Chapter 140

Tests for the Ratio of Two Within-Subject Variances in a 2×2M Replicated Cross-Over Design

Introduction

This procedure calculates power and sample size of inequality tests of within-subject variabilities from a 2×2M replicated cross-over design. This routine deals with the case in which the statistical hypotheses are expressed in terms of the ratio of the within-subject variances.

This design is used to compare two treatments which are administered to subjects in different orders. It has two treatment sequences. Here, \(M\) is the number of times a particular treatment is received by a subject. For example, if \(M = 2\), the design is a 2×4 cross-over. The two sequences would often be

- sequence 1: R T R T
- sequence 2: T R T R

It is assumed that either there is no carry-over from one measurement to the next, or there is an ample washout period between measurements.

Technical Details

This procedure uses the formulation given in Chow, Shao, Wang, and Lokhnygina (2018).

Suppose \(x_{ijkl}\) is the response in the \(i\)th sequence \((i = 1, 2)\), \(j\)th subject \((j = 1, \ldots, N_i)\), \(k\)th treatment \((k = T, C)\), and \(l\)th replicate \((l = 1, \ldots, M)\). The mixed effect model analyzed in this procedure is

\[
x_{ijkl} = \mu_k + \gamma_{ikl} + S_{ijk} + e_{ijkl}
\]

where \(\mu_k\) is the \(k\)th treatment effect, \(\gamma_{ikl}\) is the fixed effect of the \(l\)th replicate on treatment \(k\) in the \(i\)th sequence, \(S_{ij1}\) and \(S_{ij2}\) are random effects of the \(ij\)th subject, and \(e_{ijkl}\) is the within-subject error term which is normally distributed with mean 0 and variance \(V_i\).
Unbiased estimators of these variances are found after applying an orthogonal transformation matrix $P$ to the $x$’s as follows

$$z_{ijk} = P'x_{ijk}$$

where $P$ is an $m \times m$ matrix such that $P'P$ is diagonal and $\text{var}(z_{ijkl}) = \sigma_{wk}^2$.

For example, in a $2 \times 4$ cross-over design the $z$’s become

$$z_{ijk1} = \frac{x_{ijk1} + x_{ijk2}}{2} = \bar{x}_{ijk},$$

and

$$z_{ijk2} = \frac{x_{ijk1} + x_{ijk2}}{\sqrt{2}} = \bar{x}_{ijk}.$$ 

In this case, the within-subject variances are estimated as

$$\hat{\sigma}_T^2 = \frac{1}{(N_1 + N_2 - 2)(M - 1)} \sum_{i=1}^{N_i} \sum_{j=1}^{M} \sum_{l=1}^{M} (z_{ij} - \bar{z}_{i,l})^2$$

and

$$\hat{\sigma}_C^2 = \frac{1}{(N_1 + N_2 - 2)(M - 1)} \sum_{i=1}^{N_i} \sum_{j=1}^{M} \sum_{l=1}^{M} (z_{ij} - \bar{z}_{i,l})^2$$

### Testing Inequality

The following hypotheses are usually used to test for inequality

$$H_0: \frac{\sigma^2_{WT}}{\sigma^2_{WC}} = 1 \text{ versus } H_A: \frac{\sigma^2_{WT}}{\sigma^2_{WC}} \neq 1.$$

The test statistic used to test this hypothesis is $T = (\hat{\sigma}_T / \hat{\sigma}_C)$.

Upon making the usual normality assumptions, $T$ is distributed as an $F_{d,d}$ random variable where

$$d = (N_1 + N_2 - 2)(M - 1).$$

Hence the null hypothesis is rejected if $T < F_{\alpha/2,d,d}$ or $T > F_{1-\alpha/2,d,d}$.

### Power

The power of this combination of tests is given by

$$\text{Power} = P(R1(F_{\alpha/2,d,d})) + 1 - P(F < R1(F_{1-\alpha/2,d,d}))$$

where $F$ is the common $F$ distribution with the indicated degrees of freedom, $\alpha$ is the significance level, and $R1$ is the actual variance ratio.

A simple binary search algorithm can be applied to this power function to obtain an estimate of the necessary sample size.
Procedure Options

This section describes the options that are specific to this procedure. These are located on the Design tab. For more information about the options of other tabs, go to the Procedure Window chapter.

Design Tab

The Design tab contains the parameters associated with this test such as sample sizes, alpha, and power.

Solve For

Solve For

This option specifies the parameter to be solved for from the other parameters. Under most situations, you will select either Power or Sample Size.

