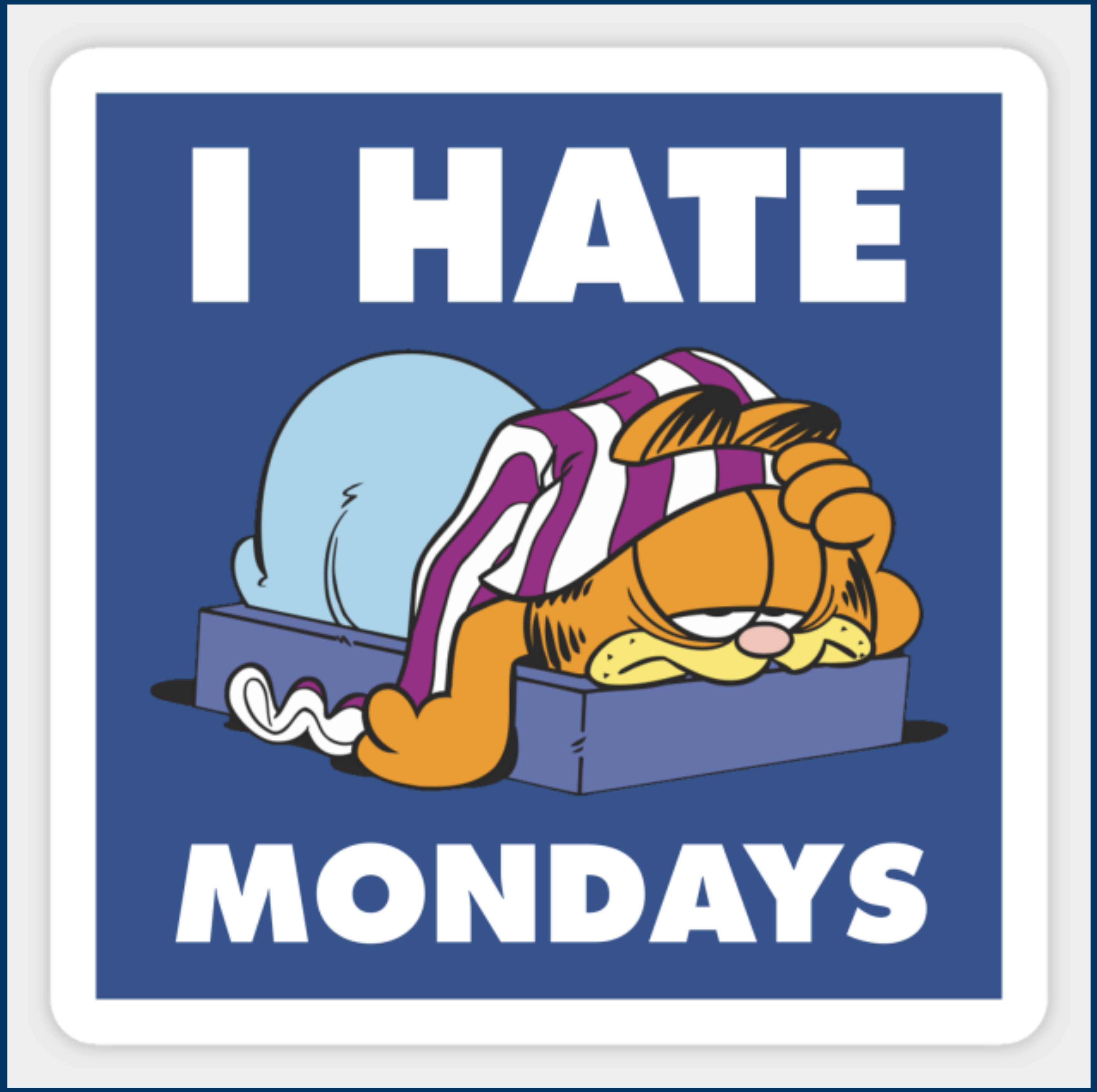
Biostatistics

Applications in Medicine

Nuno Sepúlveda, 13.10.2025


I HATE
MONDAYS


CLASSIC 80s GARFIELD
I HATE MONDAYS
JIM DAVIS


I HATE MONDAY

Syllabus

1. **General review**
   1. Population/Sample/Sample size
   2. Type of Data – quantitative and qualitative variables
   3. Common probability distributions/popular tests
2. **Applications in Medicine**
   a. Construction and analysis of diagnostic tools – Binomial distribution, ROC curve, sensitivity, specificity, Rogal-Gladen estimator
   b. Estimation of treatment effects - generalized linear models
   c. Survival analysis - Kaplan-Meier curve, log-rank test, Cox's proportional hazards model
3. **Applications in Genetic and Epigenetic Data**
   a. Genetic association studies – Hardy-Weinberg test, homozygosity, minor allele frequencies, additive model, multiple testing correction
   b. Methylation association studies – M versus beta values, estimation of biological age
4. **Applications in Serological Data Analysis**
   a. Determination of seropositivity using Gaussian mixture models
   b. Reversible catalytic models for estimating seroconversion rate
   c. Sample size calculation for estimating seroconversion rate

Prevent

Diagnose

Medicine

Improve

Treat

Develop

Diagnosis

Negative / Positive

What is the type of random variable?

