

# Non-Parametric Inference

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# 1. Tests for Measures of Central Location

# Introduction

The most commonly used methods for inference about the means of quantitative response variables assume that the variables in question have Normal distributions in the population.

Fortunately, our usual methods for inference about population means (the one-sample and two-sample procedures and analysis of variance) are quite robust. That is, the results of inference are not very sensitive to moderate lack of Normality, especially when the samples are reasonably large.

What can we do if plots of the data suggest that the population is clearly not Normal, especially when we have only a few observations? This is not a simple question.

This section concerns one type of non-parametric procedure, tests that can replace the t-tests and one-way analysis of variance when the Normality conditions for those tests are not met.

The most useful non-parametric tests are based on the rank (place in order) of each observation in the set of all the data.

The rank tests we will study concern the **center** of a population or populations. When a population has at least roughly a Normal distribution, we describe its center by the mean. When distributions are strongly skewed, we often prefer the median to the mean as a measure of center. In simplest form, the hypotheses for rank tests just replace mean by median.

- Non-parametric alternative to `t.test` and `z.test`: `wilcox.test` (Wilcoxon test and Wilcoxon-Mann-Whitney test).
- Non-parametric alternative to `aov/anova`: `kruskal.test` (Kruskal-Wallis test).
- Non-parametric alternative to `var.test`: `mood.test` and `ansari.test`.
- For testing k-sample homogeneity of variances: `bartlett.test` (sensitive to departures from Normality) and `fligner.test` (non-parametric).

# Wilcoxon Test

## *One sample / Matched pairs*

We use the one-sample procedure for inference about the mean of one population or for inference about the mean difference in a matched pairs setting. We will now meet the **Wilcoxon test**, or **Wilcoxon signed-rank test** for matched pairs and single samples. The matched pairs setting is more important because good studies are generally comparative.

### **Example 1: Vitamin loss in a food product**

Food products are often enriched with vitamins and other supplements. Does the level of a supplement decline over time, so that the user receives less than the manufacturer intended?

Here are data on the vitamin C levels (milligrams per 100 grams) in wheat soy blend, a flour-like product supplied by international aid programs mainly for feeding children. The same 9 bags of blend were measured at the factory and five months later in Haiti.

Bag	1	2	3	4	5	6	7	8	9
Factory	45	32	47	40	38	41	37	52	37
Haiti	38	40	35	38	34	35	38	38	40

We suspect that vitamin C levels are generally higher at the factory than they are five months later.



(1) We would like to test the hypotheses:

$H_0$ : vitamin C has the same distribution at both times

$H_1$ : vitamin C is systematically higher at the factory

Because these are matched pairs data, we base our inference on the **differences**.

Positive differences indicate that the vitamin C level of a bag was higher at the factory than in Haiti.

If factory values are generally higher, the positive differences should be farther from zero in the positive direction than the negative differences are in the negative direction (one-sided test).

We therefore compare the **absolute values** of the differences, that is, their magnitudes without a sign.

Bag	1	2	3	4	5	6	7	8	9
Factory	45	32	47	40	38	41	37	52	37
Haiti	38	40	35	38	34	35	38	38	40
Difference	7	-8	12	2	4	6	-1	14	-3
Absolute value	7	8	12	2	4	6	1	14	3
Rank	6	7 <sup>-</sup>	8	2	4	5	1 <sup>-</sup>	9	3 <sup>-</sup>

## Notes:

1. Tied values receive the average of their ranks.
2. If there are zero differences, discard them before ranking.

## (2) Test statistic:

$$W^+ = \sum \text{positive ranks}$$

The test statistic is the sum of the ranks of the positive differences. This is the *Wilcoxon signed rank statistic*. Its value here is  $w^+ = 34$ . (We could equally well use the sum of the ranks of the negative differences, which is 11.)

## (3) Decision:

Reject  $H_0$  for large values of the statistic  $W^+$ , since the alternative hypothesis is "vitamin C is systematically higher at the factory".

