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Incomplete Block Designs for Multiple Asymmetric Parallel Line Assays

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Abstract: A method of construction of A-optimal binary block designs for multiple asymmetrical parallel line assays has been proposed. Illustration of the method with examples has been provided. By this method two series of designs are obtained. The first series of designs have equal replication of treatments with unequal block sizes. The second series of designs have equal block sizes with unequal replications of treatments.

Keywords: Bioassays, multiple bioassay, multiple asymmetric parallel line assays, A-optimality, incomplete block designs, relative potency, efficiency

1. INTRODUCTION

Bioassays are procedures that can determine the concentration of purity or biological activity of a substance on living organisms. These are carefully designed experiments in which two stimuli are applied to subjects. One preparation of stimulus (standard preparation) is of known strength while the other preparation is of unknown strength (test preparation). Comparison is made between the activities of living organisms between these two stimuli. Purpose behind conducting bioassay is to estimate the relative potency of the test preparation relative to the standard preparation. Parallel line assay is one of the important assays used in many research experiments. In parallel line assays, the two dose-response regression lines for each of the two stimuli are taken as parallel. There are three major contrasts of interest namely, *preparation*, *combined regression* and *parallelism* are used to get a valid estimate of the relative potency. It is desirable that when a block design is used for an assay, these contrasts of interest are estimated with high efficiency. In some instances, interest of experimenter also lies in comparing several test preparations with more than one standard preparation. Such assays are called as multiple bioassays. The utility of multiple bioassays is undisputed because resource crunch is ubiquitous. Such assays are more economical and pragmatic. Advantage of multiple bioassays is that it can be conducted as separate assays, whose results will eventually be combined to get final result. Finney [8] pointed out that conducting separate experiment for each comparison is expensive as well as not practical.

Multiple bioassays need to apply the principles of assay design and the general theory of experimental design for factors at two or more levels. The number of doses (treated as treatments) increases rapidly in case of multiple bioassays. When the number of experimental units within homogeneous set is less than that of total number of doses then recourse is made to use of incomplete block designs. It is almost imperative to use an incomplete block design for conducting multiple bioassays. If the number of doses for all the preparations is same, then the assays are known as symmetric and if the number of doses of at least one preparation is different, then the assay is known as asymmetric. We are concerned about the asymmetric parallel line assay in this paper. Incomplete block design for symmetric parallel line assays for comparing a single test preparation have been investigated by several authors (see for example [6], [7], [9], [10], [11], [13], [14]).

Optimality aspects of block designs for parallel line assays were first considered by Mukerjee and Gupta [12]. They have given the A-optimality criterion of block designs for the estimation of the three contrasts of interest. Many optimal designs are now available in the literature for symmetric parallel line assay. Some references in this

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regard are due to [2], [3], [4], and [17]. However, very little work is available on incomplete block designs for asymmetric parallel line assays. Reference [10] introduced φ - designs which are equi-replicate and proper. Reference [2] extended this method to non-proper designs. Recently [16] gave two series of designs for asymmetrical parallel line assay. All these studies are confined to the case where a single test preparation is compared with a standard one. Some work on multiple symmetric parallel line assays are done by [1], [17], [15] and [18]. However, no work seems to be available for multiple asymmetric bioassays.

The purpose of this paper is to present a methodology for construction of A-optimal block designs for multiple asymmetric parallel line assays. This method is an extension of the method given by [16]. All these designs permit the estimation of three main contrasts with full efficiency. In Section 2, some preliminaries are discussed. Section three deals with A-optimality aspects of block designs for asymmetric parallel line assays. In section 4, a general method of construction has been discussed. Method is illustrated with examples. Two cases of this method have been considered. In the first case designs are obtained for equal replication of doses and in the second case designs with unequal replications are obtained. All matrices and vectors are real, vectors being written as column vectors. We denote an n-component vector of unities by $\mathbf{1}_n$ and by $\mathbf{I}_n$ an identity matrix of order n. For a matrix $\mathbf{A}$, $\mathbf{A}'$ will denote the transpose of $\mathbf{A}$.

2. CONTRASTS FOR MULTIPLE PARALLEL LINE ASSAY

Let m_i, i = 1, 2, …, c, denote number of doses of standard and test preparations. Here (for i=1) m_1 is the number of doses of standard preparation, m_2 is number of doses of first test preparation, m_3 is number of doses of second test preparation and finally m_{c+1} is number of doses of c^{th} test preparation. Let the doses of standard and that of test preparations be denoted by $\left(t^i_1, t^i_2, \ldots, t^i_{m_i}\right)$ where i = 1, 2, … , c+1. For i =1, it denotes the doses of standard preparation and for i = 2, 3, …, c+1 doses of test preparations are denoted.

Let $\boldsymbol{\tau} = \left(\tau_1, \tau_2, \ldots, \tau_{m_1}, \tau_{m_1+1}, \ldots, \tau_{m_1+m_2}, \ldots \tau_{\sum_{i=1}^{c+1} m_i}\right)'$ be a vector of $v = \sum_{i=1}^{c+1} m_i$ dose effects. Generally we are interested in three major contrasts in parallel line assay which are sufficient to get a valid estimate of relative potency.

