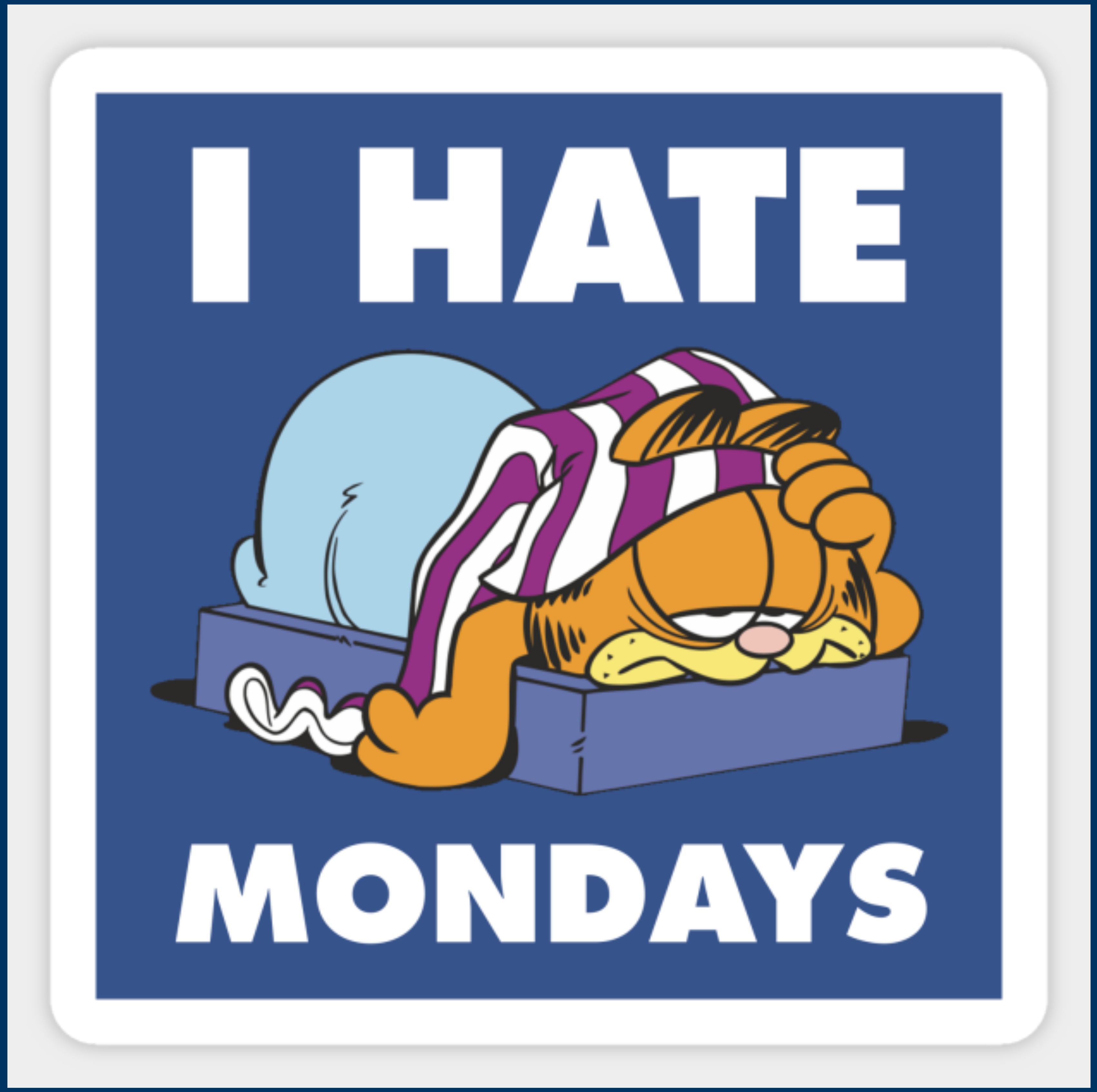
Biostatistics

Applications in Medicine

Nuno Sepúlveda, 14.10.2024


I HATE
MONDAYS


CLASSIC
80s
GARFIELD
I HATE
MONDAYS
JIM DAVIS


I HATE
MONDAY

Syllabus

1. General review
    a. What is Biostatistics?
    b. Population/Sample/Sample size
    c. Type of Data – quantitative and qualitative variables
    d. Common probability distributions
    e. Work example – Malaria in Tanzania
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 Genetics, Genomics, and other 'omics 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
    c. Gene expression studies based on RNA-seq experiments – Tests based on Poisson and Negative-Binomial
4. Other Topics
    a. Estimation of Species diversity – Diversity indexes, Poisson mixture models
    b. Serological analysis – Gaussian (skew-normal) mixture models
    c. Advanced sample size and power calculations

