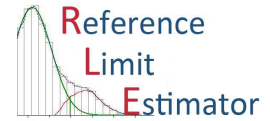


Program for the estimation  
of reference limits from the  
analysis data of clinical  
chemistry laboratories

Developed by the Working Group Guide Limits of  
the German Society for Clinical Chemistry and  
Laboratory Medicine (Registered Society)





# Introduction and Installation

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# Introduction and Installation

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## *Introduction*

A large number of measured values are defined, evaluated and documented daily in the clinical chemistry laboratory. There is a database with the measurement results and other relevant information, e.g. the sampling date, age and gender.

In general, the proportion of pathological values is small for each measured variable compared to the total number of all values. In this case, the data distribution of pathological values and the data distribution of non-pathological values can be separated with certain model assumptions and with statistical methods. From the distribution of the non-pathological values, the reference limits can then be calculated as the 2.5th and 97.5th percentiles. If the data are previously filtered according to age or gender, age and gender reference limits can be determined.

The advantage over the conventional method, in which a previously selected reference collective is examined, is enormous: the data are already available in the databases of the laboratories and the lengthy selection and examination of subjects of a reference collective are no longer necessary. Furthermore, for reasons of organization, costs and time it is very difficult to find so many people to carry out further stratifications (e.g. by age), with the exception of gender. In addition, there are often doubts about the representativeness of a selected collective. If the measuring method is poorly standardized, the reference limits determined in a laboratory with the help of volunteers cannot be adopted by other laboratories. If, on the other hand, the measured data are used from the laboratory's own database, laboratory-related reference limits are defined. Generally valid reference limits are obtained with these methods if several laboratories participate in the definition and the measuring procedure has a high standardization degree (1-6).

The program is used via a Microsoft Excel interface, which was developed with Excel 2003/2010 32 Bit and can also be used with the more recent versions of Excel. Since the statistical calculations are very extensive and partially complex, the statistical analysis is performed with the statistics program "R" and using some additional modules (packages). The statistical results and graphics representations are then transferred back into the Excel interface.

This Handbook is modular and consists of several files with continuous updates. In meantime it is possible that improvements of the program may not be described.

Please consider the version description of any part of the handbook.

# Introduction and Installation

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## Improvements

In this chapter the relevant changes are described in comparison with the last versions. The full list is found in the German handbook.

### Version 20180511 (RLE49)

Following several versions without revision of the handbook now a complete new system of help files was now created. The user can define exclusion and exclusion criteria and choose between first-value or last-value.

For huge datasets the 'Classic-Mode' was reactivated. These datasets are not limited by Excel and may consist of several files. The structure of each file must be identical.

### Version 20151017

The results of the evaluations are additionally stored as a copy in a subdirectory. The drift analysis has been extended by a regression curve with confidence intervals. Another R package (main package mgcv) is required.

For the case of very large samplings, the core density estimation was modified.

The software evaluates independently whether the predominantly pathological values are to be expected in the high or low range.

The program has new status windows and displays the required calculation time. In addition, minor errors have been corrected (for example, there may be spaces in the csv files)

### Version 20150307

Important extensions for finding and interpreting errors have been introduced in the R-codes as well as in Excel.

The drift effect analysis now uses the order date (with the date format) instead of the previous indication of month and year. Numerous minor programming improvements have been made

### Version 20141017

Renaming the tool in "Reference Limit Estimator" (previously: Guide Limit Calculator); Automatic recognition of the current R program version; Revising the graphics legends; Graphics format selectable; Graphics are permanently imported into the tool (Excel 2010); Manual as PDF file.

# Introduction and Installation

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## Installation of R under Windows

Installation of R needs administrator rights on the computer involved. The programming language R is free and can be downloaded from different platforms.

To do this, for example, you can select under <http://cran.r-project.org/mirrors.html> a nearby "CRAN mirror" (server) e.g. under "Germany" <http://mirrors.softliste.de/cran/> (The web page can be opened by pressing the "Ctrl" key and clicking on the link in blue color above this text line at the same time. On the website "Download R for Windows" will then be clicked.

The installation file is downloaded and executed.

Then the default settings suggested by the installation program can be used.

The Reference Limit Estimator can be used with either the 32bit or the 64bit R version. The R version 3.4.4 is the latest version tested at the time of publication of the manual.

