RIDA®SEEK

User manual – Art. No. ZRIDASEEK

CE
IVD

User manual

RIDA®SEEK

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User manual

Content

1 Introduction to RIDA®SEEK

1.1 Intended use

For *in vitro* diagnostic use. RIDA®SEEK is an interpretation software that enables qualitative result interpretation from raw data generated by RIDA®GENE / RIDA®UNITY real-time PCR assays in conjunction with real-time PCR instruments. The product is intended for use by professionals.

1.2 RIDA®SEEK, an introduction

RIDA®SEEK is a platform on which R-Biopharm AG hosts assay-specific “Plug-In” applications that provide support in processing and interpreting real-time PCR data analysis.

The software enables a complex automated analysis of the raw fluorescence data. The results of the analysis are interpreted automatically according to the criteria specified in the instructions for use (IFU) of the assay. The analysis workflow begins with the input of the raw data files and ends with the results of the samples. In order to be indicative of a patient result, these sample results should be seen in the full medical context of a patient and the assay performed.

In RIDA®SEEK, all analyses and samples are displayed as hyperlinks and can be accessed with a single select. Just like a browser, multiple tabs can be opened to analyse and compare multiple runs at the same time. All available or ongoing analyses can be accessed from the start screen. The uppermost panel contains separate RIDA®SEEK modules devoted to ANALYSES, DEVICES, ASSAYS, QC, ARCHIVE, USERS, SETTINGS and HELP.

The Software is available in English, German, French, Italian or Spanish. The user will be able to choose a language at the login screen (Fig. 1, Fig. 2).

The software can be used either with a laptop or with a touch screen. In the SETTINGS module, User settings, the user can activate or deactivate the touch function (Fig. 22). When using the touch function, a keyboard integrated in the software is visible on the screen.

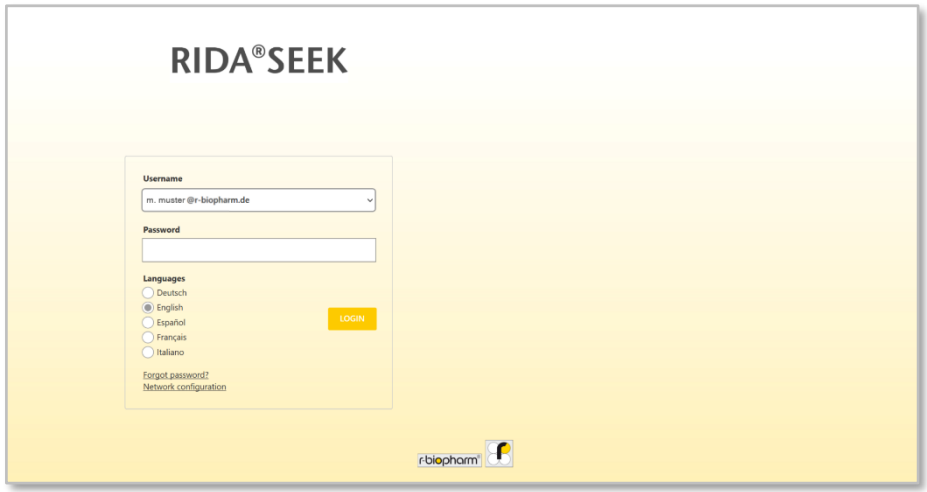


Fig. 1: Login screen: Choose a language.

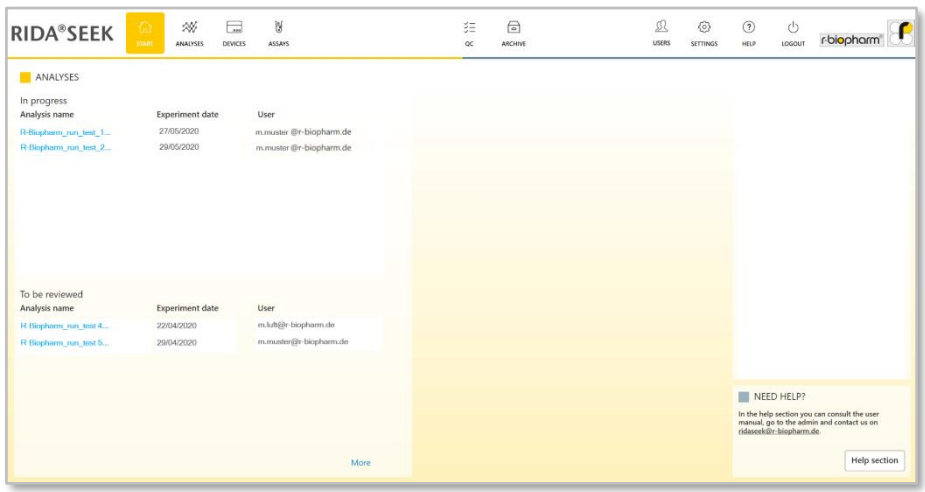
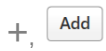


Fig. 2: Start screen: All available or ongoing analyses can be accessed.

Tab. 1: The following symbols are used in RIDA®SEEK:

Modules	
 START	Start screen
 ANALYSES	ANALYSES module
 DEVICES	DEVICES module
 ASSAYS	ASSAYS module
 QC	QC module
 ARCHIVE	ARCHIVE module
 USERS	USERS module
 SETTINGS	SETTINGS module
 HELP	HELP module
 LOGOUT	Logout
Functions and settings	
	Edit function
	Select
	Confirm function (ANALYSES module) and/or approved function (ARCHIVE module)
	Authorised function (ANALYSES module) and/or default function (ASSAYS module)

	Reset
	Set filters
	One click analyses
	Regular assay
	Audit trail comment
	Reject sample
	Approve sample
	To be reviewed
	To be repeated
	Search
	Chart display settings
	Add (See USERS module)
	Delete
	Restricted
	Drop down menu
	Resolve item(s)
	Zoom settings
	Inconclusive result

	Exclamation mark (e.g. USERS module, if two-step validation is enabled and/or in assays module if assay lot is not defined)
	Information
	Date of manufacture
	Manufacturer
	Consult instructions for use
	Serial number
	CE marking of conformity with *in vitro* diagnostic medical device
	Article number

Tab. 2: Key concepts:

Assay Plug-In:	The piece of software that 'plugs in' to RIDA®SEEK and contains, but is not limited to, the **algorithm** and **decision tree** (see below). Plug-Ins are developed and managed centrally by R-Biopharm AG.
Algorithm:	The data analysis component of an **Assay Plug-In** that is trained on historic data together with human interpretations and determines the Cp values of the curves.
Decision tree:	The part of the **Assay Plug-In** that determines how the results (Cp values) of the assay are interpreted by RIDA®SEEK. It receives the results of the **algorithm** and decides what the correct result should be based on the criteria specified in the IFU of the assay.

Resolve items:	Samples for which there is ambiguous data or an unexpected result are listed in the RESOLVE tab for the user to provide an interpretation. Interpretation of **Resolve items** is mandatory to proceed with the analysis.
Laboratory environment:	The shared workspace for all RIDA®SEEK users in the laboratory, regarding to a single database and a set of users managed centrally from the admin module (see section **2.1 Managing users and permissions**).

1.3 System requirements

Server

- Windows Server 2019 LTSC
- CPU: Intel® Xeon® E-2244G 3.8GHz
- RAM: 32GB (2 x 16GB 2666MT/s DDR4 ECC UDIMM)
- Disks: 2x 480GB Solid State Disk SATA 6Gbit/s (RAID-1)
- Network connection: 100/1000 Mbit/s Ethernet
- Other: iDRAC Port Card

Laptop

- OS: Windows 10 Pro, 64 bit
- CPU: Intel® Core™ i5-8265U
- RAM: 8GB, DDR4 Memory, non-ECC
- Disk: 256 GB M.2 SATA Solid State Disk
- Network connection: 100/1000 Mbit/s Ethernet
- Display: 15.6" FHD (1920x1080) Non-Touch Anti-Glare

Optional Client Minimum Hardware Requirements

- OS: Windows 7 or higher, 32 or 64 bit, with .NET Framework 4.5.2
- CPU: 32 or 64 bit, 2GHz[1] or faster
- RAM: 2GB
- Disk: 10 GB free disk space
- Network connection: 100/1000 Mbit/s Ethernet
- Display: min. 1440x900

The product is intended for use by professionals. The guidelines for work in medical laboratories must be observed. The operating instructions for software must be strictly observed. It is recommended to set up the server in a server room.

Systems must be correctly disposed at the end of their life cycle. Please observe the applicable national regulations for disposal.

[1] CPU performance equivalent to Intel® Core™2 Duo Processor T7250 or better

1.4 Supported PCR cyclers and file types

Tab. 3: Devices and Filetype(s).

Device(s)	Filetype(s)
R-Biopharm AG RIDA®CYCLER	.rcyclerrun
Applied Biosystems ABI® 7500 Fast	.sds (software v1.x), .eds (software v2.x)
BioRad® CFX 96	.pcrd
BMS MIC qPCR	.micrun
Roche LightCycler® 480 Type II	.ixo
QIAGEN Rotor-Gene Q	.rex

1.5 R-Biopharm AG customer support

For technical support in setting up and operating RIDA®SEEK, please contact *ridaseek@r-biopharm.de*. As the RIDA®SEEK is not an open platform, it is restricted to RIDA®GENE and RIDA®UNITY real-time PCR assays only.

2 Setting up RIDA®SEEK laboratory environment

2.1 Managing users and permissions

2.1.1 Add and edit new users

To add a new user, select the USERS module in the uppermost pane and the Users section from the list in the left-hand pane. All users are displayed in the Users section. Select the + icon (Add function) from the overview screen to add a new user (Fig. 3, Fig. 4). Select a predefined function from the Function titled drop-down menu after entering a first and last name.
Please notice that the entered email address can be fictive e.g. “m.muster@labor123.de”. Since emails are not being sent to the entered email address it is recommended to make a note of the password (Fig. 5). After the first Login to the software, the password can be changed.

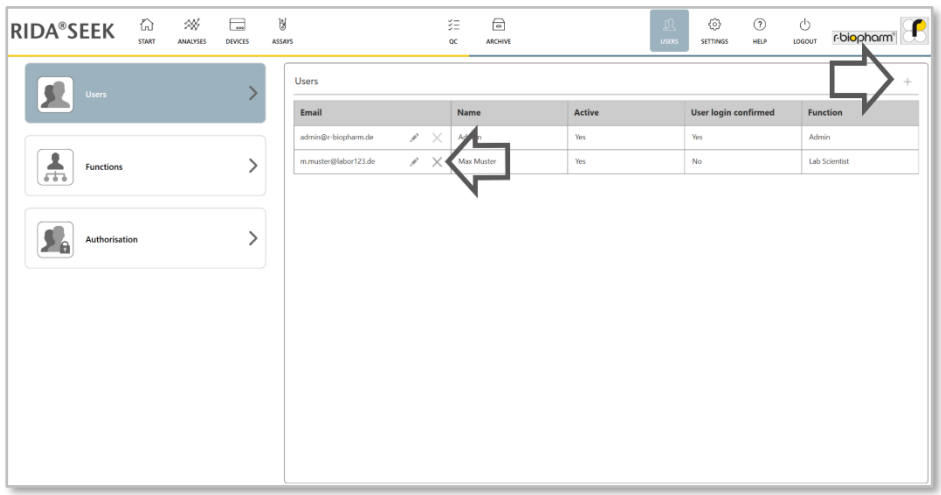


Fig. 3: USERS module and Users section: To add new user select the + icon (Add function) next to the headline Users. To edit or inactivate existing user select the Pencil icon (Edit function) next to the email address.

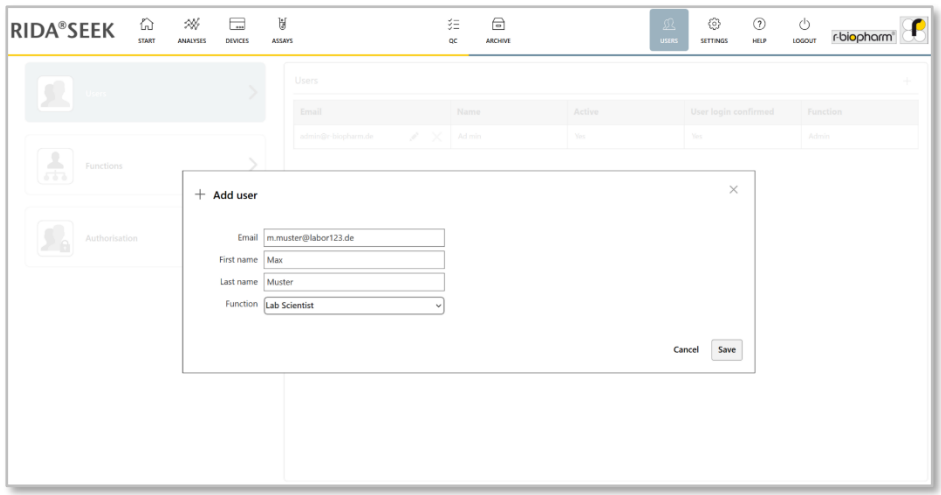


Fig. 4: USERS module and Users section: Enter information to add a new user.

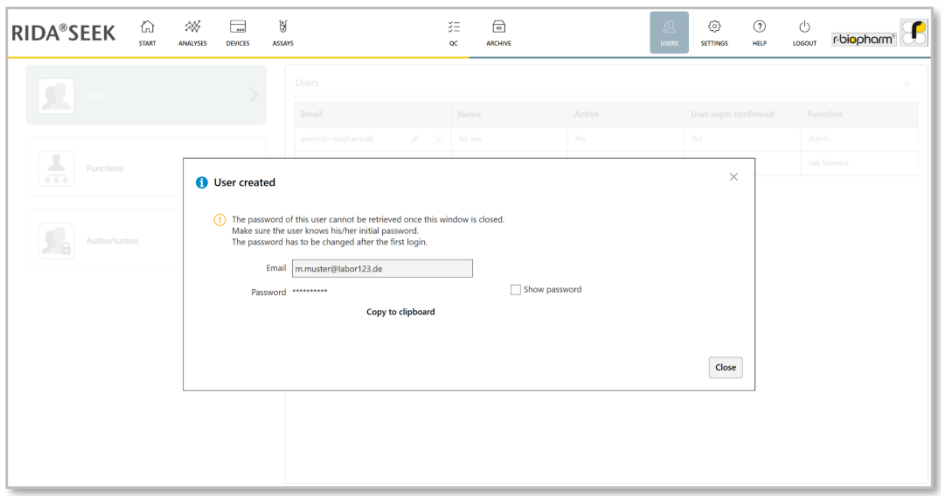


Fig. 5: USERS module and Users section: If a new user is added a password is created. It is recommended to make a note of the password, as emails are not being sent to the entered email address. This password has to be changed after the first login.

