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Efficient block designs for symmetric parallel line assays

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Abstract: Three methods of constructions of efficient block designs for symmetric parallel line assays have been proposed. These methods are based on balanced incomplete block design (BIBD). Through these designs, all contrasts of interest can be estimated free from block effect and with high efficiency. Any BIB design for which *v*, the number treatments is strictly greater than twice of block size can be converted into a design that can be used for conducting a symmetric parallel line assay. All methods of construction are demonstrated with some examples.

Subjects: Mathematics & Statistics; Science; Statistical Theory & Methods; Statistics; Statistics & Probability

Keywords: bioassay; parallel line assay; block design; A-optimality; BIBD; efficiency

1. Introduction

Bioassay is a planned experiment in which two stimuli, one with known preparation known as standard and another with unknown preparation known as test are applied to subjects. The comparison is made on the basis of two sets of doses, one from standard preparation and the other from test preparation such that they produce the same response. The main purpose of bioassay is to estimate the relative potency of the test preparation relative to the standard preparation. Further, it is also assumed that the response is quantitative. In bioassay, if the dose–response relationship is not linear, often a transformation of the dose is made so that the relationship between the transformed

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LalMohan Bhar is a scientist working at Indian Agricultural Statistics Research Institute, New Delhi, India. Primarily, he is interested in application of advanced statistical tools in agricultural research. He is engaged in developing various statistical techniques for application in agriculture like statistical models for forecasting various aspects in agriculture, construction of appropriate experimental designs, and their analysis. Recently, he is engaged in developing statistical techniques for prediction genomic values required for plant and animal breeding programs. He has provided many method of construction of designs required for agricultural research including designs for bioassays. He also provided many methods for detection of outliers in designed experiments. He extensively worked in the area of robust designs against the presence of missing observations and outliers. He published a good number of research articles in international journals of repute. He also guided many MSc And PhD students in Agricultural Statistics.

PUBLIC INTEREST STATEMENT

Bioassay is a planned experiment in which strength of test preparation is compared with a standard one on the basis of two sets of doses. The main purpose of bioassay is to draw statistically valid estimate on the relative potency of the test preparation relative to the standard preparation. One of the most popular assays is symmetric parallel line assays in which some specialized comparisons among the doses are made. Therefore, designing aspect in bioassays is very important. Experiments in bioassays should be laid in such a way that it can give statistically sound estimate of relative potency. It can be achieved through proper designing so that the estimates of various kind of comparisons required for can be obtained with minimum error. The present study is aimed to achieve this goal. Some designs are proposed for used in practice for symmetrical parallel line assays.

dose and response is nearly linear. If a log transformation is used, then the assay is known as parallel line assays. In parallel line assays, the two dose–response regression lines (one each for standard and test preparations) are taken as parallel. Further, it is assumed that doses are equi-spaced on logarithm scale. If the number of doses for both the preparations is same, then the assay is known as symmetric, otherwise it is known as asymmetric assay. In the present paper, we are concerned about the symmetric parallel line assays only. For a detailed account of the subject, one may refer to Finney (1978, 1979). For a good survey of the subject one may refer to Kshirsagar and Yuan (1992, 1993). Contrasts to be estimated in parallel line assays depend on the purpose. Here, our main concern is to estimate relative potency. The contrasts of major interest are *preparation* and *combined regression*. However, we include the contrast of *parallelism* also in the present study which is often required to test the validity of the assumption that two regression lines are parallel.

If the number of homogeneous experimental units is same as the number of doses, then the experiment can be conducted using randomized complete block (RCB) design. For large number of doses, however, incomplete block designs are to be used. In bioassays, incomplete block designs may be used profitably keeping in mind efficient estimation of contrasts of major importance. Das and Kulkarni (1966) obtained some series of incomplete block designs for symmetric parallel line assays that estimate *contrasts* of interest with full efficiency and free from block effects. If in an equi-replicate block design for symmetric parallel line assays, all three contrasts are estimated with full efficiency, then the design is known as L-design. L-designs are studied extensively in the literature. However, L-designs so constructed are confined to even number of blocks except for a work due to Chai, Das, and Dey (2003). Chai et al. (2003) provided a class of designs for symmetric parallel line assays for odd number of blocks. Kyi and Dey (1980) proposed block designs both for symmetric as well as asymmetric parallel line assays and these designs estimate all the three contrasts of interest free from block effects. These designs are termed as nearly L-designs. Some more work in this field are due to Nigam and Bhoopathy (1985), Das (1985), Das and Saha (1986), Puri and Gupta (1986), Gupta (1989), Chai et al. (2001) and Shekhar and Bhar (2013, 2016). Mukerjee and Gupta (1995) first studied optimality aspect of block designs for parallel line assays. They presented A-optimal/efficient designs for the estimation of these three contrasts in the context of symmetric parallel line assays.

In the present paper, we propose a general methodology for construction of such optimal block designs for parallel line assays. All vectors and matrices used in this paper are real. Vectors are written as column vectors; for a vector $\mathbf{a}$, $\mathbf{a}'$ denotes its transpose. An n-component vector with every element 1 is written as $\mathbf{1}_n$. For a Matrix $\mathbf{A}$, $\mathbf{A}^{-1}$ and $\mathbf{A}^{-}$ denote, respectively, its inverse and a g-inverse.

