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The Deep Phenotyping Company

MetIDQ™ Oxygen

User Manual

UM-MetIDQ-Oxygen-9

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Met*IDQ*™ Oxygen — Table of Contents

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Symbols

Symbol	*Description*
	Note: pay attention to the user manual
	Manufacturer
	Attention
	Comment
	Important information

1 Introduction

Biocrates'® proprietary Met*IDQ*™ software is an integral part of Biocrates® Kits. The software enables an automation of the assay workflow, from sample registration and data processing through data export and statistical data analysis.

1.1 Met*IDQ*™ Requirements

	Specifications and Requirements
Operating system	Windows™ 7 or higher, 64-bit architecture
Hardware	- 1 GHz processor - 4 GB RAM - 20 GB free hard disk space
Additional software requirements	- Microsoft™ .NET Framework 4.5.2 or higher - Microsoft™ Visual C++ Redistributables: 2005, 2008, 2010, 2012, 2013 Oracle® Database (*either)* - Oracle® Database 11g, 12c, or 18c - Oracle® Database 18c Express Edition (installer provided with the kit) Oracle® Database 18c Express Edition needs a minimum of 10 GB additional free hard disk space Microsoft™ .NET Framework and Microsoft™ Visual C++ Redistributable installers are provided with the USB stick and will be installed during the first Met*IDQ*™ installation.

1.2 Software Information

Data is managed and graphically presented using Biocrates' proprietary Met*IDQ*™ software. The Met*IDQ*™ software is a desktop application with two components: Met*IDQ*™ software and an Oracle® relational database called "METKIT". Kit data is stored in an Oracle® database. The USB stick in the Kit box contains both the Met*IDQ*™ software as well as files needed to install and to set up the Oracle® database. For faster installation, copy the USB stick contents onto a hard drive or server and install from there. Keep the USB stick as a backup. Met*IDQ*™ is not used during mass spectrometer data acquisition. It is used for MS data quantitation and, if required, importing result files from the LC part into the Met*IDQ*™ database after data acquisition.

To avoid data loss, **perform regular Met*IDQ*™ database backups**. Save the backup file (.dmp) at a secure place (network drive, external hard drive, USB stick) physically separated from the PC on which the database is installed.

☞ Refer to section 7.6.1 Database Backup / Export on page 143

Disclaimer – Oracle® XE

The Oracle® XE database which is provided with the Kit is from a third-party provider (Oracle®). The Oracle® XE version is free of charge and no support is provided by Oracle®. If you have higher safety requirements in your company, we recommend using a commercial version of the Oracle® database, for which updates and support are provided by Oracle®.

1.3 Video Tutorials and FAQ

In addition to this user manual, Met*IDQ*™ video tutorials are available on Biocrates® YouTube channel.

Whenever you will see the YouTube icon, a video tutorial is available for the corresponding section or topic. Click the Help button in Met*IDQ*™ or use the links provided in this manual.

Comprehensive and up to date descriptions to **F**requently **A**sked **Q**uestions can be found in Biocrates® FAQ section.

2 Install Met*IDQ*™ and Oracle®

The installation of Met*IDQ*™ and all required software is done in the following step. The Met*IDQ*™ installation wizard guides through this process.

1) Installation of Met*IDQ*™ and Oracle® (onto local PC)
2) Import of kit database into Oracle®
3) Installation of Windows™ runtime components

To update Met*IDQ*™ refer to appendix 7.5 *Met*IDQ™ *Software Update*.

Information Oracle® Database

There are two installation options (see table below).

Condition	*Option*
No Oracle® database installed.	The Oracle® Database Express Edition (Oracle® XE) can be installed during the MetIDQ™ installation. For multiple users we recommend a server installation, but you can also install Oracle® XE locally on a standard PC. ***Note***: If Oracle® XE is installed locally, an installation on the MS operating computer is not recommended. For best performance, install Met*IDQ*™ and Oracle® on a PC reserved for data analysis.
Oracle® database already installed and in use.	An Oracle® database administrator (DBA) imports the metkit database from the USB stick. Refer to appendix 7.7.

Before installing Oracle® XE make sure that an Oracle® database is **not** already installed.

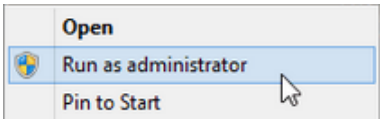
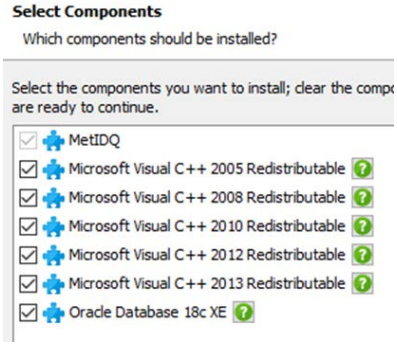
Note: **During the installation of Oracle® all existing Oracle® databases are erased!**

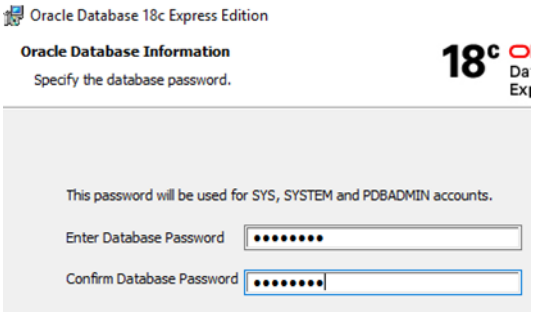
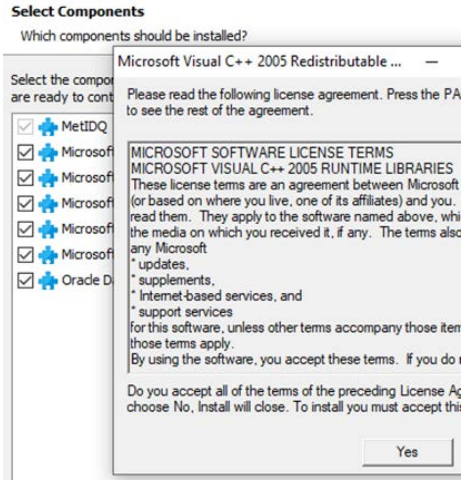
Software Support Policies

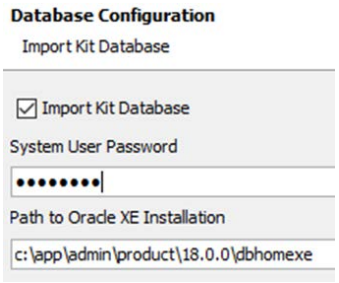
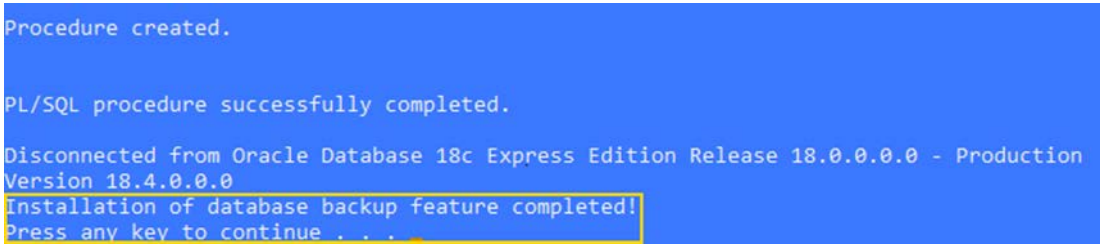
Biocrates® will offer support for software published by Biocrates®. Support for software published by third-party providers cannot be offered. Please find our support policies below.

Software	***Support***		
Met*IDQ*™ (incl. installation and setup)	Full support provided		
Oracle® database	No support provided, except initial setup during Met*IDQ*™ installation.	!	**To avoid data loss, perform regular backups according to section 7.6.1.**
Data backup and data loss	No support provided		
Software of MS vendor, e.g. Analyst®, MassLynx™, Xcalibur™, MassHunter™	No support provided		

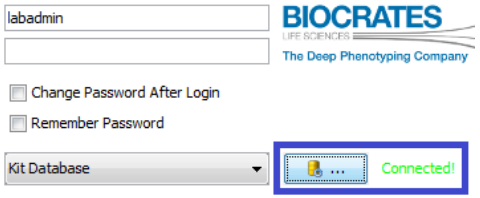
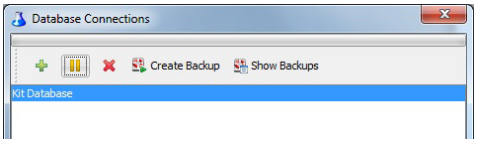
2.1 Install the Met*IDQ*™ Software

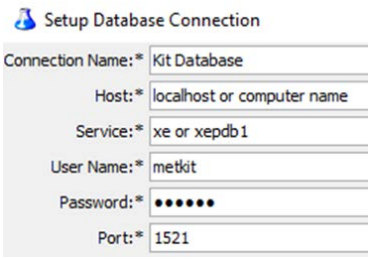
Step	Instructions	Example
!	**<u>Full</u> administrator privileges are required!**	
i	Met*IDQ*™ and all installation files are provided with the kit USB stick, folder "MetIDQ and Oracle\Installation Files"	
	Met*IDQ*™ installation	
1	Run the Met*IDQ*™ installation file with full administrator privileges. Follow the installation instructions on the screen and below.	 MetIDQ-…-install-64bit.exe 
2	A summary of the required software is shown. When Met*IDQ*™ is installed for the first time, Oracle® XE, the kit database, and Windows™ runtime components (e.g. C++ redistributable 2013) are installed.	
	Oracle® XE installation	
3	A license agreement is displayed. Confirm the default installation directory.	

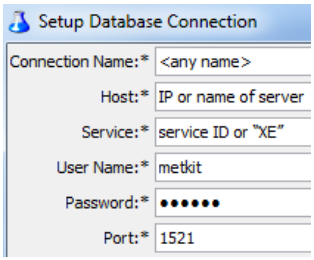
Step	Instructions	Example
4	Specify an Oralce® database password. ***Note:*** **Document this password!** You will need it for future database backups and upgrades. **The password may not contain any special characters!** Your Oracle® administrator password: _____	
5	Check the settings and start the installation.	
	Windows™ runtime components installation	
6	Required Windows™ Visual C++ Redistributable runtime components are installed one by one.	

Step	Instructions	Example
	Import of Kit Database	
7	Enter the Oracle® database password, specified in step 4. Click “Next”.	
8	An import dialog is opened. To confirm the settings type “y“ and press **Enter**. The import process starts.	
!	The import process may take several minutes. Wait until this message is displayed: “**Installation of database backup feature completed! Press any key . . .**“! 	
9	After the import process, close the window by pressing any key.	

