

# Package ‘reflimR’

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**Type** Package

**Title** Reference Limit Estimation Using Routine Laboratory Data

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## Description

Uses an indirect method based on truncated quantile-quantile plots to estimate reference limits from routine laboratory data. The principle of the method was developed by Robert G Hoffmann (1963) <doi:10.1001/jama.1963.03060110068020> and modified by Georg Hoffmann and colleagues (2015) <doi:10.1515/labmed-2015-0104>, (2020) <doi:10.1515/labmed-2020-0005>, and (2022) <doi:10.1007/978-3-031-15509-3\_31>.

**URL** <https://github.com/reflim/reflimR>

**License** GPL-2

**Encoding** UTF-8

**LazyData** true

**Imports** stats, graphics, grDevices

**Depends** R (>= 3.5.0)

**Suggests** knitr, rmarkdown, testthat (>= 3.0.0)

**BugReports** <https://github.com/reflim/reflimR/issues>

**VignetteBuilder** knitr

**RoxygenNote** 7.2.3

**NeedsCompilation** no

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adjust_digits	<i>Plausible rounding</i>
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**Description**

Rounds a quantitative laboratory result to a reasonable number of decimal places.

**Usage**

```
adjust_digits(x)
```

**Arguments**

x	numeric
---	---------

**Value**

List containing a rounded value of x and the number of decimal places

**Examples**

```
adjust_digits(0.001234)
adjust_digits(0)
adjust_digits(-12.34)
adjust_digits(5.4321)$digits
```

---

bowley	<i>Bowley skewness</i>
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### Description

Calculates a robust skewness measure for  $x$  based on the interquartile range (or any other quantile range).

### Usage

```
bowley(x, alpha = 0.25)
```

### Arguments

<code>x</code>	numeric vector
<code>alpha</code>	lower quantile of the range to be regarded (e. g. 0.25)

### Details

Bowley's quantile skewness is calculated from  $(q[1] - 2 * q[2] + q[3]) / (q[3] - q[1])$ , where  $q$  is a vector of quantiles  $\alpha$ , 0.5, and  $1 - \alpha$ . The default value for  $\alpha = 0.25$  indicates an interval from the first to the third quartile.

### Value

Bowley's quantile skewness

### References

1. Bowley, AL (1920). Elements of Statistics. London : P.S. King & Son, Ltd.
2. Klawonn F, Hoffmann G, Orth M. Quantitative laboratory results: normal or lognormal distribution. J Lab Med 2020; 44: 143–50. [doi:10.1515/labmed20200005](https://doi.org/10.1515/labmed20200005).

### Examples

```
bowley(1 : 100)
bowley(rnorm(1000, 3, 0.2))
bowley(rlnorm(1000, 3, 0.5))
```

---

`conf_int95`*Confidence intervals of estimated reference limits*

---

**Description**

Calculates 95 percent confidence intervals for the lower and upper reference limits obtained with the reflim algorithm.

**Usage**

```
conf_int95(n, lower.limit, upper.limit, lognormal = TRUE,  
           apply.rounding = TRUE)
```

**Arguments**

<code>n</code>	number of observations
<code>lower.limit</code>	positive number indicating the lower limit of the reference interval
<code>upper.limit</code>	positive number indicating the upper limit of the reference interval
<code>lognormal</code>	Boolean indicating whether a lognormal distribution should be assumed
<code>apply.rounding</code>	Boolean indicating whether the confidence limits should be rounded

**Details**

The confidence limits depend on the reference range (upper minus lower limit), and are proportional to  $1/\sqrt{n}$ .

The coefficients used in this function have been determined by 100,000 Monte-Carlo simulations for sample sizes between  $n = 200$  and  $n = 2,000$  based on a standard normal distribution.

**Value**

95 percent confidence limits and total number of observations for the lower and the upper reference limit (ranging from `lower.lim.low` to `lower.lim.upp` for the lower reference limit, and from `upper.lim.low` to `upper.lim.upp` for the upper reference limit)

**Examples**

```
conf_int95(n = 250, lower.limit = 10, upper.limit = 50)  
conf_int95(250, 135, 145, FALSE, FALSE)
```

---

Dataset: *liver*tests     *Dataset: liver*tests

---

## Description

Example data showing eight different biomarkers (laboratory tests), which are frequently measured in healthy controls and patients with liver diseases.

