Student's t test

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1 The problem

Do you pipette the same volume of liquid with a filter tip and a normal tip? If anything, one would expect that you would pipette a smaller volume with filter tips because the filter would hinder the suction. Is that the case?

To answer that question I took my P200, pipetted 100 μL of water 5 times with filter tips and 5 times with normal tips and carefully weighed the water with a precision scale. Here is the data, where the numbers represent the volume in μL.

```
> # Copy the data in your R session.
> filter_tips <- c(96.2, 99.0, 98.3, 98.0, 98.7);
> normal_tips <- c(96.6, 98.9, 97.9, 97.1, 97.7);
```

Exercise 1

Together, formulate the problem in mathematical terms. How do we approach the problem? What assumptions do we have to make? Discuss their likelihood.

2 Statistical tests

Intuitively, we would like to know if the observed difference of means pipetted volume could occur at random. For this, we need to know more about this 'at random', and in particular, we need to know more about the behaviour of the mean under the asumption of randomness.

The fundamental idea of statistical tests is to decide whether the outcome of an experiment can be produced by sheer randomness. To this end, every test has a **statistic** (note the absence of s at the end) that measures an effect. The mean and the difference of means between two samples are examples of statistics. Tests also have a **null hypothesis** that allows to predict the distribution of the statistic under the assumption of randomness. All tests also have an alternative hypothesis, the one that will be accepted if the null is rejected. Finally, tests have a decision rule allowing to decide whether the null hypothesis is accepted.

Definition 1 (statistical test)**.** A statistical test is a protocol consisting of:

1. A null hypothesis.

2. An alternative hypothesis.
3. A protocol to collect data.
4. A test statistic.
5. A decision rule.

Exercise 2

Together, discuss which are good and bad choices for these items, either in general or for the proposed problem.

3 Gaussian variables

We need to get a good view of the possible outcomes under the assumption of randomness, *i.e.* under the null hypothesis. For this, we have to look into Gaussian variables.

The function `rnorm` is used to generate Gaussian random variables. Each time you invoke it, you get a different result.

```
> rnorm(5); # Standard Gaussian sample.

[1]  1.53692414 -0.29657556 -0.75560942 -0.53001983  0.01449643

> rnorm(5); # Same input, different output.

[1]  1.129708979  0.647003595  0.007945681  0.398744351 -1.208667672

> rnorm(5, mean=1.5, sd=2.3);

[1] -5.1059392  1.3714179  3.0780552  2.1063497 -0.8249848
```

We will make extensive use of `rnorm` in what follows.

Exercise 3

Use the functions `mean` and `rnorm` to compute the mean of a random sample of size 5 from a standard Gaussian variable. Compute the difference between the means of two samples of size 5.

Exercise 4

Now do the same 10,000 times to generate a sample of size 10,000 consisting of the difference of means of two standard Gausissan samples of size 5. Store the result in a vector called `diffmeans`.
Hint: Use a `for` loop.

Exercise 5

Compute the standard deviation of a standard Gaussian random sample of size 10, 100, 1000 and 10,000. What do you observe?

Exercise 6

Compute the standard deviation of a subsample of size 10, 100, 1000 and 10,000 from the sample generated at exercise 4. What do you observe?
Hint: You can use the function `sample`.
For the aces: To what value do the numbers converge?

Exercise 7

Plot the density of a random sample of size 10,000 from a standard Gaussian random variable. Plot the density of the sample generated at exercise 4 (`diffmeans`) on the same graph. What do you observe?
Hint: `plot(density(...))` and `lines(density(...))` will help.

Exercise 8

Compute the standard deviations of samples of size 10,000 consisting of the differences of averages of sample of sizes 5 and 5, 50 and 50, 500 and 500, 5,000 and 5,000 from standard Gaussian random variables. What do you observe?

4 Estimating the standard deviation

The estimated standard deviation of a sample $(x_1, x_2, ..., x_n)$, as computed by the function `sd` is defined by the formula:

$$S = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2}, \text{ where } \bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i. \tag{1}$$

This value is an estimator of the true standard deviation, and is itself subject to random variation, as shown in exercise 5. For large samples, this value will be close to expected, but for small samples it can be far off-target.