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

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

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

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

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

$\hat{\pi}_{mle} = \dfrac{X}{n}$ Maximum likelihood estimator

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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

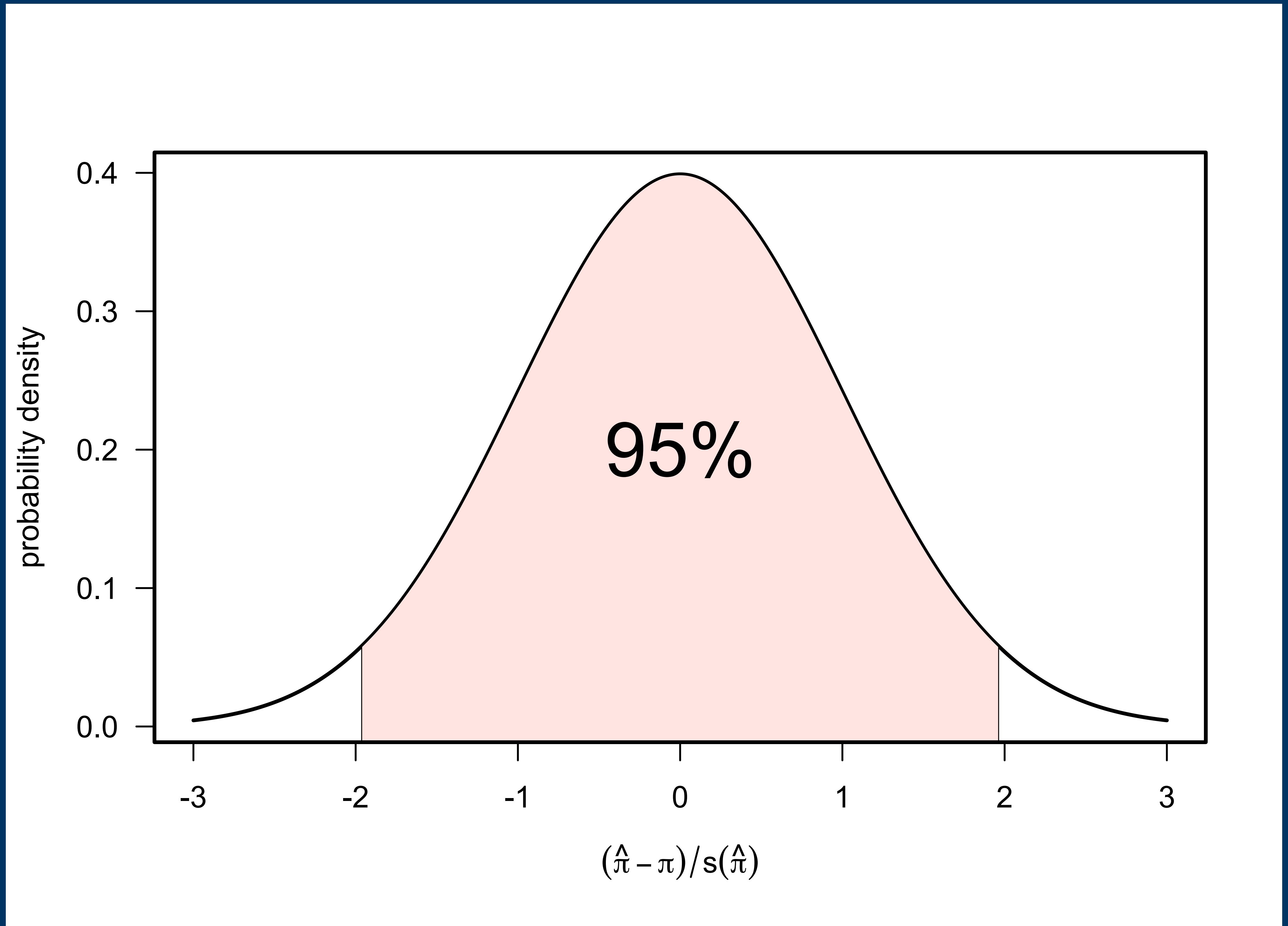
$$se\left(\hat{\pi}_{mle}\right) = \sqrt{\frac{\pi\,(1 - \pi)}{n}}$$