## Usage

```
liver
```

## Format

A data frame with 612 rows and 11 columns:

**Category** healthy reference individual or patient

**Age** age in years

**Sex** sex f = female or m = male

**ALB** albumin, g/L

**ALT** alanine aminotransferase, U/L

**AST** aspartate aminotransferase, U/L

**BIL** bilirubin,  $\mu\text{mol/l}$

**CHE** choline esterase, kU/L

**CREA** creatinine,  $\mu\text{mol/L}$

**GGT** gamma-glutamyl transferase, U/L

**PROT** total protein, mg/L

## Source

<<https://archive.ics.uci.edu/ml/datasets/HCV+data>>

## Examples

```
summary(liver)
pie(table(liver$Category), labels = c("patients", "controls"))
plot(liver$Age, liver$ALB, xlab = "Age [yr]", ylab = "ALB [g/L]")
grid()
abline(lm(liver$ALB ~ liver$Age))

che <- liver$CHE
ref <- liver$CHE[liver$Category == "reference"]
pat <- liver$CHE[liver$Category == "patient"]

hist(che, breaks = 1 : 20, col = "white", main = "cholinesterase", xlab = "kU/L")
hist(ref, breaks = 1 : 20, col = rgb(0, 1, 0, 0.5), add = TRUE)
```

```

hist(pat, breaks = 1 : 20, col = rgb(1, 0, 0, 0.5), add = TRUE)
legend("topright", fill = c(rgb(1,1,1,1), rgb(0,1,0,0.5), rgb(1,0,0,0.5)),
      legend = c("all", "controls", "patients"))

t.test(ref, pat)
var.test(ref, pat)

che.f <- livertests$CHE[livertests$Sex == "f"]
che.m <- livertests$CHE[livertests$Sex == "m"]
plot(density(che.f), xlim = c(0, 20), col = "red",
     main = "cholinesterase", xlab = "kU/L")
lines(density(che.m), col = "blue")
legend("topright", lty = 1, col = c("red", "blue"), legend = c("females", "males"))

reflim(che.m, main = "CHE (m)", xlab = "kU/L")
reflim(livertests$AST[livertests$Sex == "m"], main = "AST (m)", xlab = "U/L")

```

---

Dataset: *targetvalues* *Dataset: target values*

---

## Description

Test names (analytes), units and reference limits from a textbook.

## Usage

```
targetvalues
```

## Format

A data frame with 8 rows and 6 columns:

**analyte** short name of the analyte

**unit** measuring unit

**ll.female, ul.female** lower and upper reference limits for women

**ll.male, ul.male** lower and upper reference limits for men

## Details

The table was created from the data in the textbook (web version 2023, <https://www.clinical-laboratory-diagnostics.com>). Missing data (i.e. the lower limits for ALT, AST and GGT) were supplemented from the product sheets of the respective tests.

## Source

Thomas L. Clinical Laboratory Diagnostics, 2023

**Examples**

```
targetvalues[, 1 : 4]
reflim(livertests$ALB[livertests$Sex == "m"],
main = targetvalues[1, 1], xlab = targetvalues[1, 2],
targets = targetvalues[1, 5 : 6])
```

iboxplot

*Removal of pathological values***Description**

Iteratively truncates a vector of quantitative laboratory results until no more values outside the specified truncation interval are left.

**Usage**

```
iboxplot(x, lognormal = NULL, perc.trunc = 2.5,
apply.rounding = TRUE,
plot.it = TRUE, main = "iBoxplot", xlab = "x")
```

**Arguments**

x	vector of positive numbers
lognormal	Boolean indicating whether a lognormal distribution should be assumed (NULL means that the distribution type is defined automatically)
perc.trunc	percentage of presumably normal values to be removed from each side
apply.rounding	Boolean indicating whether the estimated reference limits should be rounded
plot.it	Boolean indicating whether a graphic should be created
main, xlab	title and x label of the graphic

**Details**

The truncated vector represents the estimated central 95 percent of values, which follow the assumed distribution (normal or lognormal). If the distribution of the reference values is unknown, medical laboratory results should be assumed to be lognormally distributed [2].