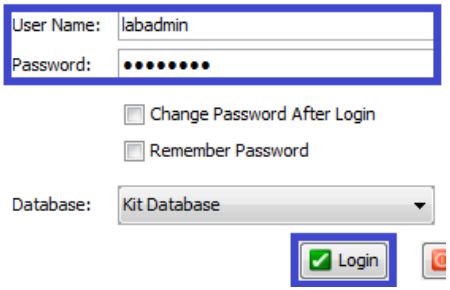
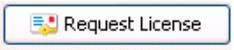
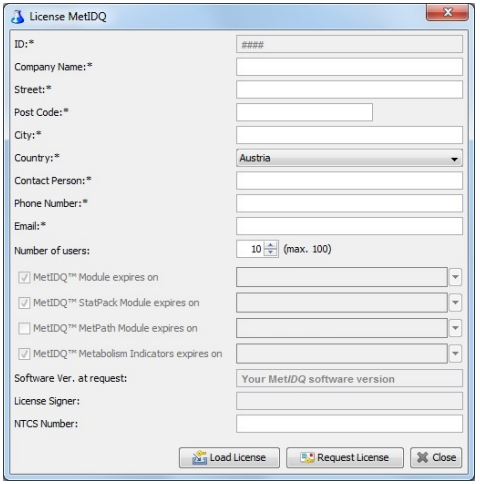
2.2 Start the Met*IDQ*™ Software

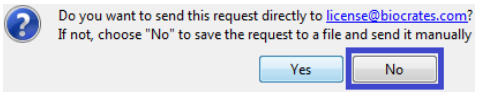
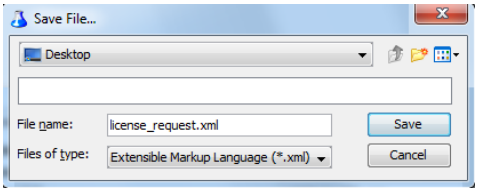
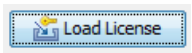
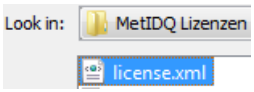
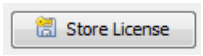
Step	*Instructions*	*Example*
1	Start Met*IDQ*™.	
2	Check the database connection: Click on the **connections** button  .	
3	Select the “Kit Database”. Click “**Edit**”  .	

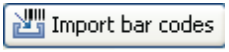
<table>
<tr><th>Step</th><th>Instructions</th><th>Example</th></tr>
<tr><td>4</td><td>
Kit Database connection settings are shown.
Oracle® installed on local PC (default)
• Host: localhost or computer name
• Service: xepdb1 for Oracle® 18c XE
• xe for Oracle® 11g XE
• Password: metkit
• Other settings, see on right.
Oracle® installed on server
• Con. Name: freely selectable
• Host: IP or DNS name of server;
localhost, for local installation
• Service: SID or service name
XE, for local installation
• User Name: default metkit
• Password: default metkit
• Port: default 1521
Support by local network administrator may be required.
- If Oracle® is installed on another PC or a server, define the IP address or name of the PC or server in the field “Host”.
- If Oracle® XE is not used, define the used Oracle® service ID in the field “Service”.
</td><td>


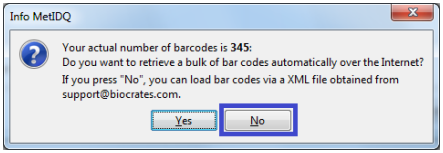
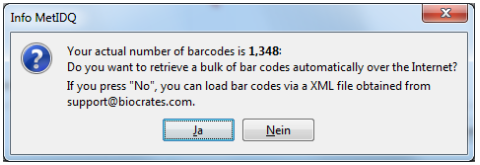
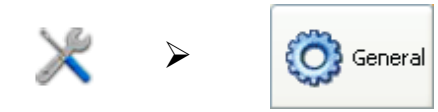
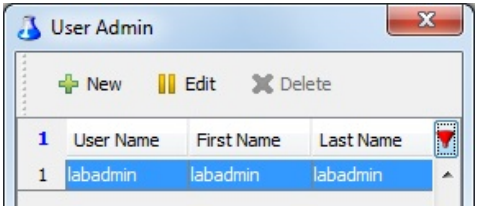


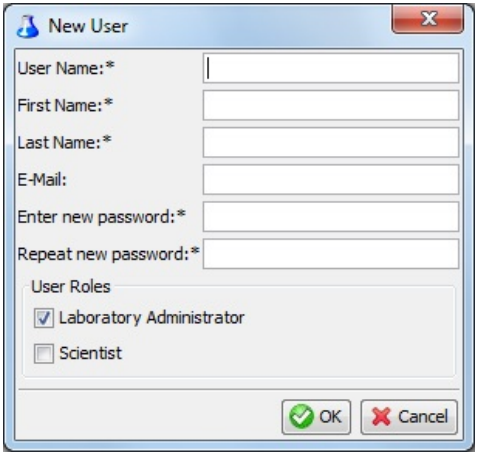
</td></tr>
</table>

Step	Instructions	Example
5	Connect with the *Kit Database*. Default log-in: Username: *labadmin* Password: *12345678*	
6	Request a Met*IDQ*™ License. The first time you start Met*IDQ*™, please request a license. Enter your contact information. Mandatory fields are marked with a "*****". Click 	

Step	Instructions	Example
7	A direct license request via internet may not be possible. Please send the license request file (.xml) by e-mail to us. Save the license request file (.xml) and send it to license@biocrates.com. Licenses are generated manually on working days in Austria (Central European Time). Please contact the Customer Support team for your personal licensing date.	 
8	Load a Met*IDQ*™ License. • After receiving a license, start Met*IDQ*™. • In the *License Window* click on "Load License". • Select the provided file "**license.xml**". • Store the license.	  

Step	Instructions	Example
9	Apply a patch. • Log-in to MetIDQ™ • Install a database patch, provided with the USB stick, folder “MetIDQ and Oracle\Patches”. • Install the appropriate patch for the kit and used MS instrument. This information are included in the patch file name: *MSmanufacturer_KIT_DB###_Patch_YYMMDD.jar* *Example:* Kit: p180 MS instrument: SCIEX Patch: SCIEX_p180_DB108_Patch_180517.jar	
	[MetIDQ: User Interface, Barcodes and Patches]	
10	For more details how to load database patches please refer to section 7.7.	
	Met*IDQ*™ uses barcodes, which are provided by Biocrates® free of charge. Barcodes are linked with samples and plates. The file “**barcodes.xml**” containing 3,000 barcodes is provided together with the license.	
11	Import barcodes. • Open the **Settings**. • Select tab **General**.	 
12	Click “Import barcodes”.	

Step	Instructions	Example
13	Click **No** in the dialogue box.	
14	Select the file "**barcodes.xml**" and click **Open**.	
!	If less than 300 barcodes are available, a warning message is shown. To request more barcodes, send an e-mail to: license@biocrates.com. i Barcodes may be available via internet, if not blocked by network security settings.	
i	To check the number of available barcodes, go to ***Settings > General***. The number of barcodes available is displayed, e.g. 346.	
15	A *Laboratory Administrator* may create new user profiles and assign user roles based on the Met*IDQ*™ tasks the user will perform. Click on the **User Admin** icon in the Met*IDQ*™ toolbar.	
16	To register a new user, click **New** in the **User Admin** window	

Step	Instructions	Example
17	To create a new user profile, fill in all fields in the **New User** window. Select one or more check boxes to define **User Roles** for the new user. The default User Role is Laboratory Administrator (recommended). Click **OK**. ***Note:*** To apply "User Roles" changes restart Met*IDQ*™.	

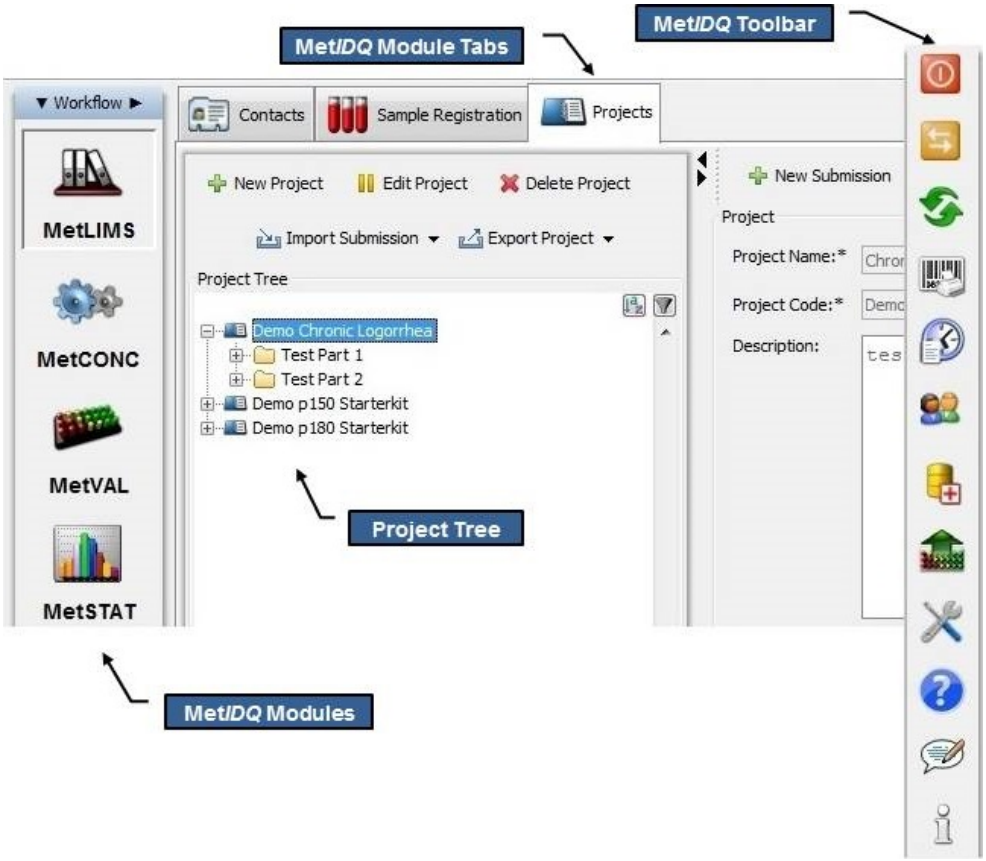
User Role	Tasks
Laboratory Administrator	Can perform all Met*IDQ*™ tasks.
Scientist	Restricted user rights.

3 Using Met*IDQ*™

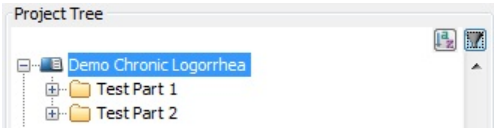
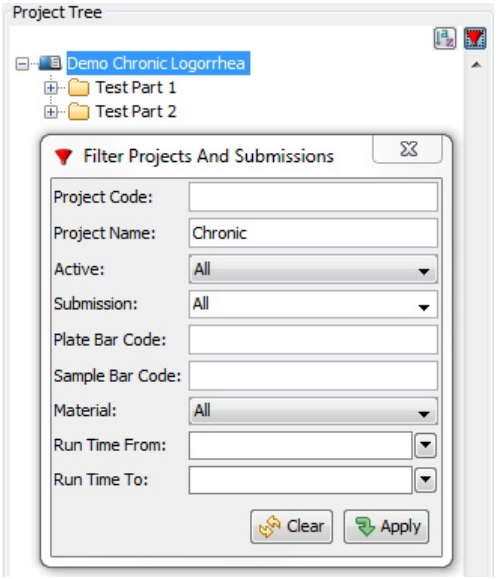
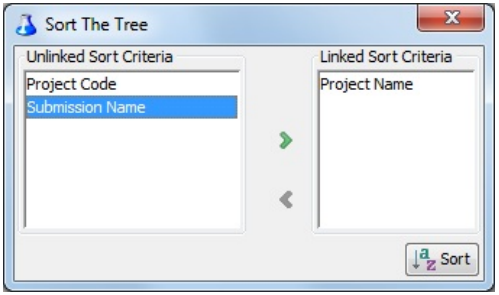
This chapter describes all essential and additional functions of Met*IDQ*™.

