A Hypothesis Testing Framework for Validating an Assay for Precision

We provide tools to convert such a procedure into a set of m hypothesis tests. This reframing motivates the m:n:q procedure, which upon completion delivers a 100q% upper confidence limit on the CV. Additionally, for a post-validation assay output of y, the method gives an "effective standard deviation interval" of log(y) plus or minus r, which is a 68% confidence interval on log(mu), where mu is the expected value of the assay output for that sample. Further, the m:n:q procedure can be straightforwardly applied to constant standard deviation assays. We illustrate these tools by applying them to a growth inhibition assay.

Package ‘testassay’

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Type Package

Title A Hypothesis Testing Framework for Validating an Assay for Precision

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Description A common way of validating a biological assay for is through a procedure, where m levels of an analyte are measured with n replicates at each level, and if all m estimates of the coefficient of variation (CV) are less than some prespecified level, then the assay is declared validated for precision within the range of the m analyte levels. Two limitations of this procedure are: there is no clear statistical statement of precision upon passing, and it is unclear how to modify the procedure for assays with constant standard deviation. We provide tools to convert such a procedure into a set of m hypothesis tests. This reframing motivates the m:n:q procedure, which upon completion delivers a 100q% upper confidence limit on the CV. Additionally, for a post-validation assay output of y, the method gives an "effective standard deviation interval" of log(y) plus or minus r, which is a 68% confidence interval on log(mu), where mu is the expected value of the assay output for that sample. Further, the m:n:q procedure can be straightforwardly applied to constant standard deviation assays. We illustrate these tools by applying them to a growth inhibition assay.

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gia  
*Growth Inhibition Assay*

**Description**

Data from a growth inhibition assay experiment. Samples were run repeatedly on different assays, for two different strains of parasites (3d7 and FVO). elisa is a measure of the amount of antibody and is measured once for each sample. sample is a unique name for each sample and is defined as paste(gia$parasite,gia$elisa,sep=" "). gia is the value of interest, and the meanAAgia is the sample level mean, which is the best estimate of the "true" gia level for that sample. varAAgia is the sample level variance.

**Usage**

```r
gia
```

**Format**

A data frame with variables: parasite, assay, elisa, gia, sample, meanAAgia, and varAAgia

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lognormConstCVCI  
*log-normal constant CV model*

**Description**

This function gets confidence intervals on $\mu=E(Y)$ assuming $Y$ is lognormal and the coefficient of variation is known.

**Usage**

```r
lognormConstCVCI(y, theta, conf.level = 0.6827)
```

**Arguments**

- `y` Observed value
- `theta` coefficient of variation (assumed known)
- `conf.level` Confidence level
Details

Let $Y$ be lognormal, so that $\log(Y)$ is normal with mean $\mu$ and variance $\sigma^2$. Then $E(Y) = e^{\mu + \sigma^2/2}$ and $\text{Var}(Y) = \sigma^2 \cdot e^{\sigma^2}$. Hence, the coefficient of variation is $\sigma/\mu = \sqrt{e^{\sigma^2} - 1}$. We want to get log-centered confidence intervals on $\mu$, meaning intervals such that $\log(y) \pm r(\theta)$, where $r(\theta)$ is a constant function of $\theta$.

Value

A list with the following components

- obs y
- lower lower confidence limit on $\mu=E(Y)$
- upper upper confidence limit on $\mu=E(Y)$

Examples

# defaults to 68.27 percent confidence level, same level as normal plus or minus 1 std dev.
lognormconstCVCI(3.4,.6)
# compare to normal constant CV model result
normConstCVCI(3.4,.6)

Description

Assume $Y$ is normal with mean $\mu>0$ and coefficient of variation $\theta$, then $Y/\mu \sim N(1, \theta^2)$. Get log-centered confidence intervals (when possible), meaning intervals such that $\log(y) \pm r(\theta)$, where $r(\theta)$ is a constant function of $\theta$.