**Value**

\$trunc	truncated vector x
\$limits	truncation points, preliminary reference limits
\$lognormal	Boolean indicating whether a lognormal distribution has been assumed
\$perc.norm	proportion of the assumed non-pathological values
\$progress	results of the iterative truncation

## References

1. Klawonn F, Hoffmann G. Using fuzzy cluster analysis to find interesting clusters. In: L.A. Garcia-Escudero et al. (eds.): Building bridges between soft and statistical methodologies for data science. Springer, Cham (2023), 231-239. doi:10.1007/9783031155093\_31.
2. Haeckel R, Wosniok W. Observed unknown distributions of clinical chemical quantities should be considered to be log-normal. Clin Chem Lab Med 2010; 48: 1393-6. doi:10.1515/CCLM.2010.273.

## Examples

```
set.seed(123)
iboxplot(rlnorm(n = 250, meanlog = 3, sdlog = 0.3))

iboxplot(rnorm(1000, 100, 10), apply.rounding = FALSE, plot.it = FALSE)$truncation.points

alb.trunc <- iboxplot(livertests$ALB, main = "ALB", xlab = "g/L")$trunc
summary(alb.trunc)
```

---

interpretation	<i>Colors and text modules to interpret deviations from given target values</i>
----------------	---

---

## Description

Creates traffic light colors green, yellow, and red as well as a textual description such as 'slightly increased'. This function is called by reflim(), if target values are available, and provides the required color information for ri\_hist().

## Usage

```
interpretation(limits, targets)
```

## Arguments

limits	vector of two numbers indicating the reference limits that have been calculated by the reflim function (or any other suitable algorithm)
targets	vector of two numbers indicating target reference limits that may have been obtained from external sources

## Details

This algorithm compares the positions and tolerance intervals of the estimated upper and lower reference limits with the tolerance limits of the respective target values.

If the estimated reference limit is within the tolerance of the target value, the dev.lim text says 'within tolerance' and the color code #00FF0080 for semi-transparent green is returned.

If the position is outside and the two tolerance limits overlap, the dev.lim text says 'slightly increased' or 'slightly decreased' and the color code #FFFF0080 for semi-transparent yellow is returned.



If the tolerance limits do not overlap, the `dev.lim` text says 'markedly increased' or 'markedly decreased' and the color code `#FF00080` for semi transparent red is returned.

### Value

`$tol.lim` and `$tol.tar`  
tolerance limits for the estimated reference limits and the respective target values. If targets are not provided, the latter tolerance limits are returned as NA.

`$col.lim` and `$col.tar`  
hexadecimal rgb values, indicating the traffic light colors green, yellow, and red

`$dev.lim`  
short text describing the deviations of the observed limit values from the target values

### Examples

```
interpretation(limits = c(10, 50), targets = c(11, 49))
interpretation(limits = c(10, 50), targets = c(8, 60))$dev.lim
```

---

lognorm	<i>Lognormal distribution model</i>
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---

### Description

Suggests lognormal modelling of a numeric vector `x` by comparing Bowley's quantile skewness for `x` and `log(x)`. Lognormality is suggested if `bowley(x) - bowley(log(x)) >= cutoff`.

### Usage

```
lognorm(x, cutoff = 0.05, alpha = 0.25, digits = 3,
        plot.it = TRUE, plot.logtype = TRUE,
        main = "Bowley skewness", xlab = "x")
```

### Arguments

<code>x</code>	numeric vector of positive numbers
<code>cutoff</code>	a skewness threshold for the suggestion of a lognormal distribution
<code>alpha</code>	lower quantile of the range to be regarded (e. g. 0.25 for IQR)
<code>digits</code>	number of digits to be displayed for the Bowley skewness
<code>plot.it</code>	Boolean indicating whether a graphic should be created
<code>plot.logtype</code>	Boolean indicating whether the distribution type should be printed in the graphic
<code>main, xlab</code>	title and x label of the graphic

## Details

If `$lognorm` is TRUE, a lognormal distribution is suggested for right-skewed density curves ( $\text{bowley}(x) > 0$ ). The decision for a lognormal distribution is based on the difference between the skewness of the original and the logtransformed values (cut-off defaults to 0.05).

In the unusual case of a left-skewed distribution ( $\text{bowley}(x) < 0$ ), a normal distribution is suggested (`lognormal = FALSE`), assuming that the left skew is caused by pathological low values rather than an unusual distribution of laboratory results.