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

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

Two-tail Wald's confidence interval

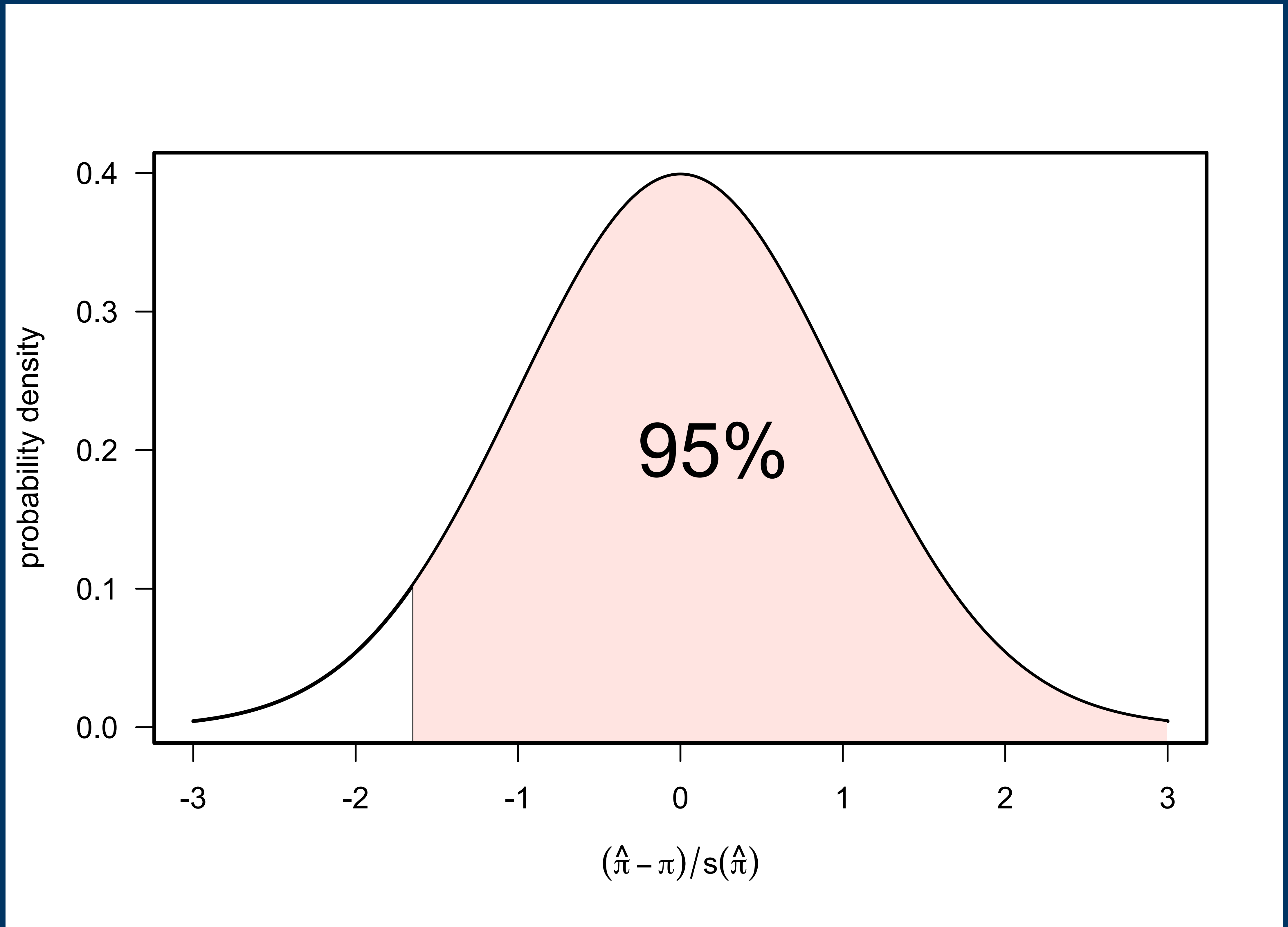
$$Y = \frac{\hat{\pi}_{mle} - \pi}{\sqrt{\frac{\hat{\pi}_{mle}\left(1 - \hat{\pi}_{mle}\right)}{n}}} \mid \pi, n \rightsquigarrow \text{Normal}\left(\mu = 0; \sigma = 1\right) \text{ For large samples}$$



