_d = \begin{bmatrix} 1 & 1 & \cdots & 1 & 0 & 0 & \cdots & 0 \\ \boldsymbol{a}_1 & \boldsymbol{a}_2 & \cdots & \boldsymbol{a}_t & \boldsymbol{b}_1 & \boldsymbol{b}_2 & \cdots & \boldsymbol{b}_t \\ \boldsymbol{c}_1 & \boldsymbol{c}_2 & \cdots & \boldsymbol{c}_t & \boldsymbol{d}_1 & \boldsymbol{d}_2 & \cdots & \boldsymbol{d}_t \end{bmatrix}, \tag{2.2}$$

where for $i = 1, 2, \ldots, t$, $\boldsymbol{b}_i = 1_m - \boldsymbol{a}_i$ and $\boldsymbol{d}_i = 1_m - \boldsymbol{c}_i$.

Observe that since for $i = 1, 2, \ldots, t$, $\boldsymbol{a}_i$ satisfies (i) of Lemma 1.1, we have

$$\sum_{j=1}^{m} a_{ij}\alpha_j = -m(m-1)/2, \quad \text{for all } i = 1, 2, \ldots, t,$$

where $\boldsymbol{\alpha} = (\alpha_1, \alpha_2, \ldots, \alpha_m)'$. Hence,

$$\sum_{j=1}^{m} b_{ij}\alpha_j = \sum_{j=1}^{m} (1 - a_{ij})\alpha_j = 0,$$

as $\sum_{j=1}^{m} \alpha_j = -m(m-1)/2$. Here, for $i = 1, 2, \ldots, t$, $\boldsymbol{b}_i = (b_{i1}, b_{i2}, \ldots, b_{im})'$. Arguing similarly for the vectors $\boldsymbol{c}_i$ and $\boldsymbol{d}_i$, it follows that the matrix N_d given by Eq. (2.2) does satisfy the conditions of Lemma 1.1 and the design d based on N_d permits the estimation of the blank and intersection contrasts free from block effects. Clearly, N_d given by Eq. (2.2) is the incidence matrix of a binary design with $b = 2t$ blocks, each dose being replicated t times. We thus have

Theorem 2.1. *Suppose $\boldsymbol{a}_1, \ldots, \boldsymbol{a}_t$ is a set of t incidence vectors for the standard preparation doses and $\boldsymbol{c}_1, \ldots, \boldsymbol{c}_t$, a set of incidence vectors for the test preparation doses, satisfying* (i) *of Lemma* 1.1 *and* (2.1). *Then using these vectors, it is possible to construct a binary, equireplicate block design with incidence matrix given by*

Eq. (2.2) *for slope ratio assays permitting the estimation of the blank and intersection contrasts free from block effects.*

Example 2.1. Let $m=7$. It can be verified that the following vector satisfies (i) of Lemma 1.1:

$$(1,1,1,0,0,0,1)'.$$

Two more vectors with the desired properties can be generated from this basic vector by switching and are

$$(1,1,0,1,0,1,0)', \qquad (1,0,1,1,1,0,0)'.$$

Taking these vectors for both the preparations and following the method of construction just described, we have the incidence matrix of a design for slope ratio assays which allows the estimation of the blank and intersection contrasts free from block effects. The incidence matrix is displayed below:

$$N_d = \begin{bmatrix} 1 & 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 1 & 0 \\ 0 & 1 & 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 & 0 & 1 \\ 1 & 0 & 0 & 0 & 1 & 1 \\ 1 & 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 1 & 0 \\ 0 & 1 & 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 & 0 & 1 \\ 1 & 0 & 0 & 0 & 1 & 1 \end{bmatrix}.$$

In the above matrix, the first row represents the incidence of the blank dose, the next seven rows indicate the incidence of the standard preparation doses and the rest, that of the test doses. The design has $b=6$ blocks, each dose being replicated $r=3$ times.