Diagnosis

Negative / Positive

What is the type of random variable?

Binary

X=0

X=1

Diagnosis

Negative / Positive

What is the type of random variable? **Binary**

What is the probability distribution associated with this random variable?

Diagnosis

Negative / Positive

What is the type of random variable? **Binary**

What is the probability distribution associated with this random variable?

Bernoulli

$$P\left[X = x \,|\, \pi\right] = \pi^{x}\left(1 - \pi\right)^{1-x} I_{\{0,1\}}\left(x\right)$$

Diagnosis

Number of Positive Tests in a Sample of n Individuals

What is the type of random variable?

Discrete

$$0, 1, \ldots, n$$

Diagnosis

Number of Positive Tests in a Sample of n Individuals

What is the type of random variable? **Discrete**

What is the probability distribution associated with this random variable?

Diagnosis

Number of Positive Tests in a Sample of n Individuals

What is the type of random variable? **Discrete**

What is the probability distribution associated with this random variable?

Hypergeometric

$$P[X = x \,|\, N, M, n] = \frac{\binom{M}{x}\binom{N-M}{n-x}}{\binom{N}{n}}$$

N is the population size

M is the size of population with a positive test

Estimation of the proportion of positive tests

$x \,|\, N, M, n \rightsquigarrow$ Hypergeometric $(N; M; n)$

N is typically known

$$P[X = x \,|\, N, M, n] = \frac{\binom{M}{x}\binom{N-M}{n-x}}{\binom{N}{n}}$$

$$\hat{M} = \,?$$

Exercise

$x \,|\, N, M, n \rightsquigarrow$ Hypergeometric $(N; M; n)$

N is typically known

$$P[X = x \,|\, N, M, n] = \frac{\binom{M}{x}\binom{N-M}{n-x}}{\binom{N}{n}}$$

$N = 700$ - Aneityum Island (Mistery Island)

$n = 50$ individuals

$x = 2$ positive malaria tests

$\hat{M} = ?$

Can you estimate this parameter by the maximum likelihood method using R?

Can you estimate this parameter by the method of moments?

Diagnosis

Number of Positive Tests in a Sample of n Individuals

What is the type of random variable? **Discrete**

What is the probability distribution associated with this random variable?

Hypergeometric

$$P[X = x \,|\, N, M, n] = \frac{\binom{M}{x}\binom{N-M}{n-x}}{\binom{N}{n}}$$

$N \to \infty$

Binomial

$$P[X = x \,|\, n, \pi] = \binom{n}{x} \pi^x (1-\pi)^{n-x}$$

Estimation of proportion of positive tests

$$x \,|\, n, \pi \rightsquigarrow \text{Binomial}\,(n; \pi)$$

$$P[X = x \,|\, n, \pi] = \binom{n}{x} \pi^x \,(1 - \pi)^{n-x}$$

$$\hat{\pi} = \,?$$

$$IC_{95\%}\,(\pi) = \,?$$

Estimation of proportion of positive tests

$x \mid n, \pi \rightsquigarrow \text{Binomial}\,(n; \pi)$

$$P[X = x \mid n, \pi] = \binom{n}{x} \pi^x (1 - \pi)^{n-x}$$

$$\hat{\pi}_{mle} = \frac{X}{n}$$

Maximum likelihood estimator

$$\hat{\pi}_{mle} = \frac{x}{n}$$

Maximum likelihood estimate

Estimation of proportion of positive tests

$$x \,|\, n, \pi \rightsquigarrow \text{Binomial}\,(n; \pi)$$

$$P[X = x \,|\, n, \pi] = \binom{n}{x} \pi^x \,(1 - \pi)^{n-x}$$

$$IC_{95\%}\,(\pi) = \hat{\pi}_{mle} \pm 1.96 \times se\,\left(\hat{\pi}_{mle}\right)$$