The plot illustrates the skewness of  $x$  and  $\log(x)$  showing density curves and boxplots with separate  $x$ -axes for the original values (bottom axis) and the log-transformed values (top axis). A skewness delta below the cut-off value means that both curves are quite symmetric. In this case,  $x$  can be approximated by a normal distribution. If the delta exceeds the cut-off, the density curve of the original values and the respective boxplot are right-skewed and become more symmetric after log-transformation.

The `plot.logtype` argument is used internally to suppress printing the automated definition of the distribution in case that the type has been set manually.

Extreme values are removed before plotting to improve the graphic.

## Value

`$lognorm` Boolean indicating whether a lognormal distribution should be assumed  
`$BowleySkewness` Bowley skewness of the original and the logtransformed values as well as the difference

## References

1. Klawonn F, Hoffmann G, Orth M. Quantitative laboratory results: normal or lognormal distribution? J Lab Med 2020; 44: 143-50. doi:10.1515/labmed20200005.

## Examples

```
lognorm(rnorm(n = 1000, mean = 20, sd = 2))
lognorm(rlnorm(n = 1000, meanlog = 3, sdlog = 0.3))
lognorm(livertests$ALB, main = "albumin", xlab = "g/L")
lognorm(livertests$BIL, main = "bilirubin", xlab = "µmol/L")
```

---

permissible\_uncertainty

*Tolerance intervals of estimated and target limits*

---

## Description

Returns the permissible uncertainty of reference limits.

## Usage

```
permissible_uncertainty(lower.limit, upper.limit, apply.rounding = TRUE)
```

## Arguments

`lower.limit` positive number indicating the lower limit of the reference interval  
`upper.limit` positive number indicating the upper limit of the reference interval  
`apply.rounding` Boolean indicating whether the tolerance limits should be rounded

## Details

The tolerance limits (also called equivalence limits) indicate the permissible uncertainty of a reference limit from a medical point of view (in contrast to the confidence interval, which reflects the statistical point of view). The calculation is based on a recommendation made by the DGKL [1, 2].

## Value

Tolerance intervals for the lower and upper reference limits (ranging from `lower.lim.low` to `lower.lim.upp` for the lower reference limit and from `upper.lim.low` to `upper.lim.upp` for the upper reference limit)

## References

1. Haeckel R et al. Permissible limits for uncertainty of measurement in laboratory medicine. Clin Chem Lab Med 2015;53:1161–71. doi:10.1515/cclm20140874.
2. Haeckel R et al. Equivalence limits of reference intervals for partitioning of population data. J Lab Med 2016; 40: 199-205. doi:10.1515/labmed20160002.

## Examples

```
permissible_uncertainty(lower.limit = 10, upper.limit = 50)
permissible_uncertainty(10, 50, FALSE)
```

---

reflim	<i>Reference limits (main function)</i>
--------	---

---

## Description

Estimation of reference limits from mixed distributions of normal and pathological laboratory results. Estimates lower and upper reference limits and provides statistical characteristics and graphics to evaluate the results.

## Usage

```
reflim(x, lognormal = NULL, targets = NULL,
       perc.trunc = 2.5, n.min = 200, apply.rounding = TRUE,
       plot.it = TRUE, plot.all = FALSE, print.n = TRUE,
       main = "reference limits", xlab = "x")
```

**Arguments**

<code>x</code>	vector of positive numbers
<code>lognormal</code>	Boolean indicating whether a lognormal distribution should be assumed (NULL means that the distribution type is defined automatically)
<code>targets</code>	vector of two numbers indicating target reference limits that may have been obtained from external sources
<code>perc.trunc</code>	percentage of presumably normal values to be removed from each side
<code>n.min</code>	minimum number of observations needed for a robust estimate of reference intervals
<code>apply.rounding</code>	Boolean indicating whether the estimated reference limits should be rounded
<code>plot.it</code>	Boolean indicating whether graphics should be created
<code>plot.all</code>	Boolean indicating whether graphics of all process steps should be created
<code>print.n</code>	Boolean indicating whether the number of cases after truncation should be printed on the graph
<code>main, xlab</code>	title and x label of the graphic

**Details**

The `reflim` function estimates reference limits from the linear part of a normal probability-probability or quantile-quantile plot [1, 2]. It combines several functions to determine the distribution type [3], to truncate the input vector [4], and to generate a truncated quantile-quantile plot [2, 4]. For details concerning the individual functions, which are called by `reflim()`, you may enter `*help(package = reflimR)*`.