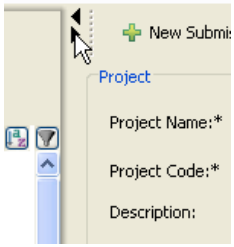
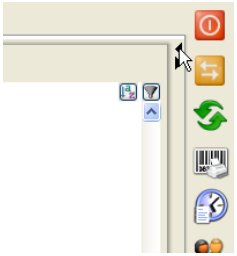
YouTube MetIDQ: User Interface, Barcodes and Patches

3.1 Met*IDQ*™ Screen Elements



3.2 General Software Features

Explanation	*Example*
Filter: If no filter is set, the Filter button is grey.  If a filter is set, the Filter button is red.  *Example*: To find a project click on the **Filter** button. You will see the **Filter Projects and Submissions** window. Define the filter criteria of your choice and click **Apply**. To clear all filter criteria, use the **Clear** button.	 
The **Sort The Tree** button sorts the project tree.  Choose a sort criterion in the **Unlinked Sort Criteria** list and move it to the **Linked Sort Criteria** list using the transfer bar. Apply with the **Sort** button.	

Explanation	*Example*
Enlarge or minimize panels by using the black arrows.   To minimize the right panel, click on the black arrow pointing right. To see the hidden panel again click on the arrow pointing left. Customize the size of the panels by drag and drop using the left mouse button. ↔	 
Help button: Link to the Met*IDQ™ YouTube* video tutorials.	

4 Processing the Kit

The Met*IDQ*™ software supports you during the entire Kit workflow. The table below describes the Met*IDQ*™ elements used in order to prepare and to run a Kit.

Element	*Short description*
MetLIMS	Laboratory Information Management System. This module is the starting point for the assay. In the **MetLIMS** module you create a project, register samples, and generate the plate layout and worklist for mass spectrometer processing.
OP	OPs (Operating Procedures) are loaded by database patches (see section 7.7) into Met*IDQ*™ and contain specific processing instructions for the assay. You must link one OP to the worklist (Kit plate) you are working with (see page 45).
Plate Report	A plate report is generated together with the worklist. It provides a graphical view of the plate layout and the location of each sample on the Kit plate. This helps you to pipette all samples correctly.
.csv/.txt file for MS data acquisition	***Analyst***®: **MetLIMS** generates a .csv file for each Kit plate. The .csv file must be loaded into Analyst® before starting the mass spectrometer run. ***MassLynx***™: **MetLIMS** generates a .txt file for each Kit plate. The .txt file must be loaded into MassLynx™ before starting the mass spectrometer run. ***Xcalibur***™: **MetLIMS** generates a .csv file for each Kit plate. The .csv file must be loaded into Xcalibur™ before starting the mass spectrometer run.

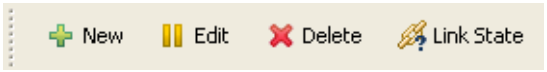
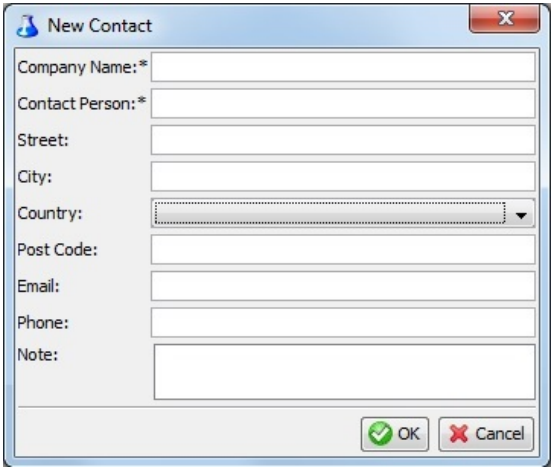
Detailed instructions for all Met*IDQ*™ steps start on the next page. Please follow these instructions in the order they are presented. It takes approximately 30 minutes to create a project, register the samples and generate the worklist in **MetLIMS**.

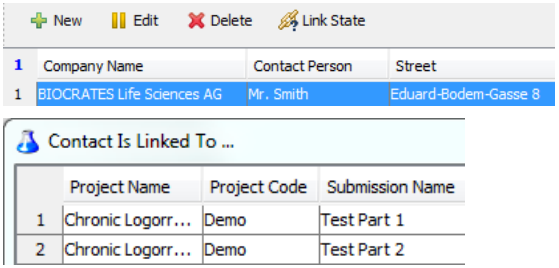
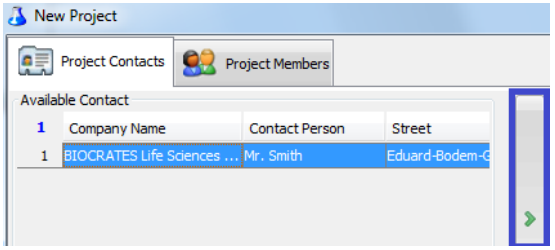
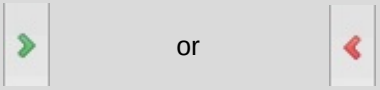
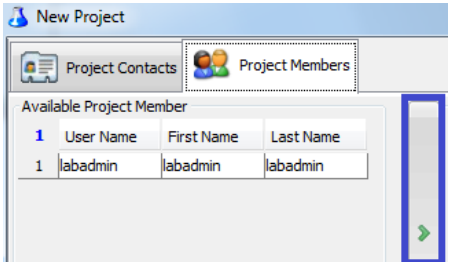
4.1 Registering the Assay in MetLIMS

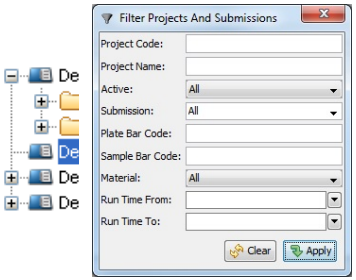
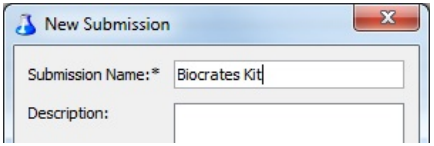
4.1.1 Registering a new Assay

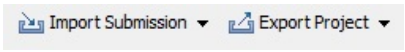
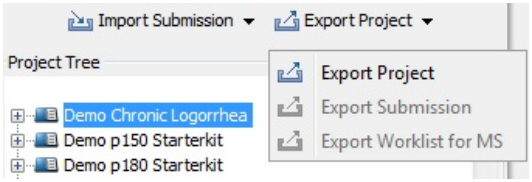
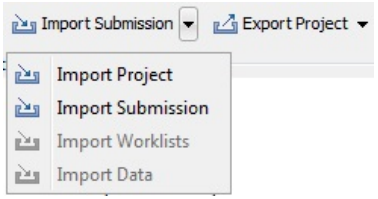
In this section you will create a new contact, project, and submission record for the assay. After this you can register and add samples to the assay.

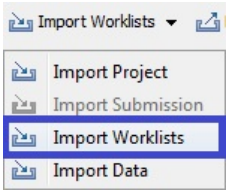
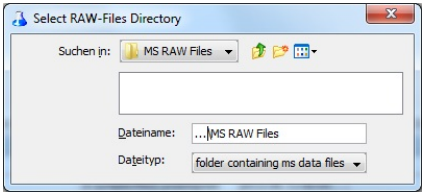
MetIDQ Project Start: Registration, Contacts and Users

Step	Instructions	Example
1	Create a new contact in the **Contacts** tab in the **MetLIMS** module. Each contact consist at least of a *Company Name* and *Contact Person*.	
2	Click **New** in the toolbar.	
3	The **New Contact** dialogue box will appear. It is up to you to keep the contact details simple or more detailed. Mandatory fields are labeled with an asterisk (*). Click **OK** when finished.	

Step	Instructions	Example
4	You will see your contact record. Use the **Edit** and **Delete** buttons above the listing to edit or delete the selected contact record. By clicking the **Link State** button, you can see all linked projects the contacts.	
5	Now you are ready to register a project. Choose the **Projects** tab. Click **New Project** in the toolbar.	
6	You will see the **New Project** record window. Choose the contact from the **Unlinked Contact** list. Move it to the **Linked Contact** list with the transfer bar.	
	The transfer bars (*add* or *remove*) are used in all Met*IDQ*™ data transfer windows. To link e.g. a contact, select it by clicking on it. Then use the green arrow to link it.	
7	Click on the **Project Members** tab to restrict access to the new project for selected Met*IDQ*™ users. By clicking the transfer bar, you can add users to the **Linked Project Members** list. ***Note:*** If no members are linked, everybody will have access to the project.	

Step	Instructions	Example
8	Give the project a unique code and name, e.g. *Demo Metabolomics*. A *Description* is optional.	
9	You will see the project listed in the **MetLIMS** project tree. With the **Filter** button you can filter your projects and submissions.	
10	Create a new submission for the project. A project may have one or several submissions. Click on the project name in the project tree, then click **New Submission**.	
	It is generally most convenient to use an individual submission for every prepared Kit.	
11	Usually all samples analyzed by one Kit are part of one submission. You will see the **New Submission** registry window. Enter a name for the submission, e.g. Biocrates Kit.	
12	You will see the project and the submission in the **MetLIMS** project tree. Now you can register your samples and add them to the submission (Kit plate). Continue with section 4.1.2 / 4.1.3.	

Step	Instructions	Example
	Backup and data transfer: You can export and import projects or submissions with the buttons shown on the right. To export data, select the project you want to export and click **Export Project**. If you want to export a submission, choose **Export Submission** by clicking on the black arrow. The **Export data window** will open and you have to name your export file. Files will be saved as **Met*IDQ*™ Export files** (.metidq). To import data, click **Import Project** / **Import Submission**. Then choose the .metidq file in your directory you want to import to Met*IDQ*™.	  
	There is no difference in the file extension (.metidq) irrespective of the exported type, *project* or *submission*! A submission can only be imported as submission and a project only as project. .metidq files are database version dependent.	