Wald's confidence interval

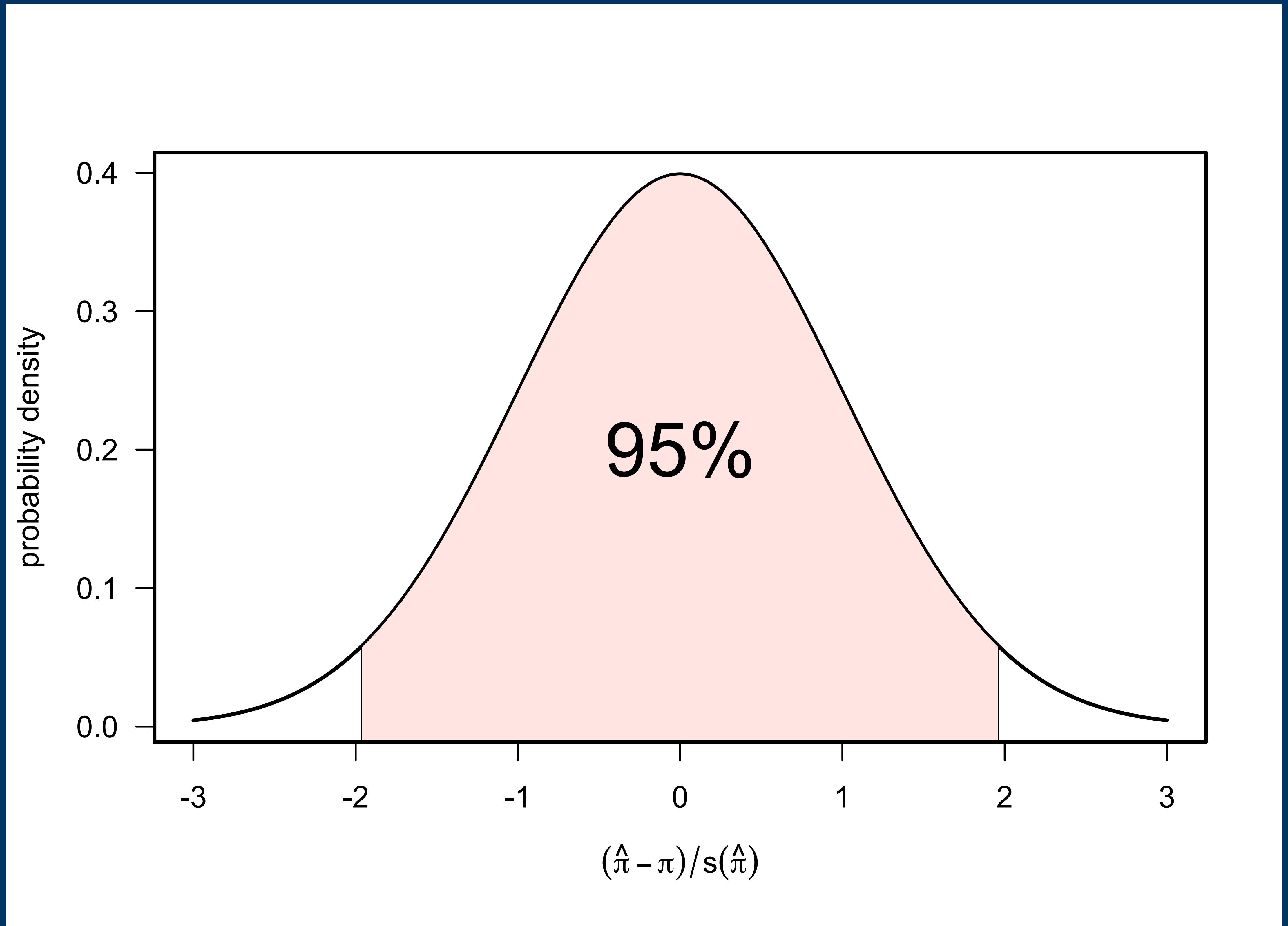
$$se\,\left(\hat{\pi}_{mle}\right) = \sqrt{\frac{\pi\,(1 - \pi)}{n}}$$

$$se\,\left(\hat{\pi}_{mle}\right) = \sqrt{\frac{\hat{\pi}_{mle}\,\left(1 - \hat{\pi}_{mle}\right)}{n}}$$