Note: Once a user is created, the user cannot be deleted from the database or from the list. This is important in order to maintain the audit trail for past analyses. Therefore, it is recommended for security reasons to inactivate users from the list as soon as they no longer require access to RIDA®SEEK.

2.1.2 Add or edit functions

To add a new function, select the USERS module in the uppermost pane and the Functions section from the list in the left-hand pane.
RIDA®SEEK will be delivered with a default setting from the manufacturer. Initially the “Lab Admin”, “Lab Scientist”, “RBio Admin” and the “Technician” role will be applied with their function to RIDA®SEEK. Functions can be edited or removed by the “Lab Admin” role. New functions can be added with the + icon (Add function) next to the Functions headline. Functions can be edited with the Pencil icon (Edit function) or deleted via the X button (Fig. 6).

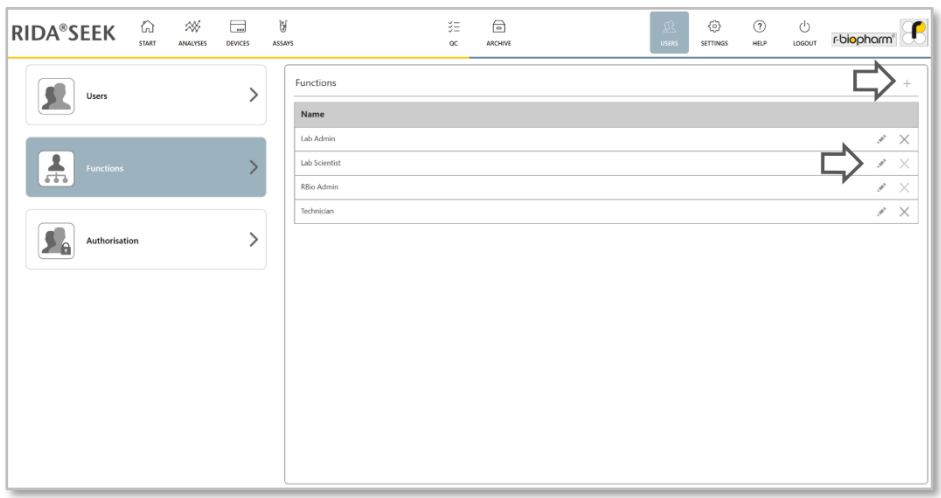


Fig. 6: USERS module and Functions section: Add functions with the + icon (Add function). Edit with the Pencil icon (Edit function) or delete via the X button.

2.1.3 Roles and functions

Roles and functions of users can be adjusted individually (Fig. 7, 8 and 9).

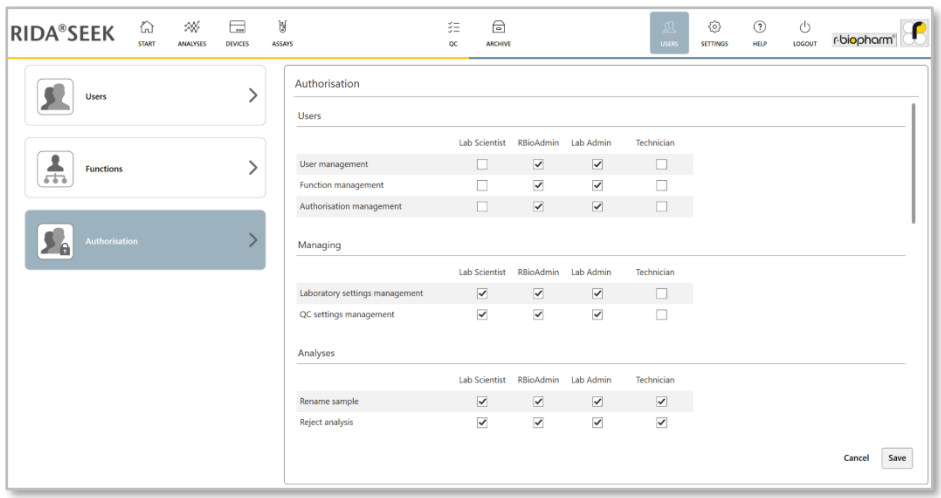


Fig. 7: USERS module and Authorisation section: Roles and functions of users can be adjusted individually.

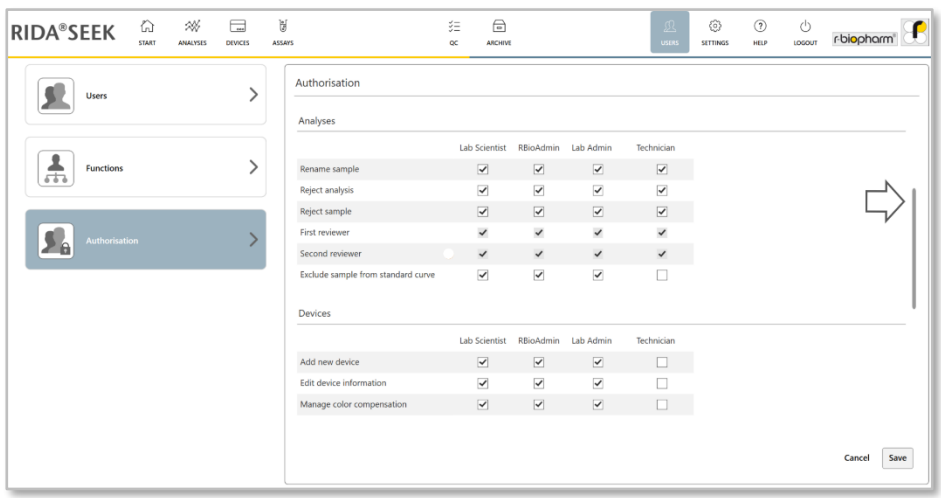


Fig. 8: USERS module and Authorisation section: More roles and functions of users can be adjusted individually.

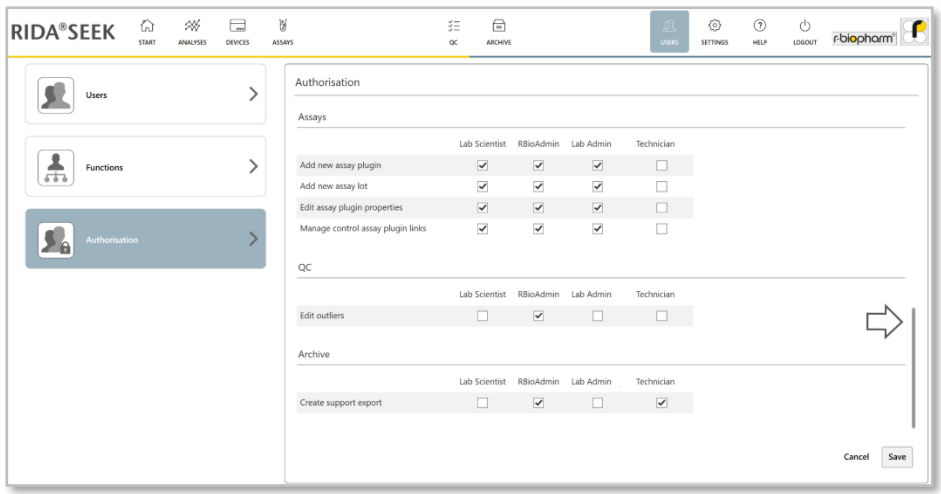


Fig. 9: USERS module and Authorisation section: More roles and functions of users can be adjusted individually.

Users in your lab have two separate sets of permissions that apply to them:

- A **role** applies to a user's permissions. Two types of a role exist: The "Lab Admin" role allows to roll out unlimited new users and to define their functions. The "Lab Scientist" users for example are not able to add and edit users and functions. A user's role is selected when users are added (see section **2.1.1 Add and edit new users**). It is recommended that routine users of the laboratory are "Lab Scientist" users.
- **Functions** reflect the permissions the laboratory team has in RIDA®SEEK itself. Functions can be added by e.g. the "Lab Admin" role (see section **2.1.1 Add and edit new users**) and permissions can be edited, see section **2.1.2 Add or edit functions**.

If a user leaves the lab, changes the role or forgets the password, the necessary settings can be edited by the "Lab Admin" role quickly. To reset the "Lab Admin" role's password, please get in touch with the R-Biopharm AG support *ridaseek@r-biopharm.de*.

2.2 Adding devices and assays

2.2.1 Add new device

Devices can be added and managed within the DEVICES module of RIDA®SEEK. RIDA®SEEK reads information directly from data files generated by the real-time PCR device. Simply select Add and browse to a file that has been output from the instrument, its device-specific information will populate automatically (Fig. 10).

In addition, information can be typed in the search bar – such as a manufacturer name (e.g. “Roche”) or device name (e.g. “LightCycler”) – to access the desired device quickly. For devices that require a CC file see section **2.3.1 Add or edit a color compensation file**.

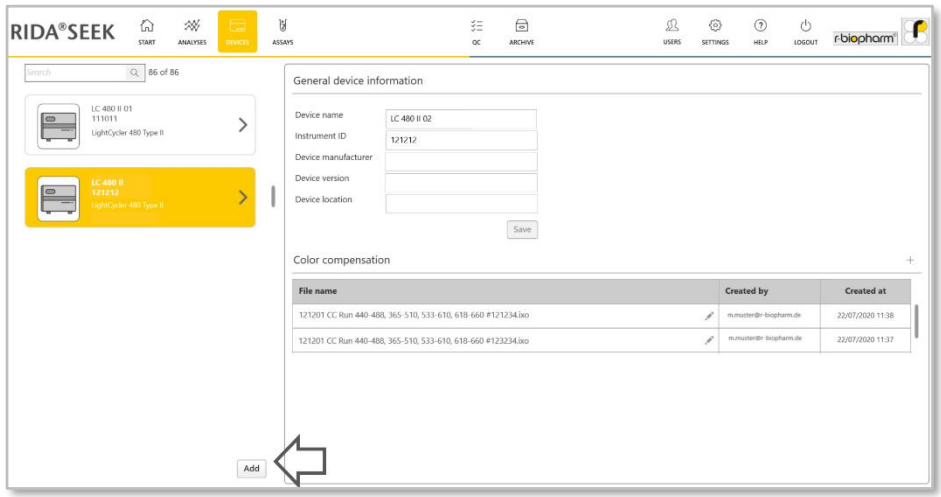


Fig. 10: DEVICES module: Add a new device with Add button.

2.2.2 Add new assays

Assays can be managed within the ASSAYS module of RIDA®SEEK. To access, select the ASSAYS module in the uppermost pane. The list in the left-hand pane shows the Assay Plug-Ins (APs) which are installed by R-Biopharm AG. Please get in touch with the R-Biopharm AG support team *ridaseek@r-biopharm.de* regarding questions to APs. User-defined information can be added via the Pencil icon (Edit function) and assay lots are added with the + icon in the Assay lot section of the AP (**see 2.3.4 Adding and managing a lot**).

2.3 Configuring devices and assays

2.3.1 Add or edit a color compensation file

Color compensation (CC) files can be managed within the DEVICES module of RIDA®SEEK. To access, select the DEVICES module in the uppermost pane. It is necessary to use a CC for Roche LightCycler® and cobas® instruments. The CC file can be added to the associated device by selecting the device from the list in the left-hand pane. Within the device’s Color compensation section select + icon (Add function) to select a file

(Fig. 11). Browse to the correct file and choose the subset name “CC” for the color compensation samples on the plate as specified in the LightCycler® software during plate setup. In the next step of the assistant, the file can be linked to any assays as required either as an available option or as the default setting during analysis configuration (see section **3.3 Section Configure assay(s)**).

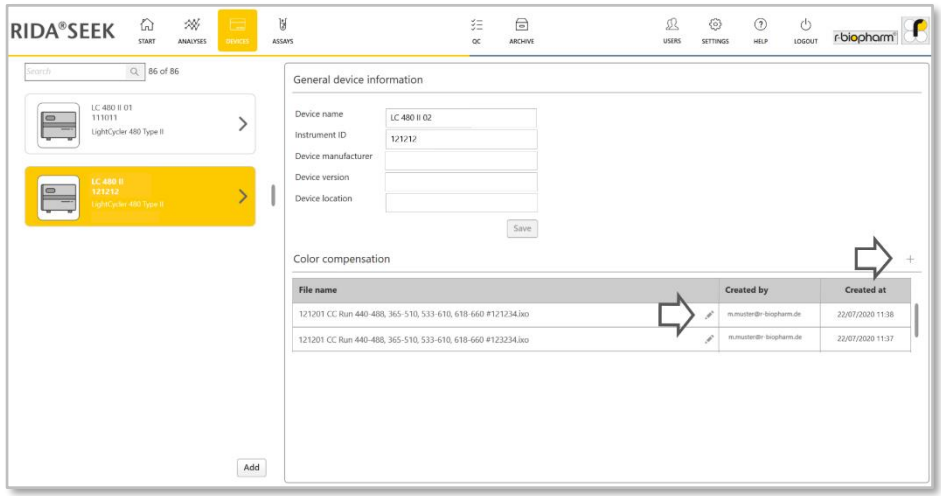


Fig. 11: DEVICE module: Add a CC file with the + icon. Edit a color compensation file with the Pencil icon (Edit function).

Multiple color compensation files can be added to devices as required and linked to one or more assays. A CC file can be retired from use by toggling the Active button to Inactive and/or can be set as default for a certain assay within the menu.

2.3.2 Sample type nametags

To distinguish categories such as controls and samples in RIDA®SEEK, specific unique **nametags** for each category are pre-defined by R-Biopharm AG. The document with the recommended nametags for the cycler software is provided by R-Biopharm AG in addition to the manual. For more information, please contact the R-Biopharm AG support address *ridaseek@r-biopharm.de*.

This corresponds to the first part of the sample name (its prefix). Sample nametags can be managed within the ASSAYS module of RIDA®SEEK. To access select ASSAYS button in the uppermost pane. Select an Assay Plug-In in the left-hand pane. To add a nametag to an assay, select the Pencil icon (Edit function) in the Sample type nametags (prefix)

window. After specifying the nametag, RIDA®SEEK will add an asterisk to the end (e.g. XXX*) – this is to allow the program to recognize the tag from the file name.

Example: If a user labels the Negative Control with “NC” and Positive Control with “POS” (below), the “NC” and “POS” should be added as sample nametags within the Negative Control and Positive control sections of the Assay Plug-In:
NC_BatchName_Assay1 → RIDA®SEEK nametag, negative control: “NC”
POS_BatchName_Assay1 → RIDA®SEEK nametag, positive control: “POS”

2.3.3 Mix definition nametags/Subsets

It is also possible to use the **subset name** instead of the sample name for this purpose (note that this applies to the LightCycler® software only). Nametags can be managed within the ASSAYS module of RIDA®SEEK. To access, select the ASSAYS button in the uppermost pane. Select an Assay Plug-In in the left-hand pane. To add a nametag to an assay, select the Pencil icon (Edit function) in the Mix definition nametags (sample name – suffix)/Mix definition nametags (subset name) section. After specifying the nametag, RIDA®SEEK will add an asterisk to the beginning (e.g. *XXX) – this is to allow the program to recognize the tag from the file name.