Step	Instructions	Example
	Import Worklist: In case you deleted an already measured project or submission by accident, you can regenerate the plate layout (Met*IDQ*™ worklist) based on the MS raw files of the measured Kit plate. 1. Select an existing Submission or create a new one 2. Select Import Worklist 3. In the dialog box select the folder containing Kit MS RAW files (e.g. all MS files of an p180 Kit measurement) 4. Select Open **>>>** A worklist is generated	1.  2.  3. 

Example: Met*IDQ*™ can import MS raw date (see section 5 Quantitation) only if the corresponding plate was registered. If a kit plate to corresponding MS data is not registered, e.g. if kit data was received from another user, use the *Import Worklist* feature to generate the worklist in Met*IDQ*™. Then import the corresponding MS data via MetCONC.

Note: When using the *Import Worklist* feature, samples that are not available in Met*IDQ*™ are registered. These newly registered samples, contain only this information:

- sample bar code, well position, injection number

Other information, e.g. “sample identification”, can be imported via *MetLIMS > Sample Registration* using the function “Import”, see 4.1.3.1 *Import Sample Information*.

MetIDQ Project Start: Sample Registration, Groups, Variables

4.1.2 Define Groups and Variables (Optional)

You can define further information about your samples in **MetLIMS**. For example, clinical information like disease state or numerical information (e.g. patient age or treatment details) can be defined as categories, groups and variables in **MetLIMS**. Later, these elements will be displayed together with the assay results. Also, the Met*IDQ*™ StatPack module uses these categories and groups for statistical data analysis.

In order to define groups or variables, follow the instructions in section 7.3. Define Groups and Variables in MetLIMS. When finished, return to section 4.1.3 Sample Registration in MetLIMS.

4.1.3 Sample Registration in MetLIMS

In this section you will register the samples for the Kit assay. Met*IDQ*™ can only calculate the metabolite concentrations of samples that are registered in **MetLIMS.** There are two ways to register new samples:

- If you have a small set of samples, you can register each sample individually. Follow the instructions in section 4.1.3.2 Register Single Samples.
- The best way to import many samples is by a .txt or .csv file import. Follow the instructions in section 4.1.3.1 Import Sample Information.

Pool or user's QC sample:

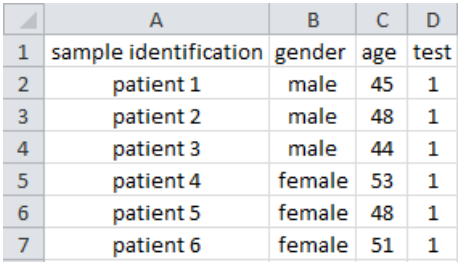
Register every sample only once. Every sample can be run in replicates on one kit plate and on different kit plates.

Example:

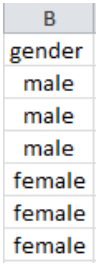
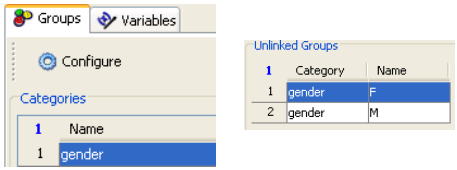
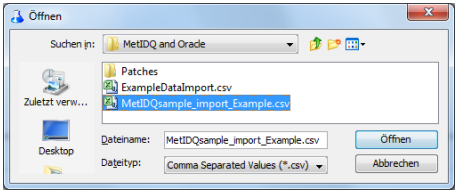
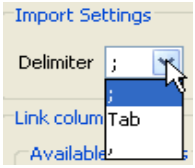
Replicates of a pooled plasma sample should be analyzed using several kit plates. Aliquots of the pooled plasma sample are available. Register this pooled plasma only once, as each aliquot consists of the sample.

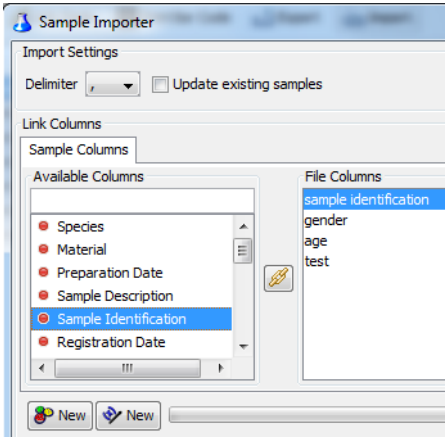
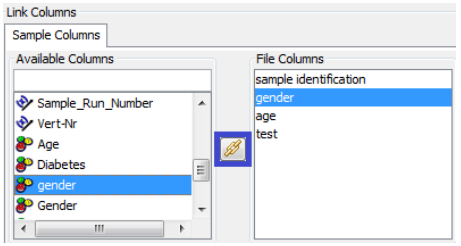
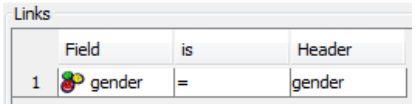
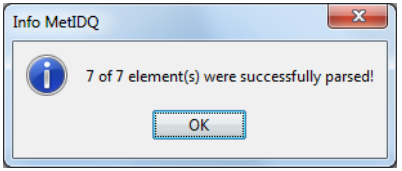
4.1.3.1 Import Sample Information

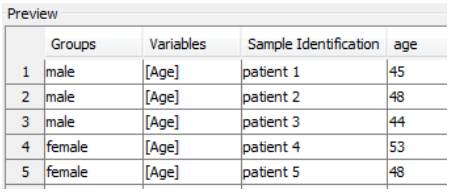
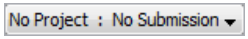
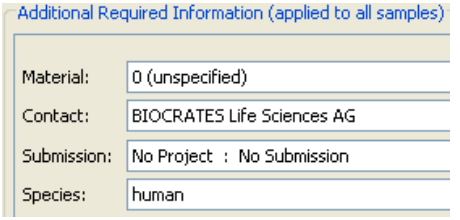
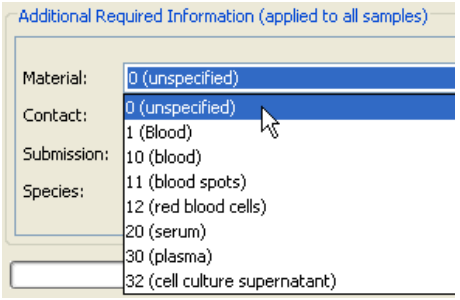
By following the instructions below, you can import the sample information of all your samples in one step. During the import process you can also link pre-defined groups and variables to the samples.

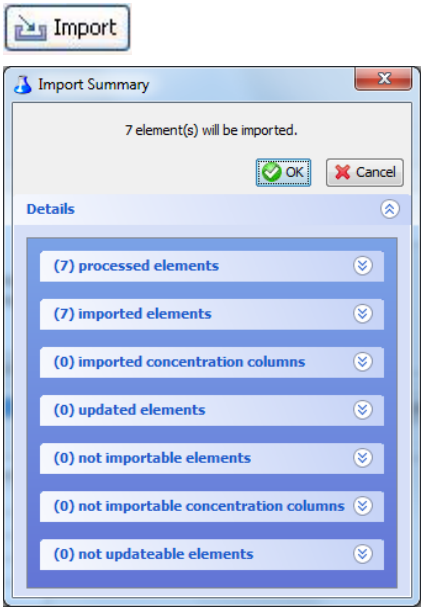
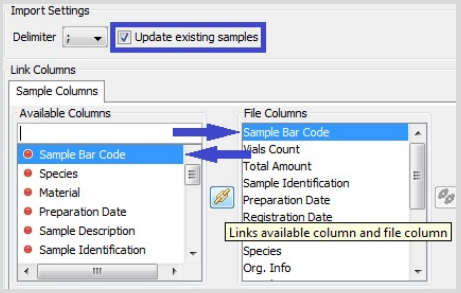
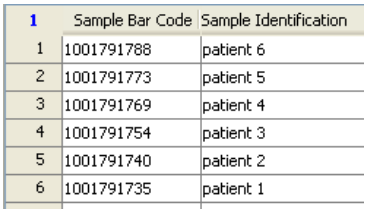
Step	***Instructions***	***Example***
1	Click on **MetLIMS**. Then click on the **Sample Registration** tab.	
2	Find the example file *MetIDQsample_import_Example* (as .txt and .csv file) on the Kit USB stick and open it with Excel™ or a similar program. Use this as template for your sample information import file. You can import columns like *sample identification* and columns containing information on groups and variables. The worksheet of the template (screenshot on the right) contains the columns • sample identification • *gender* containing the groups *male* and *female* • age ***Note:*** If using a Microsoft™ Excel™ worksheet, add a column *test* after the last column you want to import. Put any entry in each row of the column *test*, e.g. *1*.	

	A	B	C	D
1	sample identification	gender	age	test
2	patient 1	male	45	1
3	patient 2	male	48	1
4	patient 3	male	44	1
5	patient 4	female	53	1
6	patient 5	female	48	1
7	patient 6	female	51	1

Step	Instructions	Example
3	If you want to import information on groups, have the category's name in the header, e.g. *gedner*. Type the exact name of each group into the column, e.g. *male* and *female*. ***Note:*** The fields are case sensitive so the group names must match exactly between the worksheet and Met*IDQ*™. Save the worksheet as a .txt or .csv file. Supported delimiter: semicolon "**;**", tab and comma "**,**"	
4	Each category and corresponding groups, as well as each kind of variable must be defined in Met*IDQ*™ before you import your sample information. See MetIDQ section 7.3 Define Groups and Variables in MetLIMS.	
5	Click **Import** to start the sample information import.	
6	Choose the .csv or .txt file you want to import.	
7	Choose the delimiter used in your spread sheet (.txt or .csv) from the **Import Settings** drop down menu. You can use any delimiter listed: semicolon "**;**", tab or comma "**,**"	

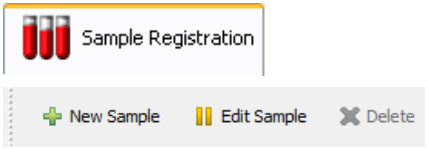
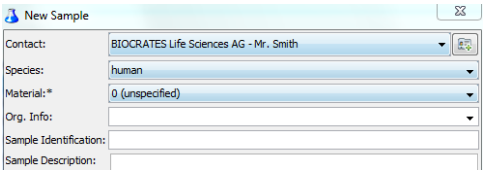
Step	Instructions	Example
8	Met*IDQ*™ will open the file in the **Sample Importer** window. Available columns are listed in the field **File Columns**.	
9	Link the data from your spread sheet with the available columns in Met*IDQ*™. To do this click on one entry in the **Available Columns** box and on the corresponding entry in the **File Columns** box. Then click on the yellow **link** button located between the two boxes.	
10	Linked entries appear in the **Links** table.	
11	Click the **Preview** button.	
12	This information box will appear when the data has been prepared for import.	

Step	Instructions	Example
13	The **Preview** table shows you the layout of the incoming data. In this example, cohorts are separated into two gender columns.	
14	Now you can add other sample specific information. Choose them in the **Additional Required Information** box. In addition, you can link the samples to a pre-defined contact and submission.  You can also choose the sample material from the drop-down menu. ***Note:*** Information such as material and species will be linked to all imported samples. In case you are analyzing samples of different material or species on one Kit plate, we recommend importing separate sample lists.	 