$se\,(\,\cdot\,) =$ standard error

Two-tail Wald's confidence interval

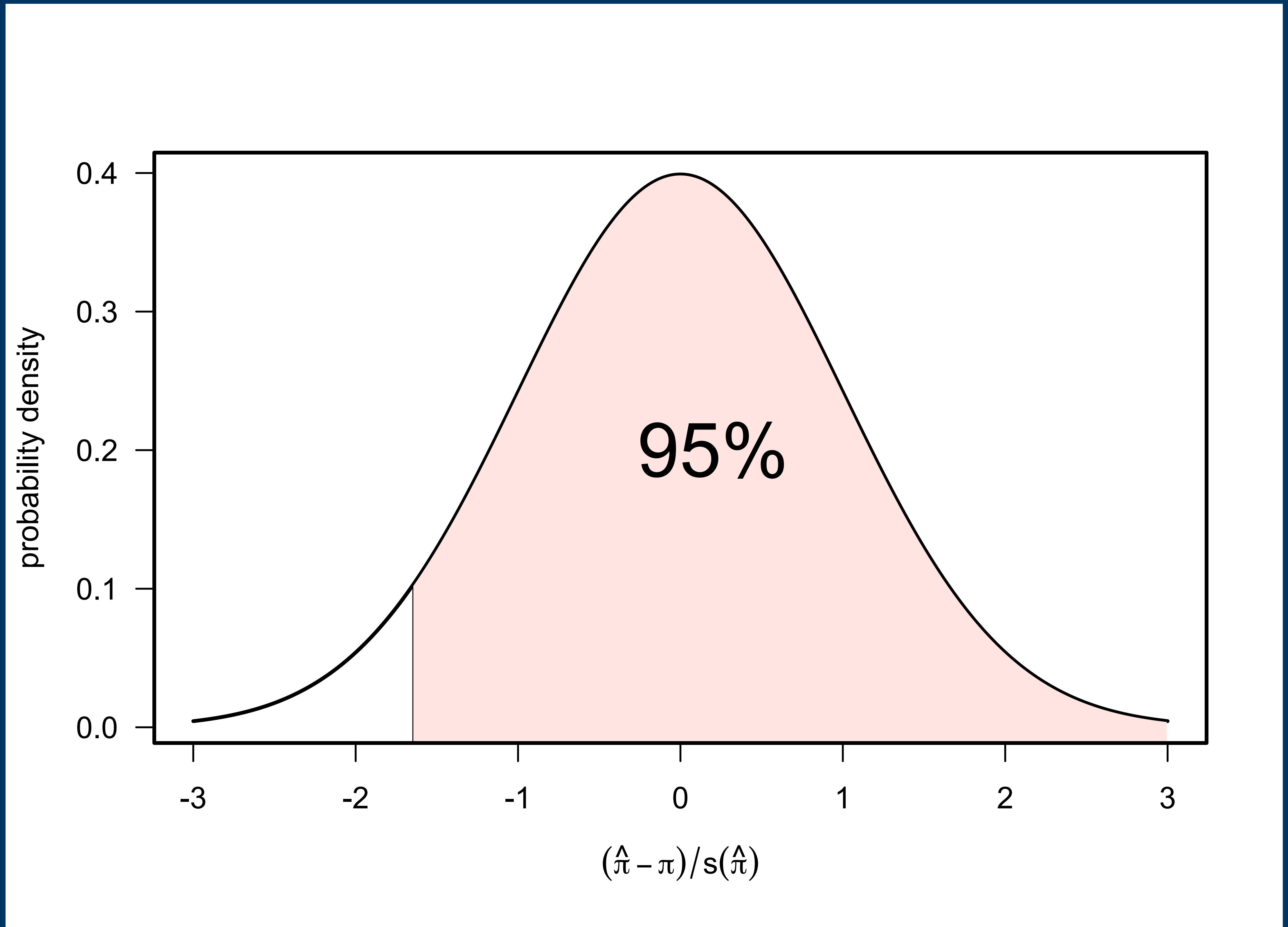
$$Y = \frac{\hat{\pi}_{mle} - \pi}{\sqrt{\frac{\hat{\pi}_{mle}\left(1 - \hat{\pi}_{mle}\right)}{n}}} \,|\, \pi, n \rightsquigarrow \text{Normal}\left(\mu = 0; \sigma = 1\right) \text{ For large samples}$$



One-tail Wald's confidence interval

$$Y = \frac{\hat{\pi}_{mle} - \pi}{\sqrt{\frac{\hat{\pi}_{mle}\left(1-\hat{\pi}_{mle}\right)}{n}}} \mid \pi, n \rightsquigarrow \text{Normal}\left(\mu = 0; \sigma = 1\right)$$