After this basic installation of R, additional modules (called "packages") have to be downloaded and installed.

The choice of the German or English version of the R console installed does not interfere with the execution of Excel tool.

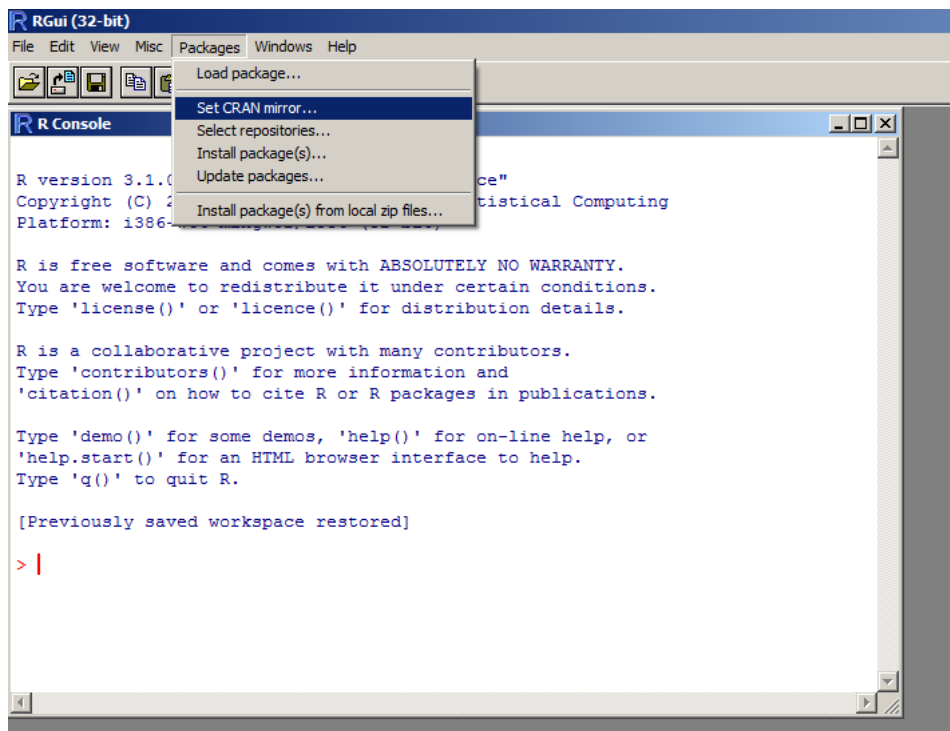
A new alternative without need of administrative rights is the USB-installation. For details please contact the developer team.

# Introduction and Installation

## Installation of additional R modules (packages)

For the installation, you must first check whether the user has written the Rights for the directory "drive / R / R-version number / library". Otherwise these rights must be established.

To add the packages to the "library" directory, R-Console must now be opened by selecting the "R" icon (located under "Programs" or on the "Desktop" of the PC). Then "Packages" is selected in the top menu bar and "Set Cran Mirror" is selected in the drop-down menu that opens (See the following illustration):



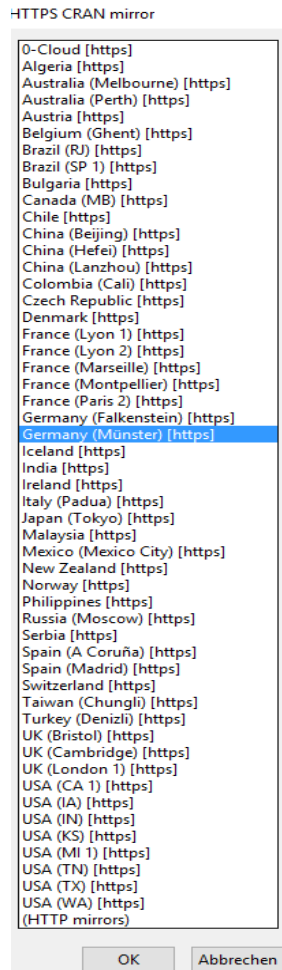
If no menu opens with a list of CRAN mirrors, a "firewall" probably prevents the process. In this case, the following text is entered directly into the R Console:

```
setInternet2()
chooseCRANmirror()
```

Other security mechanisms may also prevent the installation on the computer. In this case please contact your responsible administrators.