$H_0$  is rejected for a significance level  $\alpha$ , if  $\text{p-value} = P(W^+ \geq 34) < \alpha$ .  
P-values can be found from special tables or software like R.

```
> x<-c(45, 32, 47, 40, 38, 41, 37, 52, 37)
> y<-c(38, 40, 35, 38, 34, 35, 38, 38, 40)
> wilcox.test(x, y, alternative = "greater", paired=TRUE)
```

Wilcoxon signed rank test

data: x and y

$V = 34$ ,  $p\text{-value} = 0.1016$

alternative hypothesis: true location shift is greater than 0

In this example  $p\text{-value}=0.1016$ , thus  $H_0$  is not rejected for the usual significance levels. This small sample does not give convincing evidence of vitamin loss.

# Mann-Whitney-Wilcoxon

## *Two samples*

Although we emphasize the matched pairs setting,  $W^+$  can also be applied to a single sample. It then tests the hypothesis that the population median is zero.

To test the hypothesis that the population median has a specific value  $m$ , apply the test to the differences  $X_i - m$ . For matched pairs, we are testing that the median of the differences is zero.

The **Mann-Whitney-Wilcoxon**, or **Wilcoxon rank-sum test**, is a non-parametric alternative to the one sample  $t$ -test which is based solely on the order in which the observations from the two samples fall. We will use the following as a running example.

## Example 2: Genetic inheritance

In a genetic inheritance study discussed by Margolin (1988), samples of individuals from several ethnic groups were taken. Blood samples were collected from each individual and several variables measured.

We shall compare the groups labelled "Native American" and "Caucasian" with respect to the variable MSCE (mean sister chromatid exchange). The data is as follows:

Native American (A)	8.50	9.48	8.65	8.16	8.83	7.76	8.63		
Caucasian (C)	8.27	8.20	8.25	8.14	9.00	8.10	7.20	8.32	7.70

Here, we want to test if MSCE distribution for Native Americans is the same as that for Caucasians. Although the Native American MSCE values in the data tend to be higher, there was no prior theory to lead us to expect this so we should be doing a two-sided test.

**(1) Hypotheses:**

$H_0$ : MSCE has the same distribution for both ethnic groups

$H_1$ : MSCE has different distribution for both ethnic groups

The Wilcoxon test is based upon ranking the  $n_A + n_C$  observations of the combined sample.

Tied values receive the average of their ranks.

Native American (A)	Caucasian (C)	Rank
	7.20	1
	7.70	2
7.76		3 <sup>A</sup>
	8.10	4
	8.14	5
8.16		6 <sup>A</sup>
	8.20	7
	8.25	8
	8.27	9
	8.32	10
8.50		11 <sup>A</sup>
8.63		12 <sup>A</sup>
8.65		13 <sup>A</sup>
8.83		14 <sup>A</sup>
	9.00	15
9.48		16 <sup>A</sup>



## (2) Test statistic:

The Wilcoxon rank-sum test statistic is the sum of the ranks for observations from one of the samples. Let us use  $w_A$  for the observed rank sum and  $W_A$  to represent the corresponding random variable:

$$W_A = \sum \text{ranks for observations from } A$$

The sum of the ranks for the Native American group is

$$w_A = 3 + 6 + 11 + 12 + 13 + 14 + 16 = 75$$

### (3) Decision:

For the two-sided test, i.e., testing  $H_0: \text{median}_A = \text{median}_C$  versus the alternative  $H_1: \text{median}_A \neq \text{median}_C$ , a rank sum that is either too big or too small provides evidence against  $H_0$ .