For large samples



Estimation of proportion of positive tests

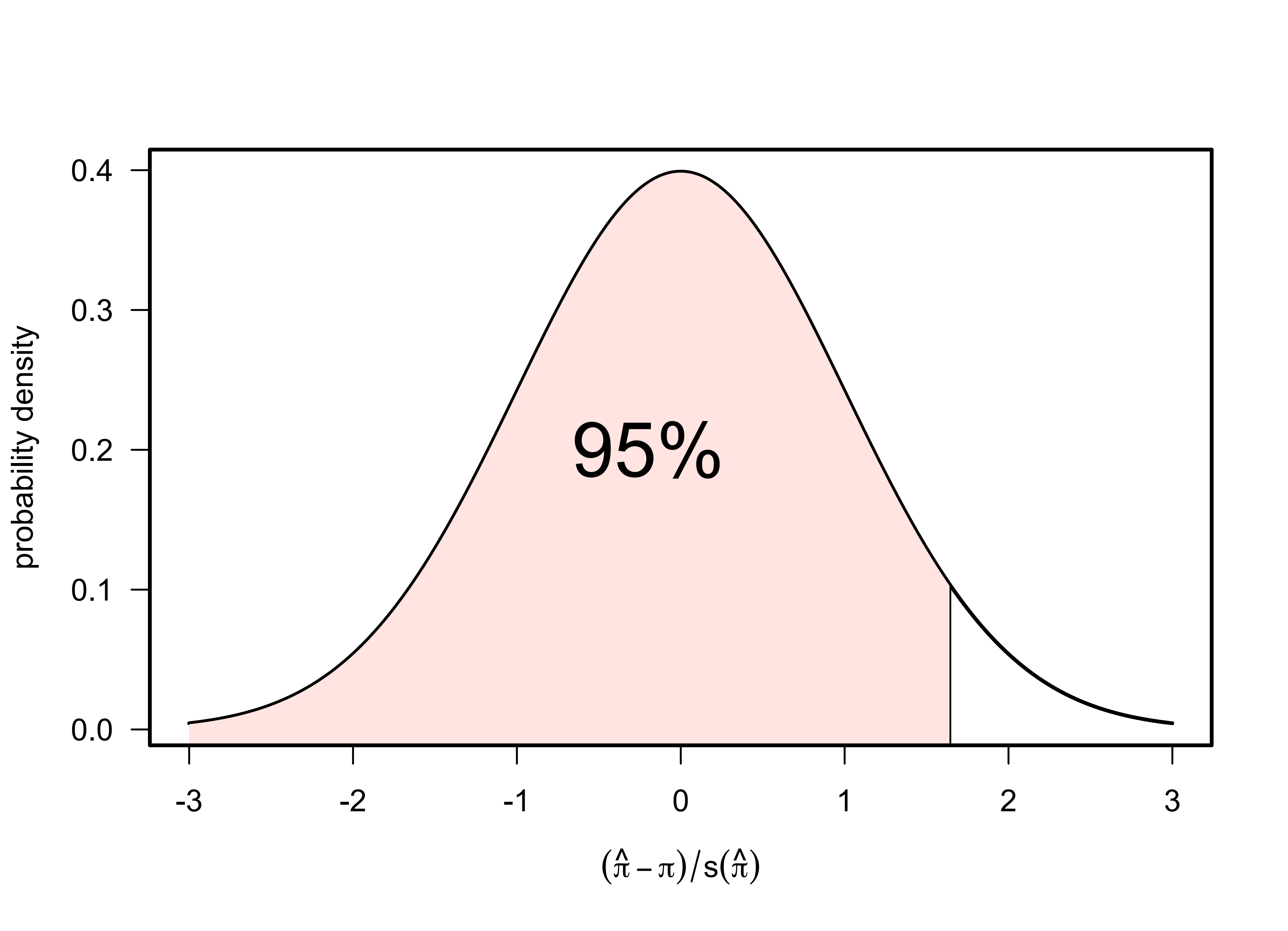
$$x \,|\, n, \pi \rightsquigarrow \text{Binomial}\,(n; \pi)$$

$$P[X = x \,|\, n, \pi] = \binom{n}{x} \pi^x (1 - \pi)^{n-x}$$

$$IC_{95\%}\,(\pi) = \left(\hat{\pi}_{mle} - 1.64 \times se\left(\hat{\pi}_{mle}\right), 1\right)$$

One-tail Wald's confidence interval

$$Y = \frac{\hat{\pi}_{mle} - \pi}{\sqrt{\frac{\hat{\pi}_{mle}\left(1 - \hat{\pi}_{mle}\right)}{n}}} \,|\, \pi, n \rightsquigarrow \text{Normal}\left(\mu = 0; \sigma = 1\right) \text{ For large samples}$$



Estimation of proportion of positive tests

$$x \,|\, n, \pi \rightsquigarrow \text{Binomial}\,(n; \pi)$$

$$P[X = x \,|\, n, \pi] = \binom{n}{x} \pi^x (1 - \pi)^{n-x}$$

$$IC_{95\%}\,(\pi) = \left(0, \hat{\pi}_{mle} + 1.64 \times se\left(\hat{\pi}_{mle}\right)\right)$$

Exercise (in the R software)

Table 1 Comparison of screening results for blood samples from community mass blood surveys and passive case detection in the Thai–Myanmar border area

	qPCR (reference)	Expert light microscopy				
	Number of samples	*P. falciparum*	*P. vivax*	*P. malariae*	Mixed *Pf* + *Pv*	Negative
Community mass blood survey						
P. vivax	21	–	2	–	–	19
P. falciparum	10	–	–	–	–	10
Mixed *Pf* + *Pv*	6	–	1	–	–	5
Mixed *Pf* + *P. ovale*	2	–	–	–	–	2
Mixed *Pf* + *Pv* + *Po*	1	–	–	–	–	1
Mixed *Pf* + *Pv* + *Po* + *P. malariae*	1	–	–	–	–	1
Negative	1306	–	–	–	–	1306
Total *n*	1347	–	3	–	–	1344
Hospital and malaria clinic PCD						
P. falciparum	5	5	–	–	–	–
P. vivax	4	–	1	–	–	3
P. malariae	1	–	–	1	–	–
Mixed *Pf* + *Pv*	22	5	14	–	–	3
Negative	265	–	–	–	–	265
Total *n*	297	10	15	1	–	271