For assays to be automatically assigned to a plate and to combine the results of multiple mixes, for each mix to be distinguished in RIDA®SEEK, unique nametags within the Assay Plug-In are pre-defined by R-Biopharm AG. The document with the recommended nametags for the cycler software is provided by R-Biopharm AG in addition to the manual. For more information, please contact the R-Biopharm AG support address *ridaseek@r-biopharm.de*.

2.3.4 Adding and managing a lot

Reagent lots must be supplied for each Assay Plug-In by selecting the + icon (Add function) in the Assay lot section (Fig. 12).

Besides the lot number and expiration date (Fig. 13), the lot dependent Cp cutoff values (if applicable for the assay) (Fig. 14) and QC tracking (Fig. 15) can be configured (see section **6 Quality Control in RIDA®SEEK**). If required by the assay, cutoffs (such as Cp cutoffs) can be defined for every target separately. Depending on the assay, cutoff values are prefilled or not and can be edited or not.
An assay lot can be set as default for a certain assay to ease the configuration steps in the “Data input” section (see **3.3 Section Configure assay(s)**). Once a lot is used for an analysis, the lot cannot be edited anymore. When a lot is not used anymore, it can be inactivated so it is not visible anymore during the during the analysis configuration. The lot can be inactivated or set as default by selecting the Pencil icon (Edit function) next to the + icon (Add function) in the Assay lot section (Fig. 12).

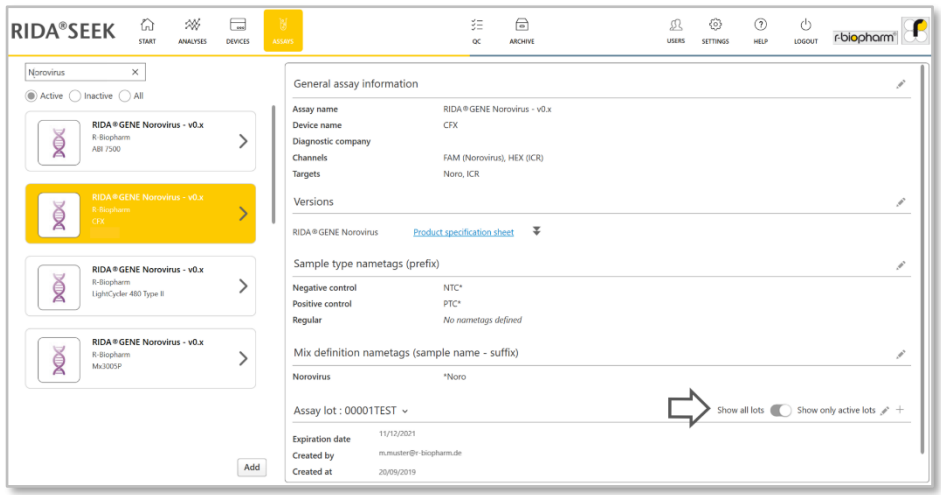


Fig. 12: ASSAYS module: Add a new assay lot with the + icon (Add function).

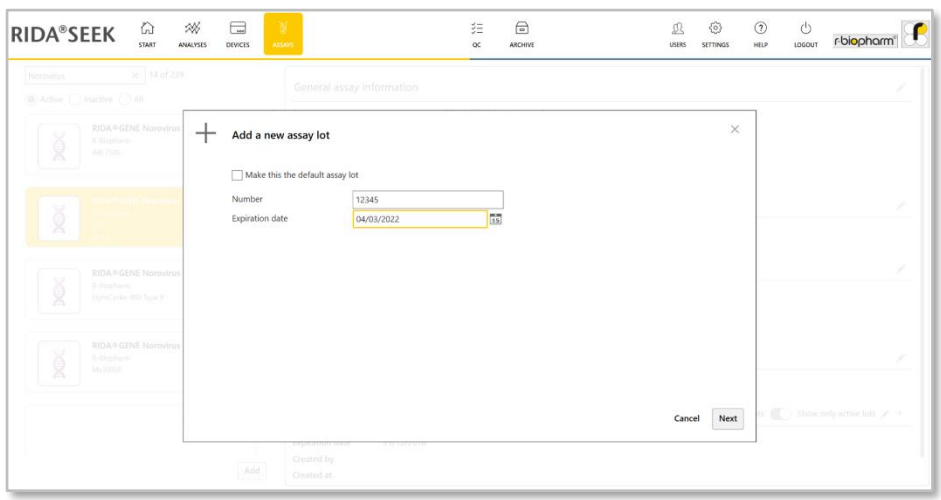


Fig. 13: ASSAYS module: Add information for a new assay lot.

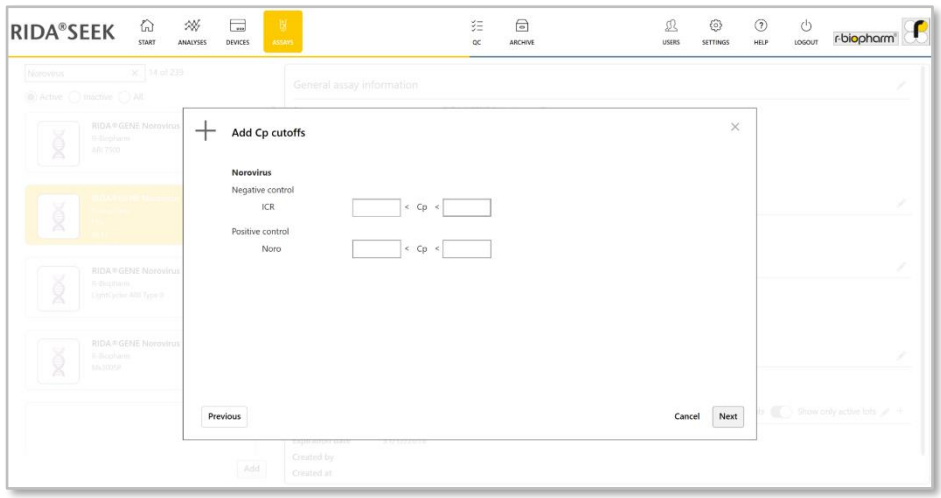


Fig. 14: ASSAYS module: Information for a new assay lot, lot dependent Cp cutoff values (if applicable for the assay).

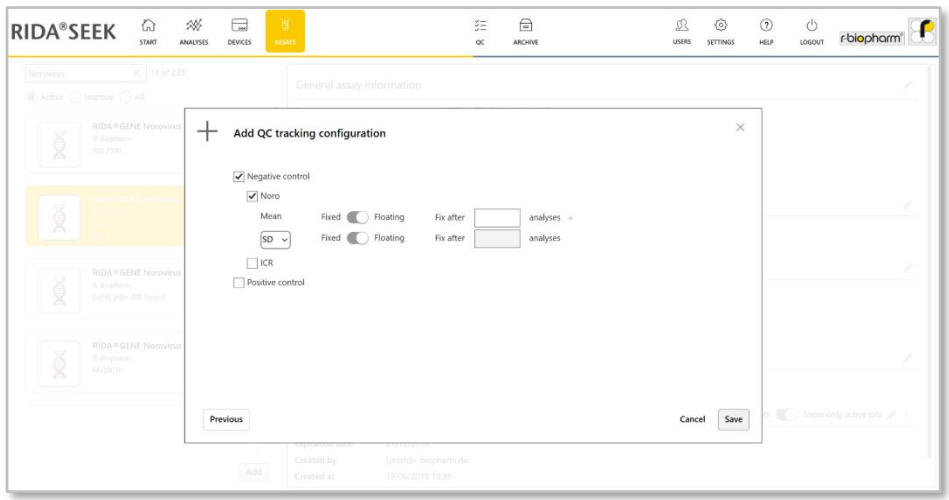


Fig. 15: ASSAYS module: Information for a new assay lot, QC tracking.

2.3.5 Assay Plug-In versions

Updated Assay Plug-Ins are released in a version controlled manner while allowing the “Lab Admin” user to control the version under current use. To inactivate an active Assay Plug-In, select the Pencil icon (Edit function) next to General assay information within the Assay Plug-In and toggle as desired. For each Assay Plug-In version a product specification sheet (PSS) is listed. The PSS document is provided by R-Biopharm AG and includes validation specifications of the Assay Plug-Ins (Fig. 16).

Note: It is highly recommended that different versions of an Assay Plug-In are not active at the same time as automated plate setup will be disabled in such cases.

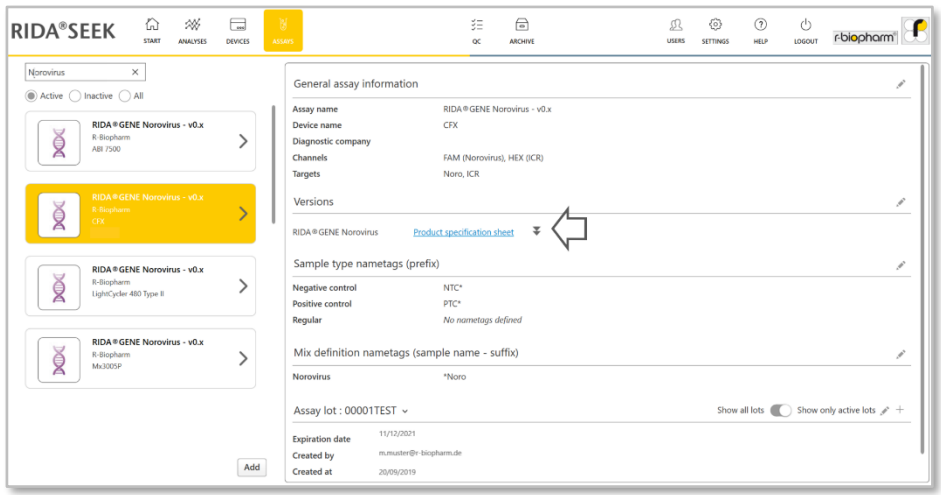


Fig. 16: ASSAYS module: To inactive an active Assay Plug-In, select the Pencil icon (Edit function) next to the General assay information and toggle as desired. To view all the inactive Assay Plug-Ins, select Inactive.

2.4 Laboratory settings

2.4.1 One and two-step validation

Depending on the laboratory protocol, all results can be reviewed and approved by a second analyst in a two-step validation process. In such a procedure, analysed data are approved by the first analyst as step one. To be reviewed analysis will appear in the To be reviewed list on the RIDA®SEEK START module. This analysis can then be opened, proceeded through a second analysis and authorisation by the second analyst.

The two-step validation workflow can be turned on or off within the SETTINGS module of RIDA®SEEK. To access, select the SETTINGS button in the uppermost pane and select Laboratory settings in the left-hand pane. Turn the Two-step validation checkbox underneath the headline General on or off accordingly (Fig. 17). Changes in this setting only affect new analyses.

Note: Results can only be authorised once all items and any QC violations have been resolved independent of the validation procedure.

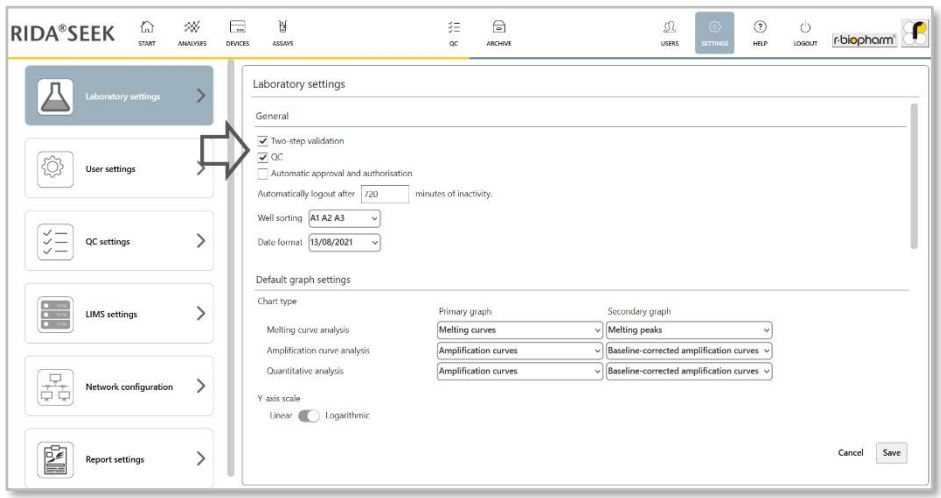


Fig. 17: SETTINGS module and Laboratory settings section: Two-step validation and QC checkbox turn on or off.

2.4.2 QC settings

RIDA®SEEK can track user-specified targets such as a negative control and positive control and apply four Westgard Rules to each analysis in real-time. The desired target and the statistical criteria by which it is tracked are provided by the user at the time of adding a new assay lot (see section **6.2.3** Fehler! Verweisquelle konnte nicht gefunden werden.).
Configuration can be set in the ASSAYS module. Select an Assay Plug-In and scroll to the Assay lot section.
QC tracking capability can be turned on or off within the SETTINGS module and Laboratory settings section of RIDA®SEEK (Fig. 18).

Each of the four Westgard Rules used by RIDA®SEEK (1_{2S}, 2_{2S}, 1_{3S} and 10_{x}) can be separately turned on or off by setting the corresponding checkboxes within the SETTINGS screen, and selecting QC settings in the left-hand pane. Note that changes in these settings

only apply for new analyses. For more information about the Westgard Rules, see section **6.3.1 Notes on Westgard Rules**.

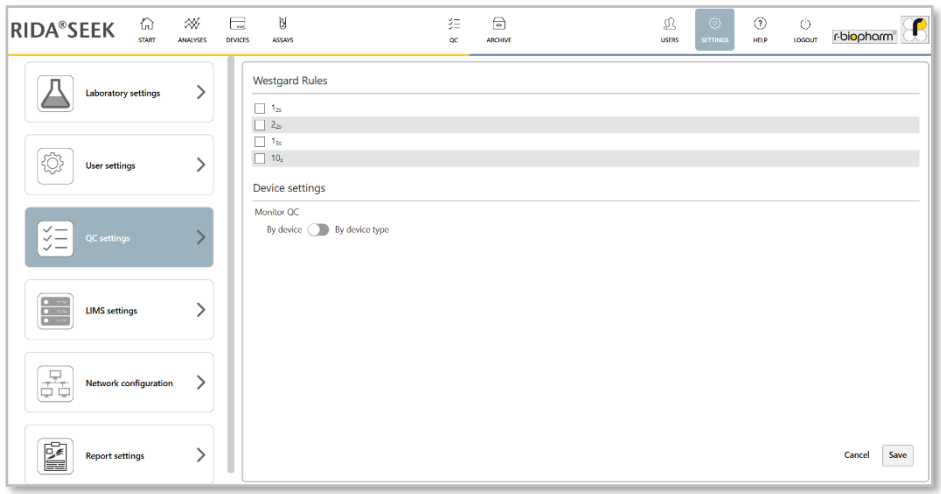


Fig. 18: SETTINGS module and QC settings section: Westgard Rules checkboxes turn on or off.