The rank sum for the Native American group was  $w_A = 75$ . We know from the ranks table that this will be in the upper tail of the distribution. The  $p$  - value is thus

$$p - \text{value} = 2 \times P(W_A \geq 75) = 0.114$$

For the usual significance levels we do not reject  $H_0$ . This suggests that median MSCE measurements are similar for Native Americans than for Caucasians.

```
> x<-c(8.50, 9.48, 8.65, 8.16, 8.83, 7.76, 8.63)
> y<-c(8.27, 8.20, 8.25, 8.14, 9.00, 8.10, 7.20, 8.32, 7.70)
> wilcox.test(x, y, alternative = "two.sided", paired = FALSE)
```

### Wilcoxon rank sum test

data: x and y

$W = 47$ , p-value = 0.1142

alternative hypothesis: true location shift is not equal to 0

### Note:

R computes the value of the statistic as the number of all pairs  $(x[i], y[j])$  for which  $y[j]$  is not greater than  $x[i]$ . Then, R gives as output

$W = 2 + 4 + 4 \times 8 + 9 = 47$  since

nr of elements of  $C$  not greater than 7.76 = 2

nr of elements of  $C$  not greater than 8.16 = 4

nr of elements of  $C$  not greater than 8.50 = 8

nr of elements of  $C$  not greater than 8.63 = 8

nr of elements of  $C$  not greater than 8.65 = 8

nr of elements of  $C$  not greater than 8.85 = 8

nr of elements of  $C$  not greater than 9.48 = 9

# Kruskal-Wallis test

The **Kruskal-Wallis test** is a rank test that can replace the ANOVA  $F$  test. The assumption about data production (independent random samples from each population) remains important, but we can relax the Normality assumption. We assume only that the response has a continuous distribution in each population.

Data considered in the Example 7, of the previous section, will be used to illustrate this test.

## Example 3: Cows' weights

Four samples of weights (kg) of cows, according to the treatments  $A$ ,  $B$ ,  $C$  and  $D$ .

## (1) Hypotheses:

$H_0$ : Weights have the same distribution in all groups

$H_1$ : Weights are systematically higher in some groups than in others

The null hypothesis is that all four samples have the same median weights. The alternative hypothesis is that not all four median weights are equal.

Like the Wilcoxon rank-sum statistic, the Kruskal-Wallis statistic is based on the sums of the ranks for the groups we are comparing. The more different these sums are, the stronger is the evidence that responses are systematically larger in some groups than in others.

Treatment					Treatment				
A	B	C	D	Rank	A	B	C	D	Rank
	21.78			1				30.40	16
	25.59			2				30.70	17
		25.85		3				30.83	18
		26.22		4		32.55			19
	26.86			5			33.40		20
		27.38		6				33.54	21
27.58				7				33.84	22
27.91				8		34.26			23
			28.82	9		35.08			24
		29.11		10			39.47		25
29.33				11					
			29.60	12					
		30.15		13					
30.28				14					
		30.39		15					

## (2) Test statistic:

There are  $N$  observations in all:

$$N = n_A + n_B + n_C + n_D = 4 + 6 + 8 + 7 = 25.$$

After ranking all  $N$  observations, let  $R_i$  be the sum of the ranks for the  $i$ th sample:  $R_A^2 = 1600$ ;  $R_B^2 = 5476$ ;  $R_C^2 = 9216$ ;  $R_D^2 = 13225$ .

The Kruskal-Wallis statistic is:

$$H = \frac{12}{N(N+1)} \sum \frac{R_i^2}{n_i} - 3(N+1)$$

The observed value of the Kruskal-Wallis statistic for this example is  $H = 2.38$ .

### (3) Decision:

The exact distribution of the Kruskal-Wallis statistic  $H$  under the null hypothesis depends on all the sample sizes, so tables are awkward. The calculation of the exact distribution is so time-consuming for all but the smallest problems that even most statistical software uses the chi-squared approximation to obtain p-values.

Here, the p-value is 0.4972, according to a chi-squared distribution with 3 (4-1) degrees of freedom.