Exercise (in the R software)

Estimate the number of positive tests by qPCR

Community

Hospital

Estimate the number of positive tests by light expert microscopy

Community

Hospital

Use binom.test function

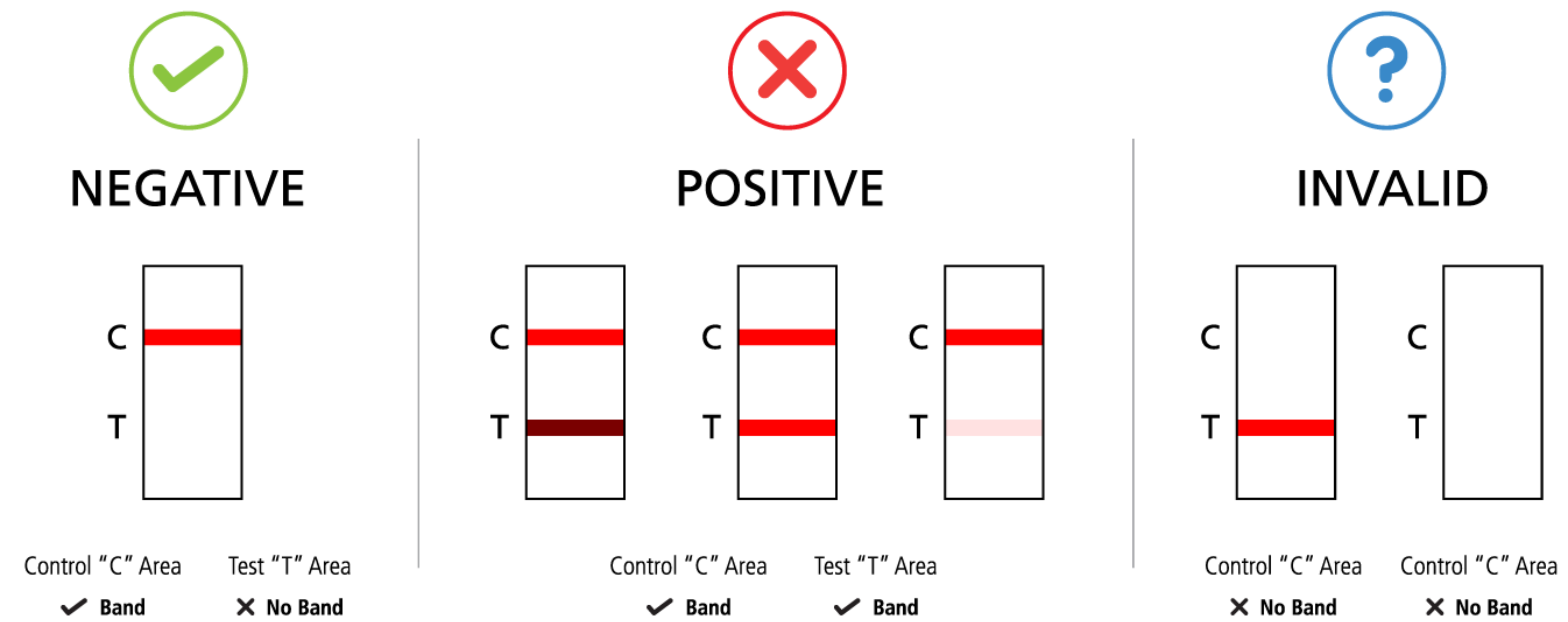
Exercise (in the R software)

Table 1 Comparison of screening results for blood samples from community mass blood surveys and passive case detection in the Thai–Myanmar border area

	qPCR (reference)	Expert light microscopy				
	Number of samples	***P. falciparum***	***P. vivax***	***P. malariae***	**Mixed *Pf* + *Pv***	**Negative**
Community mass blood survey						
P. vivax	21	–	2	–	–	19
P. falciparum	10	–	–	–	–	10
Mixed *Pf* + *Pv*	6	–	1	–	–	5
Mixed *Pf* + *P. ovale*	2	–	–	–	–	2
Mixed *Pf* + *Pv* + *Po*	1	–	–	–	–	1
Mixed *Pf* + *Pv* + *Po* + *P. malariae*	1	–	–	–	–	1
Negative	1306	–	–	–	–	1306
Total *n*	1347	–	3	–	–	1344
Hospital and malaria clinic PCD						
P. falciparum	5	5	–	–	–	–
P. vivax	4	–	1	–	–	3
P. malariae	1	–	–	1	–	–
Mixed *Pf* + *Pv*	22	5	14	–	–	3
Negative	265	–	–	–	–	265
Total *n*	297	10	15	1	–	271