Power and Alpha

Power

This option specifies one or more values for power. Power is the probability of rejecting a false null hypothesis and is equal to one minus Beta. Beta is the probability of a type-II error, which occurs when a false null hypothesis is not rejected. In this procedure, a type-II error occurs when you fail to reject the null hypothesis of equal variances when in fact the variances are unequal.

Values must be between zero and one. Historically, the value of 0.80 (Beta = 0.20) was used for power. Now, 0.90 (Beta = 0.10) is also commonly used.

A single value may be entered here or a range of values such as 0.8 to 0.95 by 0.05 may be entered.

Alpha

This option specifies one or more values for the probability of a type-I error. A type-I error occurs when a true null hypothesis is rejected. In this procedure, a type-I error occurs when you reject the null hypothesis of equal variances when in fact the variances are equal.

Values must be between zero and one. Historically, the value of 0.05 has been used for alpha. This means that about one test in twenty will falsely reject the null hypothesis. You should pick a value for alpha that represents the risk of a type-I error you are willing to take in your experimental situation.

You may enter a range of values such as 0.01 0.05 0.10 or 0.01 to 0.10 by 0.01.

Sample Size (When Solving for Sample Size)

Sequence Allocation

Select the option that describes the constraints on $N_1$ or $N_2$ or both.

The options are

- **Equal ($N_1 = N_2$)**
  
  This selection is used when you wish to have equal sample sizes in each sequence. Since you are solving for both sample sizes at once, no additional sample size parameters need to be entered.

- **Enter $N_2$, solve for $N_1$**
  
  Select this option when you wish to fix $N_2$ at some value (or values), and then solve only for $N_1$. Please note that for some values of $N_2$, there may not be a value of $N_1$ that is large enough to obtain the desired power.
• Enter $R = N_2/N_1$, solve for $N_1$ and $N_2$
For this choice, you set a value for the ratio of $N_2$ to $N_1$, and then PASS determines the needed $N_1$ and $N_2$, with this ratio, to obtain the desired power. An equivalent representation of the ratio, $R$, is

$$N_2 = R \times N_1.$$

• Enter percentage in Sequence 1, solve for $N_1$ and $N_2$
For this choice, you set a value for the percentage of the total sample size that is in Sequence 1, and then PASS determines the needed $N_1$ and $N_2$ with this percentage to obtain the desired power.

**N2 (Sample Size, Sequence 2)**

*This option is displayed if Sequence Allocation = “Enter N2, solve for N1”*

$N_2$ is the number of items or individuals sampled from the Sequence 2 population.

$N_2$ must be $\geq 2$. You can enter a single value or a series of values.

**R (Sequence Sample Size Ratio)**

*This option is displayed only if Sequence Allocation = “Enter R = N2/N1, solve for N1 and N2.”*

$R$ is the ratio of $N_2$ to $N_1$. That is,

$$R = N_2 / N_1.$$

Use this value to fix the ratio of $N_2$ to $N_1$ while solving for $N_1$ and $N_2$. Only sample size combinations with this ratio are considered.

$N_2$ is related to $N_1$ by the formula:

$$N_2 = [R \times N_1],$$

where the value $[Y]$ is the next integer $\geq Y$.

For example, setting $R = 2.0$ results in a Sequence 2 sample size that is double the sample size in Sequence 1 (e.g., $N_1 = 10$ and $N_2 = 20$, or $N_1 = 50$ and $N_2 = 100$).

$R$ must be greater than 0. If $R < 1$, then $N_2$ will be less than $N_1$; if $R > 1$, then $N_2$ will be greater than $N_1$. You can enter a single or a series of values.

**Percent in Sequence 1**

*This option is displayed only if Sequence Allocation = “Enter percentage in Sequence 1, solve for N1 and N2.”*

Use this value to fix the percentage of the total sample size allocated to Sequence 1 while solving for $N_1$ and $N_2$. Only sample size combinations with this Sequence 1 percentage are considered. Small variations from the specified percentage may occur due to the discrete nature of sample sizes.

The Percent in Sequence 1 must be greater than 0 and less than 100. You can enter a single or a series of values.
Sample Size (When Not Solving for Sample Size)

Sequence Allocation
Select the option that describes how individuals in the study will be allocated to Sequence 1 and to Sequence 2.

The options are

- **Equal (N1 = N2)**
  This selection is used when you wish to have equal sample sizes in each sequence. A single per sequence sample size will be entered.

- **Enter N1 and N2 individually**
  This choice permits you to enter different values for \( N_1 \) and \( N_2 \).