For the usual significance levels we do not reject  $H_0$ . This suggests that there are no differences between treatments.



```
> a<-c(30.28, 27.5, 27.9, 29.33)
> b<-c(34.26, 32.55, 21.78, 25.59, 35.08, 26.86)
> c<-c(39.47, 30.15, 33.40, 27.38, 30.39, 25.85, 29.11, 26.22)
> d<-c(33.54, 30.40, 29.60, 28.82, 30.70, 30.83, 33.84)
> x<-list(a,b,c,d)
> kruskal.test(x)
```

Kruskal-Wallis rank sum test

data: x

Kruskal-Wallis chi-squared = 2.3807, df = 3, p-value = 0.4972

## 2. The Chi-Squared Test

# The Chi-Squared Goodness of Fit Test

In the Example 1 of Section 3, we might have had doubts whether the geometric distribution was appropriate to model the length of sequences of consecutive *A* nucleotides.

A hypothesis test concerning the adequacy of a model is called **goodness of fit test**.

With this example we will see how to use the Pearson  $\chi^2$  statistic (a statistic which has an approximate  $\chi^2$  distribution) to test the adequacy of a model.

The null hypothesis here is that the variable of interest, say  $X$ , has a distribution  $F_X(x)$ . This distribution may be completely specified, or may be specified up to some unknown parameters. If this is the case then the parameters have to be estimated by the maximum likelihood method using the available data.

To apply the  $\chi^2$  goodness of fit test we follow the procedure:

- 1 Divide the support of the hypothesized distribution in  $k$  mutually exclusive and exhaustive intervals,  $I_1, \dots, I_k$ .
- 2 From a random sample of size  $n$ , denote by  $O_i$ , ( $i = 1, \dots, k$ ) the frequency of occurrence of data points in each interval.
- 3 Compute the expected number of points in each interval according to the hypothesized distribution, ie,  $E_i = nP(X \in I_i) = np_i$ .
- 4 Compute the statistic

$$\chi^2 = \sum_{i=1}^k \left[ \frac{(O_i - E_i)^2}{E_i} \right]$$

- 5 Under the null hypothesis and if, at least 80% of the  $E_i \geq 5$ , then the statistic  $\mathbf{X}^2$  is approximately distributed as a  $\chi^2$  with the number of degrees of freedom equal to  $k - 1 - r$ , where  $r$  is the number of parameters estimated from the data.
- 6 Reject the null hypothesis at significance level  $\alpha$  if the observed value for the  $\mathbf{X}^2$  statistic is bigger than  $\chi^2_{k-1-r}(1 - \alpha)$ , the quantile of probability  $1 - \alpha$  of a  $\chi^2$  distribution with  $k - 1 - r$  degrees of freedom.

### Example 4: Long Repeats

Suppose that we observed a very long DNA sequence and counted the number of  $A$ 's before another nucleotide appears obtaining the following data:

0	2	0	0	0	1	0	3	0	1	0
0	0	0	2	0	0	0	0	0	1	0
0	0	1	0	0	3	0	1	2	0	0
0	0	0	0	0	0	0	0	1	0	2
0	0	1	0	1	0	0	1	1	0	1
0	0	1	0	0	0	0	1	0	0	1
1	1	0	0	1	0	2	0	1	0	3
2	0	0	0	1	1	0	2	0	0	1
1	0	0	0	1	3	3	0	0	0	0
1	1	0	0	1	1	4	0	0	0	1
1	0	0	5	0	1	0	0	2	0	0
1	0	0	0	0	1					

Are these data compatible with the hypothesis that the distribution of  $X$ , the length of consecutive  $A$ 's, is geometric, i.e.,

$$P(X = x|\theta) = \theta(1 - \theta)^x, \quad x = 0, 1, 2, \dots$$

where  $1 - \theta$  is the probability of an  $A$  in any position of the DNA sequence?

The support of the geometric distribution is  $S = \{0, 1, 2, \dots\}$ .