Normalized versions of these contrasts, *viz.*, preparation, *combined regression* and *parallelism* contrasts for simple bioassays was given by [12]. We extend this for the case of multiple bioassays having one standard and c test preparations as follows:

$$\left.\begin{aligned} L_p &= \sqrt{\left(\frac{m_1 m_{i+1}}{m_1 + m_{i+1}}\right)}\left(m_1^{-1}\mathbf{1}'_{m_1}, \mathbf{0}'_{m_2}, \ldots, -m_{i+1}^{-1}\mathbf{1}'_{m_{i+1}}, \ldots \mathbf{0}'_{m_{c+1}}\right) = \mathbf{u}'_{1i}\tau, \\ L_2 &= \sqrt{\frac{12}{\left(\sum_{i=1}^{c+1}\theta_i\right)}}\left(\mathbf{w}_1', \mathbf{w}_2', \mathbf{w}_3', \ldots, \mathbf{w}_{c+1}'\right) = \mathbf{u}'_2\tau, \\ L'_p &= \sqrt{\left(\frac{12\theta_1\theta_{i+1}}{\theta_1 + \theta_{i+1}}\right)}\left(\frac{\mathbf{w}_1'}{\theta_1}, \mathbf{0}'_{m_2}, \ldots \frac{-\mathbf{w}_{i+1}'}{\theta_{i+1}}, \ldots \mathbf{0}'_{m_{c+1}}\right) = \mathbf{u}'_{3i}\tau, \end{aligned}\right\} \quad (1)$$

where

$$\mathbf{w}_i = (1, 2, \ldots, m_i)' - \left(\frac{m_i + 1}{2}\right)\mathbf{1}_{m_i},$$

$$\theta_i = m_i(m_i^2 - 1),\ i = 1, \ldots, c+1.$$

Contrasts represented above are in general format. These contrasts represent asymmetrical multiple parallel line assays with one standard and c test preparations having respective doses m_1, m_2, m_3,…, m_{c+1} . It contains contrasts of all types of parallel line assays within its ambit like symmetrical, asymmetrical, simple and multiple bioassays.

(i) These set of contrasts will reduce to simple asymmetric parallel line assays when we take c =1 and $m_1 \neq m_2$.

(ii) These set of contrasts will reduce to symmetric multiple parallel line assays with c tests when we take $m_1 = m_2 = \ldots = m_{c+1} = m$.

(iii) These set of contrasts will reduce to symmetric parallel line assays when we take c =1 and $m_1 = m_2 = m$.

We represent these three contrasts by $\mathbf{U}\boldsymbol{\tau}$, where

$$\mathbf{U} = \left[\mathbf{u}'_{11}, \mathbf{u}'_{12}, \ldots, \mathbf{u}'_{1(c+1)}, \mathbf{u}'_2, \mathbf{u}'_{31}, \mathbf{u}'_{32}, \ldots, \mathbf{u}'_{3(c+1)}\right]' . \quad (2)$$

3. A-OPTIMAL DESIGNS FOR ASYMMETRIC PARALLEL LINE ASSAYS

Use of incomplete block designs in bioassays has been affected by the rigidity of such designs. The core concern of such designs is to estimate the differences between all pairs of treatments with the same (or nearly the same) variance. But in bioassays all contrasts are not of equal importance. In parallel line assay only three contrasts, as mentioned earlier, are of major importance. Incomplete

block designs are of special significance in bioassay keeping the limited resource availability in mind. With the use of incomplete block designs in bioassay the optimality study of such designs among the class of designs is necessitated as it serves as a criterion to choose better designs.

Let us have a binary block design d with $v = \sum_{i=1}^{c+1} m_i$ treatments (doses) in b blocks and each dose of standard and test preparations are replicated r_i times. Let k_j be the block size of the j^{th} block, j = 1, …, b, $\mathbf{K}_d = diag(k_1, k_2, ..., k_b)$, $\mathbf{N}_d$ be the $v \times b$ incidence matrix of d and $\mathbf{C}_d = \mathbf{R}_d - \mathbf{N}_d \mathbf{K}_d^{-1} \mathbf{N}_d'$,

where $\mathbf{R}_d = \begin{bmatrix} r_1 \mathbf{I}_{m_1} & 0 & \cdots & 0 \\ 0 & r_2 \mathbf{I}_{m_2} & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \cdots & r_{C+1} \mathbf{I}_{m_{C+1}} \end{bmatrix}$.

Fixed effects additive model for the data collected through d is assumed here. Usual assumption that the errors are independent with zero mean and constant variance σ^2 is made. Let D be a class of all such designs in which $\mathbf{U}\boldsymbol{\tau}$ is estimable. Assume $\mathbf{V}_d$ as 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. An A-optimal design for $\mathbf{U}\boldsymbol{\tau}$ in D is one that minimize $tr(\mathbf{V}_d)$, where $tr(.)$ stands for the trace of a square matrix. It minimizes the average variance of the BLUEs of the components of $\mathbf{U}\hat{\tau}$ over the class of competing designs in D.