- **Enter N1 and R, where \( N_2 = R \times N_1 \)**
  Choose this option to specify a value (or values) for \( N_1 \), and obtain \( N_2 \) as a ratio (multiple) of \( N_1 \).

- **Enter total sample size and percentage in Sequence 1**
  Choose this option to specify a value (or values) for the total sample size (\( N \)), obtain \( N_1 \) as a percentage of \( N \), and then \( N_2 \) as \( N - N_1 \).

Sample Size Per Sequence
This option is displayed only if Sequence Allocation = “Equal (N1 = N2).”

The Sample Size Per Sequence is the number of items or individuals sampled. Since the sample sizes are the same in each sequence, this value is the value for \( N_1 \), and also the value for \( N_2 \).

The Sample Size Per Sequence must be \( \geq 2 \). You can enter a single value or a series of values.

**N1 (Sample Size, Sequence 1)**
This option is displayed if Sequence Allocation = “Enter N1 and N2 individually” or “Enter N1 and R, where \( N_2 = R \times N_1 \).”

\( N_1 \) is the number of items or individuals sampled from the Sequence 1 population.

\( N_1 \) must be \( \geq 2 \). You can enter a single value or a series of values.

**N2 (Sample Size, Sequence 2)**
This option is displayed only if Sequence Allocation = “Enter N1 and N2 individually.”

\( N_2 \) is the number of items or individuals sampled from the Sequence 2 population.

\( N_2 \) must be \( \geq 2 \). You can enter a single value or a series of values.

**R (Sequence Sample Size Ratio)**
This option is displayed only if Sequence Allocation = “Enter N1 and R, where \( N_2 = R \times N_1 \).”

\( R \) is the ratio of \( N_2 \) to \( N_1 \). That is,

\[
R = \frac{N_2}{N_1}
\]

Use this value to obtain \( N_2 \) as a multiple (or proportion) of \( N_1 \).

\( N_2 \) is calculated from \( N_1 \) using the formula:

\[
N_2 = \lfloor R \times N_1 \rfloor,
\]

where the value \( \lfloor Y \rfloor \) is the next integer \( \geq Y \).
For example, setting $R = 2.0$ results in a Sequence 2 sample size that is double the sample size in Sequence 1. $R$ must be greater than 0. If $R < 1$, then $N2$ will be less than $N1$; if $R > 1$, then $N2$ will be greater than $N1$. You can enter a single value or a series of values.

**Total Sample Size (N)**

*This option is displayed only if Sequence Allocation = “Enter total sample size and percentage in Sequence 1.”*

This is the total sample size, or the sum of the two sequence sample sizes. This value, along with the percentage of the total sample size in Sequence 1, implicitly defines $N1$ and $N2$.

The total sample size must be greater than one, but practically, must be greater than 3, since each sequence sample size needs to be at least 4.

You can enter a single value or a series of values.

**Percent in Sequence 1**

*This option is displayed only if Sequence Allocation = “Enter total sample size and percentage in Sequence 1.”*

This value fixes the percentage of the total sample size allocated to Sequence 1. Small variations from the specified percentage may occur due to the discrete nature of sample sizes.

The Percent in Sequence 1 must be greater than 0 and less than 100. You can enter a single value or a series of values.

**M (Number of Replicates)**

Enter one or more values for $M$: the number of replicates in a $2 \times 2M$ replicated cross-over design. $M$ is the number of times each treatment is measured on each subject.

For example, $M = 2$ refers to a $2 \times 4$ replicated cross-over design. The two sequences used for this design are often

*Sequence 1*: $R \ T \ R \ T$
*Sequence 2*: $T \ R \ T \ R$.

You can enter a single value such as 2, a series of values such as 2 3 4, or 2 to 8 by 1.

The range is $M \geq 2$.

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**Effect Size**

**R1 (Actual Variance Ratio)**

Enter one or more values for within-subject variance ratio assumed by the alternative hypothesis. This is the value of $\sigma^2_{wt} / \sigma^2_{wc}$ at which the power is calculated.

This value should be greater than zero. It cannot be equal to one.
Example 1 – Finding Sample Size

A company has developed a generic drug for treating rheumatism and wants to show that it is has a different within-subject variance from the standard drug. A 2 x 4 cross-over design will be used.

Company researchers set the significance level to 0.05, the power to 0.90, and the alternative variance ratios between 0.5 and 2.0. They want to investigate the range of required sample size values assuming that the two sequence sample sizes are equal.