If we follow the method of construction of designs described above, it is clear that the final design has blocks of two sizes. Henceforth, we denote by k_1 the block size of those blocks for which $n_{cj}=1$, $j=1,2,\ldots,t$ and by k_2, that of those blocks for which $n_{cj}=0$. Clearly, $k_2=2m+1-k_1$. Now, for $n_{cj}=1$, suppose $\boldsymbol{\beta}_{j1}=(1'_u,0'_{m-u})'$, $1\leqslant u<m$. Then

$$\boldsymbol{\alpha}'\boldsymbol{\beta}_{j1} = -u(4m-3u-1)/2. \tag{2.3}$$

If the right-hand side of Eq. (2.3) is larger than $-m(m-1)/2$, then clearly there is no solution in the sense that in such a case, the conditions of Lemma 1.1 can never be met, for, the sum of any u elements in $\boldsymbol{\alpha}$ (which is an increasing sequence) is at least equal to the sum of first u elements of $\boldsymbol{\alpha}$. It follows that a necessary condition for a possible solution is that

$$-u(4m-3u-1)/2 \leqslant -m(m-1)/2, \tag{2.4}$$

or, equivalently,

$$h(u) = 3u^2 - u(4m-1) + m(m-1) \leqslant 0. \tag{2.5}$$

Since the coefficient of u^2 in $h(u)$ is positive, $h(u) \geqslant 0$, unless u lies between the roots of the equation $h(u) = 0$. The roots of $h(u) = 0$ are easily seen to be $u = m$ and $u = (m-1)/3$. The larger root is trivial. Hence, we have $(m-1)/3 \leqslant u < m$. Repeating the same argument for the test dose, we arrive at the following result.

Lemma 2.1. *For a binary, equireplicate block design permitting the estimation of the blank and intersection contrasts free from block effects, it is necessary that* $k_1 \geqslant 2(m-1)/3 + 1$, *where* k_1 *is as defined above.*

We now prove the following

Lemma 2.2. *Let* $m \equiv 0 \pmod 3$. *A necessary condition for an equireplicate, binary design permitting the estimation of the blank and intersection contrasts free from block effects is that* $k_1 \equiv 1 \pmod 3$.

Proof. If a design permitting the estimation of blank and intersection contrasts exists, then for this design, we must have $n_{cj} = 1$ for at least one $j \in \{1, 2, \ldots, b\}$. Let $n_{cj} = 1$, for some $j \in \{1, 2, \ldots, b\}$. It is easy to see that if $m \equiv 0 \pmod 3$, then every element in $\boldsymbol{\alpha}$ is congruent to $2 \pmod 3$. Also, $-m(m-1)/2$ is congruent to $0 \pmod 3$. Define $S_m = \{2-2m, 5-2m, \ldots, m-1\}$. Now, if a design with the stated properties exists, then there must exist an integer k' such that the sum of some k' elements of S_m should be $-m(m-1)/2$. This means that $2k' \equiv 0 \pmod 3$, implying that $k' \equiv 0 \pmod 3$. This argument is valid for one of the doses, say standard. Repeating the same argument for the test doses, we find that k_1 must necessarily be congruent to $1 \pmod 3$. □

On similar lines, we can prove the following

Lemma 2.3. *Let* $m \equiv 2 \pmod 3$. *A necessary condition for an equireplicate, binary design permitting the estimation of the blank and intersection contrasts free from block effects is that* $k_1 \equiv 2 \pmod 3$.

In the next section, we give specific procedures for constructing designs for different values of m. We distinguish three cases, viz., (i) $m \equiv 1 \pmod 3$, (ii) $m \equiv 2 \pmod 3$ and (iii) $m \equiv 0 \pmod 3$ and give designs for each of the cases.

3. Specific designs

3.1. $m \equiv 1 \pmod 3$

When $m \equiv 1 \pmod 3$, each element in $S_m = \{2-2m, 5-2m, \ldots, -3, 0, 3, 6, \ldots, m-1\}$ is congruent to zero (mod 3). Define

$$S_y = \{2-2m, 5-2m, \ldots, -2-m\};\ S_z = \{1-m, 4-m, \ldots, -3\};$$
$$S_w = \{3, 6, \ldots, m-1\}.$$

Clearly, the cardinality of each of the sets S_y, S_z, S_w is $(m-1)/3$ and $S_m = S_y \cup S_z \cup \{0\} \cup S_w$. Also, each element in S_w has its negative in S_z, and the sum of the elements in S_y is $-m(m-1)/2$.