From Lemma 3.1 of [10], it follows that $\sigma^{-2}\mathbf{V}_d - \mathbf{U}\mathbf{R}_d^{-1}\mathbf{U}'$ is non-negative definite for any $d \in D$. Hence, for each $d \in D$

$$\sigma^{-2} tr(\mathbf{V}_d) \geq tr\left(\mathbf{U}\mathbf{R}_d^{-1}\mathbf{U}'\right). \qquad (3)$$

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

$$\sigma^{-2}\mathbf{V}_{d_0} = \left(\mathbf{U}\mathbf{R}_{d_0}^{-1}\mathbf{U}'\right), \text{ i.e.,}$$
$$\sigma^{-2} tr(\mathbf{V}_{d_0}) = tr(\mathbf{U}\mathbf{R}_{d_0}^{-1}\mathbf{U}') \ . \qquad (4)$$

It follows that if d_0 minimizes the right hand side of (3), then d_0 is A-optimal over D. From Lemma 3.1 of [10], (4) holds if and only if

$$\mathbf{U}\mathbf{R}_{d_0}^{-1}\mathbf{N}_{d_0} = \mathbf{0} \qquad (5)$$

Let in the case of multiple asymmetric parallel line assay $\mathbf{N}_{d_0}$ be the incidence matrix of d_0. We may write $\mathbf{N}_{d_0}$ as

$$\mathbf{N}_{d_0} = \begin{bmatrix} \mathbf{N}_{d_{01}} \\ \mathbf{N}_{d_{02}} \\ \vdots \\ \mathbf{N}_{d_{0(c+1)}} \end{bmatrix},$$

where $\mathbf{N}_{d_{01}}$ is $m_1 \times b$ incidence matrix for the standard preparation and $\mathbf{N}_{d_{0i}}$ is $m_i \times b$ incidence matrix for $(i-1)^{\text{th}}$ test preparation, i = 2, … , (c+1).

Using equation (5), the condition for getting fully efficient binary design d_0 for asymmetric parallel line assay is given in Lemma 3.1 of [16]. We extend that condition for multiple asymmetric parallel line assay as.

Condition for A-optimality for *preparation* contrasts

$$\begin{aligned} &\frac{k_1^{m_1}}{m_1 r_1} = \frac{k_1^{m_2}}{m_2 r_2}, \quad \frac{k_2^{m_1}}{m_1 r_1} = \frac{k_2^{m_2}}{m_2 r_2}, \cdots, \frac{k_b^{m_1}}{m_1 r_1} = \frac{k_b^{m_2}}{m_2 r_2}, \\ &\qquad \vdots \\ &\frac{k_1^{m_1}}{m_1 r_1} = \frac{k_1^{m_{i+1}}}{m_2 r_{i+1}}, \ \frac{k_2^{m_1}}{m_1 r_1} = \frac{k_2^{m_{i+1}}}{m_2 r_{i+1}}, \cdots, \frac{k_b^{m_1}}{m_1 r_1} = \frac{k_b^{m_{i+1}}}{m_2 r_{i+1}}, \\ &\qquad \vdots \\ &\frac{k_1^{m_1}}{m_1 r_1} = \frac{k_1^{m_{c+1}}}{m_{c+1} r_{c+1}}, \ \frac{k_2^{m_1}}{m_1 r_1} = \frac{k_2^{m_{c+1}}}{m_{c+1} r_{c+1}}, \cdots, \frac{k_b^{m_1}}{m_1 r_1} = \frac{k_b^{m_{c+1}}}{m_{c+1} r_{c+1}}. \end{aligned} \qquad (6)$$

The *combined regression* and *parallelism* contrasts can be estimated with full efficiency through the design d_0 if and only if

$$\left(\mathbf{w}_1', \mathbf{w}_2', \mathbf{w}_3', \ldots, \mathbf{w}_{c+1}'\right)\mathbf{N}_{d_0} = \mathbf{0}_b', \qquad (7)$$

$$\left(\frac{\mathbf{w}_1'}{\theta_1}, \mathbf{0}_{m_2}', \ldots, \frac{-\mathbf{w}_{i+1}'}{\theta_{i+1}}, \ldots \mathbf{0}_{m_{c+1}}'\right)\mathbf{N}_{d_0} = \mathbf{0}_b', \qquad (8)$$

for i = 1, 2, …, c.