One-tail Wald's confidence interval

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

For large samples



Estimation of proportion of positive tests

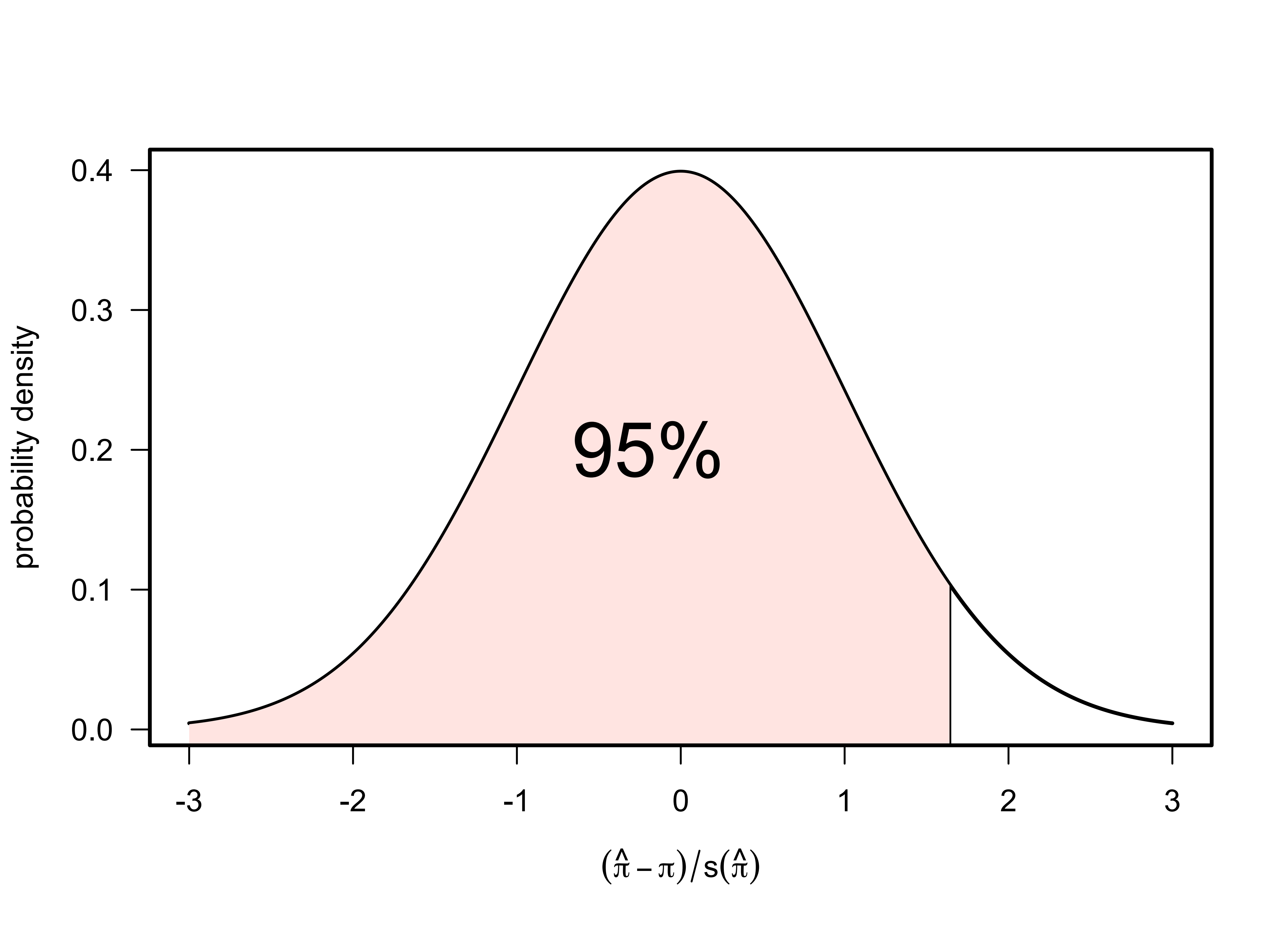
$$x \mid n, \pi \rightsquigarrow \text{Binomial}\,(n; \pi)$$