Let $n_{cj} = 1$. We define two functions f and g as follows:

$f : S_m \to \{0, 1\}$ such that

(1) $f(x) = 1\ \forall x \in S_y$,

(2) for $x \in S_w$, $f(x) = 1 \Leftrightarrow f(-x) = 1$,

(3) $f(0) = 0$,

(4) $|\{x: f(x) = 1,\ x \in S_w\}| = s_1$, where s_1 is a nonnegative integer.

$g : S_m \to \{0, 1\}$ such that $g(x)$ satisfies the conditions (1)–(3) of $f(x)$ with f replaced by g and

(5) $|\{x: g(x) = 1, x \in S_w\}| = s_2$, where s_2 is a nonnegative integer.

The basic incidence vectors are then given by

$$\boldsymbol{w}_s = f(S_m) = (f(2-2m), f(5-2m), \ldots, f(-3), f(0), f(3), \ldots, f(m-1))',$$
$$\boldsymbol{w}_t = g(S_m) = (g(2-2m), g(5-2m), \ldots, g(-3), g(0), g(3), \ldots, g(m-1))',$$

for the standard and test preparation doses respectively. Note that these incidence vectors satisfy (i) of Lemma 1.1.

Clearly, $k_1 = 2(m-1)/3 + 2(s_1 + s_2) + 1$. Also, $0 \leqslant s_1, s_2 \leqslant (m-1)/3$; $(s_1, s_2) \neq (0, 0)$. Once the basic incidence vectors are constructed, other incidence vectors can be obtained by switching. We thus have the following

Theorem 3.1. *For $m \equiv 1 \pmod 3$, there exists an equireplicate incomplete block design for slope ratio assays with $k_1 = 2(m-1)/3 + 2(s_1 + s_2) + 1$ which permits the estimation of the blank and intersection contrasts free from block effects, where s_1, s_2 are integers, satisfying $0 \leqslant s_1, s_2 \leqslant (m-1)/3$, $(s_1, s_2) \neq (0, 0)$.*

Example 3.1. Let $m = 7, s_1 = 0, s_2 = 1$. This choice gives rise to a design with $k_1 = 7$. With $n_{cj} = 1$, following are the basic incidence vectors, satisfying (i) of Lemma 1.1:

$(1, 1, 0, 0, 0, 0, 0)'$ for standard; $(1, 1, 1, 0, 0, 0, 1)'$ for test.

From the first of these, no more vectors can be obtained by switching. However, the following two vectors can be obtained by switching from the basic vector for test

preparation:

$$(1,1,0,1,0,1,0)', \qquad (1,0,1,1,1,0,0)'.$$

The roles of the standard and test preparations can of course be interchanged. Combining these vectors suitably and following the procedure of construction just described, one can get the final design. The incidence matrix of such a design is shown below:

$$N_d = \begin{bmatrix} 111 & 111 & 000 & 000 \\ 111 & 111 & 000 & 000 \\ 111 & 110 & 000 & 001 \\ 000 & 101 & 111 & 010 \\ 000 & 011 & 111 & 100 \\ 000 & 001 & 111 & 110 \\ 000 & 010 & 111 & 101 \\ 000 & 100 & 111 & 011 \\ 111 & 111 & 000 & 000 \\ 110 & 111 & 001 & 000 \\ 101 & 000 & 010 & 111 \\ 011 & 000 & 100 & 111 \\ 001 & 000 & 110 & 111 \\ 010 & 000 & 101 & 111 \\ 100 & 000 & 011 & 111 \end{bmatrix},$$

where the first row corresponds to the blank dose, the next seven rows correspond to the standard preparation doses and the rest are for test preparation doses. This design has $b=12$ blocks and each treatment is replicated $r=6$ times. The block sizes are $k_1=7$ and $k_2=8$.

Designs with fewer blocks can be obtained by deleting one or more columns for which $n_{cj}=1$ of the incidence matrix and also deleting the complementary column(s). For instance, deleting columns 1 and 7 of the above incidence matrix leaves a design with $b=10$ blocks.