Let $\boldsymbol{\beta}_{j1}$ (respectively $\boldsymbol{\beta}_{ji}$) be the j^{th} column of $\mathbf{N}_{d_{01}}$ (respectively $\mathbf{N}_{d_{0i}}$) for j = 1, 2,…, b and i = 2, … , (c+1). Then from equations (7) and (8) we must have

$$\mathbf{w}_1'\mathbf{N}_{d_{01}} = \mathbf{w}_i'\mathbf{N}_{d_{0i}} = \mathbf{0}_b' \qquad (9)$$

We find that (9) is satisfied if and only if

$\mathbf{w}_i'\boldsymbol{\beta}_{ji} = 0, \quad \forall\ j = 1,2,\ldots,b$ and $i = 1,2,\ldots,(c+1)$.

Summarizing, we get the following result

Lemma 3.1: *The preparation, combined regression and parallelism contrasts of multiple asymmetric parallel line assay can be estimated free from block effects and with full efficiency through a binary design d_0 if and only if*

$$(i)\quad \frac{k_1^{m_1}}{m_1 r_1} = \frac{k_1^{m_2}}{m_2 r_2}, \quad \frac{k_2^{m_1}}{m_1 r_1} = \frac{k_2^{m_2}}{m_2 r_2}, \cdots, \frac{k_b^{m_1}}{m_1 r_1} = \frac{k_b^{m_2}}{m_2 r_2},$$
$$\vdots$$
$$\frac{k_1^{m_1}}{m_1 r_1} = \frac{k_1^{m_{i+1}}}{m_2 r_{i+1}}, \quad \frac{k_2^{m_1}}{m_1 r_1} = \frac{k_2^{m_{i+1}}}{m_2 r_{i+1}}, \cdots, \frac{k_b^{m_1}}{m_1 r_1} = \frac{k_b^{m_{i+1}}}{m_2 r_{i+1}},$$
$$\vdots$$
$$\frac{k_1^{m_1}}{m_1 r_1} = \frac{k_1^{m_{c+1}}}{m_{c+1} r_{c+1}}, \quad \frac{k_2^{m_1}}{m_1 r_1} = \frac{k_2^{m_{c+1}}}{m_{c+1} r_{c+1}}, \cdots, \frac{k_b^{m_1}}{m_1 r_1} = \frac{k_b^{m_{c+1}}}{m_{c+1} r_{c+1}}.$$

$$(ii)\quad \mathbf{w}_i'\boldsymbol{\beta}_{ji} = 0, \quad \forall\ j = 1,2,\ldots,b \text{ and } i = 1,2,\ldots,(c+1)$$

In the next section, using Lemma 3.1 above, we give methods of construction of binary block designs for multiple asymmetric parallel line assays in which all three contrasts of interest can be estimated with full efficiency.

4. A METHOD OF CONSTRUCTION OF A-OPTIMAL DESIGNS FOR MULTIPLE ASYMMETRIC PLA

In order to construct designs satisfying the conditions of Lemma 3.1, first we obtain some incidence vectors for each of the incidence matrices $\mathbf{N}_{d_{0i}}$ for i = 1,2,…, c+1 such that the row sums of the incidence matrices make a complete replication. Suppose $\mathbf{a}_{ji}^i = (a_{j1}, a_{j2}, \ldots, a_{jm_i})'$, $j = 1,2,\ldots b_i$, $a_{ji} = 0,1$ is such an incidence vector for the standard preparation doses (for i =1) and that of the test preparation doses (for i =2,3,…, c+1), satisfying the conditions of Lemma 3.1. Here b_i, i = 1, 2, 3,…, c+1 is a positive constant representing the number of columns of $\mathbf{N}_{d_{0i}}$ which makes one complete replication for m_i doses. Now we consider some special cases for construction of such designs.

A. *Case 1*

$r_1 = r_2 = \ldots = r_{c+1} = r$ and $b_1 = b_2 = \ldots = b_{c+1} = g$.

In this case, we first choose $\mathbf{a}_{ji}^i$ such that

$$\mathbf{a}_{ji}^{i\,\prime}\mathbf{w}_i = 0 \text{ for } j = 1,2,\ldots,g,$$

where $\mathbf{w}_i = (1, 2,\ldots,m_i)' - \left(\frac{m_i+1}{2}\right)\mathbf{1}_{m_i}$ for i = 1, 2, …, c+1 as given in (2.1).

Thus $\mathbf{a}_{ji}^{i\,\prime}\mathbf{1}_{m_i} = k_j^{m_i}$, and $(\mathbf{a}_{j1}^{1\,\prime}, \mathbf{a}_{j2}^{2\,\prime}, \ldots, \mathbf{a}_{j(c+1)}^{c+1\,\prime})\mathbf{1}_m = \sum_{i=1}^{c+1} k_j^{m_i}$

for $j = 1,2,\ldots, g$, (10)

where $m = \sum_{i=1}^{c+1} m_i$.