2. Efficient block designs for parallel line assay

In this section, we consider construction of efficient block designs for symmetric parallel line bioassays. Let s and t denote doses of standard and test preparations, respectively, each at m levels. Therefore, there are in total $2m$ doses in the design. In bioassays, these doses are considered as treatments. Thus, there are $2m$ treatments in the design. Suppose that the doses of standard preparations are denoted by $s_1, s_2, \ldots, s_m$ and doses of test preparation by $t_1, t_2, \ldots, t_m$. These doses are equi-spaced on the logarithmic scale, the common ratio being the same for both the preparations. Let $\boldsymbol{\tau} = (\tau_1, \tau_2, \ldots, \tau_m, \tau_{m+1}, \ldots, \tau_v)'$ be the vector of $2m$ dose effects. Preparation contrasts are the difference between the totals of the standard and test preparation doses. Parallelism contrast is of importance to test whether two lines are parallel or not. Combined regression contrast is the pooled estimate of slopes, i.e. it is sum of linear contrasts of the dose totals of two preparations. *Combined regression* and *preparation* contrasts are useful for the estimation of relative potency. These contrasts are defined as follows:

$$\text{Preparation contrast } (u_1) = \sum_{i=1}^{m} T_i - \sum_{i=1}^{m} S_i, \tag{1}$$

$$\text{Combined regression contrast } (u_2) = \frac{6}{m(m^2-1)}\left[\sum_{i=1}^{m}\left(i-\frac{m+1}{2}\right)S_i + \sum_{i=1}^{m}\left(i-\frac{m+1}{2}\right)T_i\right], \tag{2}$$

$$\text{Parallelism contrast } (u_3) = \frac{12}{m(m^2-1)}\left[\sum_{i=1}^{m}\left(i-\frac{m+1}{2}\right)S_i\right] - \frac{12}{m(m^2-1)}\left[\sum_{i=1}^{m}\left(i-\frac{m+1}{2}\right)T_i\right], \tag{3}$$

where S_i (T_i) is the sum of the totals of ith dose (effects) of standard (test) preparations for $i = 1, 2, \ldots, m$. These contrasts of interest can also be written in terms of dose effects using matrix notation as (Gupta & Mukerjee, 1996) $\mathbf{U}\boldsymbol{\tau}$, where $\mathbf{U} = (\mathbf{u}_1 \vdots \mathbf{u}_2 \vdots \mathbf{u}_3)'$ and

$$\mathbf{u}_1 = \sqrt{1/2m}(\mathbf{1}'_{\mathbf{m}} \quad \vdots \quad -\mathbf{1}'_m)', \tag{4}$$

$$\mathbf{u}_2 = \sqrt{3/2(m(m^2-1))}\,(\mathbf{w}' \quad \vdots \quad \mathbf{w}')', \tag{5}$$

$$\mathbf{u}_3 = \sqrt{3/2(m(m^2-1))}\,(\mathbf{w}' \quad \vdots \quad -\mathbf{w}')', \tag{6}$$

$$\text{and} \quad \mathbf{w} = (1-m, 3-m, \ldots, m-3, m-1)'. \tag{7}$$

When an incomplete block design is used, estimation of treatment contrasts may not be free from block effects. Thus, for conducting the bioassays one should choose a block design that is capable of estimating all contrasts of interest free from block effects and with full efficiency.

Consider now a binary block design d with $v = 2m$ treatments (doses) and b blocks. Let D be the class of all designs involving v doses in b blocks and in which ith treatment is replicated r_i times, $i = 1, 2, \ldots, 2m$, $\mathbf{R}_d = diag(r_1, r_2, \ldots, r_{2m})$. We also assume that the replication of a dose for test preparation is same for the corresponding dose of standard preparation, i.e. $r_i = r_{m+i}$. Let k_j be block size of the jth block, $j = 1, \ldots, b$, $\mathbf{K}_d = diag(k_1, k_2, \ldots, k_b)$. Let also $\mathbf{N}_d$ be the $v \times b$ incidence matrix of d, i.e. the entries of $\mathbf{N}_d$ are 0 or 1. We write $\mathbf{N}_d = ((n_{ij}))$, where $n_{ij} = 1$, if the ith treatment occurs in the jth block, otherwise zero, i.e. the design considered here is binary. Under this set up the reduced normal equations for the vector of treatment effects are given by

$$\mathbf{C}_d\boldsymbol{\tau} = \mathbf{Q} \tag{8}$$

where $\mathbf{C}_d = \mathbf{R}_d - \mathbf{N}_d\mathbf{K}_d^{-1}\mathbf{N}'_d$, $\mathbf{Q} = \mathbf{T} - \mathbf{N}_d\mathbf{K}_d^{-1}\mathbf{B}$, $\mathbf{T} = (T_1, T_2, \ldots, T_v)'$, $\mathbf{B} = (B_1, B_2, \ldots, B_b)'$, where T_i and B_j denoting treatment i total and block j total, respectively. Under this setup, it can be seen that $\mathbf{C}_d\mathbf{1}_v = \mathbf{0}$. Therefore, a necessary condition for estimability of the set contrasts mentioned here is $\mathbf{U}\mathbf{1}_v = 0$. Also, we assume that the errors of the postulated model are independent with mean zero and variance σ^2. Let D_1 be a subclass of D consisting of designs in which $\mathbf{U}\boldsymbol{\tau}$ estimable. The variance–covariance matrix of $\mathbf{U}\hat{\tau}$, where $\mathbf{U}\hat{\tau}$ is the best linear unbiased estimator (BLUE) of $\mathbf{U}\boldsymbol{\tau}$ under d is given by