Usage

cmpConstCVCI(y, theta, conf.level = 0.6827, eps = .Machine$double.eps^{0.25})

Arguments

- y vector of observed values, should be positive
- theta coefficient of variation (assumed known)
- conf.level Confidence level
- eps a small number used in the algorithm (look at code before changing)
Value

A list with the following components

- obs y
- lower lower confidence limit on mu=E(Y)
- upper upper confidence limit on mu=E(Y)

Examples

# defaults to 68.27 percent confidence level, same level as Normal plus or minus 1 std dev.
normConstCVCI(3.4,.6)
# symmetric on log scale
log(normConstCVCI(3.4,.6))

predict.assaytest Construct effective standard deviation intervals for observed assay values

Description

Computes effective standard deviation intervals for observed assay results. These intervals have at least 68.27 percent coverage.

Usage

## S3 method for class 'assaytest'
predict(object, newdata, ...)

Arguments

- object An object of class "assaytest"
- newdata A vector of observed values. If missing, uses object$x.
- ... additional arguments

Details

Takes the Umax element from the assaytest object and treats it as the known precision parameter. For the constant SD model, the effective standard deviation interval for observed data value y is (y-Umax, y+Umax). For the constant CV models the effective SD interval uses either normConstCVCI (for the "normal" model) or lognormConstCVCI (for the "lognormal" model).

Although Umax is an upper bound (not an estimate) of the precision parameter, simulations have shown that treating Umax as the true precision parameter gives effective SD intervals with coverage of at least 68.27 percent (see Fay, Sachs, and Miura, 2016).
Value

A data frame with the observed values, lower, and upper confidence limits

References


print.assaytest

Print the results of an assaytest

Description

Print the results of an assaytest

Usage

## S3 method for class 'assaytest'
print(x, ...)

Arguments

x An object of class "assaytest", as created by the function testassay
... Additional arguments, currently unused

testassay

Hypothesis testing procedure for assay validation for precision

Description

Does an m:n:q procedure for assay validation for precision. Returns an object of class 'assaytest'. There is are predict and print methods for that class.

Usage

testassay(x, m, n, q = 0.9, model = "normal", constant = "SD",
   data = NULL)
Arguments

x  The vector of assay values
m  The vector of values indicating sample membership
n  The vector of values indicating replicate membership
q  The confidence level, typically 0.8 or 0.9
model  String specifying the distribution for the assay values. Valid values are "normal" or "lognormal"
constant  String specifying whether the standard deviation is assumed to be constant over the levels ("SD") or the coefficient of variation is assumed constant over the levels ("CV"). The values "sd", "var", or "variance" may be used for "SD", and "cv" may be used for "CV".
data  Data frame or environment in which to look for x

details

The m:n:q procedure uses m different samples that have different levels of the true value with n replicates for each sample. The output is a 100q percent upper limit of the bound on the precision parameter when the true values within the range of values for the m samples all follow either a a constant coefficient of variation model or a constant standard deviation model (same as a constant variance model) (see constant argument).

For example, if the 4:4:90 percent procedure using a normal model with a constant variance model returns a bound on the standard deviation (the Umax element of the assaytest class) of 7.9 then under the assumptions we have 90 percent confidence that the true SD is less than 7.9.

The predict method gives effective standard deviation intervals (i.e., 68.27 pct CIs) for the expected response from subsequent observed values from the assay.

Value

An object of class "assaytest", which is a list of components including a data frame of the relevant statistics calculated on x. Print, summary, predict, and plot methods are available. The list has the following components

- sumtabTable summarizing the experiment, includes mean values, SD or CV estimates, and upper confidence limits on those.
- UmaxThe maximum of the upper limits on the SD or CV, used in the effective SD interval calculation
- nThe number of samples per level
- mThe number of levels
- qThe confidence level
- modelThe assumed model
- constantThe parameter assumed to be constant (either 'SD' or 'CV').
- alphaThe alpha level, calculated as (1 - q)^(1 / m)
- xThe data vector supplied by the user
References


Examples

```r
# reproduce Table 3 of Fay, Sachs and Miura
I <- gia$parasite=="307" & gia$meanAGia<80
treD7.test<-testassay(x=gia, m=sample, n=assay, q=.9, 
data=subset(gia, parasite=="307" & meanAGia<80))
treD7.test
# get estimated effective standard deviation intervals (68.27 percent CIs)
# for observed values 21.4 and 65.9
# using results from testassay
predict(treD7.test,c(21.4,65.9))
```
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