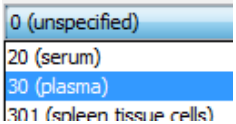
Step	Instructions	Example
15	Click **Import**. After the import, details are available by clicking on the arrows. Click **OK** to close the **Import Summary** window.	
	Existing samples in the Met*IDQ*™ database can be updated by the procedure described in steps 1 - 15. Activate the option **Update existing samples** (blue frame). For this it is necessary to link a column containing the Met*IDQ*™ Sample Bar Codes. In addition, link all columns containing information for the update process.	
16	You will see the samples listed in the **Samples** table. The samples are now registered and can be used to create a worklist.	

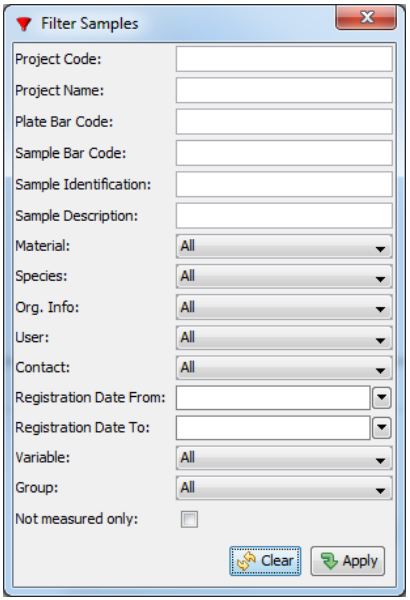
4.1.3.2 Register Single Samples

If you have a small number of samples, you can register each sample individually. To do this, follow the instructions in this section.

Step	Instructions	Example
1	Choose the Sample Registration tab. Click **New Sample** in the toolbar.	
2	The sample registry window **New Sample** will appear. Enter information at least in each field with an asterisk (*). The table below gives information needed for each field. ***Note:*** For the fields *Org. Info* and *Sample Identification* you cannot use the following special characters: / \ ? * : | " < >	

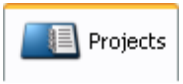
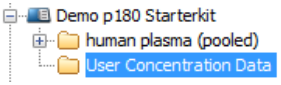
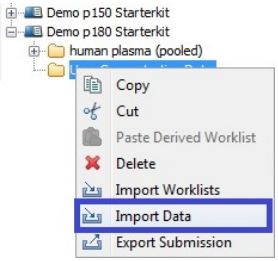
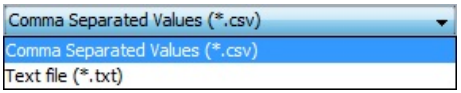
Field	Instruction
Contact	Select a contact, which was registered previously, from the drop-down menu.
Species	Choose from the drop-down menu.

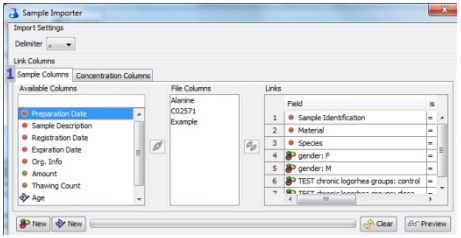
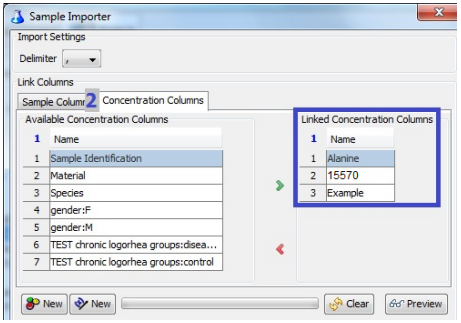
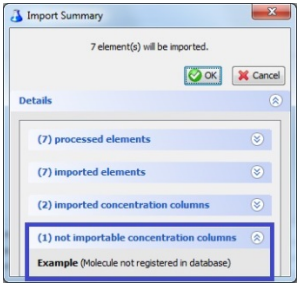
3	Choose a **Material** from the drop-down menu.	
	Field	***Instruction***
	Org. Info	Enter further sample information. This field is optional.
	Sample Identification	Enter internal lab information for each sample. This can be any sample information used to track the samples. Only this information will be shown in the plate report, worklist and assay results table.
	Sample Description	In this field you can add any information.
Step	***Instructions***	***Example***
4	If you are using the *Zebra TLP 2824 Plus* barcode printer you can print barcodes. Define the number of printed barcodes. The default number is 0.	
5	Click **OK** when finished. New sample are shown in the **Samples** list. To add more samples, click **New Sample** again.	

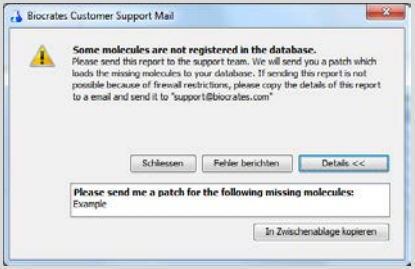
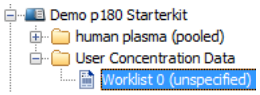
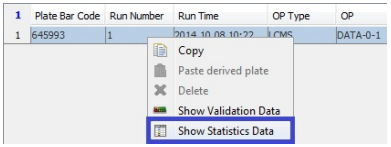
Step	Instructions	Example
6	To search for samples, use the **Filter** – button in the upper right corner	 
	After registering all samples for the assay, create a project worklist and a plate report. See **MetLIMS** section 4.1.4 *Generate Plate Layout and Worklist for MS run* (page 45).	

4.1.3.3 Import Samples containing “homebrew” concentration values

Any concentration data that were not obtained by Kit measurements can be imported together with sample information and pre-defined groups and variables.

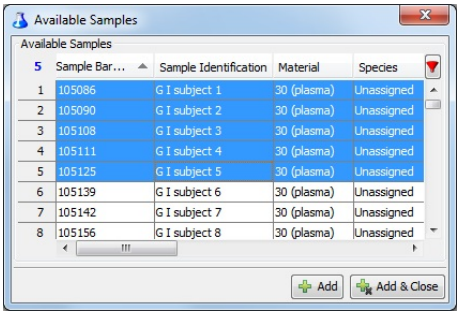
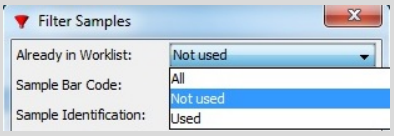
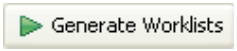
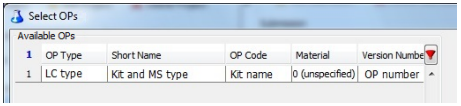
Step	*Instructions*	*Example*
1	Choose the **Projects** tab.	
2	Register a new Project and Submission or a new Submission in an existing project, e.g. “User Concentration Data” (see 4.1.1 Registering a new Assay).	
3	Right-click on a submission (e.g. “User Concentration Data”) and select **Import Data**.	
4	Select the file (*.csv or *.txt) containing sample and concentration information. Please find an example file “ExampleDataImport.csv” on the USB memory stick in the folder “MetIDQ and Oracle”.	

Step	Instructions	Example
5	The same import dialog opens which is described in section 4.1.3.1 Import Sample Information. There are two columns: • **Sample Columns (1)**: Link all columns containing sample information. • **Concentration Columns (2)**: Link all columns containing the compound names (e.g. Alanine, Example) or compound bio IDs (e.g. 15570 from a database like ChEBI). Each compound name must be defined as header name in the .csv or .txt import file (see “ExampleDataImport.csv”).	 
i	All concentration values are automatically imported with the unit µM.	
6	**Select Preview** and **Import** according to section 4.1.3.1. Analytes which are not part of the Met*IDQ*™ database are listed in the **Import Summary** in the section “not importable concentration columns”.	

Step	Instructions	Example
	When importing analytes which are not part of the database you will be asked to submit them to the BIOCRATES Support Team. We will generate a Met*IDQ*™ patch for you containing all needed analytes. You can directly request the patch by submitting this "error" to us or by copying the "error" message and sending it to support@biocrates.com.	
7	User specific data are now loaded to a newly generated worklist (OP: DATA-0-1) which belongs to the selected submission. Every sample is linked to an individual well position.	
8	To show and analyze the data, right click on the plate and select "Show Statistics Data". All data from the selected Kit plate will be shown in **MetSTAT > Display Data** (see page 117).	

4.1.4 Generate Plate Layout and Worklist for MS run

MetIDQ Project Start: Worklist Generation

Step	Instructions	Example
1	Open a project in the Project Tree. Choose the submission (created in section 4.1.1). Click on the transfer bar (**Add**) and choose the samples you want to link to the submission. Use the filter button to find the samples of your choice. Select the samples you want to add and click **Add** or **Add & Close**. The samples are added to the submission. If you have linked the samples to a submission during sample import (section 4.1.3.3), the samples will already appear in the **Linked Samples** window.	 
	To distinguish between samples that are already linked with a worklist and those that are not, use the filter option “Already in Worklist”.	
2	Click Generate Worklists.	
3	You will see the **Select OPs** window first. Please refer to the lists below to select the appropriate OP for your Kit and MS instrument.	

AbsoluteIDQ® p180 Kit

Manufacturer	**SCIEX**				
Instrument	**4000** series	**4500** series		**5500** series	
LC variant	HPLC	HPLC	UHPLC	HPLC	UHPLC
LC OP	**KIT2-0-5404**	**KIT2-0-5404**	**KIT2-0-5414**	**KIT2-0-5504**	**KIT2-0-5514**
FIA OP	**KIT3-0-5404**	**KIT3-0-5404**	**KIT3-0-5404**	**KIT3-0-5504**	**KIT3-0-5504**

Manufacturer	**SCIEX**		**SCIEX**	
Instrument	**6500** series		**6500+** series	
LC variant	HPLC	UHPLC	HPLC	UHPLC
LC OP	**KIT2-0-5604**	**KIT2-0-5614**	**KIT2-0-5704**	**KIT2-0-5714**
FIA OP	**KIT3-0-5604**	**KIT3-0-5604**	**KIT3-0-5714**	**KIT3-0-5714**

Manufacturer	**Waters Xevo**			**Thermo Fisher**	
Instrument	**TQ-S**	**TQ-S micro**	**TQ-XS**	**TSQ Vantage™**	
LC variant	UPLC	UPLC	UPLC	HPLC	UHPLC
LC OP	**KIT2-0-8115**	**KIT2-0-8215**	**KIT2-0-8315**	**KIT2-0-9004**	**KIT2-0-9014**
FIA OP	**KIT3-0-8115**	**KIT3-0-8215**	**KIT3-0-8315**	**KIT3-0-9004**	**KIT3-0-9004**

AbsoluteIDQ® p180 Kit

Manufacturer	**Agilent**
Instrument	**6470**
LC variant	UHPLC
LC OP	**KIT2-0-7111**
FIA OP	**KIT3-0-7101**