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

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

One-tail Wald's confidence interval

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



Estimation of proportion of positive tests

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

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

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

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

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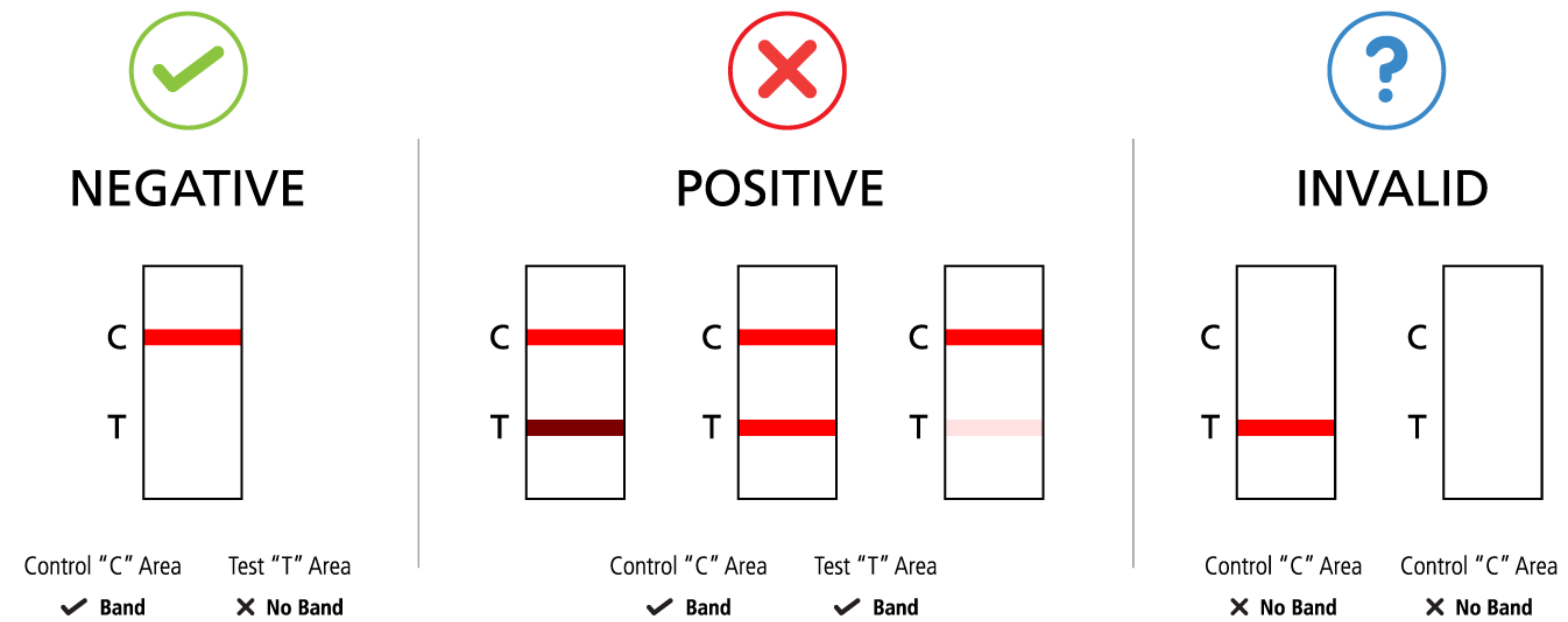
Community mass blood survey						
P. vivax	21	–	2	–	–	19
P. falciparum	10	–	–	–	–	10
Mixed *Pf* + *Pv*	6	–	1	–	–	5
Mixed *Pf* + *P. ovale*	2	–	–	–	–	2
Mixed *Pf* + *Pv* + *Po*	1	–	–	–	–	1
Mixed *Pf* + *Pv* + *Po* + *P. malariae*	1	–	–	–	–	1
Negative	1306	–	–	–	–	1306
Total *n*	1347	–	3	–	–	1344
Hospital 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