Given the data we may construct the following table of observed frequencies and expected frequencies according to the hypothesized geometric model

$x_i$	observed frequency ( $O_i$ )	expected frequency ( $E_i$ )	$(O_i - E_i)^2/E_i$
0	80	81.28	0.020
1	32	29.26	0.256
2	8	10.53	0.610
$\geq 3$	7	5.93	0.195

To compute the expected frequencies is necessary to estimate  $\theta$  from the data.

The maximum likelihood estimate of  $\theta$  is  $\hat{\theta} = n/(n + \sum x_i) = 0.64$ . Hence the probability of an  $A$  is estimated as 0.36.

The value of the  $\chi^2 = \sum_{i=1}^4 \frac{(O_i - E_i)^2}{E_i}$  statistic is 1.081.

The obtained p-value is 0.582, meaning that there is strong evidence that the model is adequate. In fact, we can never say that the model is "the true model". The fact that we do not reject a specific model does not even mean that it is the best model. It is only a **possible model**.

**Note:** For the calculation of the p-value we consider a  $\chi^2$  distribution with  $4 - 1 - 1 = 2$  degrees of freedom since one parameter has been estimated.



```
> x<-c(0,1,2)
> obs.freq<-c(80,32,8,7)
> prob<-dgeom(x,0.64)
> prob[4]<-1-sum(prob)
> exp.freq<-sum(obs.freq)*prob
> exp.freq

[1] 81.280000 29.260800 10.533888  5.925312
```

```
> res<-chisq.test(obs.freq,p=prob)
> res
```

Chi-squared test for given probabilities

data: obs.freq

X-squared = 1.081, df = 3, p-value = 0.7817

```
> 1-pchisq(res$statistic,4-1-1)
```

X-squared  
0.5824514

# The Chi-Squared Test of Homogeneity

Suppose that we have observed the frequencies of the four nucleotides  $A, G, C, T$  in two DNA sequences

	$A$	$G$	$C$	$T$	Total
sequence 1	273	258	233	236	1000
sequence 2	281	244	246	229	1000
Total	554	502	479	465	2000

The question of interest here is the following:

"Is it true that the two sequences are drawn from 'populations' with identical nucleotide frequencies?"

To simplify notation let us codify the nucleotides in the following manner:  $A, B, C, B$  are codified as 1, 2, 3, 4, respectively.

If we denote by  $p_{ij}$  the probability of occurrence of nucleotide  $j$  in the  $i$  sequence ( $i = 1, 2$ ), the previous question can be translated into a hypothesis testing framework as

$$H_0 : p_{1j} = p_{2j}, \forall j = 1, 2, 3, 4 \quad v.s. \quad H_1 : \exists j \quad p_{1j} \neq p_{2j}$$

This is a test of **homogeneity of populations**.

### Example 5:

Representing by  $X_{ij}$  the random variable which counts the number of nucleotides of type  $j$  in the  $i$  sequence, and by  $E_{ij}$  the expected number of nucleotides of type  $j$  in the  $i$  sequence (under the null hypothesis), we can construct the  $\mathbf{X}^2$  as before

$$\chi^2 = \sum_{i=1}^2 \sum_{j=1}^4 \left[ \frac{(X_{ij} - E_{ij})^2}{E_{ij}} \right]$$

and compare the observed value with the adequate quantile of a  $\chi^2$  distribution with we have  $(r - 1) \times (c - 1) = 1 \times 3 = 3$  degrees of freedom.