$$\mathbf{V}_d = \mathbf{U}\mathbf{C}_d^{-}\mathbf{U}'\sigma^2. \tag{9}$$

From Lemma 3.1 of Gupta and Mukerjee (1996), it follows that $\sigma^{-2}\mathbf{V}_d - \mathbf{U}\mathbf{R}_d^{-1}\mathbf{U}'$ is non-negative definite for any $d \in D_1$. Hence, for each

$$d \in D_1, \sigma^{-2}(\mathbf{V}_d) \geq \mathbf{U}\mathbf{R}_d^{-1}\mathbf{U}'. \tag{10}$$

Now suppose that there is a design $d_0 \in D_1$ such that

$$\sigma^{-2}(\mathbf{V}_{d_0}) = \mathbf{U}\mathbf{R}_{d_0}^{-1}\mathbf{U}'. \tag{11}$$

From Lemma 3.1 of Gupta and Mukerjee (1996), (11) holds if and only if

$$\mathbf{U}\mathbf{R}_{d_0}^{-1}\mathbf{N}_{d_0} = \mathbf{0}, \tag{12}$$

where $\mathbf{N}_{d_0}$ is the incidence matrix of d_0. Further we write $\mathbf{N}_{d_0}$ as follows:

$$\mathbf{N}_{d_0} = \begin{bmatrix} \mathbf{N}_{d_{01}} \\ \mathbf{N}_{d_{02}} \end{bmatrix}, \tag{13}$$

where $\mathbf{N}_{d_{01}}$ is the $m \times b$ incidence matrix for the m doses of the test preparation and $\mathbf{N}_{d_{02}}$ is corresponding matrix for the standard preparation.

Note that if (12) satisfied, then $\sigma^2\mathbf{U}\mathbf{R}_{d^{-1}}\mathbf{U}'$ is also the covariance matrix of BLUE of $\mathbf{U}\boldsymbol{\tau}$ in a completely randomized (unblocked) design with the same replication numbers and the same error variance as the block design under consideration. The condition (12) is necessary and sufficient for the estimation of $\mathbf{U}\boldsymbol{\tau}$ orthogonally to block effects. As one can intuitively anticipate, under this condition the BLUE of $\mathbf{U}\boldsymbol{\tau}$ can be estimated as what one would have obtained in a completely randomized design ignoring the block effects. For any single treatment contrast $\mathbf{u}'_i\boldsymbol{\tau}$, $i = 1, 2, 3$, which is estimable in the block design, it is well known that $\text{var}(\mathbf{u}'_i\boldsymbol{\tau}) = \sigma^2\mathbf{u}'_i\mathbf{C}_d^{-}\mathbf{u}_i$, hence from (10) $\text{var}(\mathbf{u}'_i\boldsymbol{\tau}) \geq \sigma^2\mathbf{u}'_i\mathbf{R}_d^{-}\mathbf{u}_i$. Then, the efficiency factor for $\mathbf{u}'_i\boldsymbol{\tau}$ in the block design under consideration is defined as $e_i = \mathbf{u}'_i\mathbf{R}_d^{-1}\mathbf{u}_i/\mathbf{u}'_i\mathbf{C}_d^{-}\mathbf{u}_i$, which is relative to a completely randomized (unblocked) design with the same replication numbers as the block design. If for a design condition (12) satisfied, then

$$\text{var}(\mathbf{u}'_i\boldsymbol{\tau}) = \sigma^2\mathbf{u}'_i\mathbf{R}_d^{-1}\mathbf{u}_i \text{ for all } i = 1, 2 \text{ and } 3. \tag{14}$$

Thus, $e_i = 1$ for all $i = 1, 2$ and 3 and we say that $\mathbf{u}'_i\boldsymbol{\tau}$ is being estimated in the block design with full efficiency or full information. On the other hand, an A-optimal design is defined as one that estimates the contrasts of interest with minimum average variance among a class of designs. Any design d^* out of a class of design C_p(say) is said to be A-optimal among the class of such designs if

$$trace\,(\mathbf{V}_{d*}) \leq trace\,(\mathbf{V}_d) \tag{15}$$

for any other design d belonging to the class C_p. Here, $\mathbf{V}_d$ ($\mathbf{V}_{d*}$) represents the variance covariance matrix of estimate of contrast using design d (d^*). For an A-optimal design condition (12) may or may not be satisfied and therefore, efficiency factors e_i's may or may not be equal to 1. Thus, A-optimal design does not ensure estimation of contrasts free from block effects and with full efficiency. On the other hand, for an arbitrary design, if condition (12) is satisfied, it also does not ensure that the design is A-optimal. Because we may still get a design in that class that satisfies condition (12) and at the same time average variance of contrasts is minimum in that class of designs. However, if the design is equi-replicate and proper and satisfies the condition (12), then the design is A-optimal, because in this case this is the only design for a given number of experimental units and also it attains the lower bound of variance. In the present paper, we consider construction of designs that satisfy the condition (12). That is, we construct designs that estimate all contrasts of interest free from block effects and with full efficiency. Some of these designs are also A-optimal. We first prove the following theorem:

THEOREM 1 The preparation, combined regression and parallelism contrasts can be estimated free from block effects and with full efficiency through a binary block design d_0 for symmetric parallel line assays if and only if the following conditions are satisfied:

(i) $(\frac{1}{r_1}, \frac{1}{r_2}, \dots, \frac{1}{r_m})'\boldsymbol{\beta}_{j1} = (\frac{1}{r_1}, \frac{1}{r_2}, \dots, \frac{1}{r_m})'\boldsymbol{\beta}_{j2}$,

(ii) $\left(\frac{1-m}{r_1}, \frac{3-m}{r_2}, \dots, \frac{m-1}{r_m}\right)'\boldsymbol{\beta}_{j1} = \left(\frac{1-m}{r_1}, \frac{3-m}{r_2}, \dots, \frac{m-1}{r_m}\right)'\boldsymbol{\beta}_{j2}$,

(iii) $\left(\frac{1-m}{r_1}, \frac{3-m}{r_2}, \dots, \frac{m-1}{r_m}\right)'(\boldsymbol{\beta}_{j1} + \boldsymbol{\beta}_{j2}) = 0$,

where $\boldsymbol{\beta}_{j1}$ (respectively, $\boldsymbol{\beta}_{j2}$) be the jth column of $\mathbf{N}_{d_{01}}$ (respectively, $\mathbf{N}_{d_{02}}$) for $j = 1,2, \dots, b$.

Proof The preparation, combined regression, and parallelism contrasts can be estimated free from block effects and with full efficiency through a binary block design d_0 if and only if condition (12) is satisfied, i.e.

$$\mathbf{U}\mathbf{R}_{d_0}^{-1}\mathbf{N}_{d_0} = \mathbf{0}.$$

Hence, using (4), (5), and (6) we get the conditions for the contrasts of interest to be estimated free from block effects and with full efficiency through the design d_0 as follows:

$$(\mathbf{1}'_m, -\mathbf{1}'_m)\mathbf{R}_{d_0}^{-1}\mathbf{N}_{d_0} = \mathbf{0}' \tag{16}$$

$$(\mathbf{w}'_m, -\mathbf{w}'_m)\mathbf{R}_{d_0}^{-1}\mathbf{N}_{d_0} = \mathbf{0}'. \tag{17}$$

$$(\mathbf{w}'_m, \mathbf{w}'_m)\mathbf{R}_{d_0}^{-1}\mathbf{N}_{d_0} = \mathbf{0}'. \tag{18}$$

Now using $\boldsymbol{\beta}_{j1}$ and $\boldsymbol{\beta}_{j2}$, for $j = 1, 2, \dots, b$, we get the conditions as stated in Theorem 1. Hence, the proof.

In the next section, we use this result to construct binary block designs for symmetric parallel line assays which estimate all three contrasts of interest free from block effects and with full efficiency.

3. Methods of construction

In this section, we give some general methods of construction of efficient binary block designs for parallel line assays. The methods are based on balanced incomplete block (BIB) designs. A BIB design is defined as an incomplete block design with v^* treatments distributed over b^* blocks, each of size k^*, where k^* is less than v^* such that each treatment occurs in r^* block, no treatment occurs more than once in a block and each pair of treatments occurs together in λ blocks. The symbols v^*, b^*, k^*, r^*, and λ are called parameters of the designs. We give some methods of construction using BIB designs for which $2k^* < v^*$. Note that the elements of the first half of $\mathbf{w}$ in (7) are the mirror image of the second half with the middle most value equal to 0, when m is odd. This property has been utilized for construction of such designs.

Method 1: (v^* even). Consider a BIB design with parameters v^*, b^*, r^*, k^*, λ, where v^* is even. Now in order to construct the design, we replace the ith treatment by a set of treatments $\{i, v^* - i + 1\}$. Each block of a BIB design contains k^* treatments. Thus, due to this replacement scheme, each block will now receive k^* new treatments along with its original k^* treatments, making the block size $2k^*$. However, while replacing the ith treatment by $(v^* - i + 1)$th treatment, we may find that in some blocks the $(v^* - i + 1)$th treatment is already present in that block. There are λ such blocks in which the treatments i and $(v^* - i + 1)$ occur together. In these blocks, we replace the ith treatment by another pair of treatment, say $\{i, j\}$ and $(v^* - i + 1)$th treatment by $\{(v^* - i + 1), \{(v^* - j + 1)\}$ such that none of the treatments j and $\{(v^* - j + 1)$ occurs in that block. We do it for all $i = 1, 2, \dots, v^*$. The contents of the blocks are now treated as the doses (treatments) of the test preparation. We denote the incidence matrix for these block contents by $\mathbf{N}_{d_1}$. We use the same block contents as the block contents

of the standard preparation and denote its incidence matrix by $\mathbf{N}_{d_2}$. Finally, combining these two incidence matrices, we get the incidence matrix of the design and denote it by $\mathbf{N}_d$, i.e. $\mathbf{N}_d = \begin{bmatrix} \mathbf{N}_{d_1} \\ \mathbf{N}_{d_2} \end{bmatrix}$. Note that by this method of construction, all conditions stated in Theorem 1 are satisfied and thus the design so constructed is capable of estimating all three contrasts free from block effects and with full efficiency. The parameters of the newly constructed design for symmetric parallel line assay would be $v = 2v^*$, $b = b^*$, $r = 2r^*$, and $k = 4k^*$. Since the design is equi-replicate and proper, this is also A-optimal.