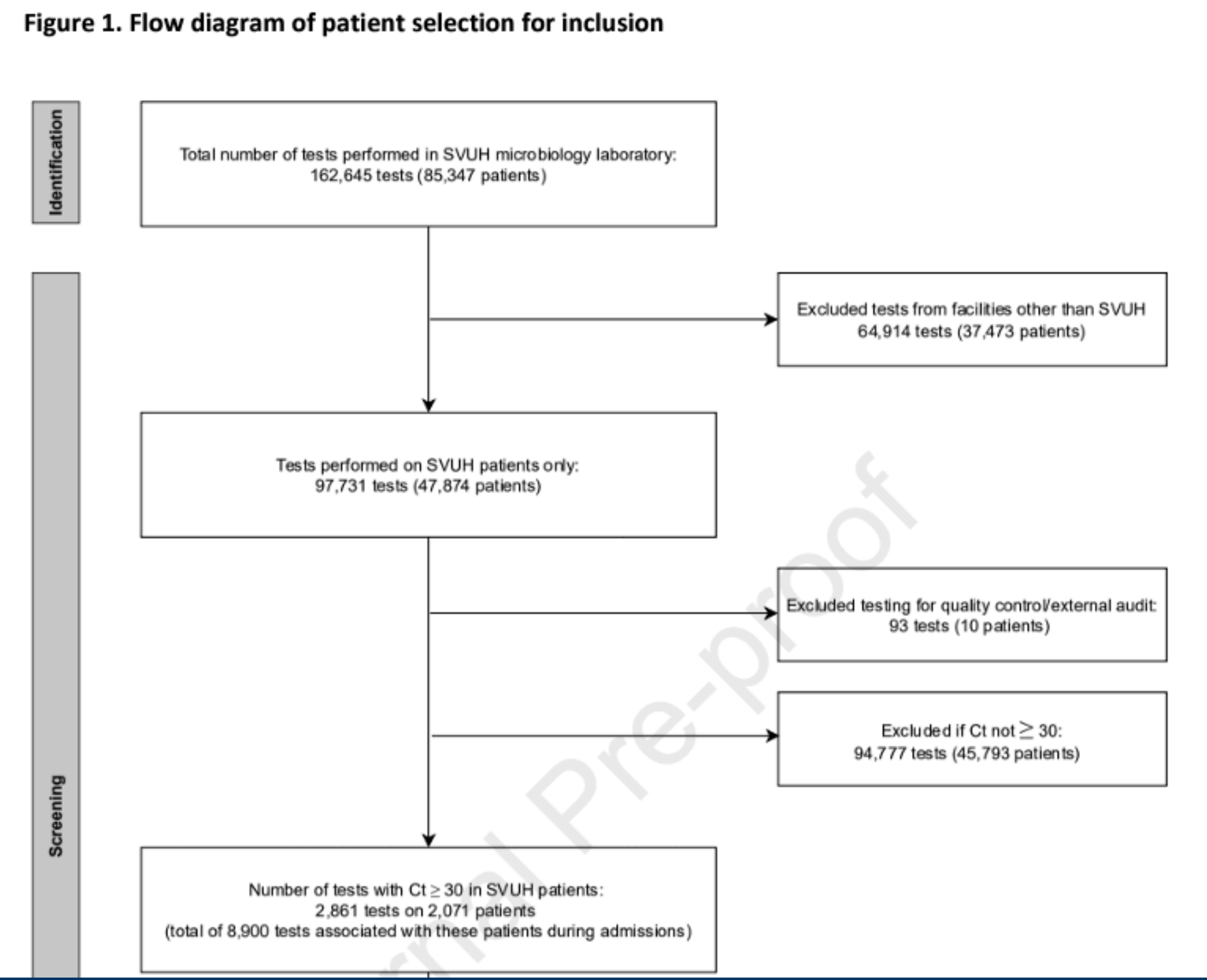
Break

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

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

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

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

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

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

$$2.24 = \Phi^{-}1\left(\frac{0.975}{2}\right)$$

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

$$\Phi^{-}1\left(\frac{1-\gamma}{2p}\right)$$

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 indeterminate tests and calculate the respective 95% confidence intervals