3.2. $m \equiv 0 \pmod 3$

Let $m=3u$, where u is a positive integer. By Lemma 2.1, we have in this case, $k_1 \geqslant 2u+1$ and by Lemma 2.2, a necessary condition for a design with desired properties is that $k_1 \equiv 1 \pmod 3$.

Let $n_{cj}=1$ for some $j \in \{1,2,\ldots,b\}$. Suppose in the incidence vector for this j there are r_1 (respectively, r_2) unities corresponding to the standard (respectively, test) preparation doses. Then $k_1 = r_1 + r_2 + 1$. Also, the sum of $r_1(r_2)$ entries in $S_m = \{2-2m, 5-2m, \ldots, m-1\}$ must be $-m(m-1)/2$. But $-m(m-1)/2 \equiv 0 \pmod 3$, as $m \equiv 0 \pmod 3$. However, when $m \equiv 0 \pmod 3$, each entry in S_m is congruent to $2 \pmod 3$. It follows therefore that each of r_1 and r_2 must be congruent to $0 \pmod 3$. Note that with

$r_1, r_2 \equiv 0 \pmod 3$, $k_1 \equiv 1 \pmod 3$, which by Lemma 2.2 is a necessary condition for a design with desired properties to exist.

Now, the sum of first r_1 entries in S_m is equal to $r_1(-12u + 3r_1 + 1)/2$. In order that there is a feasible solution to a basic vector (for the standard doses), this sum must not exceed $-m(m-1)/2 = (-9u^2 + 3u)/2$. Thus we must have

$$r_1(-12u + 3r_1 + 1)/2 \leqslant (-9u^2 + 3u)/2, \tag{3.1}$$

or,

$$w(r_1) = 3r_1^2 - r_1(12u - 1) + 9u^2 - 3u \leqslant 0. \tag{3.2}$$

Clearly, $w(r_1)$ is nonpositive only when r_1 lies between the roots of $w(r_1) = 0$. The roots of $w(r_1) = 0$ are easily seen to be $r_1 = u - 1/3$ and $3u$. The larger root is trivial, and since r_1 is an integer, for a binary design we must have $r_1 \geqslant u$. Repeating the same argument for the test preparation doses, we must have $r_2 \geqslant u$. We therefore have the following

Lemma 3.1. *Let $m \equiv 0 \pmod 3$. Then a set of necessary conditions for a binary, equireplicate block design with $k_1 = r_1 + r_2 + 1$, permitting the estimation of blank and intersection contrasts free from block effects to exist, is that r_1, r_2 satisfy $r_1, r_2 \equiv 0 \pmod 3$ and $r_1, r_2 \geqslant m/3$.*

The next question to ask is: can we construct a design with desired properties and k_1 attaining the lower bound given by Lemma 3.1, i.e., with $k_1 = 2u+1$? Such a design is possible if and only if $r_1 = r_2 = u$. But by Lemma 3.1, we must have $r_1, r_2 \equiv 0 \pmod 3$. This is achievable only when $u \equiv 0 \pmod 3$ or, equivalently, $m \equiv 0 \pmod 9$. When $m \equiv 0 \pmod 9$, one can indeed get a basic vector which yields a design with $k_1 = 2u+1$. This basic vector for both the preparation doses is given by

$$(1'_{\frac{m-3}{3}}, 0'_{\frac{m}{9}}, 1, 0'_{\frac{5m}{9}})'.$$

From this basic vector, other vectors can be generated by the procedure of switching. We thus have

Theorem 3.2. *Let $m \equiv 0 \pmod 9$. Then there exists an equireplicate binary block design permitting the estimation of blank and intersection contrasts free from block effects with $k_1 = 2u + 1$ where $u = m/3$. Further, $2u + 1$ is the smallest value of k_1 for a binary design with the desired properties.*

Example 3.2. Let $m = 18$, so that $u = 6$. Following the method of construction described above, we get the following basic vector:

$$(1, 1, 1, 1, 1, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)'.$$

The following vector can be obtained from this vector by switching:

$$(1, 1, 1, 1, 0, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)'.$$

Taking these vectors for both the standard and test preparation doses, one can build a design with $k_1 = 13$. The incidence matrix of such a design is shown below:

$$N_d = \begin{bmatrix} 1 & 111110010 & 000000000 & 111110010 & 000000000 \\ 1 & 111101100 & 000000000 & 111101100 & 000000000 \\ 0 & 000001101 & 111111111 & 000001101 & 111111111 \\ 0 & 000010011 & 111111111 & 000010011 & 111111111 \end{bmatrix}',$$

where the first row of N_d represents the incidence of the blank dose, the next 18 rows that of the standard preparation doses and the rest, that of test preparation doses. This design has four blocks and each treatment is replicated twice.