Exercise 9

Assume independent sampling of $(x_1, ..., x_n)$ from a distribution with standard deviation σ. What is the standard deviation of the estimated sample mean? Suggest an estimator for the standard deviation of the mean of a sample.
Hint: Recall that $Var(aX) = a^2 Var(X)$. Besides, if X and Y are independent, $Var(X + Y) = Var(X) + Var(Y)$.

Exercise 10

Generate 10,000 standard Gaussian random samples of size 5 and computre their estimated standard deviations. Plot the histogram of these estimated standard deviations. Does this look Gaussian?
Hint: Take inspiration from exercise 4 to generate the sample, and use `hist`.

5 Effect size

We are confronted with the difficulty of estimating the distribution of the mean with an imprecise value of the standard deviation.

Exercise 11

Together, suggest a way to go around this difficulty. If estimating two parameters at the same time is too difficult, isn't there a way to work with a single number?

Exercise 12

Generate 10,000 standard Gaussian samples of size 5 and compute their effect size. Plot the density of their distribution. Is it Gaussian?

Exercise 13

In the case of two samples $(x_1, ..., x_n)$ and $(y_1, ..., y_n)$ sampled from a distribution with the same standard deviation, what is the effect size for the difference of means?
Hint: What is the variance of the mean? What are the variance and standard deviation of the difference of means?
For the aces: Can you compute the effect size if the samples have different sizes?

Exercise 14

Suppose $(x_1, ..., x_n)$ and $(y_1, ..., y_n)$ are sampled from the same population with mean 0 and variance 1. Let's transform the samples by adding a constant a, *i.e.* $\tilde{x}_i = x_i + a$ and $\tilde{y}_i = y_i + a$. How does this change the effect size? Let's further transform the sample by multiplying the values by a constant b, *i.e.* $\mathring{x}_i = b\tilde{x}_i$ and $\mathring{y}_i = b\tilde{y}_i$. How is the effect size changed this time?
Hint: Remember that the variance (or estimated variance) of $X + a$ is the variance (or estimated variance) of X.

6 The null hypothesis

We now have a statistic for the test with a very useful property: **it is invariant by shifting and scaling**. This means that we can study the effect size on a standard Gaussian distribution with mean 0 and variance 1. All the Gaussian distributions are obtained by shifting and scaling the standard Gaussian, so the distribution of the effect size computed from any Gaussian distribution will be exactly the same as the distribution of the effect size computed from the standard Gaussian.

Let's make sure of that, and see what happens when the null hypothesis is not true.

Exercise 15

Generate a random sample of size 10,000 consisting of the effect size designed in exercise 13 for two standard Gaussian samples of size 5. Store the result in a vector called `esize`.

Exercise 16

Repeat the task of exercise 15 with other Gaussian random variables *i.e.* change the `mean` and `sd` parameters of `rnorm`, but keep the parameters equal

for both samples (*i.e.* assume that the null hypothesis is true). Overlay the obtained densities of the effect size with the one obtained for the standard Gaussian.
For the aces: Use different parameters for both populations (*i.e.* assume that the null hypothesis is false).
Note: Keep a sample size of 5.

7 The decision rule

We have almost everything. We just need to find a decision rule that makes sense. Several criteria have been proposed by different statistical schools. We will use the widespread frequentist approach.

The frequentist approach is to choose a level of risk, usually called α, that is the probability of rejecting the null hypothesis, given that it is actually true. The most commonly encoutered values for α are 0.05 and 0.01, but both are abitrary (there is no particular reason why these values are better than others). Based on that, we delineate a **rejection region** at level α, *i.e.* a set of statistic values that has a probability of occurrence equal to α if the null hypothesis is true.

Definition 2 (level of a test)**.** The level of a test is the probability of rejecting the null hypothesis given that it is true.

Exercise 17

Set $\alpha = 0.05$. What is the most meaningful rejection region for the effect size? Give a numerical estimate of that region.
Hint: Use the results of exercise 15.