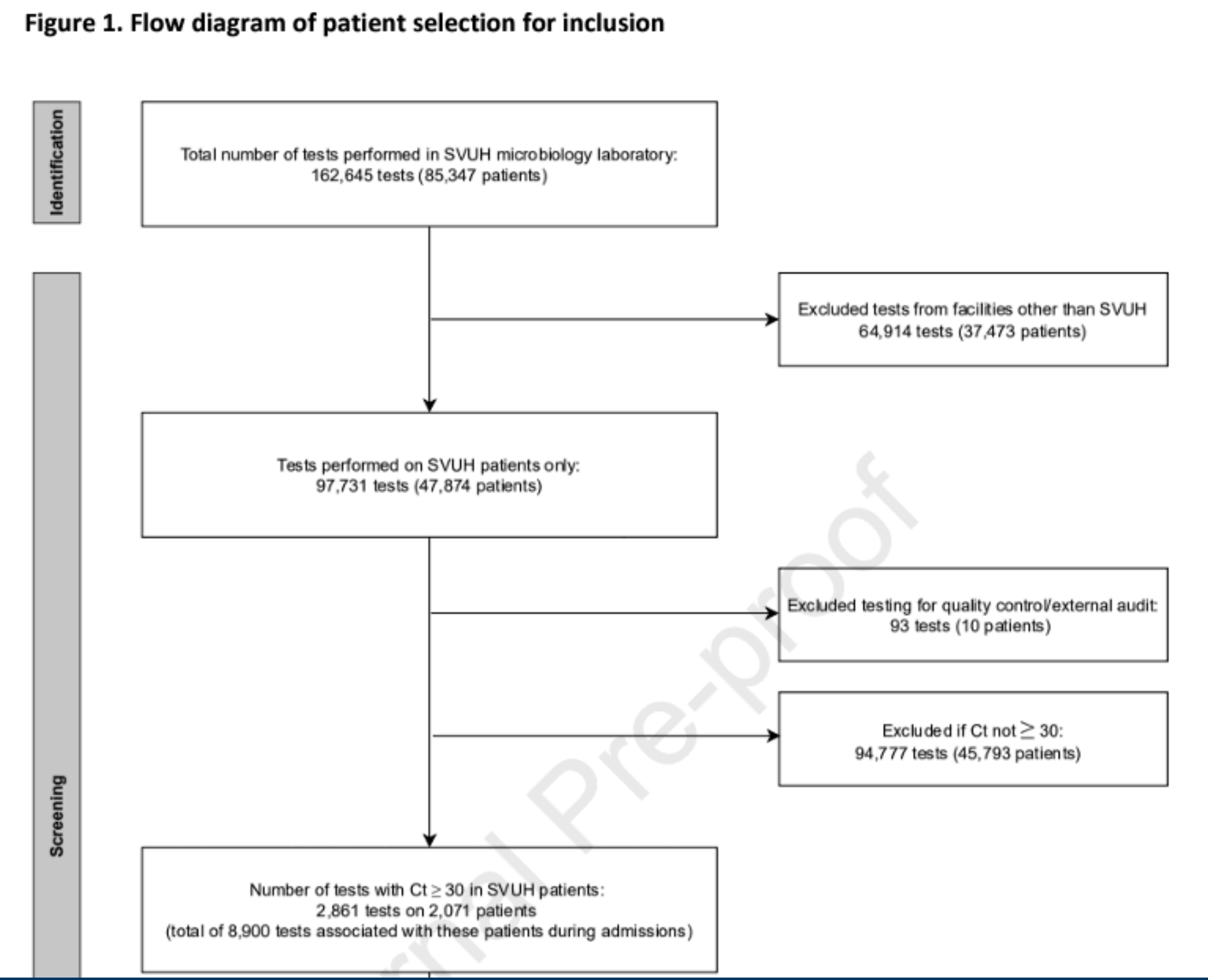
Break

Diagnosis (more complex situation)



Rapid diagnostic test for SARS-CoV-2

Diagnosis (more complex situation)



Molecular test for SARS-CoV-2 detection

Invalid

Positive

Indeterminate

Dolan & Fitzgerald (2023). Journal of Hospital Infection

Diagnosis (more complex situation)

Invalid / Indetermine / Negative / Positive

What is the type of random variable?

Categorical

Diagnosis (more complex situation)

Invalid or Indetermine / Negative / Positive

What is the type of random variable? **Categorical**

What is the probability distribution associated with this random variable?