2.4.3 Automatic approval and authorisation

RIDA®SEEK can automatically approve and authorise analyses if desired. When this option is enabled (see SETTINGS and Laboratory settings "Automatic approval and authorisation" Fig. 17) an analysis that does not have any samples to resolve or QC events that require your attention, will automatically be approved, if applicable, and authorised. This functionality in combination with automatic creation of LIMS export, allows fast release of analysis results to the LIMS.

2.4.4 Well sorting

In the laboratory general settings, the preferred well sorting can be selected. The user can choose between a row based sorting (A1 A2 A3) and a column based sorting (A1 B1 C1) in the SETTINGS module and the Laboratory settings section (Fig. 19). All tables where there is a well column will be impacted (e.g. the result tables, assign assay(s) table view, reports). The new settings will be applied on new analyses and analyses that are opened from the archive. For analyses that are already open the sorting will only apply when the well column is sorted by clicking on the header.

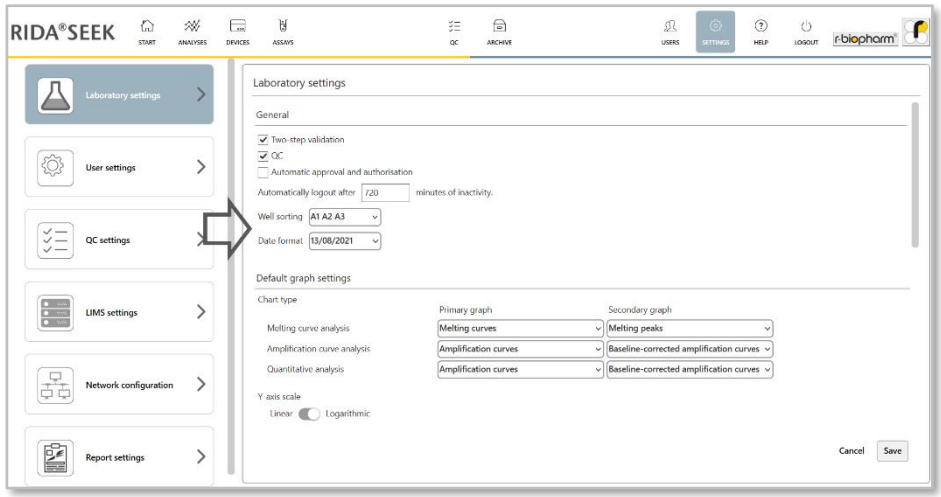


Fig. 19: SETTINGS module and Laboratory settings: Well sorting and date format can be adjusted.

2.4.5 Configuring logout time

If RIDA®SEEK has a long period of inactivity while the user is logged in, the user is logged out automatically for security reasons. The duration of inactivity after the software logs off automatically can be configured between 2 and 720 minutes within the SETTINGS module and the Laboratory settings section (Fig. 19).

2.4.6 LIMS export settings

It is possible to export the analysis results to a Laboratory Information Management System (LIMS) using a file-based transfer automatically. See SETTINGS module and the LIMS settings section “Automatically create LIMS export upon authorisation” (Fig. 20). Supported LIMS file formats: ASTM and HL7. For more information see **5.2.2 LIMS export**.

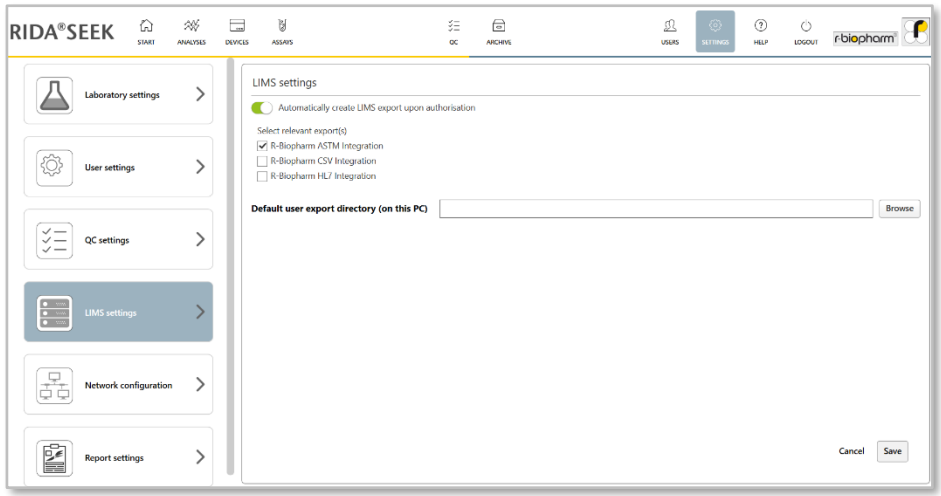


Fig. 20: SETTINGS module and LIMS settings.

2.4.7 Report settings

In the report settings, a logo can be uploaded and laboratory information can be added (Fig. 21). Both the logo and the lab info will be visible on the first page of the pdf report (see **5** Fehler! Verweisquelle konnte nicht gefunden werden.). The logo cannot exceed 1 MB.

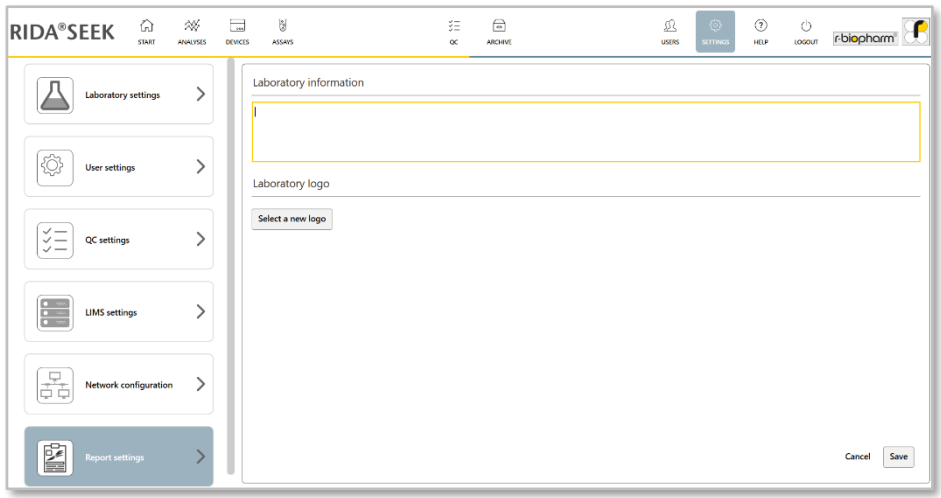


Fig. 21: SETTINGS module and Report settings.

2.4.8 User settings

The software can be used either with a laptop or with a touch screen. In the SETTINGS module, User settings, the user can activate or deactivate the touch function. When using the touch function, a keyboard integrated in the software is visible on the screen.

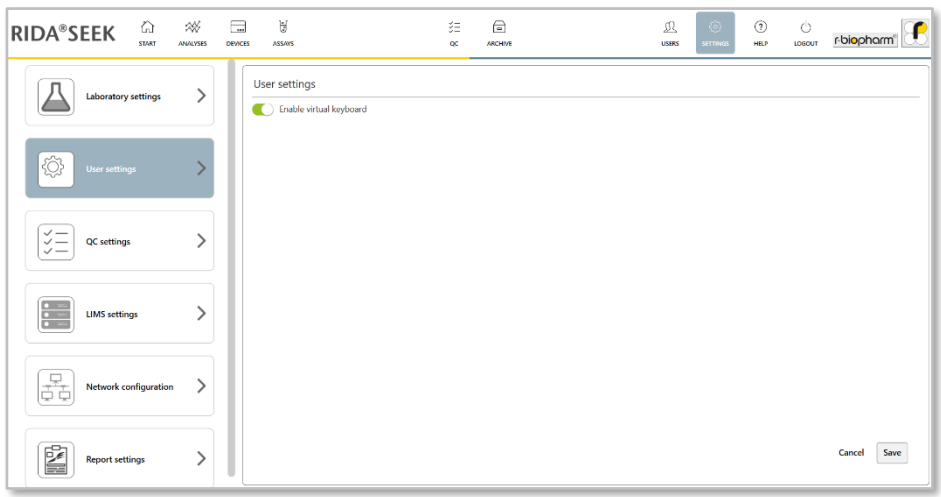


Fig. 22: SETTINGS module and User settings.

3 Performing an automated analysis

3.1 RIDA®SEEK: an overview

Only five actions are required of a user to complete an analysis:

1) Check the plate and experimental configuration
2) Choose a lot and color compensation
3) Confirm sample results
4) Approve the run
5) Create the content of the PDF report and LIMS integration file

All the steps above are performed within the ANALYSES module of RIDA®SEEK following an analysis workflow of the sections: DATA INPUTS, RESULTS, EXPORTS, REPORTS and REPORT VIEWER.

3.2 Section Data Inputs

3.2.1 Finding and filtering data files

Starting an analysis in RIDA®SEEK can be done in two ways:

- The data files from the cyclers are output to a central location on a network/computer and this folder is linked to the RIDA®SEEK File watcher. This directory can be configured in the SETTINGS module, by selecting Laboratory settings and in the File watcher section Enabled/Disabled. When enabled all files in this directory (and sub-directories) are listed on the RIDA®SEEK start screen. The files are automatically transferred to a second folder after they are uploaded in RIDA®SEEK to prevent multiple analyses of the same data file. **Make sure this second folder is not a sub-folder of the file watcher folder.**
- A file can be selected manually in the (1) Select data file(s) section, which is the first window shown when opening the ANALYSES module. Simply select the Browse button in the Current directory box and navigate to the desired folder. This location will be remembered each time the user logs in until it is changed. If available, a data file list appears. This data file list can be searched via the search bar underneath the Current directory box. It has been designed to assist users who maintain a large list of data files and who need to filter it quickly. To upload a second file, open an additional tab in the ANALYSES module using the + button (Add function) (Fig. 23).

When there is an automated plate setup and default assay configuration, a one-click analysis can be performed by clicking on the icon on the right side of each row with a data file. You will immediately be redirected to the results of the analysis.

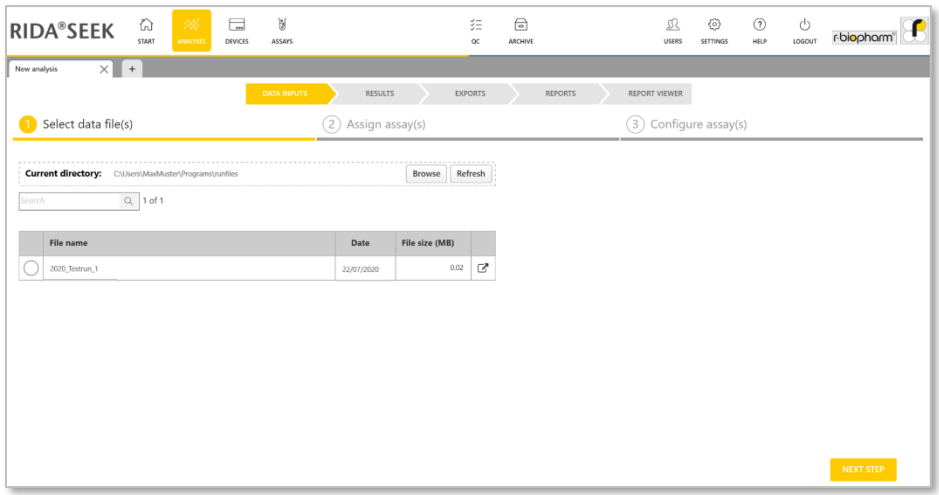


Fig. 23: ANALYSES module: Browse for data file and upload a file. Upload a second file by using the + button (Add function). The one-click analysis is available with the icon on the right side of each row with data file.

3.2.2 Plate setup

After selecting the desired data file in the (1) Select data file(s) section, RIDA®SEEK will display in the section (2) Assign assay(s) an interactive plate diagram with the diagram and detected assays listed on the right-hand pane.

Tab. 4: The following symbols are used during plate setup:

	RIDA®GENE/RIDA®UNITY assays
S	Regular sample
N	Negative control
P	Positive control
?	Unknown sample type

S	Replicate samples

Changing the view

In step (2) Assign assay(s) of the DATA INPUTS section the file is displayed either as a plate or a list. Select the Plate or Table options to choose between the two views (Fig. 24, 25).

Should a blank plate be displayed, check the contents of the data file by well in the Table view.

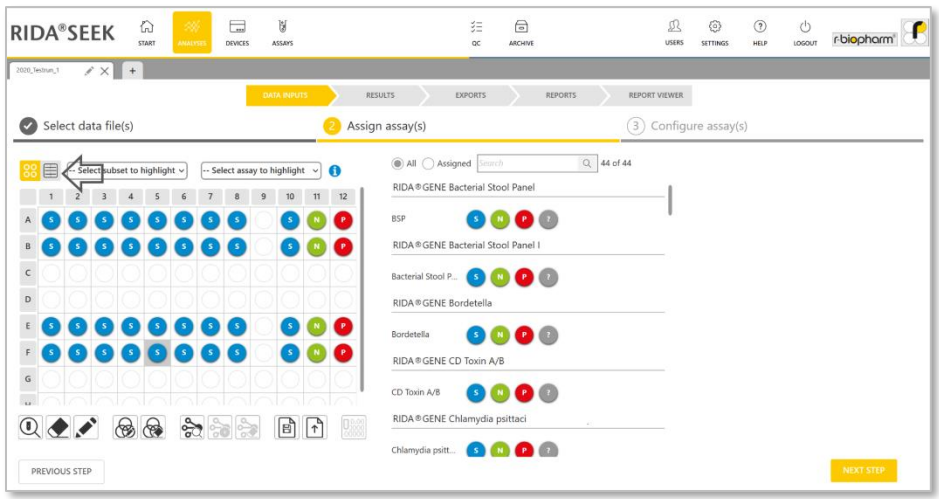


Fig. 24: ANALYSES module: (2) Assign assay(s) overview. Select the Plate or Table options to choose between the two views.