Exercise 18

Compute the effect size from the measurements and store the result in a vector called `t`. Apply the decision rule and conclude whether or not to accept the null hypothesis.

8 The p-value

You may have noticed that the p-value is not an ingredient of a statistical test (see definition 1). The conclusion of a statistical test is always to accept or reject the null hypothesis, never to be in doubt, borderline significant, call for more data or let the readers judge by themselves. In that sense, true statistical tests are a rarity of scientific litterature.

The idea of the p-value is to let the audience judge by themselves if a test should be rejected or not. If two statisticians work at different levels α, there is no reason why one should impose her conclusions on the other. The introduction of the p-value of a test came with the advent of computers. When only statistical tables were available, it was tedious to compute it and therefore accepted to use the level α as a reference.

By definition, the p-value of a test is the probability of observing a larger test statistic than the one you measured, assuming that the null hypothesis is true. Here, 'larger' is usually defined by the context. For example, in the case of the t test designed previously, it means 'larger in absolute value'.

Exercise 19

Suppose that a test has a statistic θ, and that the rejection region is always of the form $\theta > s(\alpha)$, where the threshold depends on the level α. If the p-value of the test is 0.03, what does that mean for the decision rule at level 0.05 and 0.01? What is the largest level α for which the null hypothesis would be accepted?

Exercise 20

Give an estimate of the p-value of the test.
Hint: Use the results of exercise 15.

Exercise 21

The `R` command to perform a t test is `t.test`. Use it to test the null hypothesis and compare the results with the ones you obtained previously.
Hint: Don't forget to check `?t.test` or `help(t.test)` if in doubt.

9 Power of the test

We have seen how statistical tests work if the null hypothesis is true. But what if the null hypothesis is false? Well, if there is a risk α, one might expect that there also exists a risk β, and that is the probability of accepting the null hypothesis given that it is false.

The level α is always easy to know, or at least to approximate, but the risk β is a lot more difficult to control. Indeed, there is usually one way the null hypothesis can be true, and infinitely many ways it can be false. So the risk β always depends on **how** the null hypothesis is false.

For this reason, there is not a single risk β associated with a statistical test, but many. Actually the power of a test is not a number, but a function.

Definition 3 (power of a test)**.** The power of a statistical test is a function of hypotheses. It is the probability of rejecting the null hypothesis given that this particular hypothesis is true.

Exercise 22

Say that a test has power W, function of hypotheses denoted H. What is $W(H_0)$? What does that tell you?

Exercise 23

Call Δ the difference of volume between filter tips and normal tips. Estimate the power of the test for $\Delta = 1\mu$L, 5μL, and 10μL. To this end, generate random samples of 10,000 values of the effect size computed from two Gaussian samples of size 5, one with a mean of 100, the other with a mean of $100 - \Delta$. Use the estimate of the standard deviation from the data.
Hint: Use the threshold of the rejection region determined at exercise 17.

10 Robustness, exactness

The power of a statistical test says whether it has good chances to detect small effects *i.e.* small effet sizes in our case. Robustness is another property that is much more loosely defined. A test is said to be robust relative to a particular hypothesis if the value of the test statistic does not vary much if this hypothesis is violated. Whether a test is robust depends on your degree of tolerance, and also on how the hypothesis is violated.

An exact test gives an exact distribution of its statistic (if the null hypothesis is true). If a test is not exact, it is approximate.

Exercise 24

Is the t test exact? Suppose that the variables are not prefectly Gaussian. Is the t test still exact?

Exercise 25

We will see whether the t test is robust to the assumption of normality. For this we will use exponential variables, generated by `rexp`. You can check that the distribution of these variables is far from Gaussian. Replace Gaussian by exponential variables in the generation of a sample of the effect size (`esize`) and compare the two distributions by overlaying their density.

Answer of Exercise 1

We need to formulate **a null hypothesis** H_0. In that particular case, a good hypothesis is the following:

1. Both datasets are sampled from a Gaussian distribution.
2. The parameters are unknown, but equal in both cases.
3. Sampling is IID (**Independent** with Identical Distribution).