Now let $\mathbf{N}_{d_0}^*$ be the incidence matrix for the design d_0 for a single replication and $\mathbf{N}_{d0i}^*$ be the corresponding partitioned matrices. Then the incidence matrix of the design having a single replication is given by

$$\mathbf{N}_{d_0}^* = \begin{bmatrix} \mathbf{N}_{d_{01}}^* \\ \mathbf{N}_{d_{02}}^* \\ \vdots \\ \mathbf{N}_{d_{0(c+1)}}^* \end{bmatrix} = \begin{bmatrix} \mathbf{a}_1^1, \mathbf{a}_2^1, \ldots, \mathbf{a}_g^1 \\ \mathbf{a}_1^2, \mathbf{a}_2^2, \ldots, \mathbf{a}_g^2 \\ \vdots \\ \mathbf{a}_1^{c+1}, \mathbf{a}_2^{c+1}, \ldots, \mathbf{a}_g^{c+1} \end{bmatrix} \quad (11)$$

Now for r replications the incidence matrix for a single replication $\mathbf{N}_{d_0}^*$ is repeated r times. Finally the incidence matrix of an equireplicate design for parallel line assay permitting the estimation of all three contrasts of interest with full efficiency is given by

$$\mathbf{N}_{d_0} = \left(\mathbf{N}_{d_0}^* \quad \mathbf{N}_{d_0}^* \quad \ldots \quad \mathbf{N}_{d_0}^*\right) \quad (12)$$

Clearly, $\mathbf{N}_{d_0}$ given in (12) is the incidence matrix of a binary design with $b = rg$ blocks, each dose being replicated r times. We thus have the following result.

Theorem 4.1: *Suppose $\mathbf{a}_1^i, \mathbf{a}_2^i, \ldots, \mathbf{a}_g^i$ is a set of g incidence vectors for the standard and test preparation doses, satisfying the conditions of Lemma 3.1. Then using these vectors, it is possible to construct a binary block design with incidence matrix given by (4.3) for multiple asymmetric parallel line assays permitting the estimation of all three major contrasts of interest with full efficiency.*

Example 4.1: Let m_1=5, m_2= 10, m_3=15. That is, this assays has one standard and two test preparations. Let r be the replications for standard and test preparation doses. For a single replication, *i.e.*, $r_1 = r_2 = r_3 = r = 1$ we choose the incidence vectors as follows:

$\mathbf{a}_1^1 = (01010)'$, $\mathbf{a}_2^1 = (10101)'$,

$\mathbf{a}_1^2 = (1100000011)'$, $\mathbf{a}_2^2 = (0011111100)'$ and

$\mathbf{a}_1^3 = (010101000101010)'$

$\mathbf{a}_2^3 = (101010111010101)'$.

Here the value of g is 2. Thus for a single replication the incidence matrix becomes

$$\mathbf{N}_{d_0}^* = \begin{bmatrix} \mathbf{N}_{d_{01}}^* \\ \mathbf{N}_{d_{02}}^* \\ \mathbf{N}_{d_{03}}^* \end{bmatrix} = \begin{bmatrix} \mathbf{a}_1^1, \mathbf{a}_2^1 \\ \mathbf{a}_1^2, \mathbf{a}_2^2 \\ \mathbf{a}_1^3, \mathbf{a}_2^3 \end{bmatrix} = \begin{bmatrix} 01010 : 1100000011 : 010101000101010 \\ 10101 : 0011111100 : 101010111010101 \end{bmatrix}' \quad (13)$$

Clearly $k_1^{m_1} + k_1^{m_2} + k_1^{m_3} = 12$ and $k_2^{m_1} + k_2^{m_2} + k_2^{m_3} = 18$. Suppose now that we want a design with three replications, then the above incidence matrix is repeated three times and the incidence matrix for the design is given by

$$\mathbf{N}_{d_0}^* = \begin{bmatrix} \mathbf{a}_1^1, \mathbf{a}_2^1, & \mathbf{a}_1^1, \mathbf{a}_2^1, & \mathbf{a}_1^1, \mathbf{a}_2^1 \\ \mathbf{a}_1^2, \mathbf{a}_2^2, & \mathbf{a}_1^2, \mathbf{a}_2^2, & \mathbf{a}_1^2, \mathbf{a}_2^2 \\ \mathbf{a}_1^3, \mathbf{a}_2^3, & \mathbf{a}_1^3, \mathbf{a}_2^3, & \mathbf{a}_1^3, \mathbf{a}_2^3 \end{bmatrix} = \begin{bmatrix} 01010 : 1100000011 : 010101000101010 \\ 10101 : 0011111100 : 101010111010101 \\ 01010 : 1100000011 : 010101000101010 \\ 10101 : 0011111100 : 101010111010101 \\ 01010 : 1100000011 : 010101000101010 \\ 10101 : 0011111100 : 101010111010101 \end{bmatrix}' \quad (14)$$

In the above matrix, the first five columns represent the incidence of the standard preparation doses, next ten columns represent the incidence of the first test preparation and last fifteen columns represent the incidence of second test preparation. The design has b = 6 blocks, three of them are of size 12 and three are of size 18.