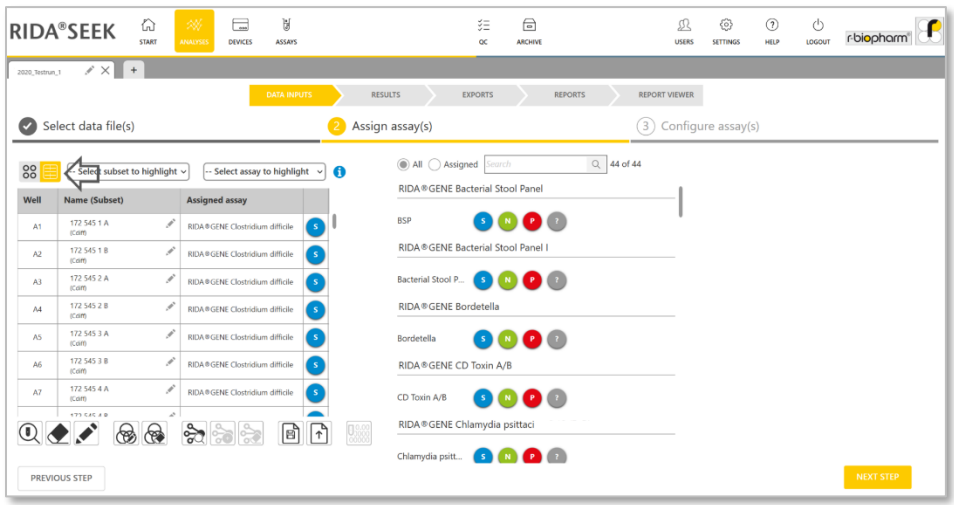


Fig. 25: ANALYSES module: (2) Assign assay(s) overview. Select the Table option.

Assign assay toolbar

Below the plate/table, a toolbar is offering quick access to the actions required for the assay assignment.

Tab. 5: The following functionalities are offered by the assign assay toolbar:

	Detect all assays
	Clear selected sample(s)
	Edit selected sample name(s)
	Edit subset(s) in selection
	Delete subset(s) in selection

	Detect all replicates
	Mark selection as replicates*
	Clear replicates in selection
	Save template
	Load plate template
	Define concentration

* note that if the names of samples that are replicates are not the same, RIDA®SEEK will assign the group of replicate samples a name that is matching the original samples' names as close as possible (e.g. in case the individual sample names are "sample1a", "sample1b" and "sample1c", the name of the group of samples will become "sample1").

3.2.3 Editing sample names

Edit a sample names in RIDA®SEEK by selecting the associated well and selecting Edit sample name(s) in the Plate view. Select multiple wells to display sample names as an editable list or choose Rename all selected samples to change the names of all selected samples at once (Fig. 26). In order to edit the sample name, select in the Table view the Pencil icon (Edit function) next to the sample.

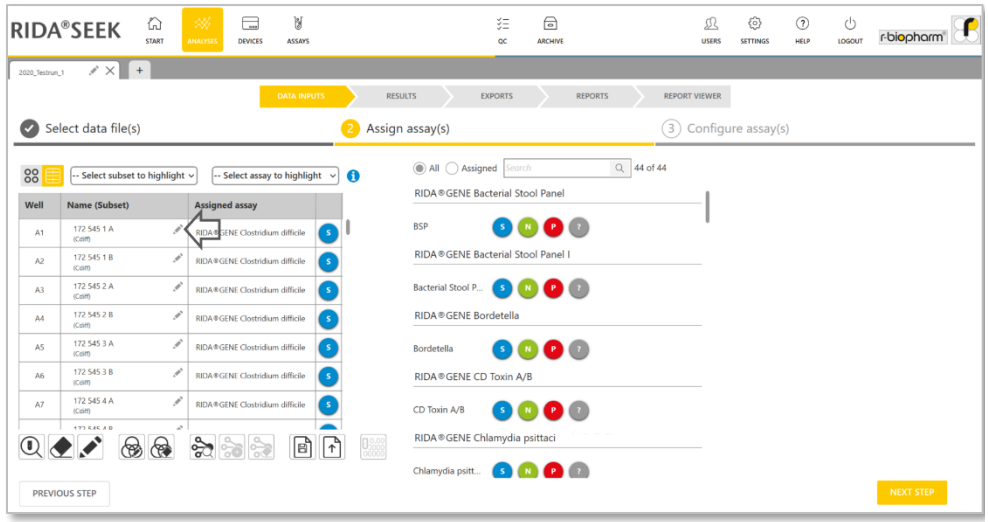


Fig. 26: ANALYSES module: Rename samples.

3.2.4 Manual plate configuration

Each sample in the Plate view can be edited by dragging it to an empty well position, cleared by selecting Clear selected or replaced by selecting a sample category icon in the right-hand pane for the assay. Select entire columns or rows by selecting the character describing it (such as “4” for column 4) and the entire plate by selecting the grey notch on the upper left-hand corner of the plate (Fig. 27). In order to reset the changes to default select Detect assays.

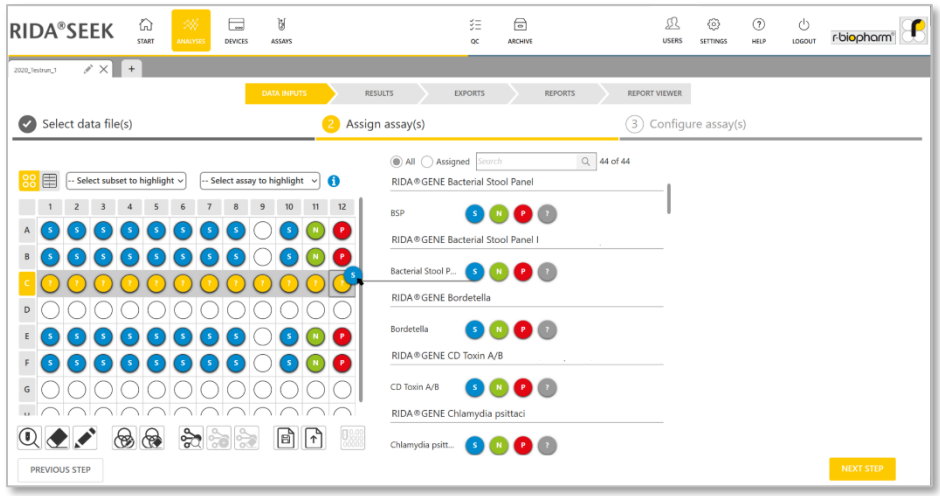


Fig. 27: ANALYSES module: Select entire columns or rows and drag assay to well position.

For users that always use the same Plate setup, templates can be used. In order to make a template assign the plate manually as desired, and save the template using the Save template function (Fig. 28). The user can choose to overwrite an existing template or enter a unique name for a new template. The template can be reused by uploading a file and select Load plate template. It is possible to choose to only assign the assay/mix or assign the sample types. Previously made assignments are always replaced by the loaded template.

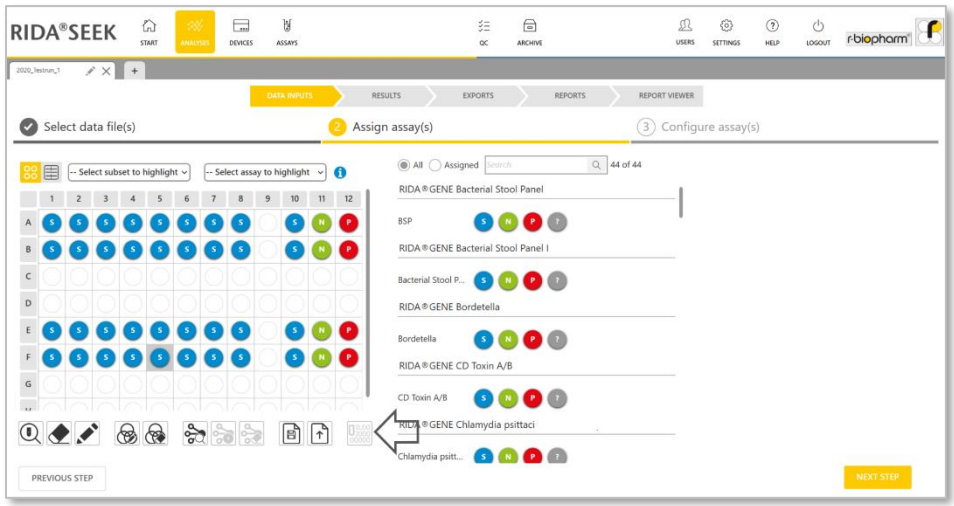


Fig. 28: ANALYSES module: Save template function.

In the event that only the assay or mix is known, to populate the plate a placeholder can be chosen, the sample type is represented by a question mark symbol "?".
When the "?" is assigned on a plate, the sample type is determined using the name tags defined in the Assay Plug-In and the sample name. If no match is possible, it is thereafter assigned as "regular sample" in the assay. However, if there is no single "regular sample" the "?" is displayed and a warning is shown preventing the user from proceeding.

3.2.5 Subsets

A plate can be divided into different subsets. When using LightCycler® files the subsets are also imported as defined in the LightCycler® software. Subsets can be highlighted in RIDA®SEEK by selecting the subset using the menu on the left corner of the Plate view.

Note: To highlight certain assays use the menu on the right corner of the Plate view. When combining both filters, a union of both is shown.

By selecting one or multiple samples, the Edit subset(s) button becomes visible. A new subset can be created or the user adds the selected samples to an existing subset. If the samples are added to an existing subset, but they are already part of another subset, a list of impacted samples is shown. It is not possible to add one sample to multiple subsets. Every subset can be configured in another way later (see section **3.3.3 Configuring subsets**).

3.3 Section Configure assay(s)

3.3.1 Selecting color compensation file

For Roche LightCycler® and cobas® instruments a CC file is loaded into the associated device and linked to an Assay Plug-In (**2.3.1 Add or edit a color compensation file**). It will thereafter be made available as a drop-down menu option in step (3) Configure assay(s) of the DATA INPUTS section in the ANALYSES module (Fig. 29). In this way, a user needs to only configure a CC per device and per assay once. It is recommended to update the color compensation files in accordance with the assay's IFU.
When the assay is configured as required select the ANALYSE button. Data analysis will proceed automatically.

Should a CC file be used routinely, it can be specified as default, avoiding the need to select it each time. Files added without being made default will be available from the list at the discretion of the user.

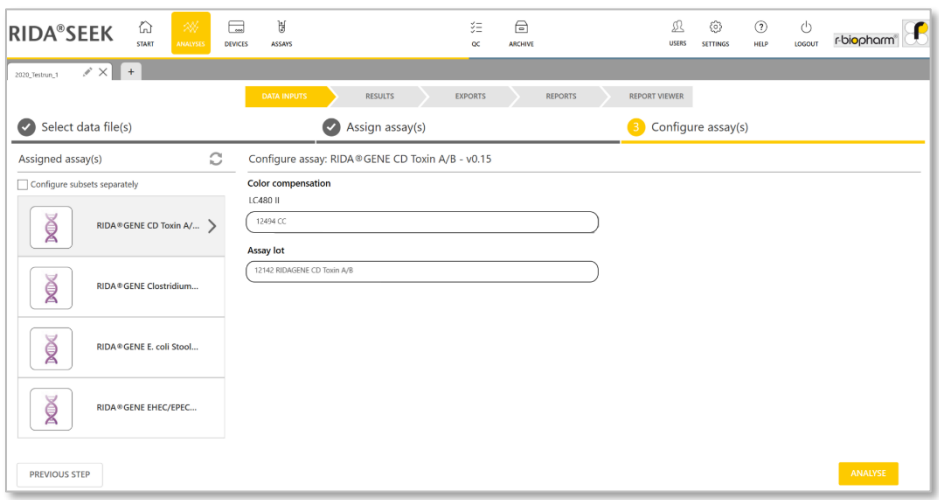


Fig. 29: ANALYSES module: (3) Configure assay(s) section, selecting a CC file.

3.3.2 Using assay lots

After adding a reagent lot into the relevant Assay Plug-In (see **2.3.4 Adding and managing a lot**), it will be made available as a drop-down menu option in step (3) Configure assay(s) of the DATA INPUTS workflow. In this way, a user only needs to add a lot once upon opening it.

Should a reagent lot be used routinely, it can be specified as default, avoiding the need to select it each time. Lots added without being made default will be available from the list as an opt-in for other lots that remain in use.

3.3.3 Configuring subsets

Subsets are user-defined subcategories that allow multiple assay configurations for different samples within an analysis for the same assay. Such configurations can include reagent lots.
When subsets are defined on the plate, it is possible to configure them separately by enabling this option above the list of assays in the step (3) Configure assay(s). When this option is enabled, every subset can be configured differently in every assay. Should a subset be defined but not used, it will be ignored in RIDA®SEEK.
Example: A user uses two different assay lots for different samples on the same plate by selecting samples for lot 1 and defining the subset “LOT1” and repeating for “LOT2”. The two subsets can be assigned different lots in the following (3) Configure assay(s) section.

3.4 Section Results

3.4.1 Result overview tab

The RESULTS section displays in the Overview tab a table of the complete set of analysed results. From this page, you are able to access the data for any sample by clicking its sample name. To view multiple samples in a single plot, select and filter the results table in the Well details tab (Fig. 30). Compare all positive or negative curves in the Target details tab. Samples to resolve are reviewed in the Resolve tab while QC violations can be reviewed in the QC overview tab.

Only after the minimum criteria for authorisation have been met a user may authorise an analysis. These criteria relate to Resolve items encountered during the workflow. Such items are denoted by a **red badge** above the Resolve/To be reviewed or QC Overview tab of the RESULTS section (see section **3.4.3 Resolving samples** for more information).

To complete the analysis, select in the AUTHORISE ANALYSIS tab.

In a **two-step validated workflow** the first analyst approves the results and the second analyst authorises the results. Also, in a two-step validated workflow, the analysis will become available in the To be reviewed list in the RIDA®SEEK START module page. If a single-step validation is set, the user can proceed to the EXPORTS section.

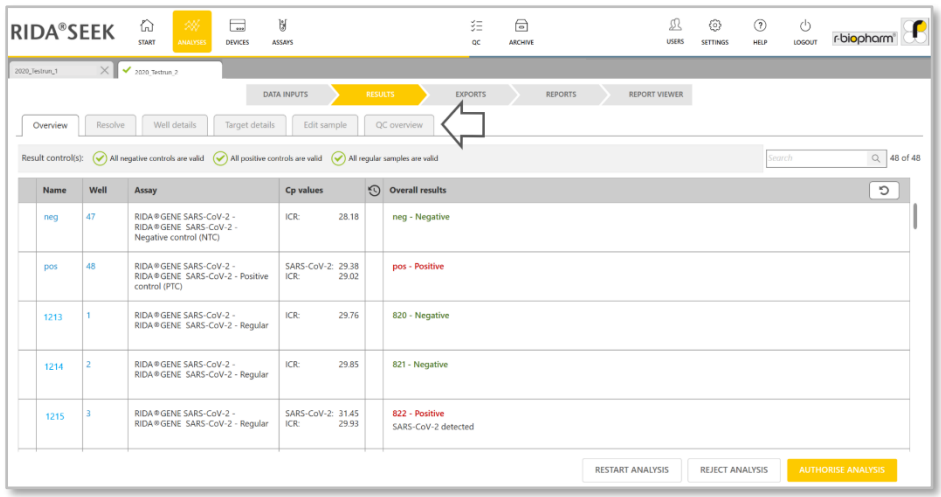


Fig. 30: ANALYSES module: To compare all positive or negative curves select the Target details tab or the Well details details tab.