This method is demonstrated by the following example.

Example 1 Consider the following BIB design with parameters $v^* = 16$, $b^* = 20$, $k^* = 4$, $r^* = 5$, $\lambda = 1$. The block contents of the design are

Block no.	Content	Block no.	Content	Block no.	Content	Block no.	Content
1	(1, 2, 3, 4)	6	(2, 6, 10, 14)	11	(3, 8, 9, 14)	16	(2, 8, 11, 13)
2	(5, 6, 7, 8)	7	(3, 7, 11, 15)	12	(4, 7, 10, 13)	17	(1, 8, 10, 15)
3	(9, 10, 11, 12)	8	(4, 8, 12, 16)	13	(1, 7, 12, 14)	18	(2, 7, 9, 16)
4	(13, 14, 15, 16)	9	(1, 6, 11, 16)	14	(3, 5, 10, 16)	19	(3, 6, 12, 13)
5	(1, 5, 9, 13)	10	(2, 5, 12, 15)	15	(4, 6, 9, 15)	20	(4, 5, 11, 14)

We now construct a design d (say) using this design for conducting symmetric parallel line assay. For this we apply the replacement scheme as described above to each of the blocks. For example, due to this replacement scheme, the block contents of the first block would be (1, 2, 3, 4, 13, 14, 15, 16). However, while applying the replacement scheme as described above, we find that in block numbers 9, 10, 11, and 12, for the ith treatment, the replacement treatment $v^* - i + 1$ already exits. For these blocks, we apply the alternative replacement scheme. For example, in block number 9, treatment 1 is replaced by {1, 2}, treatment 16 is replaced by {16, 15}, treatment 6 is replaced by {6, 7}, and treatment 11 is replaced by {11, 10}. Thus, the block contents of 9 would be (1, 2, 6, 7, 10, 11, 15, 16). For other blocks usual replacement procedure is adopted. After replacement in all blocks, we treat the block contents as the treatments of test preparation. We denote the incidence matrix of this test preparation as $\mathbf{N}_{d_1}$. This incidence matrix can be written as follows:

$$\mathbf{N}_{d_1} = \begin{bmatrix} 1&0&0&1&1&0&0&1&1&0&0&1&1&1&0&0&1&1&0&0 \\ 1&0&0&1&0&1&1&0&1&1&0&0&0&0&1&1&1&1&0&0 \\ 1&0&0&1&0&1&1&0&0&1&1&0&1&1&0&0&0&0&1&1 \\ 1&0&0&1&1&0&0&1&0&0&1&1&0&0&1&1&0&0&1&1 \\ 0&1&1&0&1&0&0&1&0&1&0&1&1&1&0&0&0&0&1&1 \\ 0&1&1&0&0&1&1&0&1&1&0&0&0&0&1&1&0&0&1&1 \\ 0&1&1&0&0&1&1&0&1&0&1&0&1&1&0&0&1&1&0&0 \\ 0&1&1&0&1&0&0&1&0&0&1&1&0&0&1&1&1&1&0&0 \\ 0&1&1&0&1&0&0&1&0&0&1&1&0&0&1&1&1&1&0&0 \\ 0&1&1&0&0&1&1&0&1&0&1&0&1&1&0&0&1&1&0&0 \\ 0&1&1&0&0&1&1&0&1&1&0&0&0&0&1&1&0&0&1&1 \\ 0&1&1&0&1&0&0&1&0&1&0&1&1&1&0&0&0&0&1&1 \\ 1&0&0&1&1&0&0&1&0&0&1&1&0&0&1&1&0&0&1&1 \\ 1&0&0&1&0&1&1&0&0&1&1&0&1&1&0&0&0&0&1&1 \\ 1&0&0&1&0&1&1&0&1&1&0&0&0&0&1&1&1&1&0&0 \\ 1&0&0&1&1&0&0&1&1&0&0&1&1&1&0&0&1&1&0&0 \end{bmatrix} \tag{19}$$

We use the same incidence matrix for standard preparation ($\mathbf{N}_{d_2}$). Finally, the incidence matrix ($\mathbf{N}_d$) of the design d is obtained as follows:

$$\mathbf{N}_d = \begin{bmatrix} \mathbf{N}_{d_1} \\ \mathbf{N}_{d_2} \end{bmatrix} \tag{20}$$

If we denote doses of standard preparation by s_i, and the doses of test preparations by t_j, for $i = 1, 2, \ldots, 16$, then the design thus constructed will be as follows:

$(t_1, t_2, t_3, t_4, t_{13}, t_{14}, t_{15}, t_{16}: s_1, s_2, s_3, s_4, s_{13}, s_{14}, s_{15}, s_{16})$,
$(t_5, t_6, t_7, t_8, t_9, t_{10}, t_{11}, t_{12}: s_5, s_6, s_7, s_8, s_9, s_{10}, s_{11}, s_{12})$,
$(t_5, t_6, t_7, t_8, t_9, t_{10}, t_{11}, t_{12}: s_5, s_6, s_7, s_8, s_9, s_{10}, s_{11}, s_{12})$,
$(t_1, t_2, t_3, t_4, t_{13}, t_{14}, t_{15}, t_{16}: s_1, s_2, s_3, s_4, s_{13}, s_{14}, s_{15}, s_{16})$,
$(t_1, t_4, t_5, t_8, t_9, t_{12}, t_{13}, t_{16}: s_1, s_4, s_5, s_8, s_9, s_{12}, s_{13}, s_{16})$,
$(t_2, t_3, t_6, t_7, t_{10}, t_{11}, t_{14}, t_{15}: s_1, s_4, s_5, s_8, s_9, s_{12}, s_{13}, s_{16})$,
$(t_2, t_3, t_6, t_7, t_{10}, t_{11}, t_{14}, t_{15}: s_1, s_4, s_5, s_8, s_9, s_{12}, s_{13}, s_{16})$,
$(t_1, t_4, t_5, t_8, t_9, t_{12}, t_{13}, t_{16}: s_1, s_4, s_5, s_8, s_9, s_{12}, s_{13}, s_{16})$,
$(t_1, t_2, t_6, t_7, t_{10}, t_{11}, t_{15}, t_{16}: s_1, s_2, s_6, s_7, s_{10}, s_{11}, s_{15}, s_{16})$,
$(t_2, t_3, t_5, t_6, t_{11}, t_{12}, t_{14}, t_{15}: s_2, s_3, s_5, s_6, s_{11}, s_{12}, s_{14}, s_{15})$,
$(t_3, t_4, t_7, t_8, t_9, t_{10}, t_{13}, t_{14}: s_3, s_4, s_7, s_8, s_9, s_{10}, s_{13}, s_{14})$,
$(t_1, t_4, t_5, t_8, t_9, t_{12}, t_{13}, t_{16}: s_1, s_4, s_5, s_8, s_9, s_{12}, s_{13}, s_{16})$,
$(t_1, t_3, t_5, t_7, t_{10}, t_{12}, t_{14}, t_{16}: s_1, s_3, s_5, s_7, s_{10}, s_{12}, s_{14}, s_{16})$,
$(t_1, t_3, t_5, t_7, t_{10}, t_{12}, t_{14}, t_{16}: s_1, s_3, s_5, s_7, s_{10}, s_{12}, s_{14}, s_{16})$,
$(t_2, t_4, t_6, t_8, t_9, t_{11}, t_{13}, t_{15}: s_2, s_4, s_6, s_8, s_9, s_{11}, s_{13}, s_{15})$,
$(t_2, t_4, t_6, t_8, t_9, t_{11}, t_{13}, t_{15}: s_2, s_4, s_6, s_8, s_9, s_{11}, s_{13}, s_{15})$,
$(t_1, t_2, t_7, t_8, t_9, t_{10}, t_{15}, t_{16}: s_1, s_2, s_7, s_8, s_9, s_{10}, s_{15}, s_{16})$,
$(t_1, t_2, t_7, t_8, t_9, t_{10}, t_{15}, t_{16}: s_1, s_2, s_7, s_8, s_9, s_{10}, s_{15}, s_{16})$,
$(t_3, t_4, t_5, t_6, t_{11}, t_{12}, t_{13}, t_{14}: s_3, s_4, s_5, s_6, s_{11}, s_{12}, s_{13}, s_{14})$,
$(t_3, t_4, t_5, t_6, t_{11}, t_{12}, t_{13}, t_{14}: s_3, s_4, s_5, s_6, s_{11}, s_{12}, s_{13}, s_{14})$,

The above design has total 32 treatments, 16 for test preparation and 16 for standard preparation. Each treatment is replicated 10 times and block size of each block is 16. Thus, by this method we always get a design that is equi-replicate and proper. For this design the vectors $\mathbf{u}_1$, $\mathbf{u}_2$ and $\mathbf{u}_3$ are given by

$$\mathbf{u}_1 = 0.176^*(1\ 1\ 1\ 1\ 1\ 1\ 1\ 1\ 1\ 1\ 1\ 1\ 1\ 1\ 1\ 1 -1\ -1\ -1\ -1\ -1\ -1\ -1\ -1\ -1\ -1\ -1\ -1\ -1\ -1\ -1\ -1)',$$

$$\mathbf{u}_2 = 78.23^*(-15\ -13\ -11\ -9\ -7\ -5\ -3\ -1\ 1\ 3\ 5\ 7\ 9\ 11\ 13\ 15\ -15\ -13\ -11\ -9\ -7\ -5\ -3\ -1\ 1\ 3\ 5\ 7\ 9\ 11\ 13\ 15)' \text{ and}$$

$$\mathbf{u}_3 = 78.23^*(-15\ -13\ -11\ -9\ -7\ -5\ -3\ -1\ 1\ 3\ 5\ 7\ 9\ 11\ 13\ 15\ 15\ 13\ 11\ 9\ 7\ 5\ 3\ 1\ -1\ -3\ -5\ -7\ -9\ -11\ -13\ -15)',$$

respectively. The matrix $\mathbf{R}_d$ is given by $\mathbf{R}_d = 10\mathbf{I}_{32}$, then it can easily be seen that the design satisfies all the three conditions in Theorem 1. Hence, the design constructed allows estimation of treatment contrasts free from block effects and with full efficiency. The efficiency factor of each contrast is 1. The design is also A-optimal. For the same gain if we would have used RCB design then we would have required a block of size 32. This is the advantage to use this design.