The default value of `perc.trunc` is 2.5 meaning that 2.5 percent of the assumed non-pathological values are truncated on both sides of the quantile-quantile plot. By increasing `perc.trunc` (e.g. to 5 percent), a stronger cut can be applied to reduce the influence of potentially overlapping pathological values.

The argument `plot.it` is used to compare the observed and the theoretical distribution curves graphically. If target values have been specified, the tolerance intervals of the calculated reference limits will be drawn as colored vertical lines. Green means that the calculated limit is within the tolerance interval of the respective target, yellow means that the calculated limit falls outside but the two tolerance intervals overlap, and red means that there is no overlap between the tolerance intervals.

More detailed graphs of the three underlying steps can be generated by setting `plot.all = TRUE`. When `plot.all` is set to `TRUE`, `plot.it` is automatically set to `TRUE` as well.

**Value**

<code>\$stats</code>	mean and sd (or <code>meanlog</code> and <code>sdlog</code> ) of the truncated vector, number of cases before and after truncation
<code>\$lognormal</code>	Boolean indicating whether a lognormal distribution has been assumed
<code>\$limits</code>	estimated reference limits with tolerance intervals
<code>\$targets</code>	target values with tolerance intervals
<code>perc.norm</code>	estimated percentage of non-pathological values

\$confidence.int	95 percent confidence intervals for the estimated reference limits (depends on n)
\$interpretation	short text describing the deviation of observed limits from target values
remarks	short text describing potential reasons why the reflim function could not be executed

## References

1. Holmes D, Buhr K. Widespread incorrect implementation of the Hoffmann method, the correct approach, and modern alternatives. *Am. J. Clin. Pathol.* 2018; 151:328-36. doi:10.1093/ajcp/aqy149.
2. Hoffmann G, Lichtinghagen R, Wosniok W. Simple estimation of reference intervals from routine laboratory data. *J Lab Med* 2015; 39: 389-402. doi:10.1515/labmed20150104.
3. Klawonn F, Hoffmann G, Orth M. Quantitative laboratory results: normal or lognormal distribution? *J Lab Med* 2020; 44: 143-50. doi:10.1515/labmed20200005.
4. Klawonn F, Hoffmann G. Using fuzzy cluster analysis to find interesting clusters. In: L.A. Garcia-Escudero et al. (eds.): *Building bridges between soft and statistical methodologies for data science*. Springer, Cham (2023), 231-239. doi:10.1007/9783031155093\_31.

## Examples

```
x <- c(rnorm(800, 100, 10), rnorm(100, 70, 15), rnorm(100, 125, 15))
reflim(x, targets = qnorm(c(0.025, 0.975), 100, 10))

x.f <- subset(livertests, livertests$Sex == "f")
reflim(x.f$AST)
reflim(x.f$AST, targets = c(13, 40))
reflim(x.f$AST, targets = targetvalues[3, 3 : 4],
main = "AST/GOT", xlab = targetvalues[3, 2])
reflim(x.f$AST, plot.all = TRUE, main = "AST/GOT", xlab = "U/L")$limits
reflim(x.f$ALB, targets = targetvalues[1, 3 : 4],
plot.all = TRUE, main = "ALB", xlab = "g/L")$limits
```

---

ri\_hist

*Histogram with density plots and reference limits*


---

## Description

Creates a graphic illustrating the results of the reflim function.

## Usage

```
ri_hist(x, lognormal, stats, limits, perc.norm,
targets = NULL, remove.extremes = TRUE,
main = "reflim", xlab = "x")
```

**Arguments**

x	vector of positive numbers
lognormal	Boolean indicating whether a lognormal distribution should be assumed
stats	vector of mean and sd, or meanlog and sdlog, respectively
limits	vector of reference limits calculated by the reffim function (or any other suitable algorithm)
perc.norm	estimated percentage of non-pathological values (usually provided by the ibox-plot function)
targets	vector of target reference limits obtained from external sources
remove.extremes	Boolean indicating whether extreme values should be removed to improve the graphic
main, xlab	title and x label of the graphic

**Details**

ri\_hist is called by the reffim function, but it can also be used to illustrate the results of other software packages (e. g. refineR), if the required arguments are available (see details).

