The Labyrinth of Multiple Testing: How to avoid the pitfall of false positives

Introduction to Hypothesis testing

12th SISMEC National Congress 2023

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Outline

1. Introduction

2. Hypothesis testing

2.1 Individual hypothesis testing

2.2 Multiple hypothesis testing

American Statistical Association's Ethical Guidelines for Statistical Practice

Recognize that any frequentist statistical test has a random chance of indicating **significance** when it is **not really present**.

Selecting the one "significant" result from a multiplicity of parallel tests poses a grave risk of an **incorrect conclusion**.

Failure to disclose the full extent of tests and their results in such a case would be highly misleading.

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e.g. VaxGen's AIDSVAX trial ...

VaxGen's AIDSVAX trial

VaxGen announced the results of the **first-ever efficacy trial** of an AIDS vaccine on 24 February 2003:

The vaccine prevent HIV infection?

	Total	Infected		
All subjects	1679	96	5.8%	PLACEBO
	3330	191	5.7%	VACCINE

"We saw absolutely no difference between the vaccine and placebo groups. Everyone was pretty depressed."

but the next day...

VaxGen's AIDSVAX trial

...by **broking the data down into racial groups** – which they say was part of the original design – the vaccine appeared to have worked in blacks:

	Total	Infected		Fisher's exact test
White	1508	81	5.4%	$p_W = 0.898$
	3003	179	6.0%	
Black	111	9	8.1%	$p_B = 0.015$
	203	4	2.2%	
Asian	20	2	10.0%	$p_A = 0.301$
	53	4	3.8%	
Other	40	6	15.0%	$p_O = 0.345$
	71	6	8.5%	

"The numbers were small, which concerned us, but the result was highly statistically significant. They were pretty incredible results."

Criticisms

1. **Failure to account for multiplicity**

   "The p-values were not adjusted."

2. **Selective reporting (data snooping)**

   "It's all murky because it's all post hoc analysis. They might as well do a subgroup analysis based on signs of the zodiac."

   If you torture your data long enough, they will confess to you whatever you want to hear!

Revived interest in multiple testing

"-omics"

e.g., genomics experiments with microarray data: which genes are differentially expressed?

model selection

e.g., multiple regression: which coefficients matter?

...

Clinical trials

sources of multiplicity

- multiple endpoints
- several treatments
- multiple time points
- subgroup analysis
- interim analysis
- ...

regulatory guidelines

- statistical principles for clinical trials (ICH E9)
- points to consider on multiplicity issues in clinical trials (EMEA)
- ...

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Hypothesis Testing: One Single Test

Two Hypotheses under comparison

- H_0: two groups are **Equal**, no relationship between X and Y.
- H_1: two groups are **Different**, there is a relationship between X and Y.

Each test produces a p-value p:

if $\boxed{p \leq .05}$ ($\alpha = .05$), we **reject** H_0 (and lean towards H_1).

Errors

		Null hypothesis	
		False (two groups are different)	True (two groups are equal)
Test	Rejected	True discovery	Type I error
	Not rejected	Type II error	True negative

- **Type I** (false positive): **Reject** H_0 when it is **True**
  $\mathbb{P}(\text{Type I Error}) = \mathbb{P}(p \leq .05 | H_0) = .05$
- **Type II** (false negative): **Fail** to reject H_0 when it is **False**
  $\mathbb{P}(\text{Type II Error}) = \mathbb{P}(p > .05 | H_1)$

  Power: $\mathbb{P}(p \leq .05 | H_1) = 1 - \mathbb{P}(p > .05 | H_1) = 1 - \mathbb{P}(\text{Type II Error})$

Asymmetric Importance of Errors

Control $\mathbb{P}$(Type I Error) (e.g., ≤ 0.05)
and
find the test with the maximum **Power** (minimum Type II Error)

It's important to remember that:

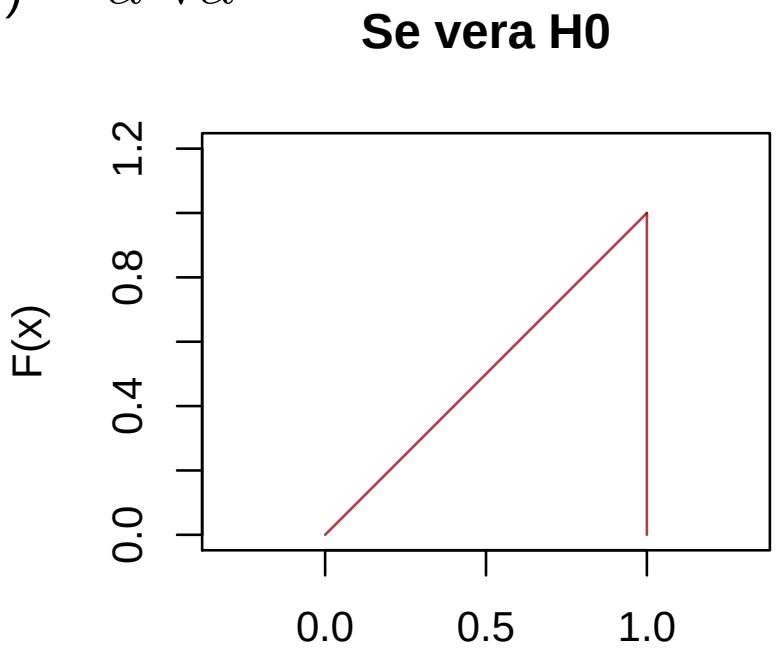
- **A significant p-value** ($p \leq \alpha$) allows us to think that H_1 is true, while
- **A non-significant p-value** ($p > \alpha$) does NOT allow us to think that H_0 is true; we simply don't have enough evidence to reject it.

Type I Error

Suppose $H_0 : \mu_1 - \mu_2 = 0$ and $H_1 : \mu_1 - \mu_2 < 0$