Method 2: (v^*, k^* even). The above design is obtained in original b^* blocks. However, number of treatments and size of each block size are increased. One may be interested to have lesser number

of treatments. This can be achieved by the present method. In this method, we can get a design with original number of treatments of BIBD. Note that due to the replacement scheme adopted, the last $v^*/2$ rows of the incidence matrix in (20) becomes the mirror image of the first $v^*/2$ rows. Each column in either of the halves contains k^* elements as 1, i.e. k^* treatments. Now by Method 2, from each column from the first half, we make a column of size k^*such that $k^*/2$ elements of this column are 1. We then take a mirror image of this column from the second half and append it at the bottom of the first column. Thus, we get a column of size $2k^*$, k^* elements of which are 1. Taking all the columns thus made together, we get an incidence matrix of a design for $v^*/2$ treatments in b^* blocks each of which is of size k^* and each treatment is replicated $2r^*$ times. Taking this incidence matrix for the both the preparations, we get a design for symmetric parallel line assay. The parameters of the design would be $v = v^*$, $b = b^*$, $k = 2k^*$, and $r = 2r^*$.

Example 2 Consider the BIB design as considered in Example 1, applying the replacement scheme as described in Method 1, we get an incidence matrix as given in (20). By Method 2, we get a new incidence matrix (say, for standard preparation) as follows:

$$\mathbf{N}_{d_1} = \begin{bmatrix} 1&0&0&1&1&0&0&1&1&0&0&1&1&1&0&0&1&1&0&0 \\ 1&0&0&1&0&1&1&0&1&1&0&0&0&0&1&1&1&1&0&0 \\ 0&1&1&0&1&0&0&1&0&0&1&1&1&1&0&0&0&0&1&1 \\ 0&1&1&0&0&1&1&0&0&1&1&0&0&0&1&1&0&0&1&1 \\ 0&1&1&0&0&1&1&0&0&1&1&0&0&0&1&1&0&0&1&1 \\ 0&1&1&0&1&0&0&1&0&0&1&1&1&1&0&0&0&0&1&1 \\ 1&0&0&1&0&1&1&0&1&1&0&0&0&0&1&1&1&1&0&0 \\ 1&0&0&1&1&0&0&1&1&0&0&1&1&1&0&0&1&1&0&0 \end{bmatrix} \tag{21}$$

Now using this incidence matrix ($\mathbf{N}_{d_2}$) for the test preparation, we get the incidence matrix ($\mathbf{N}_d$) of the design as follows:

$$\mathbf{N}_d = \begin{bmatrix} \mathbf{N}_{d_1} \\ \mathbf{N}_{d_2} \end{bmatrix} \tag{22}$$

In terms of the coded doses, the block contents can be written as it was done in Example 1. In this design, number of treatment and block size becomes half of the previous example. Other parameters of the design remain unchanged. Thus, one can choose a design according to his/her need.

Method 3: (v^* odd). Consider a BIB design again with parameters v^*, b^*, r^*, k^*, λ, where v^* is odd. In order to construct the design, we replace the ith treatment by a set of treatments $\{i, v^* - i + 1\}$, for all $i = 1, 2, \ldots, v$ except $i = (v^*+1)/2$. Thus, each block will now receive k^* new treatments along with its original k^* treatments, making the block size $2k^*$, except those blocks in which the$(v^*+1)/2th$ treatment occurs. There are r such blocks in which a treatment is $(v^*+1)/2$th. For these blocks, the new block size would be $2k^*-1$. However, as in the case of Method 1, while replacing the ith treatment by $(v^* - i + 1)$th treatment, we may find that in some blocks the $(v^* - i + 1)$th treatment is already present in that block. There are λ such blocks in which the treatments i and $(v^* - i + 1)$ occur together. In these blocks, we replace the ith treatment by another pair of treatment, say $\{i, j\}$ and $(v^* - i + 1)$th treatment by $\{(v^* - i + 1), \{(v^* - j + 1)\}$ such that none of the treatments j and $\{(v^* - j + 1)$ occurs in that block. We do it for all $i = 1, 2, \ldots, v^*$. The new block contents are treated as the block contents of the test preparation and corresponding incidence matrix is denoted by $\mathbf{N}_{d_1}$. The incidence matrix $\mathbf{N}_{d_2}$ for standard preparation is same as $\mathbf{N}_{d_1}$. Finally, combining both the incidence matrices, we get the incidence matrix of the design. The parameters of the design (after rearranging the columns) are $v = 2v^*$, $b = b^*$, $k_1 = k_2 = \ldots = k_r = 2(k^* - 1)$, $k_{r+1} = k_{r+2} = \ldots = k_b = 4k^*$, $r_i = 2r^*$ for all i except $i = (v^*+1)/2$ and $r_{(v^*+1)/2} = r^*$.