AbsoluteIDQ® p150 Kit

Manufacturer	**SCIEX**		**Waters Xevo**		**Thermo Fisher**
Instrument	**4000** series	**5500** series	**TQ MS**	**TQ-S**	**TSQ Vantage™**
LC variant	FIA	FIA	FIA	FIA	FIA
OP	**KIT1-0-5**	**KIT1-0-5505**	**KIT1-0-8006**	**KIT1-0-8106**	**KIT1-0-9005**

MxP® Quant 500 Kit

Manufacturer	**SCIEX**		**SCIEX**	
Instrument	**5500** series		**6500** series	
LC variant	UHPLC	HPLC	UHPLC	HPLC
LC OP	**MxP500L-0-5511**	**MxP500L-0-5501**	**MxP500L-0-5611**	**MxP500L-0-5601**
FIA OP	**MxP500F-0-5501**	**MxP500F-0-5501**	**MxP500F-0-5601**	**MxP500F-0-5601**

	SCIEX		**Waters**
	6500+ series		**Xevo® TQ-S**
	UHPLC	HPLC	UHPLC
	MxP500L-0-5711	**MxP500L-0-5701**	**MxP500L-0-8111**
	MxP500F-0-5701	**MxP500F-0-5701**	**MxP500F-0-8101**

AbsoluteIDQ® Stero17 Kit

Manufacturer	**SCIEX**		**Waters Xevo**	
Instrument	**5500** series		**TQ-S**	**TQ-S micro**
LC variant	HPLC	UHPLC	UPLC	UPLC
OP	**ST17-0-5502**	**ST17-0-5512**	**ST17-0-8112**	**ST17-0-8212**

AbsoluteIDQ® p400 HR Kit

Manufacturer	**Thermo Fisher**			
Instrument	**Q Exactive™ Focus**	**Q Exactive™**	**Q Exactive™ Plus**	**Q Exactive™ HF**
LC variant	UHPLC	UHPLC	UHPLC	UHPLC
LC OPs	**KIT2-0-1011** **KIT2-0-1012**	**KIT2-0-1111** **KIT2-0-1112**	**KIT2-0-1211** **KIT2-0-1212**	**KIT2-0-1311** **KIT2-0-1312**
FIA OPs	**KIT3-0-1011** **KIT3-0-1012**	**KIT3-0-1111** **KIT3-0-1112**	**KIT3-0-1211** **KIT3-0-1212**	**KIT3-0-1311** **KIT3-0-1312**

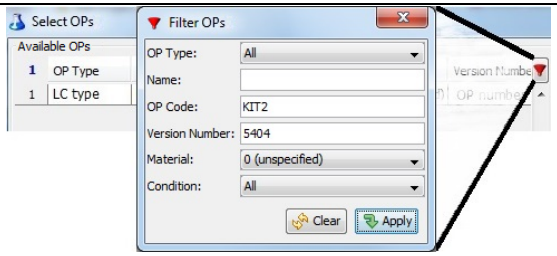
Biocrates® Bile Acids Kit

Manufacturer	**SCIEX**			
Instrument	**4000** series	**5500** series		**6500** series
LC variant	HPLC	HPLC	UHPLC	UHPLC
OP	**BA02-0-5402**	**BA02-0-5502**	**BA02-0-5512**	**BA02-0-5612**

Manufacturer	**Waters Xevo**		**Thermo Fisher**
Instrument	**TQ-S**	**TQ-S micro**	**TSQ Vantage™**
LC variant	UPLC	UPLC	UHPLC
OP	**BA02-0-8112**	**BA02-0-8212**	**BA02-0-9012**

The use of the filter in **Select OPs** is explained by the exemplary OP KIT2-0-5404:

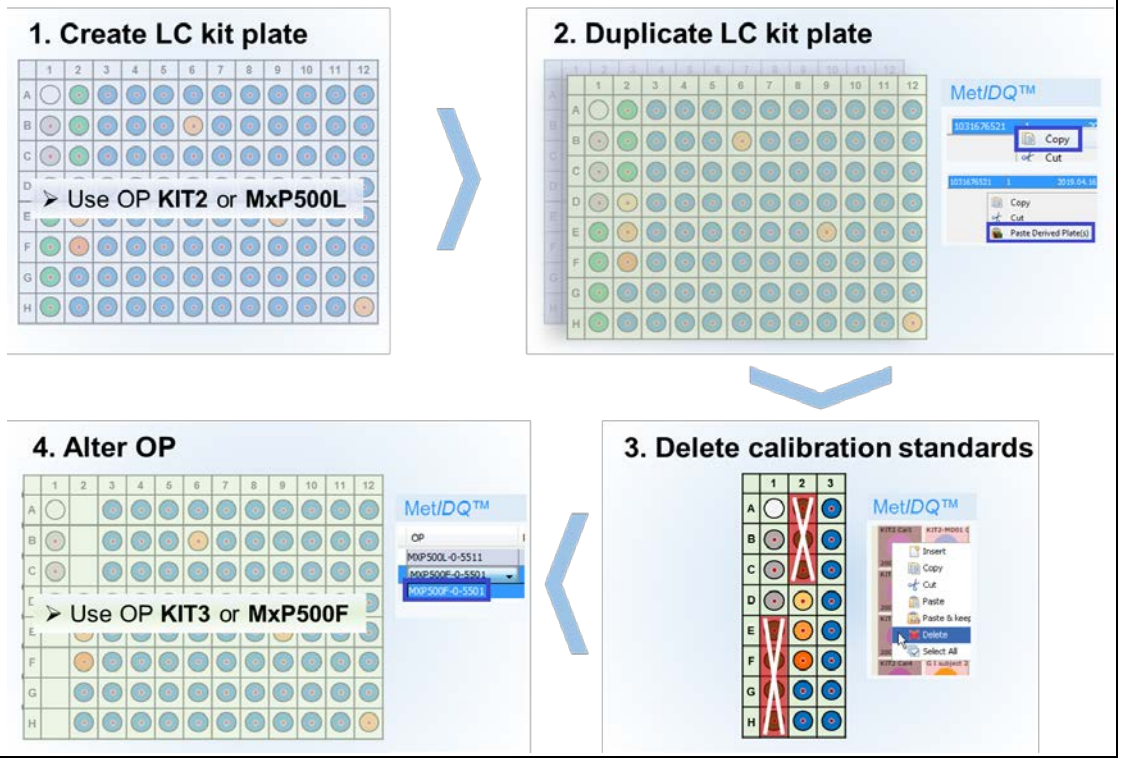
KIT2 – 0 – 5404
OP Code – Material – Version Number

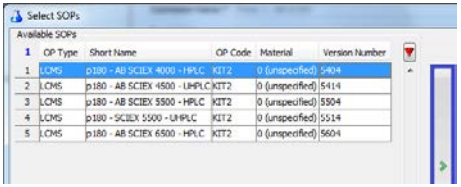
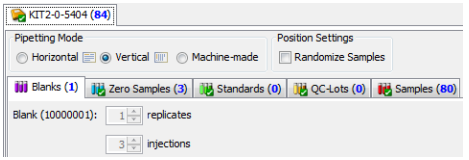
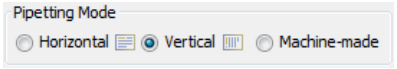


p180, p400 HR, and MxP® 500 Kits:

LC plate: Register the plate layout with zero, blank, calibration standards, QCs, and samples, using the appropriate KIT2 OP (KIT2-0-xxxx) or MxP500L OP (MxP500L-0-xxxx).

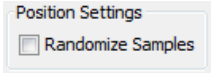
FIA plate: Duplicate LC plate, delete all calibration standards and alter the OP, see page 59.

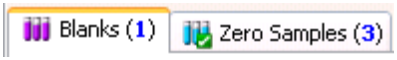


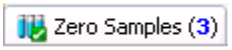
Step	Instructions	Example
4	Transfer the selected OP to the **Linked OPs** list with the transfer bar, e.g. KIT2-0-5404.	
5	Click **Next**.	
6	You will see the second worklist wizard. Here, you can configure the plate layout.	
7	The table overleaf explains the three options.	

Choice	Explanation
Horizontal	The plate will be pipetted horizontally.
Vertical	The plate will be pipetted vertically, default setting.
Machine-made (optional)	Use with Hamilton® Robotics system.

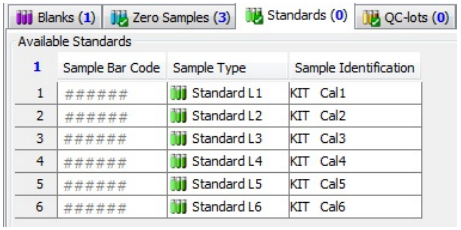
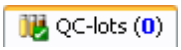
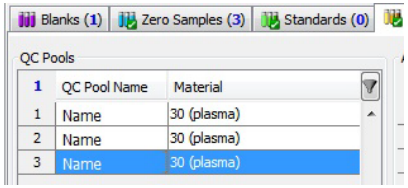
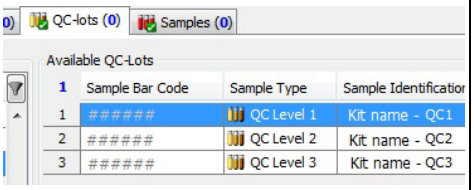
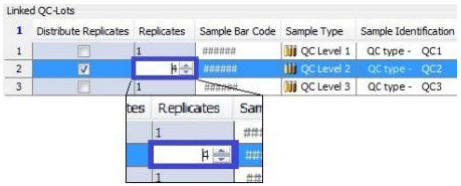
8	If you want to randomize the sample sequence click **Randomize Samples**. Blank, zero sample, standard and QC well positions will not be randomized.



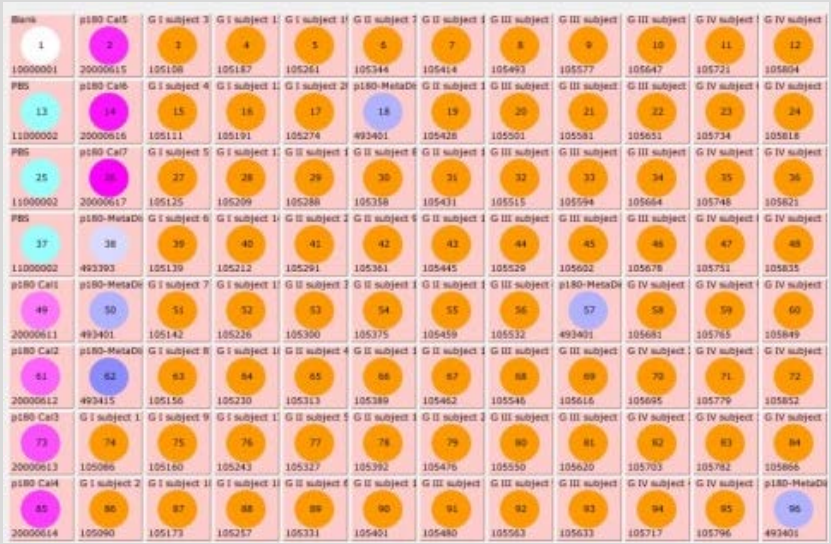
Step	Instructions	Example
9	Look at the first two tabs. The number in brackets corresponds to the number of samples that are linked to the Kit plate. See the table below for more information on the tabs.	