Remark 4.1: Alternatively, if we choose

$\mathbf{a}_1^1 = (00100)'$, $\mathbf{a}_2^1 = (10001)'$, $\mathbf{a}_3^1 = (01010)'$,

$\mathbf{a}_1^2 = (0000110000)'$

$\mathbf{a}_2^2 = (1010000101)'$

$\mathbf{a}_3^2 = (0101001010)'$ and

$\mathbf{a}_1^3 = (000001010100000)'$,

$\mathbf{a}_2^3 = (101010000010101)'$,

$\mathbf{a}_3^3 = (010100101001010)'$

as the incidence vectors for standard and test preparations, respectively, then the incidence matrix for a single replication becomes

$$\mathbf{N}_{d_0}^* = \begin{bmatrix} \mathbf{N}_{d_{01}}^* \\ \mathbf{N}_{d_{02}}^* \end{bmatrix} = \begin{bmatrix} \mathbf{a}_1^1, \mathbf{a}_2^1, \mathbf{a}_3^1 \\ \mathbf{a}_1^2, \mathbf{a}_2^2, \mathbf{a}_3^2 \\ \mathbf{a}_1^3, \mathbf{a}_2^3, \mathbf{a}_3^3 \end{bmatrix} = \begin{bmatrix} 00100 : 0000110000 : 000001010100000 \\ 10001 : 1010000101 : 101010000010101 \\ 01010 : 0101001010 : 010100101001010 \end{bmatrix}' \quad (15)$$

For three replications the incidence matrix of the design is given by

$$\mathbf{N}_{d_0} = \begin{bmatrix} \mathbf{N}_{d_0}^* & \mathbf{N}_{d_0}^* & \mathbf{N}_{d_0}^* \end{bmatrix} = \begin{bmatrix} 00100 : 0000110000 : 000001010100000 \\ 10001 : 1010000101 : 101010000010101 \\ 01010 : 0101001010 : 010100101001010 \\ 00100 : 0000110000 : 000001010100000 \\ 10001 : 1010000101 : 101010000010101 \\ 01010 : 0101001010 : 010100101001010 \\ 00100 : 0000110000 : 000001010100000 \\ 10001 : 1010000101 : 101010000010101 \\ 01010 : 0101001010 : 010100101001010 \end{bmatrix}' \quad (16)\text{T}$$

he present design has total 9 blocks. Some of the blocks have now reduced sizes than the earlier one. However, number of blocks increases from 6 to 9. Through this method number of blocks and/or block sizes can be determined keeping in mind the need of the hour and resource availability.

The above example can be easily extended for more than two test preparations.

In the present case $m_1 k_j^{m_1} = m_i k_j^{m_1}$, i = 2, 3, …, (c+1), $j = 1,2,\ldots,g$. Thus if we take m_i as a constant proportion of m_1, then $k_j^{m_i}$ would be of same proportion of $k_j^{m_1}$. This will ensure the fulfillment of condition (i) of Lemma 3.1. The construction of designs through this method also depends on the appropriate choice of the vectors $\mathbf{a}_j^i$, $j = 1,2,\ldots,g$, i =1, 2, …. ,(c+1). Note that $\mathbf{w}_i'\mathbf{1} = 0$ and last ($m_i/2$) elements when m_i is even and $(m_i - 1)/2$ elements when m_i is odd are the mirror image of the first $m_i/2$ or $(m_i - 1)/2$ elements with opposite sign, for i =1, 2,…, c+1. Using this property [16] proposed a general methodology for construction of such designs for simple bioassays. This method can easily be extended for multiple bioassays. We do not repeat this procedure here, one may refer to [16] for details.

B. Case 2: Unequal replications

In this case, we first choose the incidence vectors $\mathbf{a}_j^i$ such that $\mathbf{a}_j^{i\prime}\mathbf{w}_i = 0$ for all i and j. Thus, $\mathbf{a}_j^1\mathbf{1}_{m_1} = k_j^{m_1} = k_1$ (say), for $j = 1,2,\ldots,b_1$, $\mathbf{a}_j^2\mathbf{1}_{m_2} = k_j^{m_2} = k_2$ (say), for $j = 1,2,\ldots,b_2$, and so on and lastly $\mathbf{a}_j^{c+1}\mathbf{1}_{m_{c+1}} = k_j^{m_{c+1}} = k_{c+1}$ (say), for $j = 1,2,\ldots,b_{c+1}$.

In order to have replication numbers to be an integer value we first workout least common multiple of b_i's (say λ). We take λ as the total number of blocks in the design *i.e.*, the number of blocks in all $\mathbf{N}_{d_{0i}}$ are λ. In such situation

standard and test preparations should have ($r_i = \lambda / b_i$) replications.