3.4.2 Sorting the Overview Tab

The user is presented with a tabular view of all sample and assay details per well, the overall result and other information in the RESULTS section. To sort the table, select the column header (e.g. “Assay”, “Name”) and the table will update in ascending order. For quick access to a specific sample, type the sample name in the search bar on the right-hand pane. RIDA®SEEK will then filter the table dynamically.

The table in the Overview tab can be customised individually. To configure the desired information, select the SETTINGS module and the Laboratory settings section and scroll down to the Results overview settings section (Fig. 31). The information Sample name, Audit Trail and Overall results are always shown in the table of the RESULTS section’s Overview tab.

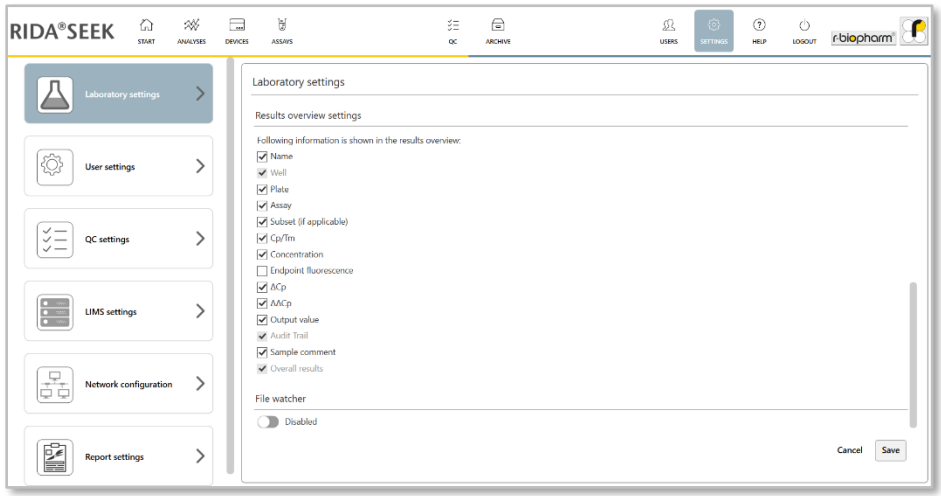


Fig. 30: SETTINGS module: To configure the desired information.

3.4.3 Resolving samples

If a sample shows ambiguous or unexpected data according to the assay's IFU the software provides an interpretation that has to be confirmed by the user. The number of items to resolve is specified in a **red badge** above the Resolve tab header (e.g. if 1 items has to be resolved see Fig. 32).

Within the Resolve tab, samples to be resolved are displayed as a list in the left-hand pane. To view the data, select the relevant sample whereupon the target in question will display a question mark in the left column; select the target to update the graphs and analyse the data.

The RIDA®SEEK algorithm will always produce an interpretation of each target, including ambiguous results, even at low confidence for resolve items. To agree with the interpretation provided, select the confirm button (Approved function) next to the Pencil icon (Edit function) and then select Go to next sample. To override the result, select the Pencil icon (Edit function) in the adjacent column, provide the interpretation and select Resolve sample, providing a comment as required. Alternatively, to reject the sample, select Reject sample at the bottom of the page. Any comments and changes made to the results are logged in the RIDA®SEEK audit trail and will be available in the PDF report (Fig. 33).

If an analysis is opened by another user, the name of the user is shown when hovering over the lock symbol.

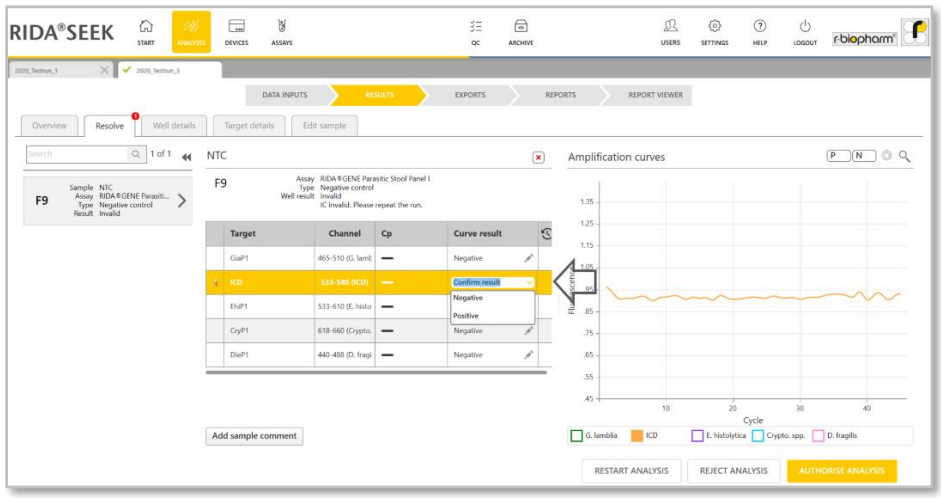


Fig. 31: ANALYSES module: Resolve tab.

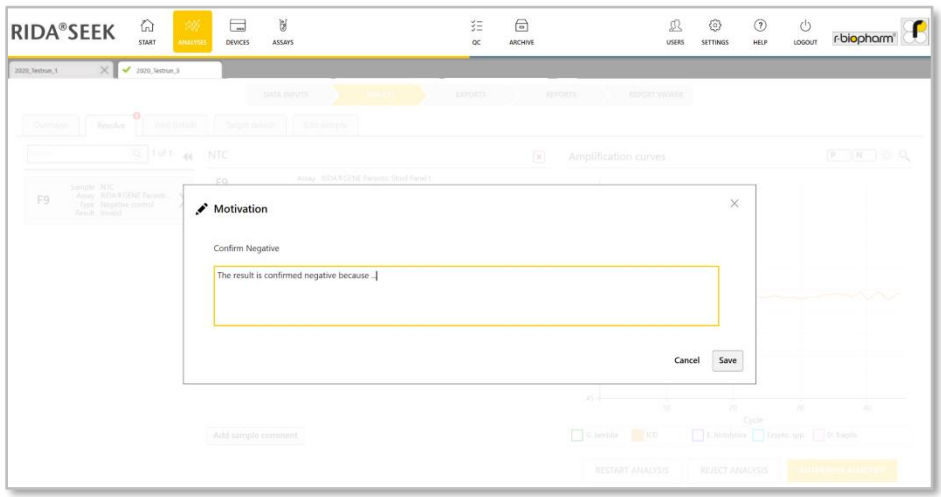


Fig. 32: ANALYSES module: Resolve tab.

3.4.4 Editing sample information

It remains possible to edit the Cp value and result or provide comments for a sample following analysis in RIDA®SEEK. To do so, select the Pencil icon (Edit function) next to the relevant sample in the Name column of the result Overview tab or in the Sample column in the Details tab. The Details tab opens if a single sample is selected. It is also possible to reject samples from a plate, e.g. when it is clear the reaction failed due to technical reasons. A sample can be rejected by the Reject sample button in the Resolve and Edit sample tab. A comment is always required (Fig. 34). It is also possible to reject multiple samples at the same time, if they all have the same sample name. When this option is enabled, RIDA®SEEK gives automatically an information which wells are impacted. Please note that samples can also be rejected by the QC module, as described in section **6 Quality Control in RIDA®SEEK**.

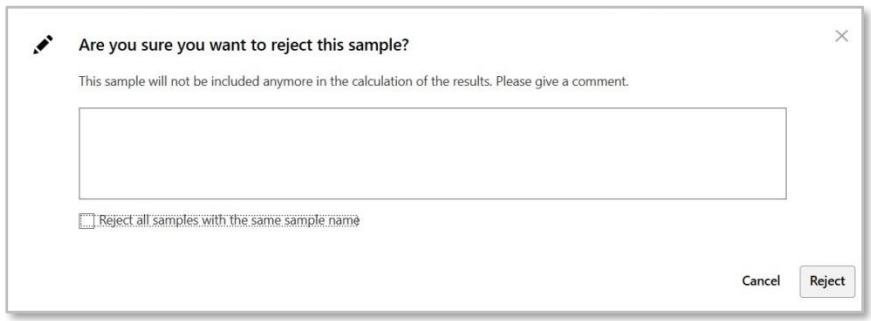


Fig. 33: ANALYSES module: RESULTS section, by selecting Reject sample button in the Resolve and Edit sample tab a window Are you sure you want to reject this sample? will open. A comment is always required.

3.4.5 Marking samples

In RIDA®SEEK, samples can be marked. There are two marking options:

- **Mark for review**: When **two-step validation** is enabled, the edit sample and resolve view have an additional column for every channel indicated by the eye icon ( ). By simply clicking in this column next to the channel of interest, the channel is marked for review and is added to the To be reviewed tab for the second reviewer, even without any changes to the result. This can be useful when there is doubt of a specific channel result. A comment can always be provided via the sample comment button.
- **To be repeated**: Sometimes, the results of a certain sample are not as qualitative as expected and a rerun of the full PCR reaction is necessary. By marking a sample as to be repeated, this information is added to the report. This functionality is only available

for regular samples and not for control samples. For more information on the to be repeated flag in your LIMS coupling, please contact your dedicated support team.

3.4.6 Resolving QC events

All QC events are reported in the QC module including the corresponding Levey-Jennings curve if the QC module is enabled. The newest data point added to this curve will be shown as a pulsating blue dot. This data point is not visible in the QC module until the analysis is approved/authorised. Choose between all QC results and violations only using the switch icon above the table. To accept a QC violation, select the X icon, doing so will automatically reject all results regarding to that control. To override a QC violation and allow the associated results to pass, select the grey tick symbol (Approved function). A comment is always required. The QC module is only displayed if QC tracking has been configured correctly as described in section **6 Quality Control in RIDA®SEEK**.

3.4.7 Warning and audit trail alerts

All changes performed by users within RIDA®SEEK are audit trailed. Should a change be made to a sample, a comment button (Audit trail icon) will be visible for the edited sample and available in the PDF report.

Warnings are always shown in the first column of tables and/or next to certain results. By hovering over the warning icon, more information about the warning is shown.

3.4.8 Reject or authorise results

There are four possible outcomes for an analysis in RIDA®SEEK:

- **Approved analysis** is available when the first reviewer completes its analysis, in a **two-step validation** workflow after which it requires a second review before the analysis can be finally authorised. A temporary PDF export can be generated, but no LIMS exports are possible yet. Approved analyses are marked with a grey tick symbol (Approved function).
- **Authorised analysis** will proceed to EXPORTS and will be marked with a green tick symbol in the ARCHIVE. Authorised analyses cannot be edited anymore and all exports are available (as configured for the lab).
- **Restart analysis** will reset the analysis back to the beginning of the workflow. Assay assignments stay the same but can be edited.
- **Reject analysis** will complete the analysis marking it as rejected. Rejected analyses are permanently displayed as such in the ARCHIVE with a red X.

4 Viewing data

4.1 Visualising the data

4.1.1 Viewing a single sample

RIDA®SEEK is designed to automate as much of your analysis workflow as possible. All data for the samples and controls in a run can be accessed on demand. Default graph settings can be configured in the SETTINGS module in the uppermost pane, select Laboratory settings section and Default graph settings section.

In the Overview tab, sample names act as hyperlinks to that sample's data. To access it, click the link to populate the Well details tab. Click the sample entry in the centre pane to view the graphs in the right-hand pane. The graph pane can be expanded by dragging the double lines (seen when hovering over the border) and adjusting as required. To adjust the graph settings, select the ⚙ button (Set function) in the top right-hand corner of the graph pane and select amongst the following options:

Tab. 6: Graph settings.

• Chart type	○ Amplification ○ Baseline-corrected amplification
• Chart curve	○ Linear ○ Logarithmic
• Chart data	○ Raw ○ Smoothed

Individual targets are selected via the dropdown menu beneath the graph using the check box to select targets as required. Several types of curves are available to be imposed over the data as a visual aid:

- **Positive control curves** are accessed via the P button in the graph pane and pertain to the positive control on the plate. It is displayed as a dashed line. If multiple controls are available on the plate, a curve can be selected via the expand icon P ⌄.
- **Negative control curves** are accessed via the N button in the graph pane and pertain to the negative control on the plate. It is displayed as a dotted line. If multiple controls are available on the plate, a curve can be selected via the expand icon N ⌄.
- **Replicate curves** are accessed via the Rep button in the graph pane and pertain to wells that are replicates of the selected well. Replicate curves are displayed in a reduced line thickness. The Rep button is only available in case the selected well has replicates.

A right mouse click on the curve will display more information about the selected curve (Fig. 35).

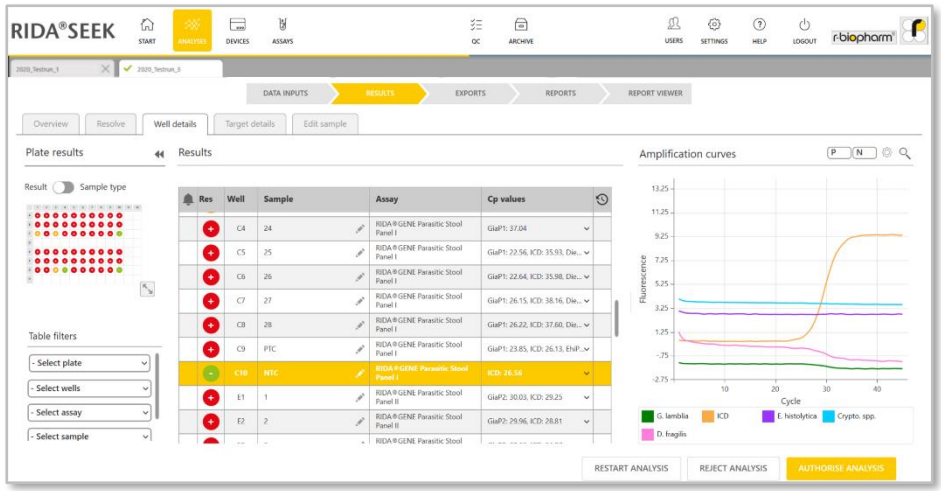


Fig. 34: ANALYSES module: Viewing a single graph, to adjust the graph settings select the ⚙ in the top right-hand corner of the graph pane.

4.1.2 Viewing multiple samples

It is possible to view a single fluorescence channel from multiple wells combined into one graph. To access, hold down “Ctrl” or “Shift” on the keyboard and select all wells as required, either from the plate view in the left-hand “Table filters” pane, or from the list view in the center pane. (De)activate channels using the buttons below each graph.

4.1.3 Filtering the plate

Selecting all samples from one assay or result outcome is accomplished by applying filters to the plate from the four dropdown menus or plate view in the left-hand pane of the Well details view. Filters can be combined to refine filtering as required. Having selected the filtering criteria, click Set filters (▽) to update the centre pane with all applicable samples (Fig. 36).