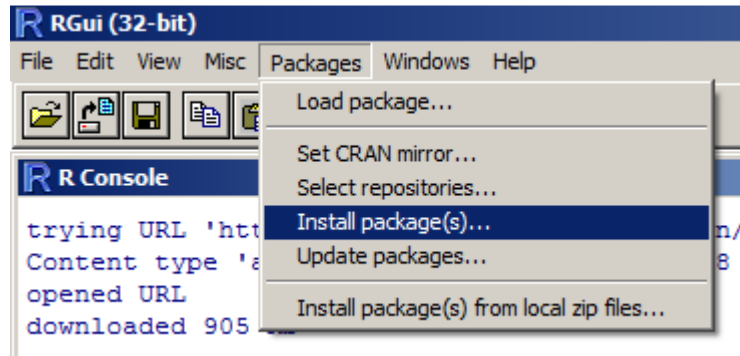
# Introduction and Installation

The CRAN mirror should now open, from which "Germany (Berlin)" is selected (see the following illustration):



# Introduction and Installation

Afterwards, "Packages" is selected in the R console in the top menu bar and "Install Packages" in the pull-down menu that opens (See the following illustration):



A long list of packages, from which "geoR" is first selected, opens. The installation is done automatically. Then the package "msm" is installed in the same way. The packages installed in this way require further packages dependent on this. Follow the installation recommendations of the software.

If the above security mechanisms of your computer prevent the installation, you can also install all the necessary packages manually. In preparation, download all necessary packages as a ZIP file from the above website and save the files.

Select "install package (s) from local zip files ...." from the "Packages" menu and select the packages required for the R program one after the other.

In addition to the basic installation, the following packages are required (version with short description).

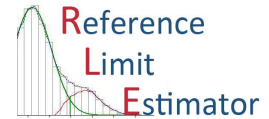
## main packages

geoR	1.7.5.2	Analysis of Geostatistical Data
mgcv	1.8-18	Mixed GAM computation Vehicle (Smoothness Estimation)
msm	1.6.4	Multi-state Markov and Hidden Markov Models in Continuous Time

## dependent packages (installation through main packages)

mass	7.3-47	Support Functions and Datasets for Venables and Ripley's MASS
mvtnorm	1.0.6	Multivariate Normal and t Distributions
nlme	3.1.131	Linear and Nonlinear Mixed Effects Models
expm	0.999.2	Matrix exponential
RandomFields	3.1.50	Simulation and Analysis of Random Fields
sp	1.2.4	Classes and Methods for Spatial Data
splancs	2.1.40	Spatial and Space-Time Point Pattern Analysis
survival	2.41.3	Survival Analysis





# Introduction and Installation

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## Installation of the „Reference Limit Estimator“

### Installation of the Excel tool „Reference Limit Estimator“ on the PC

All directories, programs and functions are located in a Zip folder, which must first be downloaded from the Internet. As a source, we recommend the website of The Working Group Reference values of the DGKL ([www.dgkl.de](http://www.dgkl.de)).

This download zip folder is then placed in a local directory, e.g. "C: / RL35 /".

The tool can be installed and used in any local directory.

### Windows and Excel versions

The Excel tool is opened as an Excel folder (xlsm). We recommend that you use Microsoft Excel 2010. Older versions of Excel can also be used, but in this case the different handling of graphics must be considered. The tool was developed under Microsoft Windows.

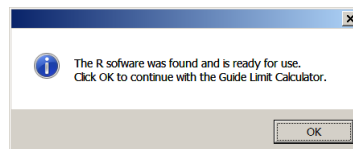
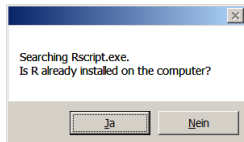
Excel 64Bit is not supported yet. The 64bit Excel use a new VBA version. The development team is working on a version for 64bit Excel.