In the alternative hypothesis item 2 is replaced by: 'The parameters are unknown, the standard deviations are equal but the means are different.'

Answer of Exercise 3

```
> mean(rnorm(5));
> mean(rnorm(5)) - mean(rnorm(5));
```

Answer of Exercise 4

```
> diffmeans <- rep(NA, 10000);
> for (i in 1:10000) {
+    diffmeans[i] <- mean(rnorm(5)) - mean(rnorm(5));
+ }
```

Answer of Exercise 5

```
> sd(rnorm(10));
> sd(rnorm(100));
> sd(rnorm(1000));
> sd(rnorm(10000));
```

The values converge to 1. This is the theoretical standard deviation of a standard Gaussian random variable.

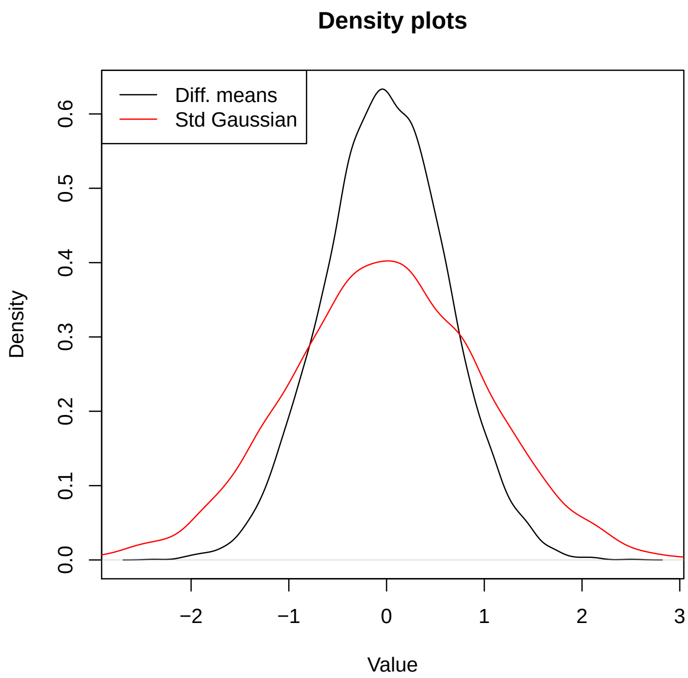
Answer of Exercise 6

```
> sd(sample(diffmeans, size=10));
> sd(sample(diffmeans, size=100));
> sd(sample(diffmeans, size=1000));
> sd(sample(diffmeans, size=10000));
```

The values converge to some number smaller than 1. You can check that it is close to $\sqrt{2/5}$.

Answer of Exercise 7

```
> plot(density(diffmeans), main="Density plots", xlab="Value");
> lines(density(rnorm(10000)), col="red");
> legend(x="topleft", lwd = 1, col=c("black", "red"),
+    legend=c("Diff. means", "Std Gaussian"));
```



Both density plots have the same shape. Actually, they are both Gaussian, but the density of differences of means has smaller standard deviation, as shown by the fact that it is narrower.

Answer of Exercise 8

```
> samples <- list();
> for (i in c(5, 50, 500, 5000)) {
+    for (j in 1:10000) {
+       samples[[as.character(i)]][j] <- mean(rnorm(i))-mean(rnorm(i));
+    }
+ }
> lapply(samples, sd);
```

The standard deviation converges to 0. It has to be clear for you that the standard deviation of a Gaussian random sample does **not** converge to 0 as the sample size increases. It is **the standard deviation of the estimated mean**, or the **standard deviation of the difference of estimated means** that converges to 0. The estimate of the difference of means is more and more accurate as the sample size increases.