test statistic $T = \frac{\bar{x}_1 - \bar{x}_2}{\hat{\sigma}}$ ($\hat{\sigma}$ estimate of the std dev of $\bar{x}_1 - \bar{x}_2$)
under H_0: $T \sim t_{n_1+n_2-2}$, then

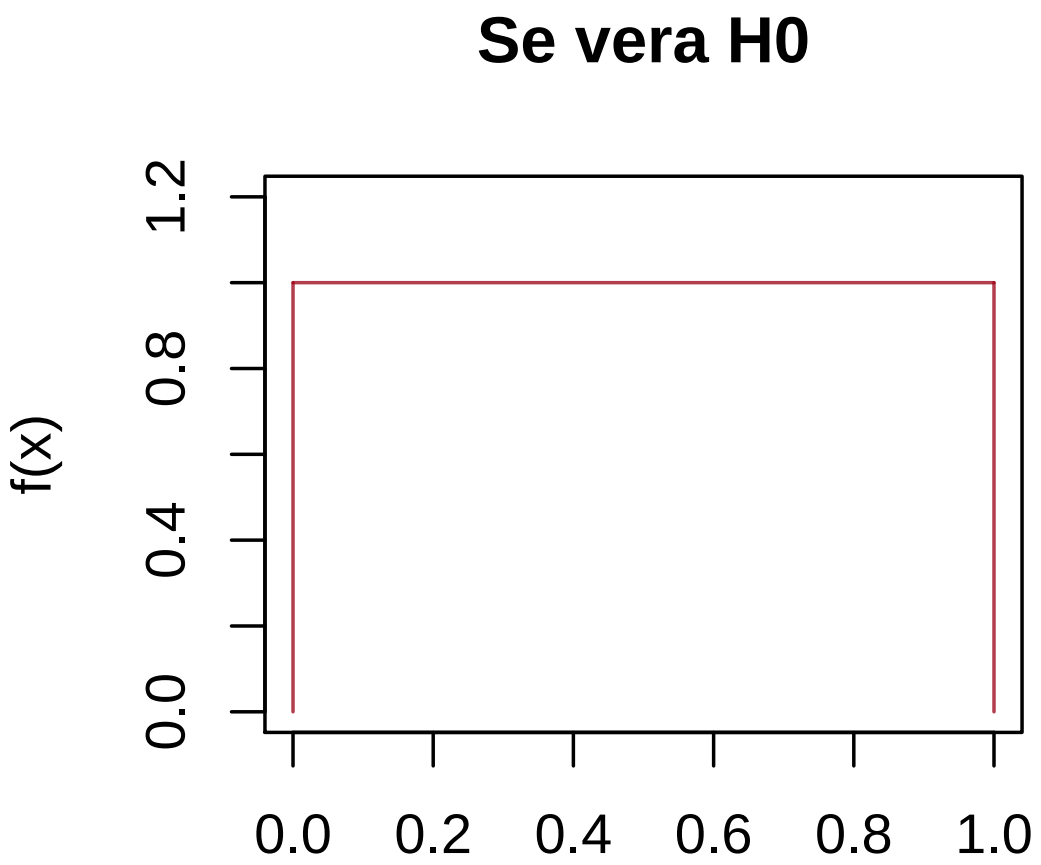
$$\mathbb{P}(T \leq t_\alpha | H_0) = \alpha \ \forall \alpha$$
$$\mathbb{P}(F(T) \leq F(t_\alpha) | H_0) = \alpha \ \forall \alpha$$
$$\mathbb{P}(P \leq \alpha | H_0) = \alpha \ \forall \alpha$$

