# An Extended Youden Design for Biological Assays

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## Abstract

A biological assay experiment (or bioassay) measures the potency of a test preparation by comparing its effect on cells, tissues, or living animals to a standard preparation. The complications of building an efficient design result from its test/standard setup and multiple blocking effects. We proposed an Extended Youden Design(EYD) that achieves optimal estimation efficiency and eliminates 2 blocking factors. We also provide a construction with algorithmic implementation of EYD.

## Introduction

**Cell-based Bioassay:**

- Within each row of the plate, each well contains different doses of the same preparation.[4]
- At least one row of standard preparation is present in each plate.

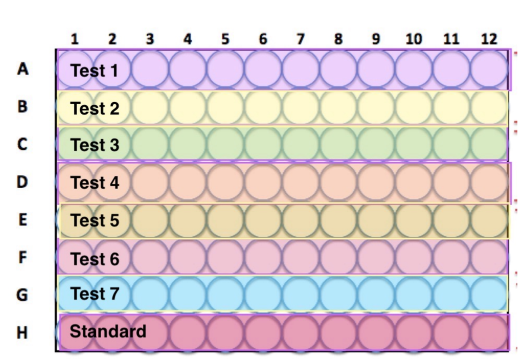


Figure 1: A 96-well plate with 7 test and 1 standard.

- **Nuisance Factors**: inconsistency of the plates, ("*Plate effect*") and different placement of samples within the plate, ("*Location effect*")
- **Design Goal**:
  - Efficient estimation of test-standard contrast ("low cost")
  - Elimination of two nuisance factors ("reduce bias")

## Model Setup

- Consider a bioassay with $v_0$ tests and 1 standard, $b$ plates each with $k$ locations. One location of each plate is standard preparation.
- **The model**

$$Y = 1\mu + X\beta + Z\rho + W\pi + \epsilon. \quad (1)$$

where $\beta \in \mathbb{R}^{v_0+1}$ is preparation effect, $\rho \in \mathbb{R}^k$ is location effect and $\pi \in \mathbb{R}^b$ is plate effect. $Y \in \mathbb{R}^{bk}$. Error $\epsilon \sim N(0, \sigma^2 I)$.

## Motivation

- An optimal design 'maximizes' the information matrix w.r.t some optimality criteria.
- Denote $C_d$ as information matrix of Model (1). The inequality

$$C_d \leq \tilde{C}_d \quad (2)$$

holds, where $\tilde{C}_d$ is information matrix of the 1-way elimination Model (3)

$$Y = 1\mu + X\beta + W\pi + \epsilon. \quad (3)$$

- The '=' in (2) holds when preparations are allocated to different locations evenly[2]. The treatment effect is orthogonal to location effect and the 2-way elimination problem boils down to a 1-way problem.
- For 1-way elimination problem, A-optimal designs for estimation of test-standard contrast is a Balanced Treatment Incomplete Block Design (BTIB).[3]
- **BTIB Design:** Let $\lambda_{i_1,i_2}$ denote the total number of times that the $i_1$th preparation appears with the $i_2$th preparation in the same plate over the whole design ($i_l \neq i_2$; $0 \leq i_l$, $i_2 \leq v_0$). For a design $d \in D(b, k, v_0)$ to be a BTIB, all $\lambda_{i_1,i_2}$ are equal for $1 \leq i_1 \neq i_2 \leq v_0$ and all $\lambda_{0,i_2}$ are equal for $1 \leq i_2 \leq v_0$
- A BTIB design with treatment effect orthogonal to location effect is desired.

## Methodology

**Extended Youden Design (EYD)**
Given a block design $D(b, k, v_0, (r, r_0))$ with two blocking factors: column effect and row effect, assume column effect has $b$ levels and row effect has $k$ levels. Parameter $r = r_1 = \ldots = r_{v_0}$ is replication of treatment and $r_0$ is replication of control. If the following conditions are satisfied, then $D(b, k, v_0, (r, r_0))$ is called an Extended Youden Design.

(a) The columns of design $D$ form a BTIB design.

(b) Control occurs in each column of $D$.

(c) The replication $r = mk + t$ , $m$ and $t$ are both non-negative integers. $0 \leq t < k$. Occurrence of each treatment $1, \ldots, v_0$ in each row is $m$ or $m + 1$. Specifically, if $t = 0$, each treatment occurs $m$ times in each row.

(d) The replication $r_0 = m_0 k + t_0$ , $m_0$ and $t_0$ are both non-negative integers. $0 \leq t_0 < k$. Occurrence of control in each row is $m_0$ or $m_0 + 1$. Specifically, if $t_0 = 0$, control occurs $m_0$ times in each row.