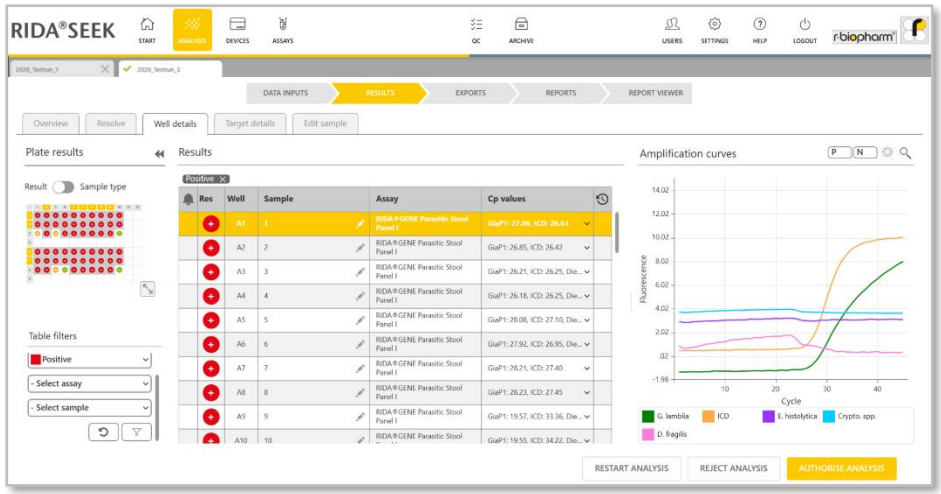


Fig. 35: ANALYSES module: Filtering the plate by applying filters to the plate from the three dropdown menus or plate view in the left-hand pane.

Example: A user would like to view all positive samples on their plate. Select “Positive” from the result dropdown. Upon selecting Set filters ( ) the center pane will populate with all wells containing a positive result (Fig. 36).

4.1.4 Comparing target curves

In the Target details tab, all targets are shown as separate rows. By multiselecting these rows, several curves can be compared. To view all positive or negative curves at once, the buttons on top of the page can be used.

5 Exports and reporting

5.1 Section Exports

Exporting from RIDA®SEEK

The EXPORTS section allows to generate a PDF report and LIMS integration file to be exported from RIDA®SEEK as desired (Fig. 37). Modify the report in the Standard report settings and select EXPORTS. In the REPORTS section, each export item is represented by an icon. Select the icon to save or preview. For LIMS files, select the icon and LIMS Export. The workflow finishes with the REPORT VIEWER section.

An export item, such as standard report (pdf) and the LIMS file, can be generated by configuring the options in the left-hand pane. Upon selecting the EXPORTS button, the files are created and displayed as icons in the REPORTS section that follows.

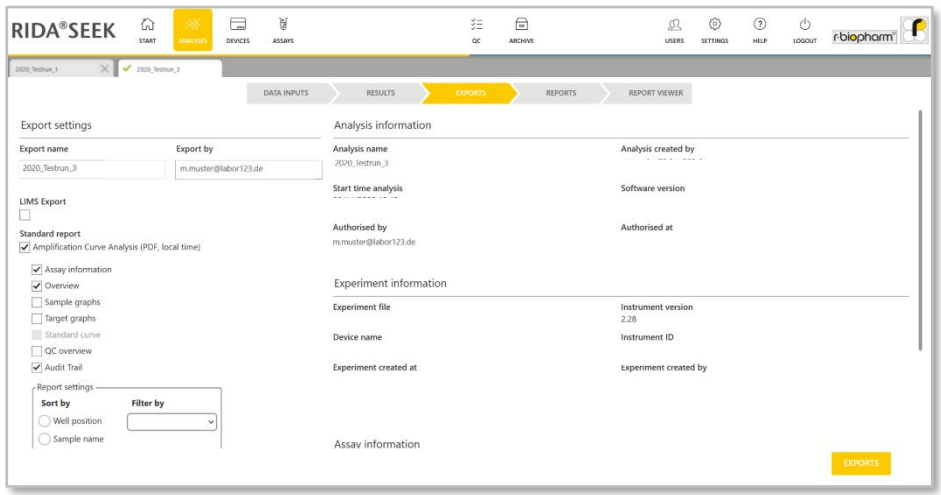


Fig. 36: ANALYSES module: Overview of Export settings in the EXPORTS section.

The Save Pencil button (save function) within each generated item allows to save the file to a specified location. For LIMS export items, a separate window will pop up previewing the LIMS file content. Select Send to LIMS to proceed. Any LIMS export can only be sent once to the LIMS system.

5.2 Section Reports and Report Viewer

5.2.1 Reports and Report Viewer

Following the EXPORTS section, the user is directed to the REPORTS section. The **standard report** is a PDF document containing both default and user-defined information and is generated in RIDA®SEEK (Fig. 38). The default information is as follows:

- **Experiment information** relates to the data file and device details.
- **Analyses information** contains the user name, time stamps and software information relating to the analysis.
- **Reports information** contains the user name and time stamp for the user who created the report.

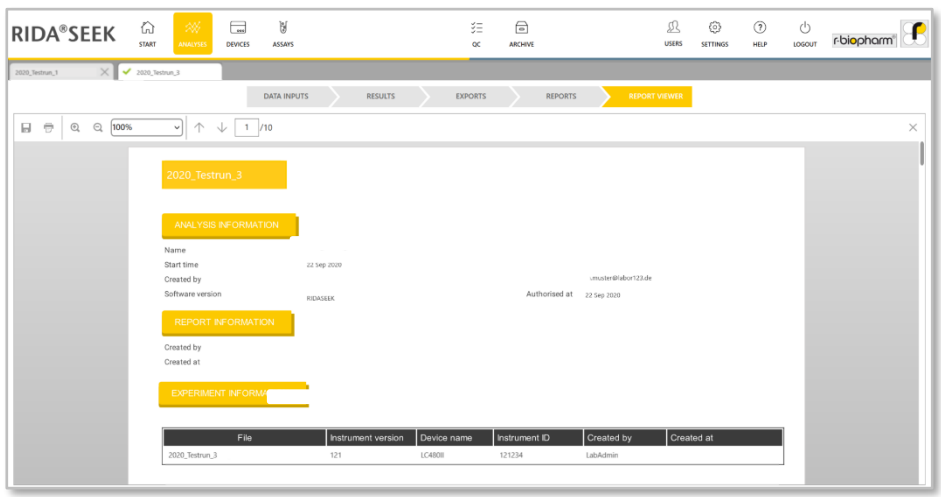


Fig. 37: ANALYSES module: Overview of REPORT VIEWER section of ANALYSES module**.**

The **optional** information for the report can be summarized as follows:

- **Assay information** contains all information about the Assay Plug-In such as the version used, lot number, color compensation files etc.
- **Results overview and legend** outputs the contents of the Overview tab with a legend to explain the fields and symbols therein.
- **Sample graphs** will output an auto-scaled graph per target, per sample. Please note that selecting the graphs option for all samples will significantly increase the run time for compiling the report. As an option, it is possible to add the positive and negative control

curve on all graphs. Choose between a linear or logarithmic scale and the amplification or baseline-corrected data.
- **Target graphs** will output a graph per target containing all samples from the analysis that were tested for that specific target. Here, the same graph options are available as for normal sample graphs
- **QC overview** contains all information available in the QC overview tab during the analysis.
- **Audit trail** displays all audit trailed items time stamped and accompanied by user credentials.

By default, the report settings are configured to export all samples from an analysis and sort by assay. It is also possible to sort the results based on well position, sample name or result and it is possible to generate individual reports per sample name using the Filter by functionality.

Note: All reports generated from an analysis will remain visible as icons in the reports page and will remain retrievable from the ARCHIVE. If **two-step validation** is enabled, a preliminary PDF report may be generated after first review, but a **watermark** is applied to identify the results as not authorised. Other exports are not possible if the analysis is not authorised yet.

5.2.2 LIMS export

In order to simplify the connection to a LIMS system, the data export (ASTM and HL7) was realized file-based. The files to be exported are stored under the control of the end user in a storage location in the file system, for example on a network share. This integration is **unidirectional**, the information is only forwarded from RIDA®SEEK to the LIMS.

The output location of these files can be defined in LIMS settings. LIMS settings can be managed within the SETTINGS module of RIDA®SEEK. To access, select the SETTINGS button in the uppermost pane and select LIMS settings (Fig. 39). To specify the location for export, select the Browse button to browse to the correct directory.

For integration with LIMS to be effective, the **field mapping** must correspond to that expected by the LIMS. Configure Order target mapping section of the assay by selecting the ASSAYS module in the uppermost pane and an Assay Plug-In in the left-hand pane, go to the Versions section and select the Pencil icon (Edit function). If a run has been authorised within RIDA®SEEK, the user has the option to select the LIMS export option in the EXPORTS section of the analysis.

For more information on how R-Biopharm AG can support LIMS integration, please contact the R-Biopharm AG support address *ridaseek@r-biopharm.de*.

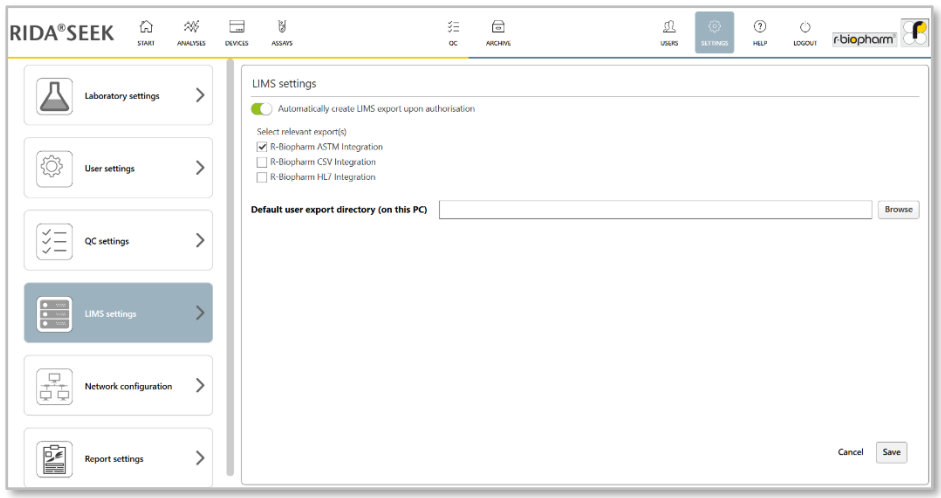


Fig. 38: SETTINGS module: Select in the SETTINGS module the LIMS settings on the left-hand pane and set the directory from which the LIMS will pick up the file.

6 Quality Control in RIDA®SEEK

6.1 QC module

6.1.1 Introduction to quality control tracking

RIDA®SEEK provides automated Statistical Process Control (SPC) per device/device type, per assay and per assay lot. Having specified which target to use as a control and how to define the mean Cp value, the variability can be tracked of an experiment and identify outlying runs in real-time. Outlying runs are Resolve items in RIDA®SEEK called **QC violations** and are based on up to four Westgard Rules. QC module is an optional feature that can be enabled or disabled in the laboratory settings.

6.1.2 Setting up the QC module

To set up the QC module, complete the following requirements to enable **automated QC tracking**:

- The QC checkbox must be ticked in the SETTINGS module in the Laboratory settings.
- Decide which Westgard rule(s) will determine a QC violation and tick in the relevant checkboxes in the QC settings.
- Decide which QC monitoring you prefer in SETTINGS module / QC settings / Device settings; per device type or per device.
- A single reference number (such as the assay kit lot number) that presents the reagents is required.
- Ensure all devices and assays are added to the RIDA®SEEK environment.
- Upon starting a new assay lot, ensure that the assay lot is added to the corresponding Assay Plug-In together with the expiry date.
- Verify that the target that will be used as a QC tracking control is specified upon adding the reagents lot, including the desired statistics (fixed or floating).

6.1.3 Viewing runs by device (type) and assay lot

Given correct configuration of the reagent lot in the Assay Plug-In, the performance of any lot of an assay on any device can be viewed in the QC module in the uppermost pane of RIDA®SEEK. The performance of an assay lot is tracked per device type or per device, depending on the Device settings in the QC settings. All devices are listed in the left-hand pane. Select the device/device type of interest to view all applicable assays and select the assay to view its QC history by lot number (Fig. 40).

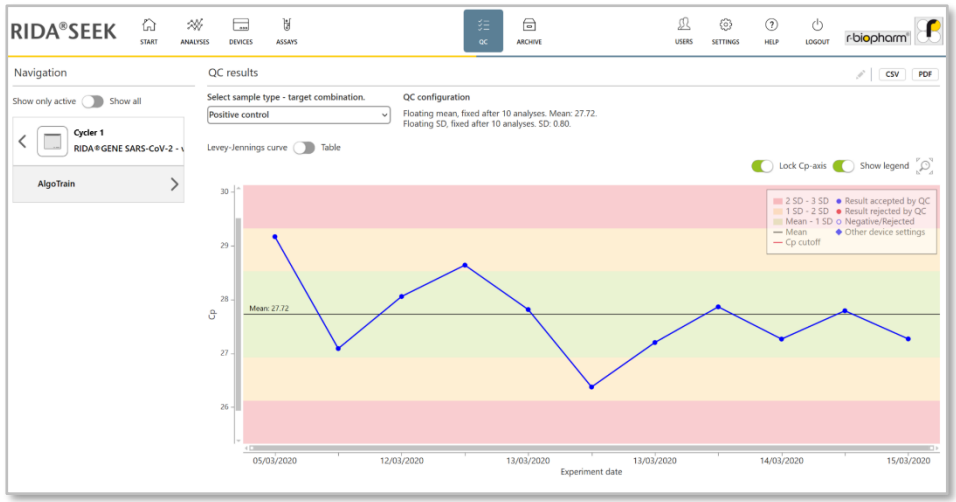


Fig. 40: QC module: Overview of QC results.

RIDA®SEEK represents Cp value variations of a control over time in a Levey-Jennings curve (see section **6.2.6 Notes on Levey-Jennings curve**) and as a list of QC events in the QC results table. To switch between the table view and Levey-Jennings curve, change the Levey-Jennings curve/Table toggle as required.

In the top left corner, there is a toggle to hide inactive assays and lots (“Show all lots” or “Show only active lots”).

In **the Levey-Jennings curve**, each data point is a single analysis with blue points for a concordant experiment, red for an accepted violation. More information for each data point can be shown by selecting it, using the right mouse button. The assay lot under selection is represented as a blue continuous trend line (through the mean of multiple similar controls if applicable) while all other lots appear behind as grey, dashed trend lines. Navigate between lots by selection from the list beneath the assay name in the left-hand pane. To adjust the Levey-Jennings curve, use the magnifying glass symbol to zoom and the Lock Cp-axis option as required or the white squares on the scrollbars.

Notes can be addressed to specific data points or at specific places on the curves. A comment can be added in the table view. This comment will be visible as an icon (Information icon) on the Levey-Jennings curve and the text in the tooltip on this icon.