Now let $u = m/3 \equiv 2 \pmod 3$ or, equivalently, $m \equiv 6 \pmod 9$ and $n_{ej} = 1$. For this case, we cannot have r_1, r_2 equal to u, as $u \equiv 2 \pmod 3$. Hence, we must have $r_1, r_2 \geqslant u+1$. If $r_1 = r_2 = u+1$, then $k_1 = 2u+3$. It is indeed possible to find a basic incidence vector with the desired properties and $k_1 = 2u+3$. The following vector may be used as a basic incidence vector for both the preparations:

$$(1'_{\frac{m}{3}}, 0'_{\frac{4m-6}{9}}, 1, 0'_{\frac{2m-3}{9}})'.$$

From this basic vector, others can be generated by switching. We thus have

Theorem 3.3. *Let $m \equiv 6 \pmod 9$. Then there exists a binary, equireplicate block design permitting the estimation of blank and intersection contrasts free from block effects with $k_1 = 2u + 3$ where $u = m/3$. Further, $2u + 3$ is the smallest value of k_1 for a binary design with the desired properties.*

Example 3.3. Let $u = 5$, $m = 15$. The basic vector for one of the preparations is as follows:

$$(1, 1, 1, 1, 1, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0)'.$$

The following are some of the vectors obtained from the above basic vector by the process of switching:

$$(1, 1, 1, 1, 0, 1, 0, 0, 0, 0, 1, 0, 0, 0, 0)', \qquad (1, 1, 1, 1, 0, 0, 1, 0, 0, 1, 0, 0, 0, 0, 0)'.$$

Following is the incidence matrix of a design based on these incidence vectors:

$$N_d = \begin{bmatrix} 1 & 111110000001000 & 111110000001000 \\ 1 & 111101000010000 & 111101000010000 \\ 1 & 111100100100000 & 111100100100000 \\ 0 & 000001111110111 & 000001111110111 \\ 0 & 000010111101111 & 000010111101111 \\ 0 & 000011011011111 & 000011011011111 \end{bmatrix}'.$$

There are $b = 6$ blocks and each treatment is replicated thrice.

Finally, let $u \equiv 1 \pmod 3$, or, $m \equiv 3 \pmod 9$. In this case, we can show that we must have $r_1, r_2 \geqslant u+2$, so that $k_1 \geqslant 2u+5$. With $r_1 = r_2 = u+2$, a basic vector with the desired properties is given by

$$(1'_{\frac{m}{3}}, 0'_{\frac{m-3}{3}}, 1, 0'_{\frac{m-3}{9}}, 1, 0'_{\frac{2m-6}{9}})'.$$

This basic vector can be used for both the preparation doses. Other vectors can be obtained from this basic vector by switching. We thus have

Theorem 3.4. *Let $m \equiv 3 \pmod 9$. Then there exists a binary equireplicate block design permitting the estimation of blank and intersection contrasts free from block effects with $k_1 = 2u+5$ where $u = m/3$. Further, $2u+5$ is the smallest value of k_1 for a binary design with the desired properties.*

Example 3.4. Let $m = 12$, so that $u = 4$. Following the construction just described, we get the following basic incidence vector with $r_1 = 6$:

$$(1,1,1,1,0,0,0,1,0,1,0,0)'.$$

The following are some (but not all) incidence vectors, obtained by switching:

$$(1,1,1,0,1,0,1,0,0,1,0,0)', \qquad (1,1,0,1,1,0,1,0,1,0,0,0)';$$

Combining these incidence vectors, an equireplicate design with the desired properties can be obtained. The incidence matrix of such a design is shown below:

$$N_d = \begin{bmatrix} 1 & 111100010100 & 111100010100 \\ 1 & 111010100100 & 111010100100 \\ 1 & 110110101000 & 110110101000 \\ 0 & 000011101011 & 000011101011 \\ 0 & 000101011011 & 000101011011 \\ 0 & 001001010111 & 001001010111 \end{bmatrix}'.$$