In the present case we have the table with the observed frequencies and, in brackets, the expected frequencies under the null hypothesis:

	$X_{i1}$	$X_{i2}$	$X_{i3}$	$X_{i4}$	Total
sequence 1	273 (277)	258 (251)	233 (239.5)	236 (232.5)	1000
sequence 2	281 (277)	244 (251)	246 (239.5)	229 (232.5)	1000
Total	554	502	479	465	2000

The observed value of  $\chi^2$  is 0.964 and the p-value is 0.8099, showing a strong evidence not to reject the hypothesis that the two sequences are homogeneous.

```
> table<-matrix(c(273,281,258,244,233,246,236,229),2,4)
> dimnames(table)<-list(c("seq1","seq2"),c("A","G","C","T"))
> chisq.test(table)
```

Pearson's Chi-squared test

data: table

X-squared = 0.9642, df = 3, p-value = 0.8099

```
> chisq.test(table)$expected
```

	A	G	C	T
seq1	277	251	239.5	232.5
seq2	277	251	239.5	232.5

# The Chi-Squared Test of Independence

The  $\chi^2$  test statistic is commonly used to test the null hypothesis that two criteria (A and B) of classification, when applied to the same set of entities, are independent. Data are organized in the form of two-way table, with  $r$  rows and  $c$  as it was previously seen.

If we represent by  $p_{ij}$  the probability that an entity falls in  $i$  category of B and  $j$  category of A,  $p_{i\bullet}$  the probability that an entity falls in  $i$  category of B and  $p_{\bullet j}$ , the probability that an entity falls in  $j$  category of A, then the hypothesis of independence is

$$H_0 : p_{ij} = p_{i\bullet}p_{\bullet j}, \quad \forall i, j$$

The alternative is that there exists at least one pair  $(i, j)$  for which the joint probability is different from the product of the marginals.

The test statistic is again

$$\chi^2 = \sum_{i=1}^r \sum_{j=1}^c \left[ \frac{(X_{ij} - E_{ij})^2}{E_{ij}} \right],$$

which, under the null hypothesis of independence, follows approximately a  $\chi^2$  distribution with  $(r - 1) \times (c - 1)$  degrees of freedom.

### Example 6: A statistical test for Markov independence

Nucleotides at adjoining DNA sites are often dependent, and a first-order Markov model fits data significantly better than the independence model. A statistical test for Markov independence is an association test in a  $4 \times 4$  contingency table.

Suppose that  $X_{ij}$  is the number of times, in a DNA sequence of interest, that a nucleotide of type  $j$  is followed by a nucleotide of type  $i$  in a sequence of length  $N$ . Then we can use the  $\chi^2$  test described before to test if there is association of nucleotides or not.

What conclusion would you have drawn if you had observed the following data?

nucleotide at site  $k+1$

		A	G	C	T	Total
nucleotide at site k	A	67	70	55	48	240
	G	80	60	70	65	275
	C	58	72	80	66	276
	T	60	74	60	82	276
	Total	265	276	265	261	1067



The table of expected frequencies under the hypothesis of independence is:

	<i>A</i>	<i>G</i>	<i>C</i>	<i>T</i>	Total
<i>A</i>	59.61	62.08	59.61	58.70	240
<i>G</i>	68.30	71.13	68.30	67.27	275
<i>C</i>	68.55	71.39	68.55	67.51	276
<i>T</i>	68.55	71.39	68.55	67.51	276
Total	265	276	265	261	1067

The observed value of  $\chi^2$  is 17.01 and p-value 0.04851. We would reject the hypothesis of independence at the 5% level but not at the 1% level.

```
> table<-matrix(c(67,80,58,60,70,60,72,74,55,70,80,60,48,65,66,82),4)
> dimnames(table)<-list(c("A","G","C","T"),c("A","G","C","T"))
> chisq.test(table)
```

### Pearson's Chi-squared test

```
data:  table
X-squared = 17.0132, df = 9, p-value = 0.04851
```

```
> chisq.test(table)$expected
```

	A	G	C	T
A	59.60637	62.08060	59.60637	58.70665
G	68.29897	71.13402	68.29897	67.26804
C	68.54733	71.39269	68.54733	67.51265
T	68.54733	71.39269	68.54733	67.51265

## Parametric tests

- Only for quantitative data.
- Assume that the data come from a population which follows a certain distribution (Normal distribution).
- Assuming equal variances and unequal variances.
- More powerful.