Setup

This section presents the values of each of the parameters needed to run this example. First, from the PASS Home window, load the Tests for the Ratio of Two Within-Subj Variances in a 2×2M Rep. Cross-Over Design procedure window. You may then make the appropriate entries as listed below, or open Example 1 by going to the File menu and choosing Open Example Template.

Option Value
Design Tab
Solve For Sample Size
Power 0.90
Alpha 0.05
Sequence Allocation Equal (N1 = N2)
M (Number of Replicates) 2
R1 (Actual Variance Ratio) 0.5 0.66667 0.8 1.25 1.5 2

Annotated Output

Click the Calculate button to perform the calculations and generate the following output.

<table>
<thead>
<tr>
<th>Actual Power</th>
<th>Sequence 1 Sample Size</th>
<th>Sequence 2 Sample Size</th>
<th>Number of Replicates</th>
<th>Actual Variance Ratio</th>
<th>Alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9049</td>
<td>46</td>
<td>46</td>
<td>92</td>
<td>0.500</td>
<td>0.050</td>
</tr>
<tr>
<td>0.9015</td>
<td>130</td>
<td>130</td>
<td>260</td>
<td>0.667</td>
<td>0.050</td>
</tr>
<tr>
<td>0.9003</td>
<td>424</td>
<td>424</td>
<td>848</td>
<td>0.800</td>
<td>0.050</td>
</tr>
<tr>
<td>0.9003</td>
<td>424</td>
<td>424</td>
<td>848</td>
<td>1.250</td>
<td>0.050</td>
</tr>
<tr>
<td>0.9015</td>
<td>130</td>
<td>130</td>
<td>260</td>
<td>1.500</td>
<td>0.050</td>
</tr>
<tr>
<td>0.9049</td>
<td>46</td>
<td>46</td>
<td>92</td>
<td>2.000</td>
<td>0.050</td>
</tr>
</tbody>
</table>

References


Report Definitions

Actual Power is the actual power achieved. Because N1 and N2 are discrete, this value is usually slightly larger than the target power.

N1 is the number of subjects in sequence 1.

N2 is the number of subjects in sequence 2.

N is the total number of subjects. N = N1 + N2.

M is the number of replicates. That is, it is the number of times a treatment measurement is repeated on a subject.

R1 is the value of the within-subject variance ratio at which the power is calculated.

Alpha is the probability of rejecting a true null hypothesis, H0.
Summary Statements
A study is being conducted to assess the inequality of within-subject variability of two groups by looking at the ratio of their variances. If the sample variance ratio is too large or too small, the group variabilities are concluded to be unequal. Sequence sample sizes of 46 and 46 achieve 90% power to reject the null hypothesis of equality at a significance level of 0.050. The variance ratio at which the power is calculated is assumed to be 0.500. The number of times each treatment measurement is repeated on a subject is 2.

This report gives the sample sizes for the indicated scenarios.

Plot Section

This plot shows the relationship between sample size and R1.
Example 2 – Validation using Chow and Liu (2014)

We will use an example from Chow and Liu (2014) page 509 to validate this procedure.

In this example, the significance level to 0.05, M is 2, the power is 0.80, and the actual variance ratio is 0.3/0.45 or about 0.667. The resulting sample size is found to be 98 per sequence.

Setup

This section presents the values of each of the parameters needed to run this example. First, from the PASS Home window, load the Tests for the Ratio of Two Within-Subj Variances in a 2×2M Rep. Cross-Over Design procedure window. You may then make the appropriate entries as listed below, or open Example 2 by going to the File menu and choosing Open Example Template.

Option  Value
Design Tab
Solve For ................................................... Sample Size
Power ...................................................... 0.80
Alpha ..................................................... 0.05
Sequence Allocation ............................... Equal (N1 = N2)
M (Number of Replicates) ............................ 2
R1 (Actual Variance Ratio) ..................... 0.667

Output

Click the Calculate button to perform the calculations and generate the following output.

<table>
<thead>
<tr>
<th>Actual Power</th>
<th>Sequence 1 Sample Size</th>
<th>Sequence 2 Sample Size</th>
<th>Number ofReplicates</th>
<th>Actual Variance Ratio</th>
<th>Alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8032</td>
<td>N1 98</td>
<td>N2 98</td>
<td>N 196</td>
<td>M 2</td>
<td>R1 0.667</td>
</tr>
</tbody>
</table>

The sample size matches Chow and Liu (2014).

Note that the result depends on the amount of rounding that is applied to R1. If R1 = 0.67, then N1 = 100. But if R1 = 0.66667, then N1 = 97.