

BM5033 Statistical Inference Methods in Bioengineering

Exam 3 Solution

- In a trial for an anti-cancer drug 10 cancer patients were selected based on some criteria. The size of the tumors in patients were measured and the drug was administered for 3 months after which the tumor sizes were measured again. Following are the measurements

Before, $x =$	22	32	27	59	22	40	31	10	38	25
After, $y =$	26	30	24	38	24	30	27	18	18	25

Table 1: Tumor size (in cc) at the start and end of the trial.

This situation will be referred to in some of the questions below.

- Some useful R functions

- `t.test(x, y = NULL, alternative = c("two.sided", "less", "greater"), mu = 0, paired = FALSE, var.equal = FALSE, conf.level = 0.95)`
- `power.t.test(n = NULL, delta = NULL, sd = 1, sig.level = 0.05, power = NULL, type = c("two.sample", "one.sample", "paired"), alternative = c("two.sided", "one.sided"))`
- `wilcox.test(x, y = NULL, alternative = c("two.sided", "less", "greater"), mu = 0, paired = FALSE, exact = NULL, correct = TRUE, conf.level = 0.95)`

Questions

- One can have higher statistical power by **(2)**
 - by increasing minimum detectable difference ✓
 - by increasing α ✓
 - by having larger population variance
 - by increasing sample size ✓
- In the experimental scenario described at the beginning of this exam, we wish to check if the use of the drug decreases tumor size. If the experiment had samples of size 20 (instead of 10) while keeping everything else the same **(2)**
 - the statistical power would low
 - nothing changes
 - Type-I error rate would be low
 - Type-II error rate would be low ✓
- Mann-Whitney U test **(2)**
 - is used to compare median values of two samples ✓
 - can be used for ordinal data ✓
 - is a non-parametric counterpart to two sample t -test ✓
 - requires samples to be normally distributed

4. For a sample with positively skewed distribution **(2)**

- (a) we can use t -test to perform one sample hypothesis testing
- (b) we cannot perform one sample hypothesis test
- (c) mean, median and mode are different ✓
- (d) mean, median and mode take the same value

5. Suppose in a statistical analysis we have rejected the null hypothesis even when it was true. Mark all the correct statements below **(2)**

- (a) We have committed type-II error
- (b) The probability of committing this error is β
- (c) We have committed type-I error ✓
- (d) The probability of committing this error is α ✓

6. In the experimental scenario described at the beginning of this exam, we wish to check if the use of the drug decreases tumor size. For this, write down the null and alternative hypotheses **(4)**

$H_0 : \mu_d = 0$, $H_a : \mu_d > 0$ where μ_d is the mean of the difference in tumor sizes $x_{\text{after}} - x_{\text{before}}$

Note: Any other variant of this answer is also acceptable.

7. Write the R command needed to estimate the statistical power if the experimentalist expects to see a decrease in tumor size by 8 cc. You can assume the population standard deviation to be 3 cc, and they only have 10 patients. **(6)**

```
power.t.test(n = 10, delta = 8, sd = 3, sig.level = 0.05, power = NULL,
type = "paired", alternative = "one.sided")
```

8. Write the R command needed to test the hypothesis described in the previous question. Your code should assume the data are stored in variables x and y . Justify your answer in 20 words. **(6)**

```
t.test(x=x, y=y, alternative="greater", mu=0, paired=TRUE)
```

We have to perform a paired comparison since the two measurements are not independent.

9. Suppose the output of the command you answered in the last question gives out the p-value to be 0.21. What is your conclusion? Be precise in your answer. **(2)**

For $\alpha = 0.05$, the p-value is $> \alpha$. We fail to reject H_0 .

10. What is your conclusion if the p-value turns out to be 0.002? Be precise in your answer. **(2)**

For $\alpha = 0.05$, the p-value is $< \alpha$. We reject $H_0 \Rightarrow$ the drug decreases tumor size.

11. Suppose during the anti-cancer trial (described at the beginning) two of the patients drop out for some undisclosed reason. The final data looks like this

Before, $x =$	22	32	27	59	22	40	31	10	38	25
After, $y =$	26	30	24	38	NA	30	27	NA	18	25

Table 2: Tumor size (in cc) at the start and end of the trial.

How will you check for the effectiveness of the drug as compared to your answer to question 8 ? **(6)**

We have to drop the x values for the subjects we do not have y values from. The rest is the same as answered in Q-8.

12. A tissue engineering lab is evaluating an electrospun synthetic polymer scaffold for vascular graft applications. To optimize cell attachment and proliferation, researchers treat the scaffold surface with one of three Plasma Surface Treatments (Oxygen, Nitrogen, or Argon) and seed the scaffolds with primary endothelial cells under two distinct Pre-Conditioning Flow Environments (Static vs. Dynamic). A total of 30 identical scaffold samples ($n = 5$ per treatment combination) are evaluated. After 72 hours, cell proliferation is measured using an MTT metabolic assay (absorbance at 570 nm). The following statistical analysis was conducted in R

```
model <- aov(Absorbance ~ Plasma * Flow, data = scaffold_data)
summary(model)
TukeyHSD(model, conf.level = 0.95)
```

which produced following output

```
> summary(model)
      Df Sum Sq Mean Sq F value    Pr(>F)
Plasma   2  1.4250   0.7125    28.50 6.12e-07 ***
Flow     1  0.8100   0.8100    32.40 6.98e-06 ***
Plasma:Flow 2  0.3600   0.1800     7.20 0.00356 **
Residuals 24  0.6000   0.0250
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

and