## Non-parametric tests:

- Do not assume Normal distribution
- No variance assumption
- Decrease effects of outliers (robust)
- Not recommended if there is less than 5 replicates per group
- Less powerful

### 3. The Multiple Testing Problem

# The multiple comparison problem

$V$ : r.v. which represents the number of false positives among  $m$  (multiple hypotheses),

$$P(V \geq 1) = 1 - P(V = 0) = 1 - (1 - \alpha)^m,$$

where  $\alpha$  is the probability of rejecting the null hypothesis when it is true (Type I Error):  $P(\text{Rej } H_0 | H_0 \text{ True})$ .

Number of hypotheses tested ( $m$ )	False positives incidence ( $m \times \alpha$ )	Probability of 1 or more false positives by chance ( $1 - (1 - 0.05)^m$ )
1	$1/20 = 0.05$	0.050
2	$2 \times (1/20) = 0.1$	0.098
20	$20 \times (1/20) = 1$	0.642
100	$100 \times (1/20) = 5$	0.994

**Problem:** When many hypotheses are tested, the probability of a type I error (false positive) increases sharply with the number of hypotheses.

# Types of error control

- $H_{01}, H_{02}, \dots, H_{0m}$  denote the null hypotheses corresponding to the  $m$  tests.
- Suppose  $m_0$  null hypotheses are true and  $m_1$  null hypotheses are false.
- $p_1, p_2, \dots, p_m$  denote the p-values corresponding to the  $m$  tests.
- $c$  denote a value between 0 and 1 that will serve as a cutoff for significance:
  - Reject  $H_{0i}$  if  $p_i \leq c$  (declare significant findings)
  - Do not reject  $H_{0i}$  if  $p_i > c$  (declare non-significant findings)

	Rejected $H_0$	Not Rejected $H_0$	
True $H_0$	$V$	$U$	$m_0$
False $H_0$	$S$	$T$	$m_1$
	$R$	$m - R$	$m$

$V$ : false positives (type I error)

$T$ : false negatives (type II error)

- **PCER:** Per-comparison error rate, the expected value of the number of Type I errors over the number of hypotheses,  $PCER = E(V)/m$ .
- **PFER:** Per-family error rate, the expected number of Type I errors,  $PFER = E(V)$ .
- **FWER:** Family-wise error rate: the probability of at least one type I error,  $FWER = P(V \geq 1)$ .
- **FDR:** False discovery rate, is the expected proportion of incorrectly rejected null hypotheses,  $FDR = E(V/R)$  for  $R > 0$ .



# FWER - Family Wise Error Rate

- ➊ Many procedures have been developed to control the Family Wise Error Rate (the probability of at least one type I error):  $P(V \geq 1)$
- ➋ Two general types of FWER corrections:
  - ➊ Single Step: equivalent adjustments made to each p-value.
  - ➋ Sequential: adaptive adjustment made to each p-value.

## Single-step approach: Bonferroni

- The Bonferroni Method is the simplest way to achieve control of the FWER at any desired level  $\alpha$ .
- Simply choose  $c = \alpha/m$ .
- With this value of  $c$ , the FWER will be no larger than  $\alpha$  for any family of  $m$  tests.

## How to do this in R?

There are several packages for multiple testing correction, as for example:

- `p.adjust` (the simplest – available at `stats` package)
- `multtest` (the most popular for gene expression data)
- `qvalue`
- `fdrtool`
- `structSSI` (for hypotheses with hierarchical or group structure)

In this example, we will apply `p.adjust`.