Answer of Exercise 9

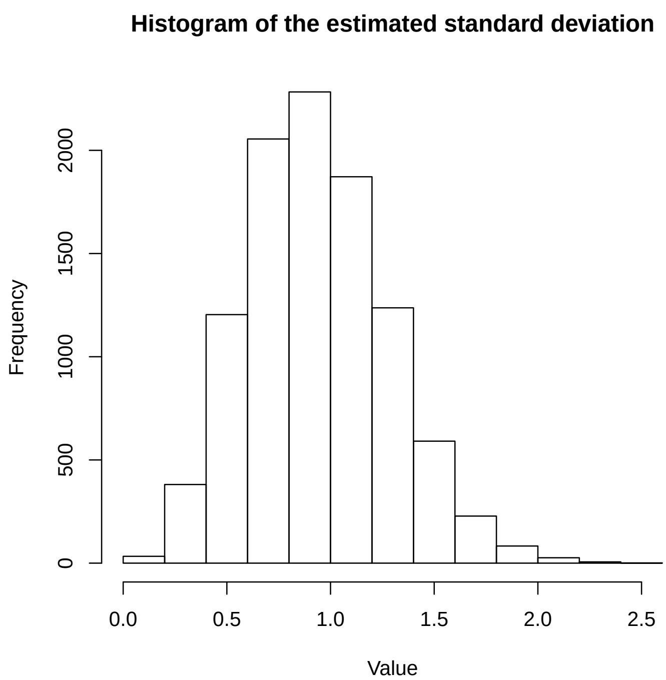
The variance of the distribution is σ^2. The variance of x_i/n is σ^2/n^2 Because of independence, the variance of $x_1/n + ... + x_n/n$ is $n\sigma^2/n^2 = \sigma^2/n$, which is the variance of the estimated mean. The standard deviation is found by taking the square root: $\sqrt{\sigma^2/n} = \sigma/\sqrt{n}$.

Based on this result, the natural estimator for the standard deviation of the mean of a sample comes as:

$$\frac{S}{\sqrt{n}} = \sqrt{\frac{1}{n(n-1)} \sum_{i=1}^{n} (x_i - \bar{x})^2}. \tag{2}$$

Answer of Exercise 10

```
> stddevs <- rep(NA, 10000);
> for (i in 1:10000) {
+     stddevs[i] <- sd(rnorm(5));
+ }
> hist(stddevs, main="Histogram of the estimated standard deviation",
+     xlab="Value");
```



The distribution of the estimated standard deviation is not Gaussian. Because the standard deviation is always positive the distribution is not symmetric.

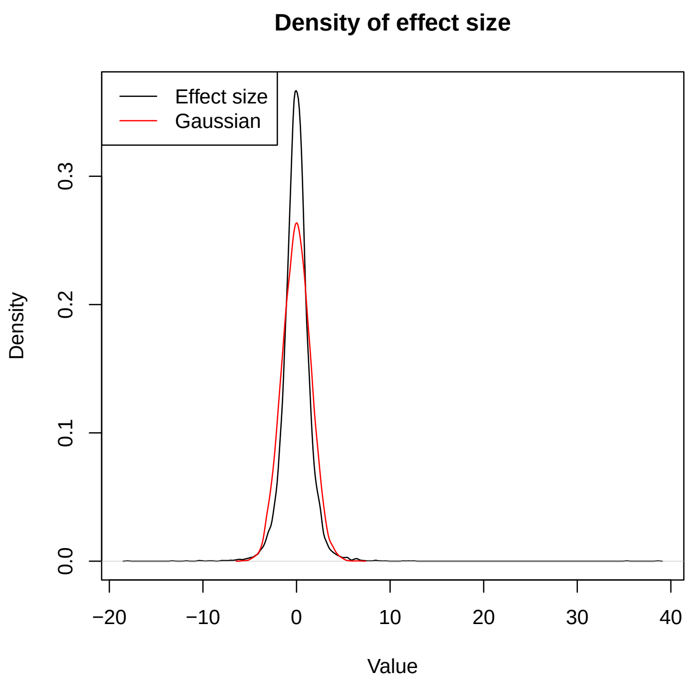
Answer of Exercise 11

As suggested by the title of this section. We can work with the **effect size**, *i.e.* the mean divided by the standard deviation of the mean.