Multivariate Bernoulli

$$P\left[\mathbf{X} = (x_1, x_2, x_3) \,|\, (\pi_1, \pi_2, \pi_3)\right] = \Pi_{i=1}^{3} \pi_i^{x_i}$$

with the restrictions: $x_i \in \{0,1\}, \sum_{i=1}^{3} x_i = 1$ and $\pi \in (0,1), \sum_{i=1}^{3} \pi_i = 1$

Diagnosis (more complex situation)

Number of Invalid or Indetermine / Negative / Positive

What is the type of random variable?

Diagnosis (more complex situation/less common)

Number of Invalid or Indetermine / Negative / Positive

What is the type of random variable? **Multivariate Categorical**

Diagnosis (more complex situation/less common)

Number of Invalid / Indetermine / Negative / Positive

What is the type of random variable? **Multivariate Categorical**

What is the probability distribution associated with this random variable?

Diagnosis (more complex situation/less common)

Number of Invalid or Indetermine / Negative / Positive

What is the type of random variable? **Multivariate Categorical**

What is the probability distribution associated with this random variable?

Multivariate Hypergeometric (small population sizes)

$$P[(n_1, n_2, n_3) \,|\, n, N, (M_1, M_2, M_3)] = \frac{\binom{M_1}{n_1}\binom{M_2}{n_2}\binom{M_3}{n_3}}{\binom{N}{n}}$$

with $\sum_{i=1}^{3} n_i = n$ and $\sum_{i=1}^{3} M_i = N$

Diagnosis (more complex situation/less common)

Number of Invalid or Indetermine / Negative / Positive

What is the type of random variable? **Multivariate Categorical**

What is the probability distribution associated with this random variable?

Multinomial (large population sizes)

$$P[(n_1, n_2, n_3)\,|\,n, (\pi_1, \pi_2, \pi_3)] = \frac{n!}{n_1!n_2!n_3!}\pi_1^{n_1}\pi_2^{n_2}\pi_3^{n_3} \text{ with } \sum_{i=1}^{3} n_i = n \text{ and } \sum_{i=1}^{3} \pi_i = 1$$

Estimation of the proportions

$$\hat{\pi}_1 = ?$$

$$\hat{\pi}_2 = ?$$

$$\hat{\pi}_3 = 1 - \hat{\pi}_1 - \hat{\pi}_2$$

$$IC_{95\%}(\pi_1) = ?$$

$$IC_{95\%}(\pi_2) = ?$$

$IC_{95\%}(\pi_3)$ – no need

Estimation of the proportions

$$\hat{\pi}_1 = \frac{n_1}{n}$$

$$\hat{\pi}_2 = \frac{n_2}{n}$$

$$\hat{\pi}_3 = 1 - \hat{\pi}_1 - \hat{\pi}_2$$

Estimation of the proportions

$$\hat{\pi}_1 = \frac{n_1}{n}$$

$$IC_{95\%}(\pi_1) = ?$$

$$\hat{\pi}_2 = \frac{n_2}{n}$$

$$IC_{95\%}(\pi_2) = ?$$

$$\hat{\pi}_3 = 1 - \hat{\pi}_1 - \hat{\pi}_2$$

Estimation of the proportions

$$\hat{\pi}_1 = \frac{n_1}{n} \qquad IC_{95\%}\left(\pi_1\right) = \hat{\pi}_1 \pm 2.24 \times se\left(\hat{\pi}_1\right)$$

$$\hat{\pi}_2 = \frac{n_2}{n} \qquad IC_{95\%}\left(\pi_2\right) = \hat{\pi}_2 \pm 2.24 \times se\left(\hat{\pi}_2\right)$$

$$\hat{\pi}_3 = 1 - \hat{\pi}_1 - \hat{\pi}_2 \qquad 2.24 = \Phi^{-1}\left(\frac{0.025}{2}\right)$$

$$IC_{\gamma\%}\left(\pi_1\right) = \hat{\pi}_1 \pm \Phi^{-1}\left(1 - \frac{\gamma}{2}\right) \times se\left(\hat{\pi}_1\right) \qquad \Phi^{-}1\left(\frac{1-\gamma}{2p}\right)$$

Bonferroni's method

p is the number of estimated parameters

$$P\left[\cup_{i=1}^{n} A_i\right] \leq \sum_{i=1}^{n} P\left[A_i\right]$$

Exercise (in the R software)

Cliff et al (2019). Frontiers in Medicine

Herpesvirus	Seronegative	Indeterminate	Seropositive
Cytomegalovirus	254	7	133
Epstein-Barr virus (VCA)	46	4	344
Epstein-Barr virus (EBNA1)	83	15	296
Herpesvirus simplex 1	195	20	179
Herpesvirus simplex 2	232	12	150

Estimate the proportion of positive and negative tests and calculate the respective 95% confidence region