

Example 20
Two-way Analysis of Variance (ANOVA)
Comparing more than 2 samples and
more than 2 independent variables
Setup

A botanist is trying to grow a tissue culture. She suspects that both the temperature and the type of agar that the tissue is grown on influences the growth of the tissue. She has measured the mass (mg) of tissue grown on two types of agars and at two temperatures.

Temperature	Media	Mass
Warm	Gold	238
Warm	Gold	250
Warm	Gold	288
Warm	Gold	293
Warm	Gold	320
Warm	Clear	213
Warm	Clear	262
Warm	Clear	358
Warm	Clear	391
Warm	Clear	402
Cool	Gold	100
Cool	Gold	108
Cool	Gold	110
Cool	Gold	143
Cool	Gold	145
Cool	Clear	127
Cool	Clear	128
Cool	Clear	140
Cool	Clear	165
Cool	Clear	184

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Solution

1. State your question: Does agar type, temperature, or their interaction influence tissue culture growth?
 - a. Is it a good scientific question? Definable, measurable, and controllable
 - b. Identify your population: Growth of tissue cultures
 - c. Identify your dependent variable: Culture growth
 - d. Identify your independent variable: Agar type and temperature
2. State your hypothesis set
 - a. Verbal hypothesis: Agar type and temperature influence culture growth.
 - b. Statistical hypothesis (H_0 , H_A).
 - H_0 : Agar type does not influence tissue culture growth
($\mu_{\text{gold}} = \mu_{\text{clear}}$)
 - H_A : Agar type does influence tissue culture growth
($\mu_{\text{gold}} \neq \mu_{\text{clear}}$)

 - H_0 : Temperature does not influence tissue culture growth
($\mu_{\text{warm}} = \mu_{\text{cool}}$)
 - H_A : Temperature does influence tissue culture growth
($\mu_{\text{warm}} \neq \mu_{\text{cool}}$)

 - H_0 : There is no interaction of agar and temperature that influences tissue culture growth.
 - H_A : There is an interaction of agar and temperature that influences tissue culture growth.
 - c. Is your hypothesis set exhaustive? Yes
 - d. Is your hypothesis set exclusive? Yes
3. State your significance level: $\alpha=0.05$
4. Select the appropriate test/
 - a. Variable scales
 - i. Dependent variable: Ratio
 - o Converted or transformed? No
 - ii. Independent variable (agar): Nominal
 - o Converted or transformed? No
 - iii. Independent variable (temperature): Nominal
 - o Converted or transformed? No
 - b. What information is given or available?
 - i. Sample data

- c. Number of samples: 4
- d. Are the data paired or unpaired: Unpaired
- e. What aspect of the variable do you want to compare?
 - i. Central tendency – Means
- f. State the test to be used: Two-Way Analysis of Variance
 - i. Are the assumptions of the test met?
 - o Random sample – Assumed
 - o Independent sample – Assumed
 - o Normally distributed populations – Tested
 - o Equal variances within treatments – Tested

Shapiro-Wilk normality test

data: TissueWG\$Mass
 W = 0.9411, p-value = 0.6736

Shapiro-Wilk normality test

data: TissueWC\$Mass
 W = 0.8811, p-value = 0.3144

Shapiro-Wilk normality test

data: TissueCG\$Mass
 W = 0.8215, p-value = 0.12

Shapiro-Wilk normality test

data: TissueCC\$Mass
 W = 0.8813, p-value = 0.3153

Levene's Test for Homogeneity of Variance (center = median)

group	Df	F value	Pr(>F)
	3	2.2891	0.1175
	16		

5. Conduct your sampling

The researcher grew five tissue cultures on the four combinations of agar type and temperature. Data is provided