consequently, $P \sim U(0, 1)$



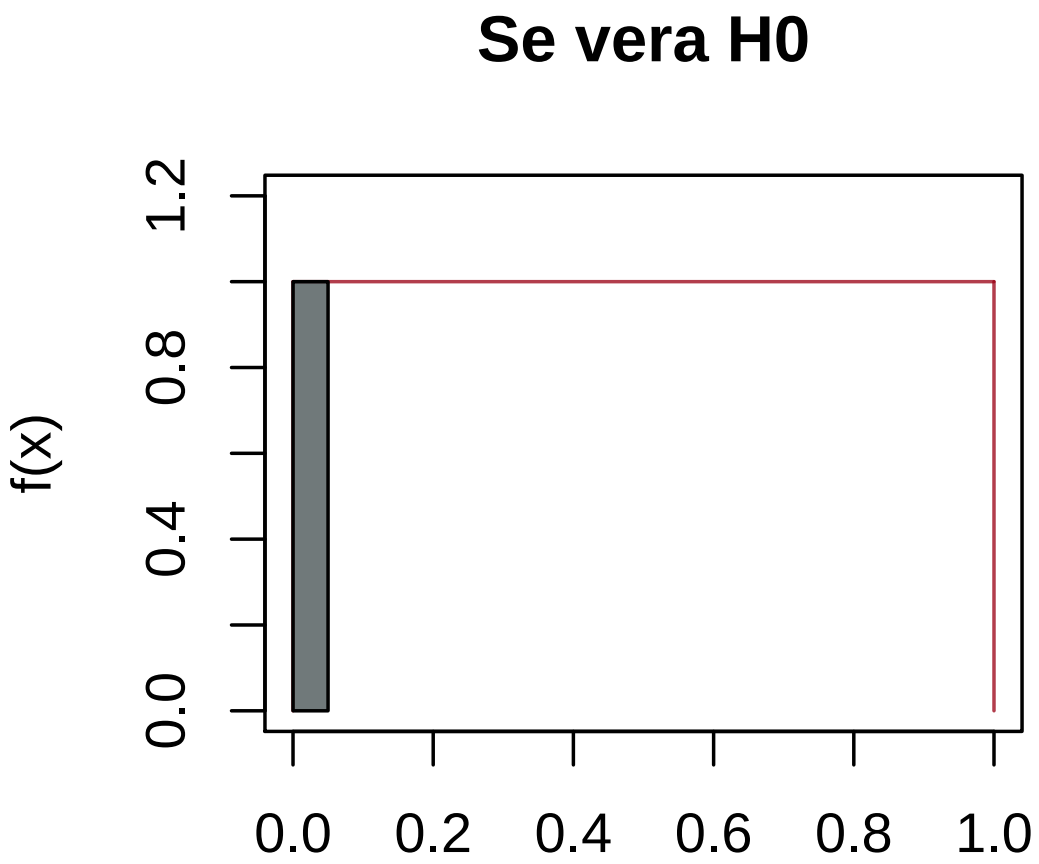
Type I Error

Under H_0, the p-value is a uniform random variable $U(0,1)$



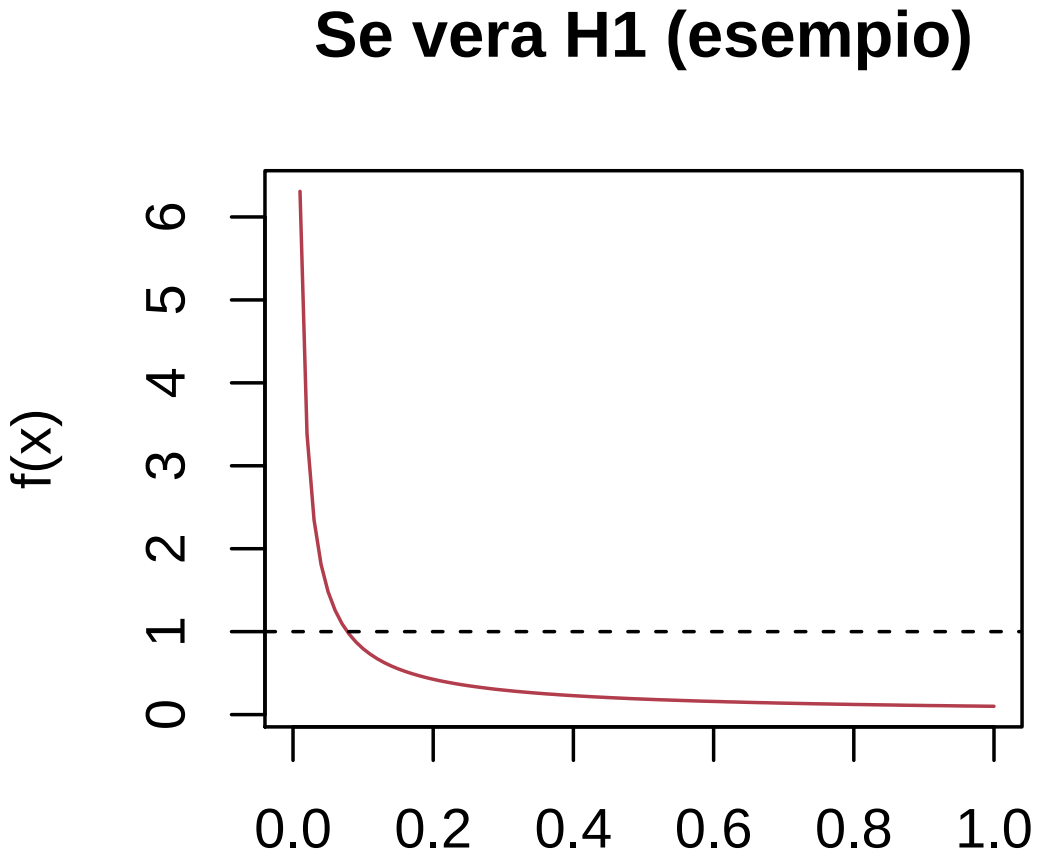
Type I Error

Type I Error: $\mathbb{P}(p \leq .05 | H_0) = .05$



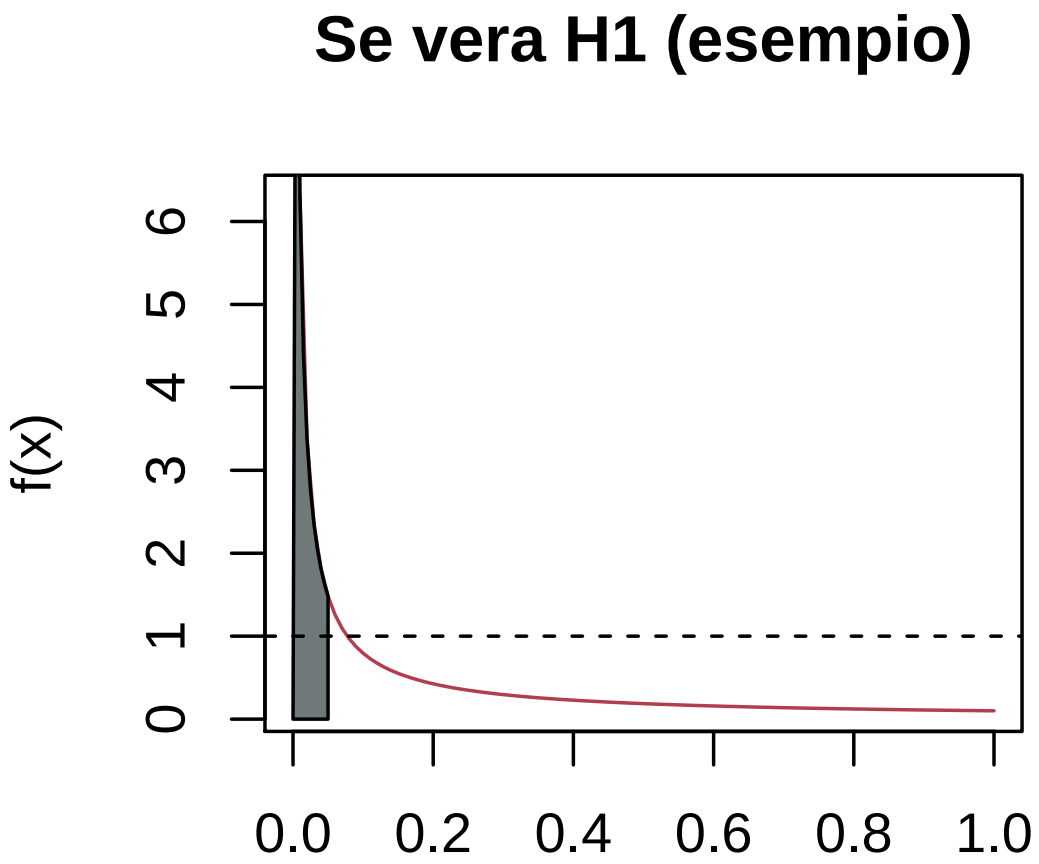
Power

Under H_1, the p-value is stochastically smaller than a uniform random variable $U(0,1)$ (No test distortion)



Power

Under H_1: $\mathbb{P}(p \leq .05 | H_1) > .05$, in our case $= .74$



Hypothesis testing: Multiple Tests

The goal is to test $m \geq 2$ hypotheses simultaneously from the same data.

Each test carries the risk of making a Type I error $\rightarrow$ the risk of having **AT LEAST one** may become unmanageable.