Answer of Exercise 12

```
> esize <- rep(NA, 10000);
> for (i in 1:10000) {
+     Gaussian.sample <- rnorm(5);
```

```
+    esize[i] <- sqrt(5)*mean(Gaussian.sample)/sd(Gaussian.sample);
+ }
> plot(density(esize), xlab="Value", main="Density of effect size");
> lines(density(rnorm(10000, mean(esize), sd(esize))), col="red");
> legend(x="topleft", lwd = 1, col=c("black", "red"),
+    legend=c("Effect size", "Gaussian"));
```



The distribution is symmetric and bell-shaped but it is not Gaussian. The red line on the previous graph is the density of the distribution of a Gaussian variable with same mean an standard deviation as the effect size. The distribution of the effect size has so called 'heavy tails', which are usually visible because of the presence of outliers far off the bulk of values around 0. The outliers occur in samples where all 5 values are close to each other, which gives a very small estimated standard deviation and therefore an inflated effect size ratio.

Answer of Exercise 13

Denote S_x^2 and S_y^2 the estimated variances of the first and second samples respectively. The estimated variances of the means are S_x^2/n and S_y^2/n, so that the estimated variance of the difference is $S_x^2/n + S_y^2/n$ (see hint of exercise 9), and the estimated standard deviation $\sqrt{(S_x^2 + S_y^2)/n}$. The effect size is thus

$$\frac{\sqrt{n}(\bar{x} - \bar{y})}{\sqrt{S_x^2 + S_y^2}}. \tag{3}$$

Answer of Exercise 14

$\bar{\tilde{x}} = \bar{x} + a$ and $\bar{\tilde{y}} = \bar{y} + a$. According to the hint, $S^2_{\tilde{x}} = S^2_x$ and $S^2_{\tilde{y}} = S^2_y$. So adding a constant does not change the effect size.

$\bar{\mathring{x}} = b\bar{x} + ab$ and $\bar{\mathring{y}} = b\bar{y} + ab$. $S^2_{\mathring{x}} = b^2 S^2_x$ and $S^2_{\mathring{y}} = b^2 S^2_y$. The b's in the numerator and denominator cancel out, so multiplying by a constant also does not change the effect size.

Answer of Exercise 15

```
> esize <- rep(NA, 10000);
> for (i in 1:10000) {
+    x <- rnorm(5);
+    y <- rnorm(5);
+    esize[i] <- sqrt(5)*(mean(x)-mean(y))/sqrt(var(x)+var(y));
+ }
```

Answer of Exercise 17

There is a matter of discussion whether this is a unilateral of bilateral test. Bear in mind that unilateral tests make a very strong assumption, in that case, that it is strictly physically **impossible** that you pipette more volume with filter tips, and that even if you measured a volume 10 times bigger with filter tips, you would accept the null hypothesis (thereby coming to the conclusion that there is no difference in the volume you pipette). For this kind of test, only extraordinary circumstances would call for unilateral testing. Therefore a symmetric rejection region, distant from the bulk of the values around 0 is the most meaningful.

The limits of that region can be estimated from the distribution of the effect size.

```
> abs_threshold <- quantile(abs(esize), probs=0.95);
> abs_threshold;

    95%
2.26078

> quantile(esize, probs=c(0.025, 0.975)); # is not symmetric.

     2.5%     97.5%
-2.294104  2.233898
```

Answer of Exercise 18

```
> t <- sqrt(5) * (mean(filter_tips) - mean(normal_tips)) /
+    sqrt(var(normal_tips) + var(filter_tips));
> print(t);

[1] 0.6387136

> mean(abs(esize) > abs(t));

[1] 0.5354
```

The computed effect size does not fall in the rejection region. We accept the null hypothesis and conclude that you pipette the same volume with filter tips and normal tips.

Answer of Exercise 19

Suppose that the observed value of θ is θ_0. By definition $P(\theta > \theta_0) = 0.03$. Note that $P(\theta > s(0.05)) = 0.05$, which is possible only if $\theta_0 > s(0.05)$, *i.e.* the null hypothesis is rejected at level 0.05. Symmetrically, $P(\theta > s(0.01)) = 0.01$, so we must have $\theta_0 < s(0.01)$ and the null hypothesis is accepted at level 0.01.