3.3. $m \equiv 2 \pmod 3$

Let $m = 3u+2$, where u is a positive integer. Arguing as in Section 3.2, we can show that with $n_{cj} = 1$, r_1, r_2 must satisfy the conditions

(1) $r_1, r_2 \equiv 2 \pmod 3$, and

(2) $r_1, r_2 \geqslant u+1$.

Thus the minimum values of r_1, r_2 are as given below:

(1) $r_1 = r_2 = u+2$, *when* $u \equiv 0 \pmod 3$;

(2) $r_1 = r_2 = u+1$, *when* $u \equiv 1 \pmod 3$;

(3) $r_1 = r_2 = u+3$, *when* $u \equiv 2 \pmod 3$.

For each of the above values of u, one can get a solution for the basic vector with r_1, r_2 attaining the lower bound. The basic vector when $u \equiv 0 \pmod 3$ is given by

$$(1'_{\frac{m+1}{3}}, 0'_{\frac{5m-10}{9}}, 1, 0'_{\frac{m-2}{9}})'.$$

The above basic vector may be used for both the preparation doses. Other incidence vectors with the desired properties can be obtained from the basic vector by the process of switching. We thus have

Theorem 3.5. *Let $m \equiv 2 \pmod 9$. Then there exists a binary block design permitting the estimation of blank and intersection contrasts free from block effects with $k_1 = 2u + 5$ where $u = (m-2)/3$. Further, $2u + 5$ is the smallest value of k_1.*

Example 3.5. Let $m = 11$, so that $u = 3$. Following the method of construction described above, we get the following basic incidence vector:

$$(1,1,1,1,0,0,0,0,0,1,0)'.$$

The following are some of the vectors obtained by switching:

$$(1,1,1,0,1,0,0,0,1,0,0)', \qquad (1,1,1,0,0,1,0,1,0,0,0)'$$

$$(1,1,0,1,1,0,0,1,0,0,0)'.$$

Using these vectors for both the preparations, a design with $k_1 = 11$ can be constructed. The incidence matrix of such a design is shown below:

$$N_d = \begin{bmatrix} 1 & 11110000010 & 11110000010 \\ 1 & 11101000100 & 11101000100 \\ 1 & 11100101000 & 11100101000 \\ 1 & 11011001000 & 11011001000 \\ 0 & 00001111101 & 00001111101 \\ 0 & 00010111011 & 00010111011 \\ 0 & 00011010111 & 00011010111 \\ 0 & 00100110111 & 00100110111 \end{bmatrix}'.$$

This design has $b = 8$ blocks and each dose is replicated four times.

Now let $u \equiv 1 \pmod 3$. For this case, a basic vector with $r_1 = u + 1$ is given below:

$$(1'_{\frac{m-2}{3}}, 0'_{\frac{2m-1}{9}}, 1, 0'_{\frac{4m-2}{9}})'.$$

This vector may be used as a basic vector for both the preparations. Other vectors can be obtained by switching. We thus have

Theorem 3.6. *Let $m \equiv 5 \pmod 9$. Then there exists a binary, equireplicate block design permitting the estimation of blank and intersection contrasts free from block effects with $k_1 = 2u + 3$ where $u = (m-2)/3$. Further, $2u + 3$ is the smallest value of k_1.*

Example 3.6. Let $m=14$, so that $u=4$. Following the method just described, we get the following basic incidence vector:

$$(1,1,1,1,0,0,0,1,0,0,0,0,0,0)'.$$

The following vector can be obtained by switching:

$$(1,1,1,0,1,0,1,0,0,0,0,0,0,0)'.$$

Combining these vectors we get a design with $k_1=11$. The incidence matrix of such a design is shown below:

$$N_d=\begin{bmatrix}1 & 11110001000000 & 11110001000000\\ 1 & 11101010000000 & 11101010000000\\ 0 & 00001110111111 & 00001110111111\\ 0 & 00010101111111 & 00010101111111\end{bmatrix}'.$$