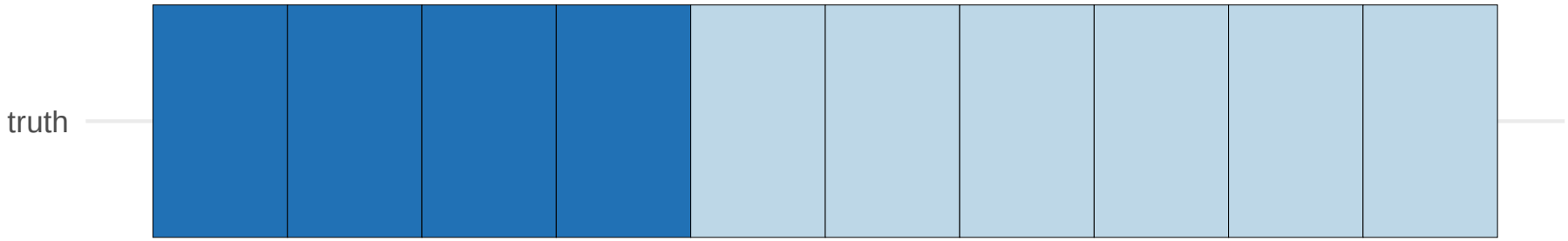
Two Tests (Independent) Case

EXAMPLE: independent tests

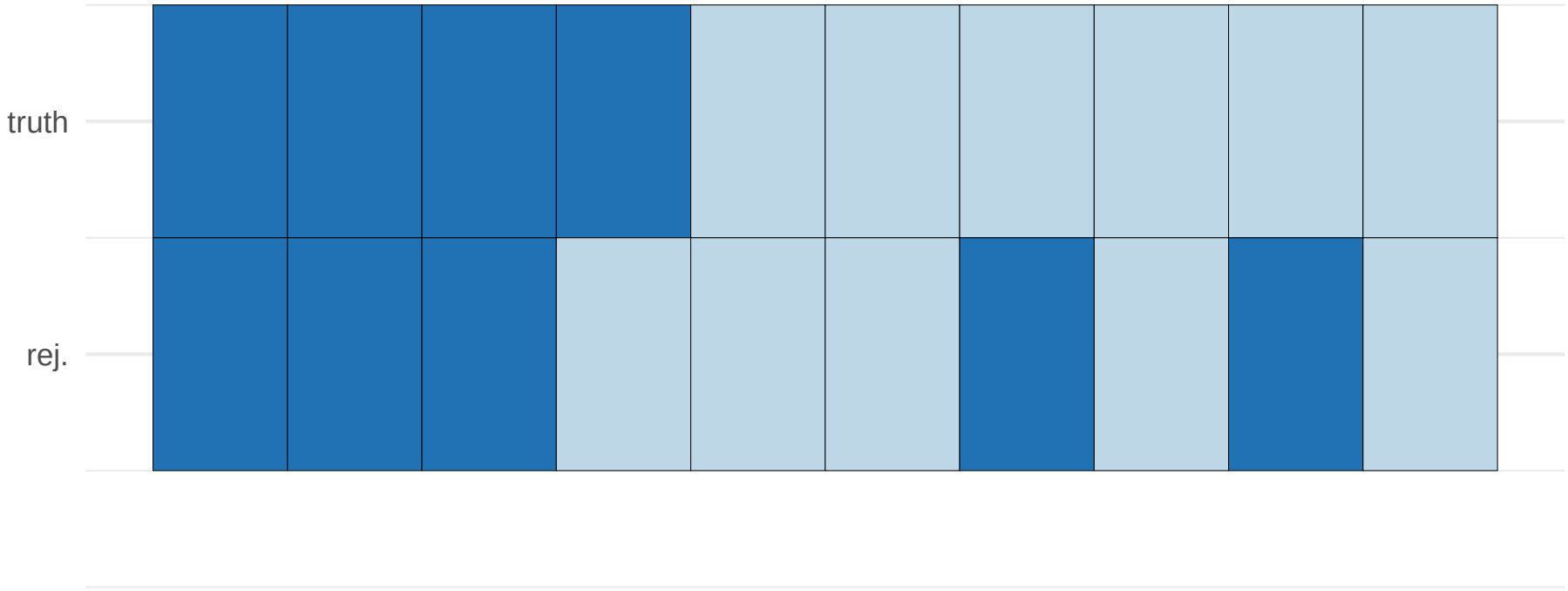
Probability of **AT LEAST one** (false) rejection?

$$\begin{aligned}\mathbb{P}(p_1 \leq .05 \cup p_2 \leq .05 | H_0) &= .05 + .05 - (.05 \cdot .05) = 1 - (1 - .05)^2 \\ &= .0975 = 1 - (1 - \alpha)^2 > \alpha\end{aligned}$$