If we had chosen the largest level α for which the null hypothesis would be accepted, observing any larger θ would involve rejecting the null hypothesis at level α. So $P(\theta > \theta_0) = \alpha$. But we know that this value is 0.03, the p-value of the test. This gives rise to an alternative definition of the p-value of a test as **the largest level α for which the null hypothesis is accepted for this value of the statistic**.

Answer of Exercise 21

```
> t.test(filter_tips, normal_tips);

        Welch Two Sample t-test

data:  filter_tips and normal_tips
t = 0.6387, df = 7.608, p-value = 0.5418
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.057203  1.857203
sample estimates:
mean of x mean of y
    98.04     97.64
```

Answer of Exercise 22

By definition, it is the probability of rejecting the null hypothesis given that the null hypothesis is true, that is α. This shows that **The power of a test always depends on the risk α**.

Answer of Exercise 23

```
> sdest <- sqrt((var(normal_tips) + var(filter_tips) / 2));
> W <- list();
> for (delta in c(1,5,10)) {
+    for (i in 1:10000) {
+       filter_t <- rnorm(5, mean=100-delta, sd=sdest);
+       normal_t <- rnorm(5, mean=100, sd=sdest);
+       W[[as.character(delta)]][i] <- sqrt(5) *
+          (mean(filter_t) - mean(normal_t)) /
+          (sqrt(var(filter_t) + var(filter_t)));
+    }
+ }
> for (x in W) {
+    print(mean(abs(x) > abs_threshold));
+ }
```

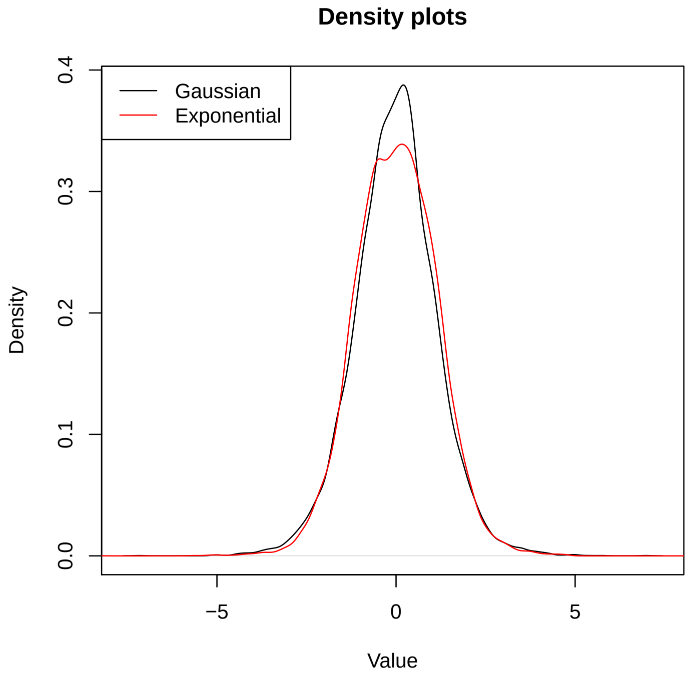
```
[1] 0.2737
[1] 0.9998
[1] 1
```

Answer of Exercise 24

Yes. No.

Answer of Exercise 25

```
> esize_exp <- rep(NA, 10000);
> for (i in 1:10000) {
+     x <- rexp(5);
+     y <- rexp(5);
+     esize_exp[i] <- sqrt(5) * (mean(x) - mean(y)) /
+         (sqrt(var(x) + var(y)));
+ }
> plot(density(esize), main="Density plots", xlab="Value");
> lines(density(esize_exp), col="red");
> legend(x="topleft", lwd = 1, col=c("black", "red"),
+     legend=c("Gaussian", "Exponential"));
```



The t test is relatively robust to the departure from normality because of the **Central Limit Theorem**. The theorem says that the average of independently sampled variables becomes progressively Gaussian as the sample size increases. A rule of thumb is that for $n > 30$, the approximation is good enough.