```
TukeyHSD(model, conf.level = 0.95)
Tukey Multiple Comparisons of Means
95% family-wise confidence level
```

```
Fit: aov(formula = Absorbance ~ Plasma * Flow, data = scaffold_data)
```

```
$Plasma
      diff      lwr      upr      p adj
Nitrogen-Argon  0.150 -0.0125814 0.3125814 0.074121
Oxygen-Argon    0.500  0.3374186 0.6625814 0.000003
Oxygen-Nitrogen 0.350  0.1874186 0.5125814 0.000048

$Flow
      diff      lwr      upr      p adj
Static-Dynamic -0.300 -0.4081658 -0.1918342 0.000007

$`Plasma:Flow`
      diff      lwr      upr      p adj
Nitrogen:Static-Argon:Static  0.000 -0.2982848 0.2982848 1.0000000
Oxygen:Static-Argon:Static    0.300  0.0017152 0.5982848 0.0482120
Argon:Dynamic-Argon:Static    0.100 -0.1982848 0.3982848 0.8924150
Nitrogen:Dynamic-Argon:Static 0.400  0.1017152 0.6982848 0.0041280
Oxygen:Dynamic-Argon:Static    0.800  0.5017152 1.0982848 0.0000010
Oxygen:Static-Nitrogen:Static  0.300  0.0017152 0.5982848 0.0482120
Argon:Dynamic-Nitrogen:Static  0.100 -0.1982848 0.3982848 0.8924150
Nitrogen:Dynamic-Nitrogen:St  0.400  0.1017152 0.6982848 0.0041280
Oxygen:Dynamic-Nitrogen:Static 0.800  0.5017152 1.0982848 0.0000010
Argon:Dynamic-Oxygen:Static   -0.200 -0.4982848 0.0982848 0.3421800
Nitrogen:Dynamic-Oxygen:Static 0.100 -0.1982848 0.3982848 0.8924150
Oxygen:Dynamic-Oxygen:Static   0.500  0.2017152 0.7982848 0.0003120
Nitrogen:Dynamic-Argon:Dynamic 0.300  0.0017152 0.5982848 0.0482120
Oxygen:Dynamic-Argon:Dynamic   0.700  0.4017152 0.9982848 0.0000040
Oxygen:Dynamic-Nitrogen:Dynam  0.400  0.1017152 0.6982848 0.0041280
```

(a) What conclusion do you draw from the output of aov? Be concise in your answer. (4)

From ANOVA output we can conclude that all plasma surface treatments do not result in the same cell proliferation. The pre-conditioning flow environment also affects cell proliferation. There is also statistical evidence for some interaction between the two.

- (b) Which specific surface treatments differ significantly from one another at $\alpha = 0.05$, and which do not? **(6)**

From post-hoc analysis we see that Oxygen treatment results in different cell proliferation as compared to that with Nitrogen and Argon.

- (c) If the engineering objective is to maximize cell proliferation while minimizing bio-processing cost, evaluate whether transitioning from Nitrogen to Oxygen plasma treatment yields a statistically significant benefit under static vs. dynamic conditions. **(8)**

For $\alpha = 0.05$, under both static and dynamic flow conditions, the difference between the cell proliferation with Oxygen and Nitrogen treatment is different (p-values 0.048 and 0.004, respectively).

Note: In static conditions, the p-value is close to α .

13. A biomedical engineering laboratory is developing an injectable, 3D-bioprinted composite hydrogel scaffold designed to accelerate critical-sized bone defect repair. A major bottleneck in scaffold design is ensuring uniform vascularization throughout the construct to prevent core necrosis before functional tissue forms. To evaluate tissue regeneration efficacy, the research team recruits 8 healthy human donors to provide bone marrow aspirates from which primary human Mesenchymal Stem Cells (hMSCs) are isolated to represent the broader human patient population. For each individual donor, 3 independent 3D-bioprinted scaffold constructs are fabricated. Each scaffold construct is then prepared under one of three distinct polymer matrix formulations (GelMA-only, GelMA/Alginate, and GelMA/Nanoclay) and combined with one of two growth factor delivery conditions (Control [No VEGF] vs. VEGF-Enriched), resulting in 6 unique treatment combinations per scaffold. In total, 144 experimental runs (8 cell donors $\times$ 3 scaffold constructs per donor $\times$ 3 matrix formulations $\times$ 2 growth factor conditions) are conducted in an in vitro bioreactor. After 21 days of culture, the primary outcome metric recorded for every sample is the Total Capillary Network Length (mm/mm³) inside the scaffold core.

- (a) List the factors involved in the study. **(6)**

polymer matrix formulation, growth factor delivery conditions, human subject

- (b) Is/are there any random factor in this study? If yes, name it/them. **(4)**

Yes. cell donor

- (c) What is the factorial nature (full factorial, nested factorial, etc.) of the design. Justify your answer. **(8)**

This is full factorial design where all values of the the two fixed factors have been combined with each other.