Thus we have

$$\sum_{i=1}^{c+1} \mathbf{a}_j^{i\prime} \mathbf{1}_{m_i} = \sum_{i=1}^{c+1} k_j^{m_i} = k \text{ (say)}, \quad \text{for } j = 1,2,\ldots,\lambda \ . \qquad (17)$$

Let $\mathbf{N}_{d_0}^*$ be the incidence matrix for the design d_0 for a single replication and $\mathbf{N}_{d_{0i}}^*$ are the corresponding partitioned matrices. These incidence matrices for a single replication are then given by

$$\mathbf{N}_{d_{0i}}^* = \left(\mathbf{a}_1^i \quad \mathbf{a}_2^i \quad \ldots \quad \mathbf{a}_{b_i}^i\right) \qquad (18)$$

In the final incidence matrix $\mathbf{N}_{d_i}^*$ is repeated r_i times *i.e.*,

$\mathbf{N}_{d_{0i}} = \left(\mathbf{N}_{d_{0i}}^* \quad \mathbf{N}_{d_{0i}}^* \quad \ldots \quad \mathbf{N}_{d_{0i}}^*\right)$,

and the incidence matrix of the design is displayed below

$$\mathbf{N}_{d_0} = \begin{bmatrix} \mathbf{N}_{d_{01}} \\ \mathbf{N}_{d_{02}} \\ \vdots \\ \mathbf{N}_{d_{0(c+1)}} \end{bmatrix}. \qquad (19)$$

In order to get designs having more replications, the same set of λ incidence vectors need to be repeated desired number of times.
Thus we have the following result.

Theorem 4.2: *Suppose* $\mathbf{a}_1^i, \mathbf{a}_2^i, \ldots, \mathbf{a}_{b_i}^i$ *be set of* b_i *incidence vectors for the standard and test preparation doses for i=1, 2, …, c+1, satisfying the conditions of Lemma 3.1. Then using these vectors, it is possible to construct a binary, proper block design with incidence matrix given by (4.18) for multiple asymmetric parallel line assays permitting the estimation of all three major contrasts of interest with full efficiency.*

Example 4.2: Let m_1=4, m_2=6 m_3=9 and r_i , for i =1, 2, 3, be the replications for standard and test preparation doses. For a single replication, we choose the following incidence vectors for all the preparations:

$\mathbf{a}_1^1 = (1001)'$, $\mathbf{a}_2^1 = (0110)'$,

$\mathbf{a}_1^2 = (010010)'$, $\mathbf{a}_2^2 = (100001)'$, $\mathbf{a}_3^2 = (001100)'$,

$\mathbf{a}_1^3 = (100010001)'$, $\mathbf{a}_2^3 = (010001100)'$ and

$\mathbf{a}_3^3 = (001100010)'$.

Thus the value of b_1, b_2 and b_3 would be 2, 3 and 3, respectively and its least common multiple is 6. Thus $k_j^{m_1} = 2$, for $j = 1,2,\ldots,b_1$ $k_j^{m_2} = 3$, for $j = 1,2,\ldots,b_2$ and $k_j^{m_3} = 3$, for $j = 1,2,\ldots,b_3$. Therefore, the value of r_1 , r_2 and r_3 would be $r_1 = \lambda / b_1 = 3$ and $r_2 = \lambda / b_2 = 2$ and $r_3 = \lambda / b_3 = 2$. $\mathbf{N}_{d_{01}}^* = \left(\mathbf{a}_1^1, \mathbf{a}_2^1\right)$, $\mathbf{N}_{d_{02}}^* = \left(\mathbf{a}_1^2, \mathbf{a}_2^2, \mathbf{a}_3^2\right)$ and $\mathbf{N}_{d_{03}}^* = \left(\mathbf{a}_1^3, \mathbf{a}_2^3, \mathbf{a}_3^3\right)$. The incidence matrix of the design is then given by

$$\mathbf{N}_{d_0} = \begin{bmatrix} \mathbf{N}_{d_{01}} \\ \mathbf{N}_{d_{02}} \\ \mathbf{N}_{d_{03}} \end{bmatrix} = \begin{bmatrix} \mathbf{a}_1^1, \mathbf{a}_2^1 : \mathbf{a}_1^1, \mathbf{a}_2^1 : \mathbf{a}_1^1, \mathbf{a}_2^1 \\ \mathbf{a}_1^2, \mathbf{a}_2^2, \mathbf{a}_3^2 : \mathbf{a}_1^2, \mathbf{a}_2^2, \mathbf{a}_3^2 \\ \mathbf{a}_1^3, \mathbf{a}_2^3, \mathbf{a}_3^3 : \mathbf{a}_1^3, \mathbf{a}_2^3, \mathbf{a}_3^3 \end{bmatrix} = \begin{bmatrix} 1001 : 010010 : 100010001 \\ 0110 : 100001 : 010001100 \\ 1001 : 001100 : 001100010 \\ 0110 : 010010 : 100010001 \\ 1001 : 100001 : 010001100 \\ 0110 : 001100 : 001100010 \end{bmatrix}'.$$