It creates a graphic, which includes a histogram and a density curve of x, as well as a theoretical density curve of presumably non-pathological values (blue) and a calculated density curve of presumably pathological values (red). Calculated reference limits and target limits are shown as vertical lines, and their tolerance intervals (i. e., the permissible uncertainties) are represented by surrounding boxes. If target values are provided, traffic light colors indicate the deviation between target and actual results.

If the arguments lognormal or perc.norm are unknown, they can be set according to the user's expertise. For example, if the distribution type is unknown, a lognormal distribution can be assumed [1]. If the percentage of non-pathological values has not been provided by a foreign algorithm (e. g. refineR), it can be roughly estimated, if density curves of normal and pathological values are available (the argument perc.norm does not influence the result; its only effect is on the shape of the theoretical density curve).

**Value**

\$lognormal	assumed distribution model
\$percent_normal	assumed percentage of non-pathological values
\$interpretation	text describing the deviation of observed limits from target values

**References**

1. Haeckel R, Wosniok W. Observed unknown distributions of clinical chemical quantities should be considered to be log-normal. Clin Chem Lab Med 2010; 48: 1393-6. doi:10.1515/CCLM.2010.273.

**Examples**

```

set.seed(123)
x1 <- rlnorm(800, 3, 0.3)
lim <- quantile(x1, c(0.025, 0.975))
ri_hist(x1, lognormal = TRUE, stats = c(3, 0.3), limits = lim, perc.norm = 100)

x2 <- rlnorm(200, 3.5, 0.4)
x <- c(x1, x2)
tar <- quantile(x, c(0.025, 0.975))
ri_hist(x, lognormal = TRUE, stats = c(3, 0.3), limits = lim, targets = tar, perc.norm = 80)

```

---

truncated\_qqplot      *Quantile-Quantile plot of truncated laboratory results*


---

**Description**

Generates and plots a quantile-quantile plot (q-q plot) with a theoretical normal distribution on the x-axis and the corresponding empirical distribution on the y-axis. Returns intercept, slope, and estimated quantiles 0.025 and 0.975 (i.e., reference limits).

**Usage**

```

truncated_qqplot(x.trunc, lognormal = NULL, perc.trunc = 2.5, n.min = 200,
  apply.rounding = TRUE, plot.it = TRUE,
  main = "Q-Q plot",
  xlab = "theoretical quantiles",
  ylab = "sample quantiles")

```

**Arguments**

x.trunc	truncated numeric vector of positive numbers, usually generated by <code>iboxplot()</code>
lognormal	Boolean indicating whether a lognormal distribution should be assumed (NULL means that the distribution type is defined automatically)
perc.trunc	percentage of values that has been removed from each side by truncation
n.min	minimal number of values in x.trunc for a robust estimate of reference limits
apply.rounding	Boolean indicating whether the reference limits should be rounded
plot.it	Boolean indicating whether a graphic should be created
main, xlab, ylab	title and labels of the graphic

**Details**

Intercept and slope of the q-q plot represent the robust mean and standard deviation of x.trunc. They serve as parameters to estimate the reference limits being represented by the quantiles 0.025 and 0.975 of a presumably normal subset of x.

**Value**

`$stats`            intercept and slope of the q-q plot, lower and upper truncation points  
`$lognormal`        Boolean indicating whether a lognormal distribution has been assumed

**References**

1. Hoffmann G et al. Simple estimation of reference intervals from routine laboratory data. J Lab Med 2016. [doi:10.1515/labmed20150104](https://doi.org/10.1515/labmed20150104).

**Examples**

```
set.seed(123)
x <- rlnorm(n = 250, meanlog = 3, sdlog = 0.3)
x.trunc <- iboxplot(x, plot.it = FALSE)$trunc
truncated_qqplot(x.trunc)

x.f <- subset(livertests, livertests$Sex == "f")
x.trunc <- iboxplot(x.f$ALT, plot.it = FALSE)$trunc
truncated_qqplot(x.trunc, n.min = length(x.trunc), main = "ALT")
```



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