Finally, let $u\equiv 2\,(\mathrm{mod}\,3)$. In this case, with $r_1=u+3$, a basic vector is as given below:

$$(1'_{\frac{m+1}{3}},0'_{\frac{4m-14}{9}},1'_2,0'_{\frac{2m-7}{9}})'.$$

Using this vector as a basic vector for both the preparations and obtaining other vectors by switching, the final design with $k_1=2u+7$ can be obtained and we have the following

Theorem 3.7. *Let $m\equiv 8\,(\mathrm{mod}\,9)$. Then there exists a binary, equireplicate block design permitting the estimation of blank and intersection contrasts free from block effects with $k_1=2u+7$ where $u=(m-2)/3$. Further, $2u+7$ is the smallest value of k_1.*

Example 3.7. Let $m=8$ so that $u=2$. The following basic incidence vector can be obtained by following the above construction:

$$(1,1,1,0,0,1,1,0)'.$$

The following vector is obtained by switching:

$$(1,1,0,1,1,0,1,0)'.$$

Combining these vectors, the incidence matrix of a design with $k_1=11$ is obtained and is displayed below:

$$N_d=\begin{bmatrix}1 & 11100110 & 11100110\\ 1 & 11011010 & 11011010\\ 0 & 00011001 & 00011001\\ 0 & 00100101 & 00100101\end{bmatrix}'.$$

Remark 3.1. The designs constructed in this paper have in general blocks of two different sizes. Therefore, these designs are applicable in situations where one can

assume that the intra-block variance is independent of the block size, especially if the two block sizes are widely different. As noted in pp. 201–202 of Finney (1978) such an assumption is more likely to be correct in experiments with animals (with litters forming the blocks) than it would be in agricultural field experiments. If the two block sizes are nearly equal, it is safer to make the assumption of equal intra-block variance. It is possible to generate designs through the present method with block sizes that are nearly equal, by choosing an appropriate value of k_1. For instance, with $m \equiv 0$ or $2 \pmod 3$, we have presented solutions of designs with the least possible value of k_1. From these, designs with larger values of k_1 can be obtained. If k_{10} is the value of k_1 of a design described in Sections 3.2 and 3.3, then starting from this design, another one with block size $k_{10} + t$ can be obtained, where $t \equiv 0 \pmod 3$. This is achieved by selecting t elements from S_m in such a manner that the sum of these elements is zero and then putting unities at those places in the incidence vector of one of the preparations which correspond to these elements. The problem of obtaining designs for slope ratio assays with the stated properties and blocks of equal sizes remains open. Similarly, as mentioned in Remark 1.1, methods of constructing designs with possibly unequal replicates of doses and permitting the estimation of the blank and intersection contrasts free from block effects are still to be explored.

Acknowledgements

The authors wish to thank the referees for useful comments on an earlier version. Part of this work was done during A. Dey's visit to the Department of Mathematical Sciences, The University of Akron. He would like to thank the department Chair, Dr. P.H. Schmidt for providing excellent facilities to carry out this work.

References

Das, M.N., Kulkarni, G.A., 1966. Incomplete block designs for bio-assays. Biometrics 22, 706–729.

Finney, D.J., 1978. Statistical Method in Biological Assay, 3rd ed. Griffin, London.

Gupta, S., Mukerjee, R., 1990. On incomplete block designs for symmetric parallel line assays. Austral. J. Statist. 32, 337–344.

Gupta, S., Mukerjee, R., 1996. Developments in incomplete block designs for parallel line bioassays. In: Ghosh, S., Rao, C.R. (Eds.), Handbook of Statistics, vol. 13. North-Holland, Amsterdam, pp. 875–901.

Kulshreshtha, A.C., 1972. A new incomplete block design for slope ratio assays. Biometrics 28, 585–587.

Kyi Win, Dey, A., 1980. Incomplete block designs for parallel line assays. Biometrics 36, 487–492.