Example 4.3: Let m_1=6, m_2=8, m_3=12 and r_i , for i=1,2,3, be the replications for standard and test preparation doses. For a single replication $\mathbf{a}_1^1 = (100001)'$, $\mathbf{a}_2^1 = (010010)'$, $\mathbf{a}_3^1 = (001100)'$, $\mathbf{a}_1^2 = (11000011)'$, $\mathbf{a}_2^2 = (00111100)'$, $\mathbf{a}_1^3 = (001100001100)'$, $\mathbf{a}_2^3 = (100001100001)'$ and $\mathbf{a}_3^3 = (010010010010)'$. Here we have $b_1 = 3$, $b_2 = 2$, $b_3 = 3$. Thus $k_j^{m_1} = 2$, for j =1, 2, …, b_1 , $k_j^{m_2} = 4$, for $j = 1,2,\ldots,b_2$ and $k_j^{m_3} = 4$, for $j = 1,2,\ldots,b_3$. Therefore the value of r_1, r_2 and r_3 would be $r_1 = \lambda / b_1 = 2$, $r_2 = \lambda / b_2 = 3$ and $r_3 = \lambda / b_3 = 2$, $\mathbf{N}_{d_{01}}^* = \left[\mathbf{a}_1^1, \mathbf{a}_2^1, \mathbf{a}_3^1\right]$ $\mathbf{N}_{d_{02}}^* = \left[\mathbf{a}_1^2, \mathbf{a}_2^2\right]$ and $\mathbf{N}_{d_{03}}^* = \left[\mathbf{a}_1^3, \mathbf{a}_2^3, \mathbf{a}_3^3\right]$. The incidence matrix of the design is given by

$$\mathbf{N}_{d_0} = \begin{bmatrix} \mathbf{N}_{d_{01}} \\ \mathbf{N}_{d_{02}} \\ \mathbf{N}_{d_{03}} \end{bmatrix} = \begin{bmatrix} \mathbf{a}_1^1, \mathbf{a}_2^1, \mathbf{a}_3^1 : \mathbf{a}_1^1, \mathbf{a}_2^1, \mathbf{a}_3^1 \\ \mathbf{a}_1^2, \mathbf{a}_2^2 : \mathbf{a}_1^2, \mathbf{a}_2^2 : \mathbf{a}_1^2, \mathbf{a}_2^2 \\ \mathbf{a}_1^3, \mathbf{a}_2^3, \mathbf{a}_3^3 : \mathbf{a}_1^3, \mathbf{a}_2^3, \mathbf{a}_3^3 \end{bmatrix} = \begin{bmatrix} 100001 : 11000011 : 001100001100 \\ 010010 : 00111100 : 100001100001 \\ 001100 : 11000011 : 010010010010 \\ 100001 : 00111100 : 001100001100 \\ 010010 : 11000011 : 100001100001 \\ 001100 : 00111100 : 010010010010 \end{bmatrix}'.$$

In last two examples replications for test and standard preparations are different. Example 4.2 has three replications for standard while in Example 4.3 we have two replications for standard preparation.

Here we just presented two examples of designs constructed through the above method. To generate more designs, we have to choose vectors $\mathbf{a}_j^i$ for j =1, 2, ..., b_i (for different values of i) for particular values of m_i's such that condition (ii) of Lemma 3.1 is satisfied.

Let m_i be even for i =1, 2, … ,c+1 , $k_j^{m_i} = k_i$, for j = 1, 2, …, b_i, and all k_i's are are even. We also assume that m_i's are divisible by respective k_i's and $b_i = m_i / k_i$. As assumed earlier λ is the least common multiple of b_i's.

Thus $r_i = \lambda/b_i$. This will ensure the fulfillment of the condition (i) of Lemma 3.1.

Now, let $m_i^* = m_i/2$, $k_i^* = k_i/2$ for $i = 1, 2, \ldots, c+1$.

Step1: Construct $\mathbf{a}_1^0 = \left(\mathbf{1}'_{k_1^*} \quad \mathbf{0}' \quad \ldots \quad \mathbf{0}'\right)'$,

$\mathbf{a}_2^0 = \left(\mathbf{0}' \quad \mathbf{1}'_{k_1^*} \quad \ldots \quad \mathbf{0}'\right)'$, …, $\mathbf{a}_{b_1}^0 = \left(\mathbf{0}' \quad \mathbf{0}' \quad \ldots \quad \mathbf{1}'_{k_1^*}\right)'$.

Step 2: Obtain the vectors $\mathbf{a}_j^i$ such that $\mathbf{a}_j^i = \begin{bmatrix} \mathbf{a}_j^0 \\ \mathbf{a}_j^{00} \end{bmatrix}$, where $\mathbf{a}_j^{00}$ is the mirror image of $\mathbf{a}_j^0$ for $j = 1, 2, \ldots, b_i$.

Step 3: For a single replication obtain different $\mathbf{N}_{d_{0i}}^*$ matrices.

Step 4: Finally, obtain the incidence matrix of the design $\mathbf{N}_{d_0}$ from (4.18).

The design given in Example 4.3 is obtained by applying this method. For readily use, one can get the vectors of $\mathbf{a}_j^i$ for some specific values of m_i's in [16]. From these vectors desired designs can be generated easily.

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