# Introduction and Installation

## Configuring the „Reference Limit Estimator“

The tool is configured automatically. As part of the automatic configuration, the program searches for the current version of the statistical software R at the first start:

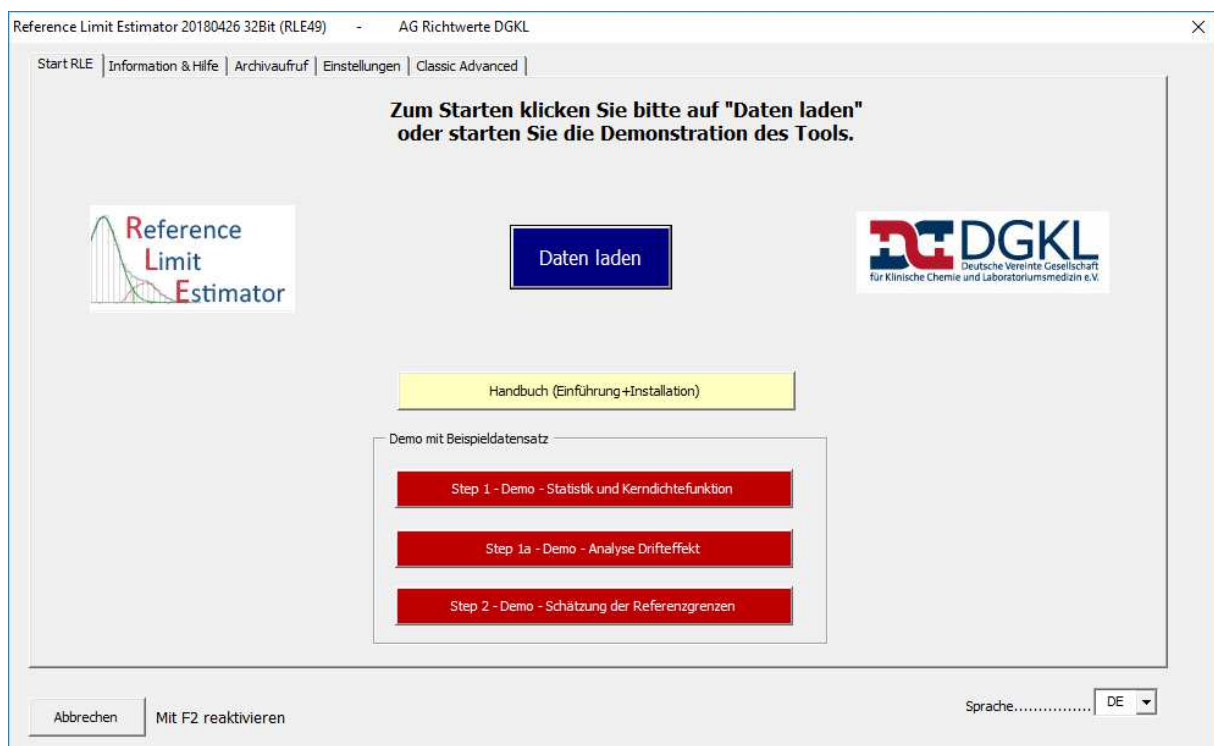
Note: The following windows appear when you open the estimator for the first time..



If the automatic recognition of the R-software fails, you have the option to enter the path to the R-software (RScript.exe) in ‚Settings‘ -> ‚Directories‘

If several R-versions are installed, it is decided which version of the Reference Limit Estimator should be used. For 64-bit installations of R, it is possible to distinguish between 32-bit and 64-bit.

The following window appears:

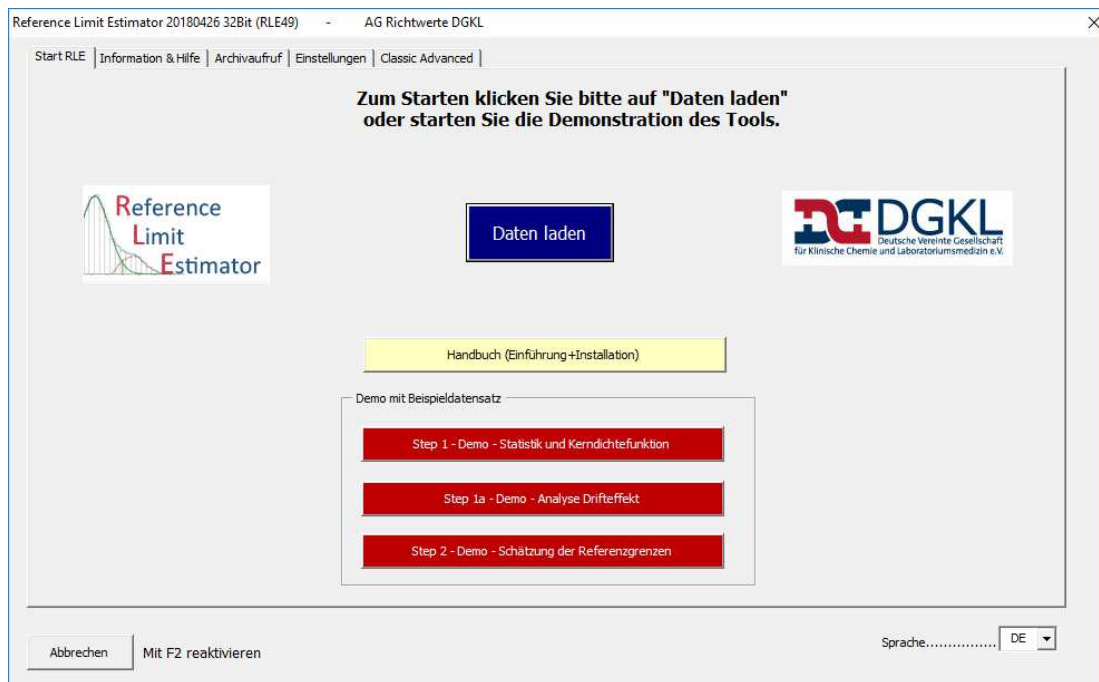


The preparations for the use of the tool are now completed.

# Introduction and Installation

## Test Run „Reference Limit Estimators“

In order to test the success of the installations and preparations, a demo sample is supplied with the download zip folder. All settings are pre-assigned for the test. To start the demo calculations, three steps can be started one after another.

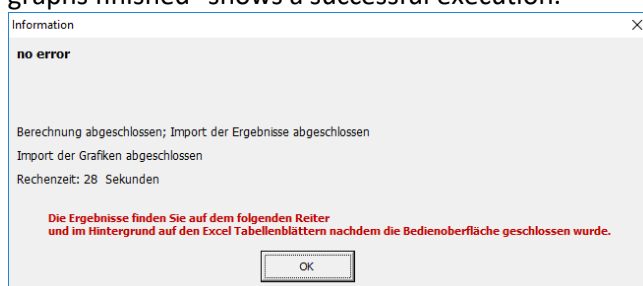


**Note:** During the calculations, a DOS window with a black background and the script of R opens temporally. Short notes show, which calculation is currently being carried out. As long as this window is visible, the statistical calculation is still running. In addition, windows with the graphics are briefly displayed simultaneously. These windows disappear after the completion of the R program.

First, the command button of "Step 1 - Demo" is pressed. The continuation of the calculation is displayed in small windows, which either shows the successful finish of the calculation or give an error message. Additionally the calculation time is displayed. "Step 1" is successfully completed when the information "Calculation finished" appears on the display. Then the results and graphics are imported into Excel. When "Import of graphs finished" appears, Step 1 is completed.

The installed demo sample also contains the sampling date. Therefore the "Step 1a Demo" can be started.

Finally "Step2 Demo" is executed. Extensive calculations are carried out, which can take a few minutes (depending on the performance of the computer used). The last information "import of graphs finished" shows a successful execution.



# Introduction and Installation

## Error Handling

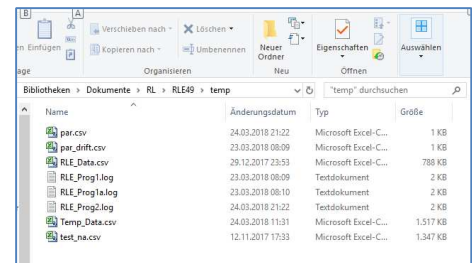
If the estimation was not finished regular, the user need to adept the configuration of the tool. The type of error is shown in the information window.

In case of abnormal termination of the Reference Limit Estimator the complete installation should be repeated.

The development team can be contacted in case of problems with the installation via email. Please report the error messages and send us the logfiles.

For every step of the estimation a logfile is saved in the subfolder \temp

RLE\_Prog1.log           = Logfile Step 1  
RLE\_Prog1a.log         = Logfile Step 1a  
RLE\_Prog2.log           = Logfile Step 2



Please consider that the RLE under Microsoft Excel 64Bit does not run!

# Introduction and Installation

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# Introduction and Installation

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# Introduction and Installation

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## Revision Level

04.2018	Version 0.1 (RLE 49 - 20180330)	Dr. Wolters
05.2018	Version 1.0 (RLE 49 – 20180511)	Dr. Wolters