Read the data:

```
> raw.p.values<-c(0.042,0.001,0.031,0.014,0.007)
> raw.p.values
[1] 0.042 0.001 0.031 0.014 0.007
```

Adjust the p-values:

```
> corr.p.values.Bonf<-p.adjust(p=raw.p.values,"bonferroni")
> corr.p.values.Bonf
[1] 0.210 0.005 0.155 0.070 0.035
```

Visualize tests corresponding to the rejected null hypotheses (position of the corrected p-value in the vector):

```
> global.alpha<-0.05
> which(corr.p.values.Bonf<global.alpha)
[1] 2 5
```

## Sequential Adjustments: Holm's method

- Let  $p_{(1)}, p_{(2)}, \dots, p_{(m)}$  denote the  $m$  p-values ordered from smallest to largest.
- Find the **largest** integer  $k$  so that:

$$p_{(i)} \leq \frac{\alpha}{m-i+1} \text{ for all } i = 1, \dots, k.$$

- If no such  $k$  exists, set  $c = 0$  (declare nothing significant).

## How to do this in R?

Adjust the p-values:

```
> corr.p.values.Holm<-p.adjust(p=raw.p.values,"holm")
```

```
> corr.p.values.Holm
```

```
[1] 0.062 0.005 0.062 0.042 0.028
```

Visualize tests corresponding to the rejected null hypotheses (position of the corrected p-value in the vector):

```
> which(corr.p.values.Holm<global.alpha)
```

```
[1] 2 4 5
```

## Some considerations

- FWER is appropriate when you want to guard against ANY false positives.
- However, in many cases (particularly in genomics) we can live with a certain number of false positives.
- FWER criteria may be too restrictive because control of false positives implies a considerable increase of false negatives.
- FWER is too conservative because it depends on the overall number of tests ( $m$ ).
- Holm's method is less conservative than the Bonferroni method.
- The methods will provide the same results for many data sets, but sometimes Holm's method will result in more rejected null hypotheses.

# FDR - False Discovery Rate

In practice, however, many biologists seem willing to accept that some errors will occur, as long as this allows findings to be made. For example a researcher might consider acceptable a small proportion of errors (say 10%, 20%) between his findings. In this case, the researcher is expressing interest in controlling the **false discovery rate** (FDR).



	Rejected $H_0$	Not Rejected $H_0$	
True $H_0$	$V$	$U$	$m_0$
False $H_0$	$S$	$T$	$m_1$
	$R$	$m - R$	$m$

$V$ : false positives (type I error)

$T$ : false negatives (type II error)

- FDR is designed to control the proportion of false positives among the set of rejected hypothesis ( $R$ )
- $$FDR = \frac{E(V)}{R}$$

## The Benjamini and Hochberg Procedure for Strongly Controlling FDR at Level $\alpha$

- Let  $p_{(1)}, p_{(2)}, \dots, p_{(m)}$  denote the  $m$  p-values ordered from smallest to largest.
- Find the **largest integer**  $k$  so that  $p_{(k)} \leq \frac{k \times \alpha}{m}$ .
- If no such  $k$  exists, set  $c = 0$  (declare nothing significant).

## How to do this in R?

Adjust the p-values:

```
> corr.p.values.BH<-p.adjust(p=raw.p.values,"BH")
```

```
> corr.p.values.BH
```

```
[1] 0.04200000 0.00500000 0.03875000 0.02333333 0.01750000
```

Visualize tests corresponding to the rejected null hypotheses (position of the corrected p-value in the vector):

```
> which(corr.p.values.BH<global.alpha)
```

```
[1] 1 2 3 4 5
```

# Some final considerations

## FWER vs FDR

- The decision of controlling FDR or FWER depends on the goals of the experiment.
- If the objective is *gene fishing*, allowing a certain number of false positives to be reasonable, then FDR is preferable.
- If instead one is working with a shorter number of hypotheses, in which we want to verify if some specific ones are significant, then FWER is the appropriate criteria.
- FDRs are more appropriate in large sets of hypotheses.

## Remarks

- Which multiple tests correction should be used? As long as the conditions you have for the data meet with the assumptions in particular multiple tests corrections, use the one that gives the highest power. **Using an FDR method is common these days.**
- 5% (or 95% confidence) is a convention, not a magic number (same to 10% or 1%). If you do not have any particular reason to favour a particular threshold, use a convention.

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