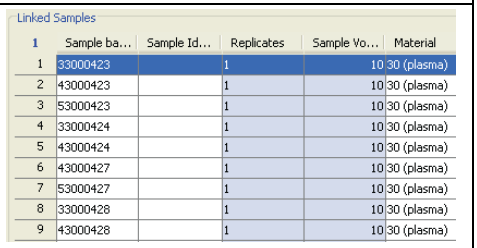
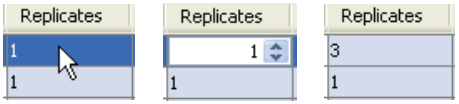
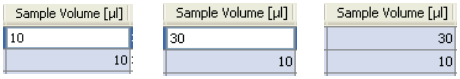
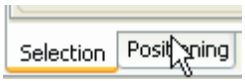
Tab	Choice	Explanation
	This is pre-configured and cannot be changed.	Well A1 on the Kit plate does not have internal standards. It is called *Blank*. This blank well is used to determine the background counts and to check your system for contaminations.
	For plasma samples, PBS is recommended as zero sample (linked by default).	Internal standards but no analytes added. Used to calculate the limit of detection (LOD) for each analyte. Three replicates are recommended for the p150, p180, and p400 HR, and MxP® 500 Kit. Bile Acids and Stero17 Kit: Change replicates from 3 to 1.

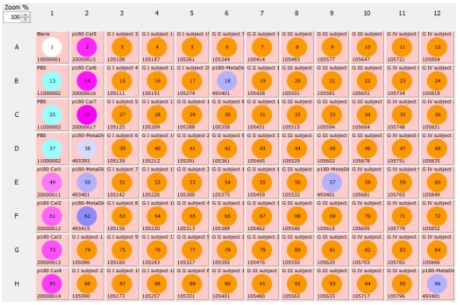
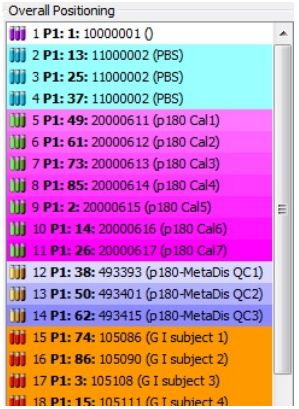
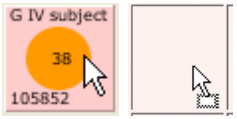
Step	Instructions	Example
10	Calibration standards and QCs are already registered in **MetLIMS**.	
11	Click on the **Standards** tab.	
	Standards are used for LC-MS/MS analytics only. No Standards will be linked in a FIA-MS/MS assay.	

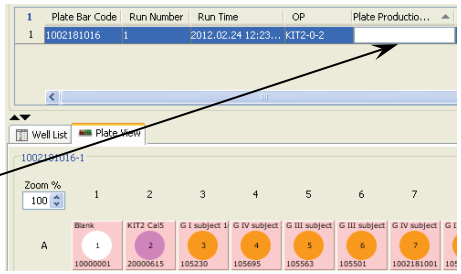
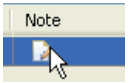
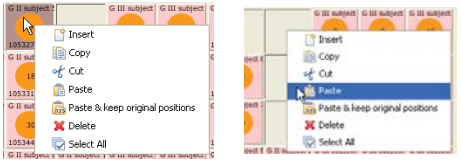
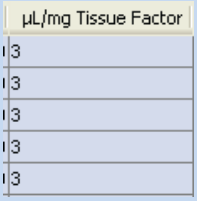
Step	Instructions	Example
12	Select the set of calibration standards of your Kit in the **Unlinked Standards** list and then use the transfer bar to move the standards to the **Linked Standards** list. Make sure that the sample barcodes of the selected standards match the barcodes on the standard vials included in the Kit!	
13	Click on the **QC lots** tab.	
14	Look at the **QC Pools** list and select the QC Pool displayed on the QC vials included in the Kit. You will see the QC lots in the **Unlinked QC-Lots** listing (as shown in the example on the right). Use the transfer bar to move all three QC lots to the **Linked QC-Lots** list. Make sure that the sample barcodes of the selected QCs match the barcodes on the QC vials!	 
15	Now QCs are linked to the submission. Distribute Replicates Recommendation: run a QC2 replicate after every 20th sample. Define the number of replicates accordingly. Activate the checkbox “Distribute Replicates”, resulting in a equal distribution over the plate (see also 7.1 Data Normalization). The last sample on the plate is a QC replicate.	

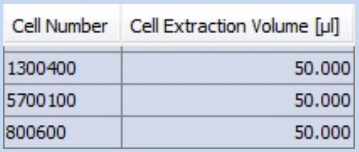
Step	Instructions	Example
	Example: Distribute QC replicates equally over a Kit plate By activating the function “Distribute Replicates” the specified QC level (here QC2) is distributed equally over all linked samples on one Kit plate. If you would like to distribute 4 QC replicates over one Kit plate, please calculate the number of available positions for your samples on the Kit plate (79 on the plate below). When linking more than 79 samples, Met***IDQ*™** will generate an additionally Kit plate automatically. Please keep in mind: • The number of QC replicates does not automatically increase when more than one Kit plate is generated. • The last well position is always a QC replicate, if “Distribute Replicates” is activated.	

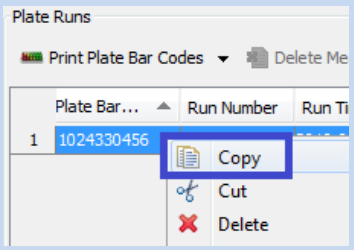
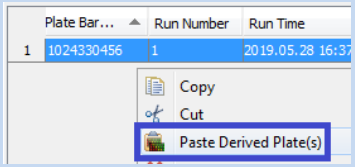
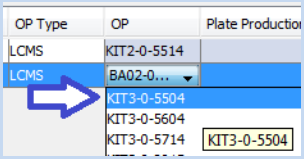
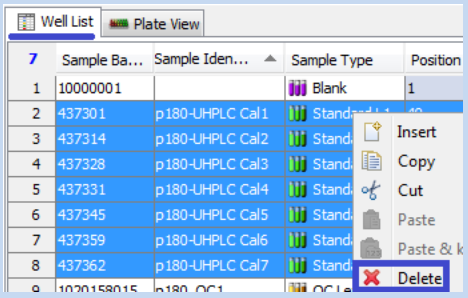


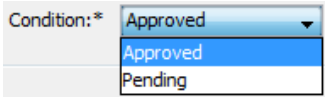
Step	Instructions	Example
16	To finish the plate layout, choose the **Samples** tab. The number in brackets corresponds to the number of samples that are linked to the Kit plate. Here it is 26.	
17	All samples linked with a submission are shown. Further samples can be added. Injection replicates per sample can be defined. Choose the sample from the **Linked Samples** list. Double click on the *Replicates* column. Use the counter to change the number of replicates. Click outside of the field to save your changes. Here, three replicates of one sample are chosen. In order to change the number of replicates for more than one sample, select the samples by holding the Shift button pressed (press Ctrl + A to select all), right mouse click in the header of the corresponding column and enter the value	 
i	The Sample Volume corresponds to the volume you pipette onto the Kit plate. This is pre-defined by the selected OP and normally 10 µL. Based on this volume, the final metabolite concentrations will be calculated by Met*IDQ*™. Before you change the pre-defined value, please contact our Customer Support.	
18	If you want to see the plate layout before you generate the final worklist, click on the **Positioning** tab at the bottom of the window.	

Step	Instructions	Example
19	The plate layout will be displayed based on your specifications. Specific colors are used for each sample type. An example plate layout as overall positioning list is shown on the right. You can move samples from one well to another using the standard drag-and-drop technique. The tab below illustrates this procedure.	 
	To move a sample on the plate: 1. Move the mouse pointer to the sample well you want to move. 2. Hold down the left mouse button. 3. Drag the sample to a new position on the plate. You will see the mouse pointer over the shape. 4. Click **Yes** to make the change.	 
20	When you are satisfied with the plate layout, click **Generate Worklist**.	

Step	Instructions	Example
21	When the worklist generation is finished, the window shows two panels. The upper panel shows the **Plate Runs**. In the panel below you can choose between the **Well List** tab and the **Plate View** tab, which shows the plate layout. Each plate has a plate production number attached (for example, P08265). Enter this number in the *Plate Production No.* field.	
i	You can also enter a note. Double click onto the **Note** symbol, type in your note and click **OK**.	
i	The plate layout can be changed, e.g. do copy-past of a well in the **Plate View**. To move or copy a sample on the plate: 1. Select a well with right mouse click. 2. Choose **Copy** or **Cut**. 3. Move the mouse to an empty well on the plate. 4. Right click and choose **Paste**. 5. The sample will be moved to the new position.	
22	<u>Usage of the Tissue Factor Tool:</u> If you are measuring tissue samples, type in your µL/mg Tissue Factor into the Well List. To update multiple samples, select the tissue samples while holding the Shift button (press Ctrl + A to select all), right-click in the header of the Tissue Factor column and enter the µL/mg factor that you used for tissue homogenization.	
	i For further information see section 7.10 Usage of the Tissue Factor Tool.	

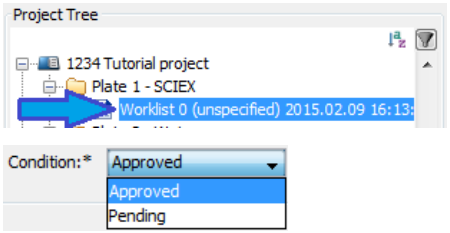
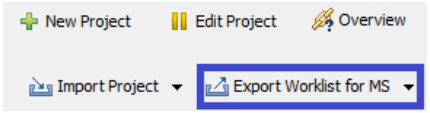
<table>
<tr><th>Step</th><th>Instructions</th><th>Example</th></tr>
<tr><td>23</td><td><u>Usage of the Cell Normalization Tool:</u>
If you are measuring cell extracts, type in your number of cells and the volume of extraction solvent [µL] in the Well List.
To enter the same number of cells or amount of extraction volume for more than one sample at the same time, select the samples while holding the Shift button (press Ctrl + A to select all), right mouse click in the header of the corresponding column and enter the value.
For further information see section 7.11 Usage of the Cell Normalization Tool.</td><td> 
</td></tr>
<tr><td>24</td><td><u>MxP® 500, p180 and p400 HR Kits:</u> now the worklist for the LC part was created. Copy the worklist to obtain the FIA worklist as described in the next step.</td><td></td></tr>
</table>

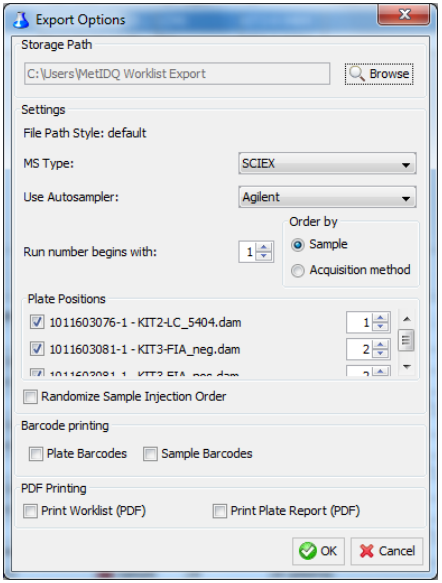
Step	Instructions	Example
25	**MxP® 500, p180, and p400 HR Kits only** – How to copy a plate	
	Use the worklist for the LC part as template. To generate the FIA worklist, right click anywhere on the LC worklist and choose copy.	
	Right click somewhere below the LC plate and select **Paste derived plate**.	
	- A copy of the LC worklist was created. - Use this copy to create the FIA worklist. - Change the OP: double click into the field OP and select the appropriate OP of the FIA part. ☞ Refer to section 4.1.4, page 45	
	FIA part: calibration standards are not used and can be deleted from the FIA plate layout. Select all calibration standards, right click and choose **Delete**.	
⚠	If you want to delete a single worklist, select the respective plate, do right click, and choose **Delete**. Choosing **Delete Worklist** will remove all plates of the currently selected worklist.	