** The EYD is defined for 'near-orthogonality' between treatment and location effect when absolute orthtogonality is not feasible.

## Construction of EYD

**Theorem 1** Let $d \in D(b, k, v; r_1, r_2, \ldots, r_v)$ with $r_i = m_i k + t_i$, $0 \leq m_i$, $0 \leq t_i \leq k-1$, $i = 1, \ldots, v$. Then the treatments can be arranged within blocks so that each treatment occurs $m_i$ or $(m_i + 1)$ times in each row. [1]

**The construction:**

1. Form a BTIB design with parameters $(b, k, v_0, (r, r_0))$,
2. According to Theorem 1, obtain proper within-plate shuffles of the treatments with Set of Distinct Representative.

## Examples

**Example 1:** $(b = 10, k = 4, v_0 = 6, r = 5, r_0 = 10)$

Step 1

| Location \ Plate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 2 | 1 | 4 | 3 | 1 | 2 | 1 | 1 | 1 | 2 |
| 3 | 5 | 4 | 5 | 4 | 3 | 3 | 3 | 2 | 2 | 3 |
| 4 | 6 | 6 | 6 | 5 | 6 | 6 | 5 | 5 | 4 | 4 |

↓ ↓ ↓ ... ↓ ↓ ↓

Step 2

| Location \ Plate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 0 | 6 | 3 | 3 | 2 | 1 | 0 | 0 | 4 |
| 2 | 2 | 1 | 0 | 0 | 6 | 3 | 0 | 5 | 4 | 2 |
| 3 | 6 | 6 | 4 | 5 | 1 | 0 | 3 | 2 | 1 | 0 |
| 4 | 0 | 4 | 5 | 4 | 0 | 6 | 5 | 1 | 2 | 3 |

**Example 2:** $(b = 5, k = 5, v_0 = 5, r = 4, r_0 = 5)$

Step 1

| Location \ Plate | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 2 | 1 | 1 | 1 | 1 |
| 3 | 3 | 3 | 2 | 2 | 2 |
| 4 | 4 | 4 | 4 | 3 | 3 |
| 5 | 5 | 5 | 5 | 5 | 4 |

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Step 2

| Location \ Plate | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 4 | 1 | 2 | 5 | 0 |
| 2 | 2 | 3 | 1 | 0 | 4 |
| 3 | 5 | 4 | 0 | 1 | 3 |
| 4 | 3 | 0 | 5 | 2 | 1 |
| 5 | 0 | 5 | 4 | 3 | 2 |

**Example 3:** $(b = 6, k = 3, v_0 = 4, r = 3, r_0 = 6)$

Step 1

| Location \ Plate | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 2 | 3 | 1 | 1 | 1 | 2 |
| 3 | 4 | 4 | 4 | 3 | 2 | 3 |

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Step 2

| Location \ Plate | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 4 | 0 | 0 | 1 | 2 | 3 |
| 2 | 0 | 4 | 1 | 3 | 0 | 2 |
| 3 | 2 | 3 | 4 | 0 | 1 | 0 |

## Efficiency

The A-efficiency of EYD for Example 1-3 are calculated, along with the theoretical upper bounds.

| | Example 1 | Example 2 | Example 3 |
|---|---|---|---|
| **EYD** | 2.0127 | 2.3611 | 2.2857 |
| **Upper bound** | 1.9765 | 2.3026 | 2.2857 |
| **Relative Efficiency** | 98.202% | 97.523% | 100% |

Table 1: A-Efficiency for Ex1-2

## Conclusion & Future Work

- EYD provides a solution to the challenges of experimental design in bioassays.
- An R package *Ext.Youden* was built as an efficient algorithmic implementation.

1. This work discusses the 2-way elimination of heterogeneity. In practice, bioassays can be complicated and solutions for multi-way elimination problem can be explored.
2. The EYD and its construction are currently based on BTIB. The optimality property of non-BTIB designs has yet to be established.

## References

[1] Chai, F.-S. (1998). A note on generalization of distinct representatives. *Statistics & Probability Letters*, 39(2):173 – 177.

[2] Hedayat, A. S. and Yang, M. (2005). Optimal and efficient crossover designs for comparing test treatments with a control treatment. *Ann. Statist.*, 33(2):915–943.

[3] Majumdar, D. and Notz, W. I. (1983). Optimal incomplete block designs for comparing treatments with a control. *Ann. Statist.*, 11(1):258–266.

[4] USP, G. C. (2010). Design and analysis of biological assays 1032-1034. *Pharm. Forum.*, 34(3):685.

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