6.1.4 Editing QC outliers

Using **floating statistics** to determine an average provides an automated way to configure a baseline against which future experiments can be judged as possible outliers (QC violations). However, the set of analyses used to define the mean can itself contain outliers, leading to a potentially misrepresentative definition of in-control results.

To amend this, it is possible to remove such outlying analyses by selecting the Pencil icon (Edit function) in the upper right pane next to the QC results headline of the QC module page of a lot. A pop-up opens indicating which samples are currently used for the calculation. Outliers can be deselected from the list and new analyses (next in time) are automatically added to the list if available. After selecting Next, provide an interpretation before selecting Save button (save function). Doing so will recalculate the mean based on the new set of experiments.

Please note that RIDA®SEEK cannot re-calculate already authorised analyses because they are potentially already sent to the LIMS. The results need to be checked manually and corrected in the LIMS if appropriate. This can be easily done by checking the Levey-Jennings curve that contains the new mean. The table view also contains a µ symbol next to all data points that were excluded from the mean/SD calculations.

6.2 Assay lot management

6.2.1 Reagent lot life cycle

A necessary prerequisite for QC tracking in RIDA®SEEK is the representation of the reagents by a single reference such as a reagent kit lot number.

In the event of multiple lot numbers per experiment it remains possible to either summarize all components as a single lot number on the stock management system or track only the most sensitive reagent.

Example: A user receives a shipment of kits under a single lot, but has a number of kits remaining under a different lot. Upon opening a new kit, the technician enters the lot number into RIDA®SEEK and sets it to default. For new runs completed with the older reagents, the appropriate lot number can be selected from the dropdown menu until the stock is depleted, whereupon the technician sets the lot status to inactive.

6.2.2 Adding a new assay lot with QC tracking

To add a new assay lot number, navigate to the correct assay and select it from the left-hand pane in the ASSAYS module in the uppermost pane of RIDA®SEEK. Select the + icon (Add function) in the Lots section in the right-hand pane. Add the lot number and expiration date in the pop-up screen that follows, choosing to set it as the default as required. Select Next.

The following screen displays a list of targets, select one or more targets to serve as the **QC tracking control**. Next, specify the mean Cp, this will serve as the baseline for measuring variation, and standard deviation (or coefficient of variation (CV)). This can be done by switching the toggle to fixed or floating (see section **6.2.3 QC tracking criteria for new assay lots**). Once satisfied, select Save button (save function) to finish.

The tracking criteria and lot name cannot be edited after it has been used; however, lots can be set as Active, Inactive or default by selecting the Pencil icon (Edit function) next to the lot name. When adding a new lot to an assay, the QC tracking configuration of the previously configured lot is automatically filled in but can be changed if necessary.

6.2.3 QC tracking criteria for new assay lots

To track performance, it is best to choose a control target that validates the run (such as a positive control).

Multiple methods can be used to determine the mean Cp value, which will serve as the baseline for measuring variation, and the standard deviation (SD) or CV value:

- **Floating statistics** fixes the mean Cp value automatically after n analyses, where n is specified by the user.
- **Fixed statistics** uses values for the mean Cp and SD or CV (%) manually provided by the user.
- **A combination of floating statistics and fixed statistics**, for example a floating mean and a fixed SD.

When making use of floating statistics for the calculation of the mean, it is possible to set multiple stages (using the + icon) after which the mean and SD is blocked until the next stage. After every stage, the values are recalculated. Unlimited intervals can be added.

6.2.4 Active and inactive assay lots

Assay lots can be made active or inactive by switching the Active switch in the edit menu of an existing lot (see ASSAYS module and Assay lot section). Use the Pencil icon (Edit function) to access a lot's edit menu. Inactive lots will no longer be available to the user at the experiment configuration stage of the RIDA®SEEK analysis workflow.

6.2.5 QC module export

The contents of the QC results table can be exported either as a PDF (including Levey-Jennings curves) or a .csv file on a device, per assay basis. To do so, select one of the export options at the top right corner of the QC module and select the export location. Note that these exports are not saved in RIDA®SEEK like the exports of an analysis, but can be re-generated at any time.

6.2.6 Notes on Levey-Jennings curve

A Levey-Jennings curve is a visual representation of the validity of a set of experiments over time as measured by their variation from the mean (usually expressed as SD). In RIDA®SEEK, the mean is represented as a solid black horizontal line through the middle of the curve. The green band represents the area within which Cp is less than 1 SD from the mean line, the yellow band is greater than 1 SD and less than 2 SD, with the outer red band representing greater than 2 SD. Further information see **6.3.1 Notes on Westgard Rules**.

6.3 QC violations

6.3.1 Notes on Westgard Rules

The **Westgard Rules** were developed to identify an analysis as in-control or out-of-control by characterizing types of measurable variation. Different rules can be described, for instance, large deviations for a small number of runs in succession or as smaller variation over a larger set of runs as part of a trend.

The Westgard Rules available in RIDA®SEEK are as follows:

- 1_{2S}: 1 control measurement exceeding absolute 2 SD from the mean
- 2_{2S}: 2 consecutive control measurements exceeding absolute 2 SD from the mean
- 1_{3S}: 1 control measurement exceeding absolute 3 SD from the mean
- 10_{x}: 10 consecutive control measurements on a single side of the mean

More information on Westgard rules is available also on https://www.westgard.com/.

6.3.2 Violation criteria

QC violations in RIDA®SEEK are defined as any violation of the Westgard rules set as active in the SETTINGS module in the uppermost pane and the QC settings on the left-hand pane by the user.

Example: A laboratory using the standard Westgard rules in RIDA®SEEK has used floating statistics to define the mean and SD from a set of 5 runs. Upon analysing a plate, they observe that a QC violation is triggered in QC overview. From the table view, they can see that the SD for the current and previous run were 2.53 and 2.73 respectively, a violation of the 2_{2S} rule. They then choose to invalidate and repeat the run before raising a nonconformity incident in their quality management system.

6.3.3 Viewing QC violations

QC violations are introduced to the user as they are detected during the analysis workflow and are available thereafter in the QC module of RIDA®SEEK.

6.3.4 Resolve and edit QC violations

In the event of a QC violation being detected, the user is prompted to resolve each violation in the QC Overview tab in the RESULTS section of the ANALYSES module. The number of violations is noted in a red badge atop the QC Overview tab heading.

To reject the associated results of a QC result, select the red X mark in the Status field of the relevant control. To accept the associated results of a QC result, select the green tick symbol. In both cases, a pop-up window will prompt the user to provide an interpretation. In case the related results were rejected, a list of impacted samples is shown. The user can deselect samples that should not be rejected. All actions (both rejecting related samples and not rejecting them) are audit trailed.

To add an action, select the + icon (Add function) in the Action field for the QC item entry. In case of two-step validation, the second analyst has to confirm (using the checkbox) the decision of the first analyst, or can overrule the decision by selecting the cross mark or tick tick symbol.

Note that it is very important to analyse the experiment in the correct order in RIDA®SEEK. When more recent experiments are analysed first in RIDA®SEEK, the QC module does not contain all required information for a correct interpretation of the results. RIDA®SEEK will give a warning if this happens but already authorised results cannot be changed because the results are potentially entered in the LIMS.
When additional data is added to the QC module during the time an analysis in RIDA®SEEK is performed, the software request a permission to re-calculate the QC violations before approving/authorising the analysis.

7 Retrieving runs from the Archive

7.1 Navigating the Archive module

7.1.1 Archive overview

The RIDA®SEEK ARCHIVE is a searchable database of all the analysed runs that can be re-opened. Also in the ARCHIVE results can be retrieved, unfinished analyses and reports can be accessed.

To access the archive, select the ARCHIVE module in the uppermost pane of RIDA®SEEK. The leftmost field of the table denotes the stage of the analysis; blank meaning analysed, the grey tick symbol meaning approved, a green tick symbol meaning authorised and the padlock symbol meaning that the analysis is currently viewed by another user (access restricted) and a red X mark denoting a rejected analysis. By default, and when no filters are selected, the last created analyses are shown first.

When going to the Active module search results are automatically refreshed and by default 20 items are shown.

7.1.2 Searching the Archive

Access a sample or analysis by applying multi-level dynamic filters from the dropdown options in the left-hand pane see Fig. 41 (ARCHIVE module). The Search results page will populate a table of hits; select the Analysis name link to open the associated analysis.

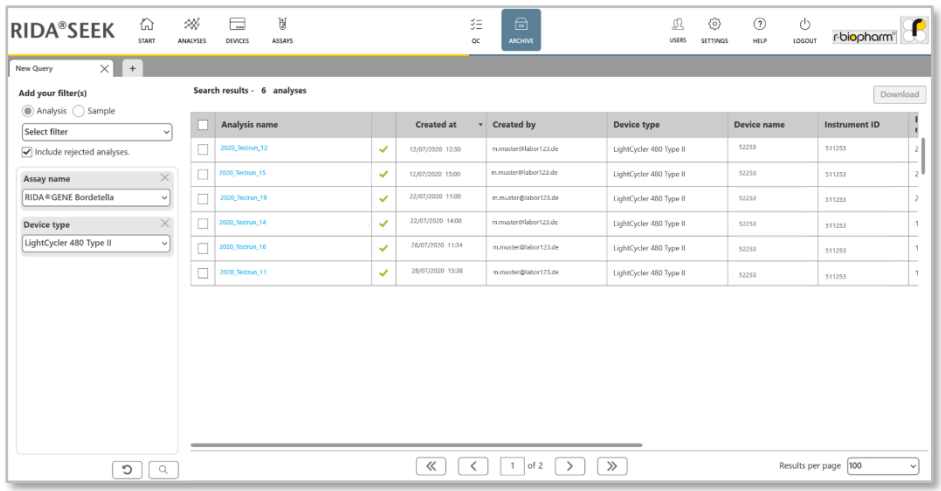


Fig. 39: ARCHIVE module: Searching the archive by applying multi-level dynamic filters from the dropdown options in the left-hand pane**.**

To filter the archive, select a criterion from the dropdown menu in the left-hand pane to add it to the list of active filters in the pane below. Filters can be removed by selecting the X button (Delete function) on the top right-hand corner of the icon. Select another criterion to add as an “and” combined search filter.

To search a sample name, select the Add your filter(s) / Sample option and select sample name. It is possible to search sample names based on contains, equals, starts with and ends with criteria from the available drop-down menu.

Example: A laboratory user is concerned that a device has been faulty for the past week and would like to retrieve all analysis that were run on this device. The user can add a filter “Device name” and select the device in question from the dropdown list. A second filter “Analysis created at” set to past week will automatically retrieve all analyses from this device in the past week.

8 Help module

Overview of the help module

In the RIDA®SEEK HELP module the user manual, the license agreement and the release notes are deposited. To access, select the HELP module in the uppermost pane of the RIDA®SEEK. Four fields are displayed with the following information:

- User manual – the IFU for the RIDA®SEEK software.
- About – Splash Screen with information of the software's developer.
- License agreement – Conditions of use of the platform.
- Release notes – Information about the release version.

To access the HELP module, choose the Help section button in the START module in the right hand pane. For further questions please contact the R-Biopharm AG support team *ridaseek@r-biopharm.de*.

9 Advanced topics and troubleshooting

For users in the European Union: Serious incidents related to the product must be reported to R-Biopharm AG and the national authority.

9.1 Data import/export strategies

Standard ASTM and HL7 integration

The following describes the standard ASTM and HL7 interface that is available off-the-shelf to integrate R-Biopharm AG's RIDA®SEEK system with a laboratory's LIMS. A file-based transfer, where files are stored to a system location such as a network share, under the control of the end-user has been chosen.

Integration is **unidirectional** in the sense that information flows only from RIDA®SEEK out to the LIMS. Specifically, when a run has been authorised inside RIDA®SEEK the user has the possibility to check the LIMS export option inside the Exports tab. To generate ASTM or HL7 messages, the sample name on the plate in the PCR file that is analysed by RIDA®SEEK is assumed to depict the order number. RIDA®SEEK then generates an ASTM or HL7 file per analyses, containing the results per original order for which it was able to find the results. For configuration see chapter **5.2.2 LIMS export**.

9.2 Proxy settings

There are three options for network proxy configuration:

- **System proxy**: Uses the proxy settings as defined by the user's system
- **No proxy**: No proxy settings applied
- **Manual proxy**: Manual settings for which the proxy URL and port are required inputs. Authentication can be toggled as manual or automatic. If manual, supply the domain, username and password.

9.3 Troubleshooting

Empty plate in automated plate setup

If a data file is uploaded into RIDA®SEEK and an empty plate is displayed, automated plate set up has failed. In such an event, please check the following:

- More than one version of the Assay Plug-In is active at the same time. Check the list of active Assay Plug-Ins in the ASSAYS module (in the uppermost pane). For any active Assay Plug-Ins of the same name, select the older version and the Pencil icon (Edit function) in its General assay information section to toggle inactive.

- If the plate is empty but the correct assay is listed in the right-hand pane, the sample nametags and/or mix definition nametags may be missing in the sample names. Switch to the Table view and check the correct prefix and suffix is present in all sample names.

- If the plate is filled with question mark symbols or only regular samples, RIDA®SEEK knows which assay/mix needs to be assigned but does not know which sample types need to be assigned. If there is only one possible regular sample, the plate is assigned with regular samples. If there are multiple regular samples possible, the question marks are assigned. In this case, check if the sample type nametags are correct.

For more information on sample nametags and mix definition nametags, please consult sections **2.3.2 Sample nametags** and **2.3.3 Mix definition nametags/Subsets**.

10 Manual version number

10.1 Version overview

Tab. 7: Version number.

User manual version number	Date	Chapter and designation
1.0	2019-04-16	Release version
2.0	2021-08-11	Revision of chapter: 1.1 Intended Use 1.4. System requirements 1.4 Supported PCR cyclers and file types 2.1 Managing users and permissions 2.2 Adding devices and assays 2.3 Configuring devices and assays 2.4 Laboratory settings 3.1 RIDA®SEEK: an overview 3.2 Section Data Inputs 3.3 Section Configure assay(s) 3.4 Section Results 4.1 Visualising the data 5.1 Section Exports 5.2 Section Reports and Report Viewer 6.1 QC module 7.1 Navigating the Archive module

10.2 Software updates

Information about the software updates will be provided by the RIDA®SEEK team from R-Biopharm AG. The implementation of the update, if available, will be supported by the RIDA®SEEK team of R-Biopharm AG.
For further information please contact *ridaseek@r-biopharm.de*.

List of abbreviations

Tab. 8: List of abbreviations.

ASTM	American society for testing and materials
AP(s)	Assay Plug-In(s)
CC	Color compensation
Cp	Crossing point
CV	Coefficient of variation
HL7	Health level 7
IFU	Instructions for use
LIMS	Laboratory information management system
OS	Operating system
PCR	Polymerase chain reaction
PDF	Portable document format
PSS	Product specification sheet
QC	Quality control
SD	Standard deviation
SOP	Standard operating procedure
SPC	Statistical process control

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