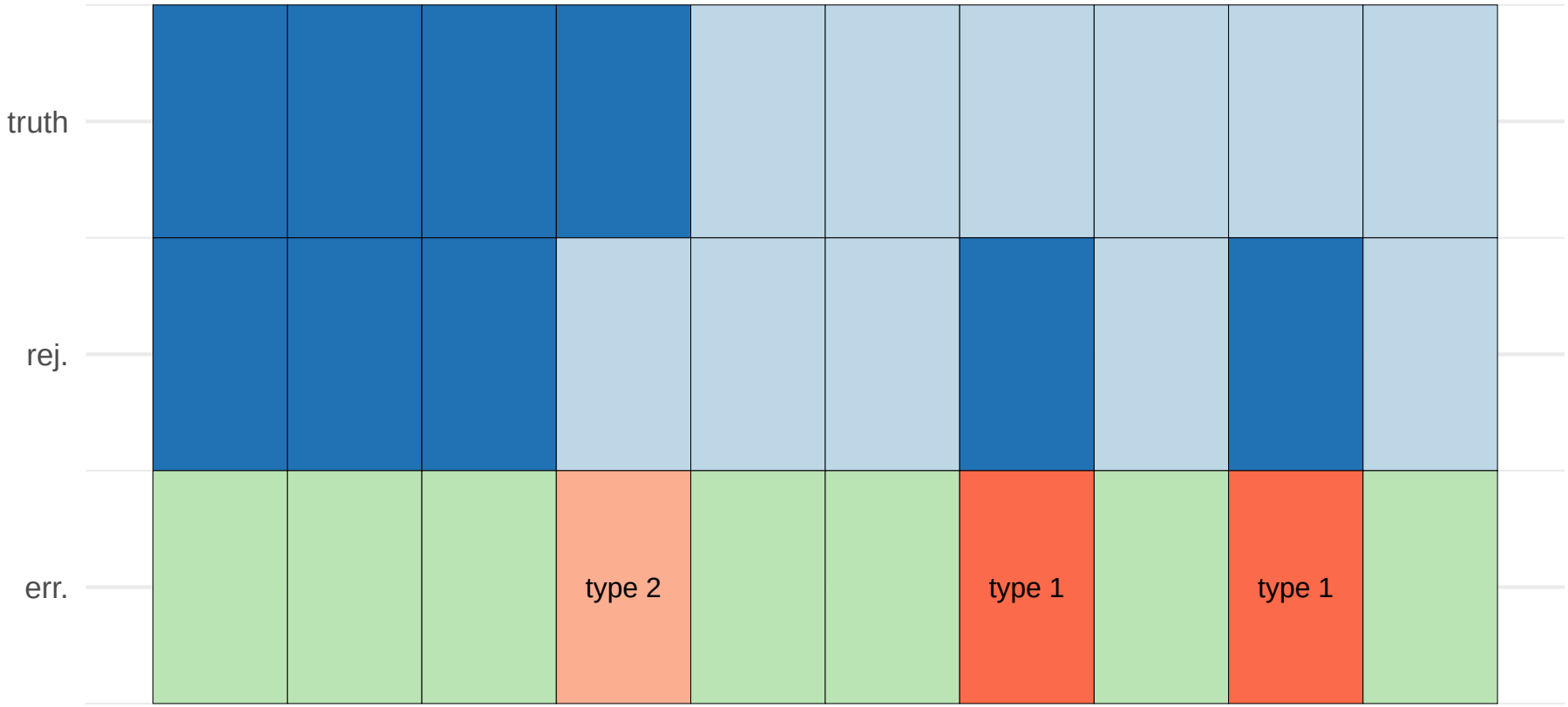
Multiple Tests



Multiple Tests



Multiple Tests



Error control

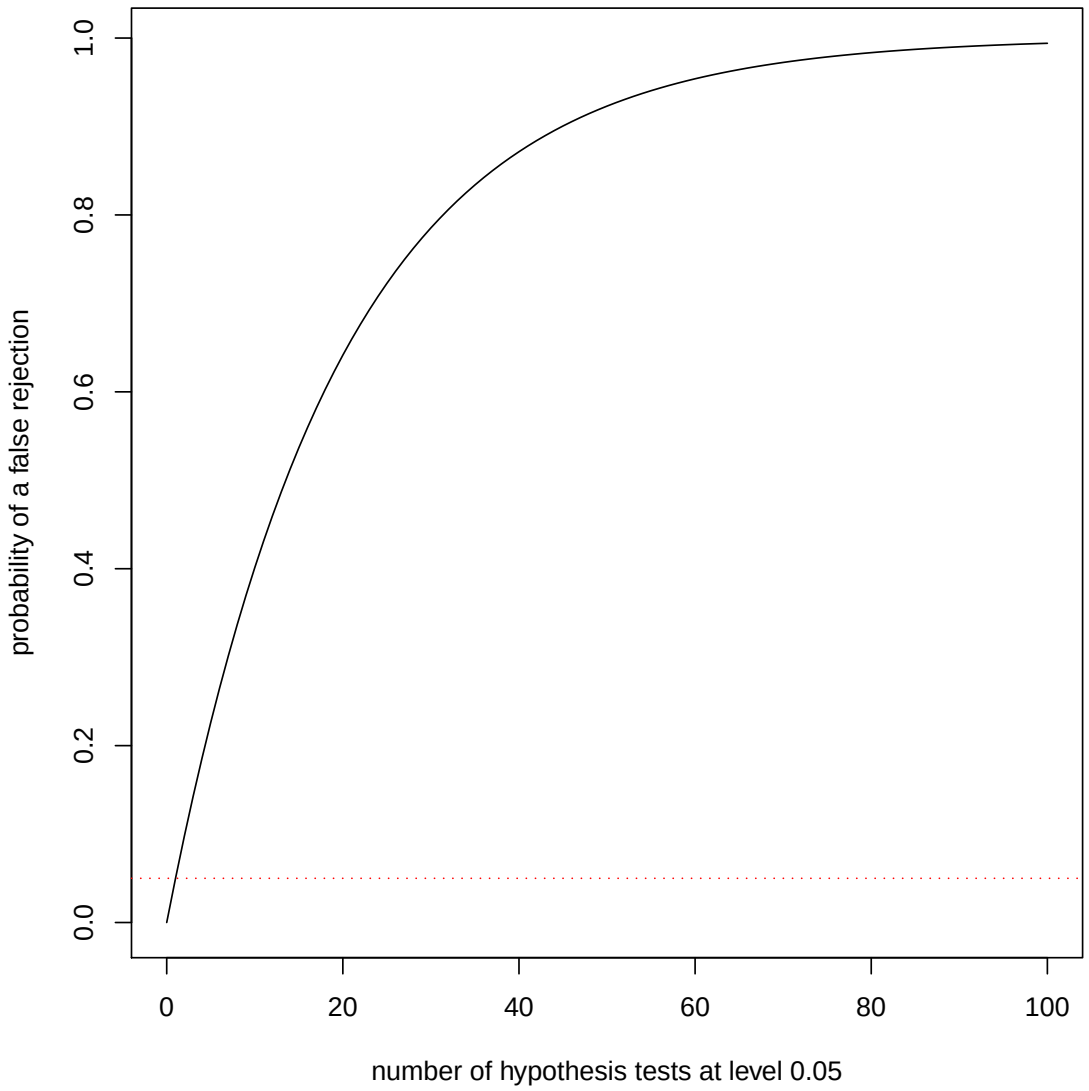
		Null hypothesis		
		False	True	Tot
Test	Rejected	S	V (false discoveries)	R
	Not rejected	T	U	$m - R$
	Tot	m_1	m_0	m

Probability of AT LEAST one false rejection (independent tests):

$$\mathbb{P}(V > 0) = 1 - (1 - \alpha)^m$$

This quickly becomes a problem if m becomes large ...

Error control



Type I error

- How to **define** the Type I error when there are many hypotheses?
- Which procedures **control** this error?