6. Graph the data

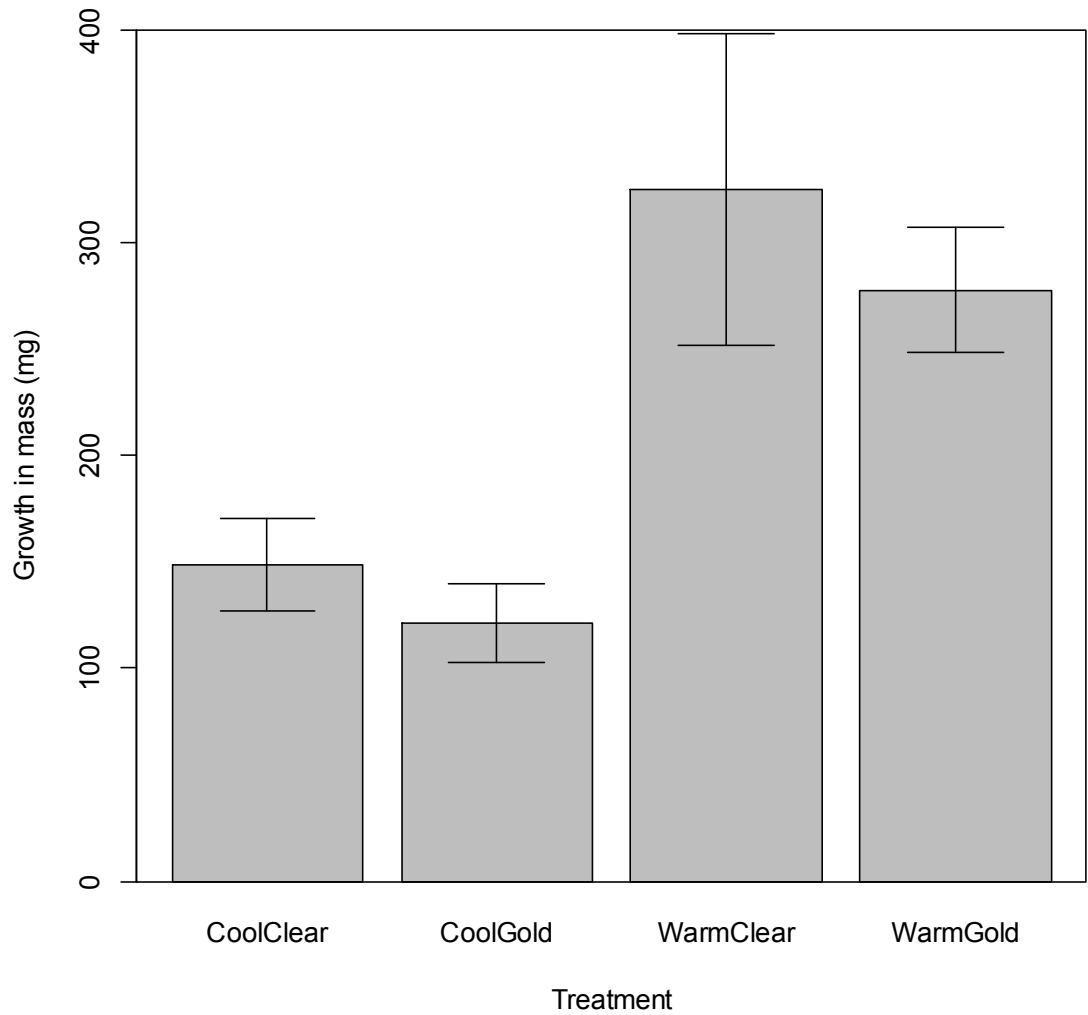


Figure 1. Tissue cultures growth in mass (mg) on two different media (clear and gold) and incubated at two temperatures (cool and warm).

7. Summarize the data.

Cool Clear

$n = 5$

$\bar{x} = 148.8$ mg

$s = 24.0$ mg

Cool Gold

$n = 5$

$\bar{x} = 121.2$ mg

$$s = 21.2 \text{ mg}$$

Warm Clear

$$n = 5$$

$$\bar{x} = 325.2 \text{ mg}$$

$$s = 83.5 \text{ mg}$$

Warm Gold

$$n = 5$$

$$\bar{x} = 277.8 \text{ mg}$$

$$s = 33.4 \text{ mg}$$

8. Calculate your test statistic.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Tissue\$Media	1	7031	7031	3.071	0.0989 .
Tissue\$Temperature	1	138611	138611	60.534	7.94e-07 ***
Tissue\$Media:Tissue\$Temperature	1	490	490	0.214	0.6499
Residuals	16	36637	2290		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

9. Retain or reject your null hypothesis based on your test statistic.

Since the calculated p-value (0.0989) is greater than the significance level (0.05), we retain the media null hypothesis and reject the media alternate hypothesis.

Since the calculated p-value (7.49e-07) is less than the significance level (0.05), we reject the temperature null hypothesis and retain the temperature alternate hypothesis.

Since the calculated p-value (0.6499) is greater than the significance level (0.05), we retain the interaction null hypothesis and reject the interaction alternate hypothesis.

10. Interpret the results in biological terms.

While temperature does influence the growth of the tissue culture (F=60.53, df=1, 16, p<0.001), the media tested did not (F=3.07, df=1, 16, p=0.099) and there was no significant interaction between temperature and media type (F=0.21, df=1, 16, p=0.650).