Example 3 For example, consider the following BIB design with parameters $v^* = 7$, $b^* = 7$, $k^* = 3$, $r^* = 3$, $\lambda = 1$. The block contents of the design are as follows:

(1, 2, 4); (2, 3, 5); (3, 4, 6); (4, 5, 7); (1, 5, 6); (2, 6, 7); (1, 3, 7)

The incidence matrix of the design can be written as follows:

$$\mathbf{N} = \begin{bmatrix} 1 & 0 & 0 & 0 & 1 & 0 & 1 \\ 1 & 1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 1 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 1 & 1 \end{bmatrix} \tag{23}$$

The columns of the above matrix represent the blocks. In block numbers 1, 3, and 4, the treatment (v^*+1)/2*th* treatment, i.e. 4*th* treatment occurs. Now applying the replacement scheme as described above, we get the block contents of the new blocks as follows:

$$\begin{bmatrix} 1 & 0 & 1 \\ 1 & 1 & 0 \\ 0 & 1 & 1 \\ 1 & 1 & 1 \\ 0 & 1 & 1 \\ 1 & 1 & 0 \\ 1 & 0 & 1 \end{bmatrix}$$

Applying the same replacement scheme, we get the new block contents for other blocks. Finally, we get the incidence matrix for the test preparation as follows:

$$\mathbf{N}_{d1} = \begin{bmatrix} 1 & 0 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 0 & 1 & 1 & 1 & 1 \\ 0 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 0 & 1 & 1 & 1 & 1 \\ 1 & 0 & 1 & 1 & 1 & 1 & 1 \end{bmatrix} \tag{24}$$

We treat this incidence matrix as the incidence matrix for standard preparation also. Thus, incidence matrix of the design would be

$$\mathbf{N}_d = \begin{bmatrix} \mathbf{N}_{d_1} \\ \mathbf{N}_{d_2} \end{bmatrix} \tag{25}$$

In terms of the doses the block contents of the designs can be written as follows:

$(t_1, t_2, t_4, t_6, t_7, s_1, s_2, s_4, s_6, s_7)$;
$(t_2, t_3, t_4, t_5, t_6, s_2, s_3, s_4, s_5, s_6)$;
$(t_1, t_3, t_4, t_5, t_7, s_1, s_3, s_4, s_5, s_7)$;
$(t_1, t_2, t_3, t_5, t_6, t_7, s_1, s_2, s_3, s_5, s_6, s_7)$;
$(t_1, t_2, t_3, t_5, t_6, t_7, s_1, s_2, s_3, s_5, s_6, s_7)$;
$(t_1, t_2, t_3, t_5, t_6, t_7, s_1, s_2, s_3, s_5, s_6, s_7)$;
$(t_1, t_2, t_3, t_5, t_6, t_7, s_1, s_2, s_3, s_5, s_6, s_7)$.

Six of treatments for both test preparation and standard preparation are replicated six times, whereas fourth treatment is replicated three times. Four of the blocks are of size 12, whereas three are of size 10. This design has all optimal properties described above. For this design vectors $\mathbf{u}_1$, $\mathbf{u}_2$ and $\mathbf{u}_3$ and matrix $\mathbf{R}_d$ can be worked out and it can be verified that all conditions in Theorem 1 are satisfied. Thus, this design allows estimating contrasts free from block effects and with full efficiency. However, this design may not be A-optimal, because for a fixed number of experimental units there may be a design with different replications and block sizes whose minimum variance is minimum among the class.

Note that the last four blocks of the design are identical. Naturally, one may be interested to see what effect would be on the design if the number of such block is increased or decreased. As such there would be no effect on the properties of the design described in this paper, because this block fulfills all conditions in Theorem 1. However, actual variance of the contrasts would be affected. Variance of the *i*th contrast as given in (14) is $\text{var}(\mathbf{u}'_i\boldsymbol{\tau}) = \sigma^2\mathbf{u}'_i\mathbf{R}_d^{-1}\mathbf{u}_i$. This is involved inverse of matrix $\mathbf{R}_d$, which is a diagonal matrix. Thus, increasing (decreasing) the number of blocks would increase (decrease) the number of replication and hence would naturally decrease (increase) the variance of $\mathbf{u}'_i\boldsymbol{\tau}$.

4. Discussion

The main concern of the present study was to provide efficient designs for symmetric parallel line bioassays. Since the number of treatment increases rapidly with the number of test preparations, we are compelled to advocate incomplete block designs. And once incomplete block designs are used, naturally concern of efficient estimation treatment contrasts arise because all the contrasts cannot be estimated with full efficiency in every incomplete block design. Again in case of bioassays, we are interested only in a limited number of contrasts. Therefore, there is a huge scope to generate efficient incomplete block designs in bioassays. Keeping this in mind, the present investigation aims to generate efficient incomplete block designs for symmetric parallel line bioassays. Fortunately, success has been achieved to get designs that are fully efficient. The present paper provides some ready solution for experimenters for conducting symmetric parallel line assay. The methods are based on BIB designs. BIB designs are very popular designs and are used extensively in other field of research. For conducting a symmetric parallel line bioassay, one has to get a corresponding BIB designs. BIB designs for various parametric combinations are available in many text books. However, one can get these designs from the website of Indian Agricultural Statistics Research Institute, New Delhi, India (http://iasri.res.in/design/). This site contains list of 494 BIB designs for $r \leq 30$ in case of symmetrical BIB designs and for $r \leq 20$ in case of asymmetric BIB designs.

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