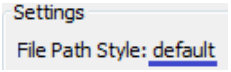
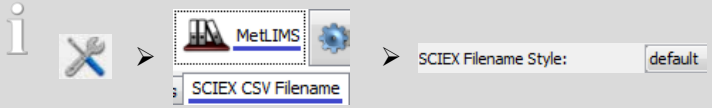
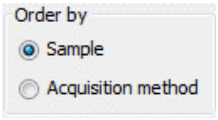
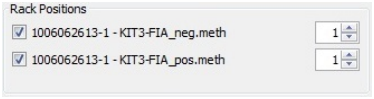
Step	*Instructions*	*Example*
26	When everything is completed, change the "Condition" from *Pending* to *Approved*.	
	After the condition has been set to *Approved*, modifications in the Plate View tab are no longer possible. To revise the plates, set the condition back to *Pending*.	
	If you want to analyze a large number of samples, watch the following video (in addition to the video *MetIDQ™ Project Start: Sample Registration, Groups, Variables*).	
	MetLIMS and Complex Projects, Bar Code Printing, Search, Filter, Import and Export	

4.1.5 Export Worklist for Kit measurement

MetIDQ Project Start: Worklist Generation

Step	Instructions	Example
1	To export a worklist for Kit measurements, the MetIDQ™ worklist(s) "Condition" must be set to *Approved*.	
2	Choose **Export Worklist for MS**, from the toolbar above the *Project Tree*.	

Step	Instructions	Example
3	You will see the **Export Options** dialogue box. Choose a folder for the *Worklist Export*. A detailed description is provided below.	

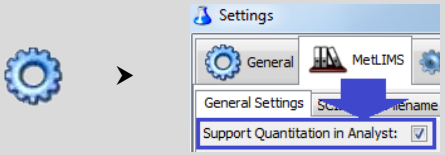
Item	Definition
	Selected style of .csv sequence file name for Analyst® is shown, e.g. “default”. The style may be defined in the settings: 
	Choose your MS type and autosampler in the drop-down menus.
	Leave the run number as **1**, except when you want to perform another run of the same Kit plate. In this case, increase the run number accordingly, as this will ensure that different MS raw files (e.g. .wiff, .raw) are created.
	Order by **Sample** is the default setting and recommended.
	Select the plates you want to export/measure and select the rack position in the autosampler  . For Thermo autosampler[1]: Rack positions G, R, B and Y are available 
	Samples will be injected randomly when activating this checkbox. Blank, zero, calibration standards, and QC samples will maintain their positions.
	To print plate barcodes and/or sample barcodes you must activate the corresponding check boxes.

For SCIEX® users – **p180, Bile Acids, Stero17 Kits only**:

With Met*IDQ*™, it is possible to perform the quantitation in the Quantitation Wizard of the Analyst® software without copying the calibration standard concentrations from an Excel™ file. This feature can be activated in the Met*IDQ*™ MetLIMS settings (see screenshot below, "Support Quantitation in Analyst®"). If you use this feature, make sure you have copied your Kit quantitation method from the USB stick into the "Quantitation Methods" folder of your Analyst® project (MS operating computer). Do not rename the quantitation method! This quantitation method will be automatically selected in the "Quantitation" field in the Analyst® Acquisition Batch window when you import the csv file. During the quantitation procedure, steps 7 and 8 can be skipped, see page 32 in the manual "UM_p180_SCIEX_##.pdf".

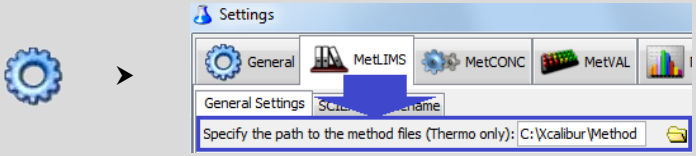
☞ **Quant 500 Kit: do not activate this option!**

Alternatively, you can find the Excel™ file with the concentrations of the calibration standards on the USB memory stick ("Calibrator_Conc_KIT2_5xx4"). If you deactivate the checkbox below, you must copy the concentrations according to the manual "UM_p180_SCIEX_##.pdf" (see step 7 and 8 on page 32).



For Thermo users:

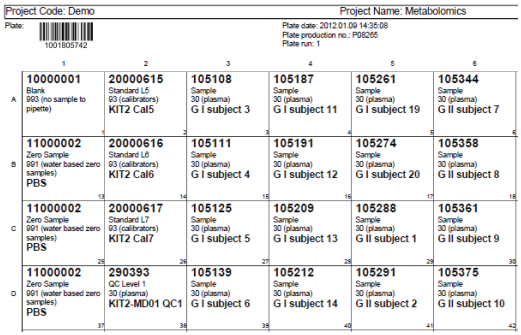
The path where the Xcalibur™ acquisition methods are located on the MS operating computer, e.g. *KIT2-LC_9014.meth*, can be included in the worklist file (.txt). For this define the path, e.g. c:*xcalibur**method*, in the ***Settings > MetLIMS*** as shown below.

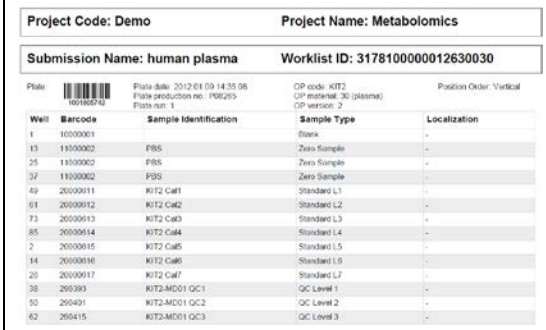


[1] The position and color code correlation may not apply to all Thermo Dionex autosampler and MS instrument combinations.

4	Met*IDQ*™ will generate the folder "*MetIDQ Worklist Export"* with the following files.

Subfolder	***Definition***
barcodes	Contains the barcode label of each plate.
csv (SCIEX) txt (Waters, Thermo)	Contains the acquisition batch and must be imported into the MS software (MassLynx™, Analyst®, Xcalibur™)
plate report	Contains the plate report and can be referenced during pipetting of the kit plate.
worklist	Contains the sample list.
Plate report	Worklist
xml	Used by Met*IDQ*™ to generate a plate report and worklist.





Please refer to the Kit user manual for processing the Kit.

Video Tutorials – Processing the Kit

In addition to the Kit user manuals, video tutorials are available. They describe the steps in the MS software and how the data is quantified. Depending on the MS instrument, watch the corresponding video tutorial: Waters, SCIEX or Thermo.

SCIEX – Analyst®

- MS Measurement: Batch File/Acquisition Method Import for AB Sciex MS Instruments
- AB Sciex MS Measurement: Quantitation, Result File Import, Validation in MetIDQ

Waters – MassLynx™/TargetLynx™

- MS Measurement: Batch File/Acquisition Method Import for Waters MS Instruments
- Waters MS Measurement: Quantitation, Result File Import, Validation in MetIDQ

Thermo Scientific™ – Xcalibur™

- MS Measurement: Batch File/Acquisition Method Import for Thermo MS Instruments
- Thermo MS Measurement: Quantitation, Result File Import, Validation in MetIDQ

5 Quantitation

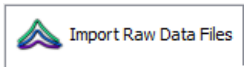
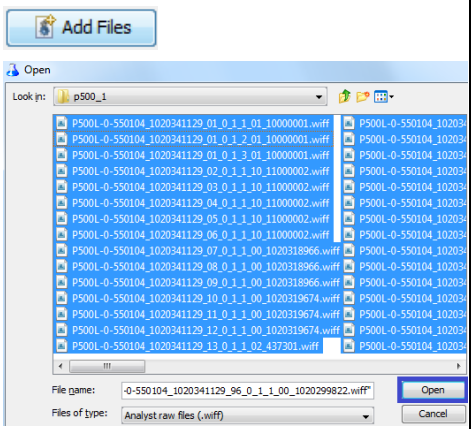
- Quantitation of LC and FIA data is performed using the Met*IDQ*™ software. The procedure is described in the following sections.
- Optionally, LC data quantitation can be performed using the MS manufacturer's software (MassLynx™, Analyst®, Xcalibur™), which is described in the kit user manual.
- Results from LC and FIA part are validated, displayed, and visualized in **MetVAL** and **MetSTAT**.

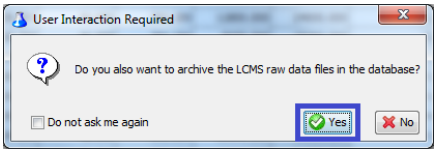
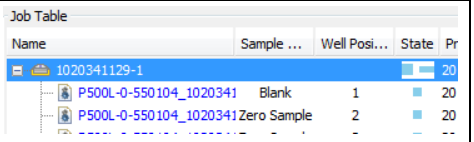
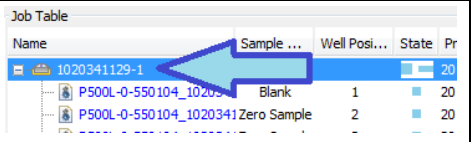
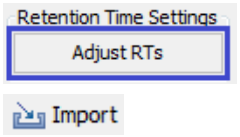
NEW **Quant 500 Kit:** LC data quantitation can be performed by Met*IDQ*™ (**recommended**) or the MS software (Analyst®, MassLynx™). For best longitudinal data comparability, we recommend continuing using the MS software for LC data quantitation, if the p180 Kit was used before.

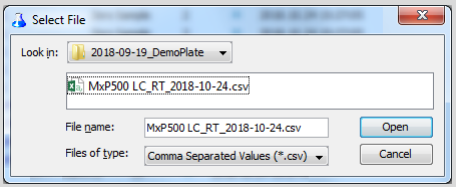
Important: To generate reproducible and longitudinal comparable data, do not change the acquisition method, quantitation method, or software version within one study. For LC data quantitation use either Met*IDQ*™ or the MS software (Analyst®, MassLynx™, Xcalibur™).

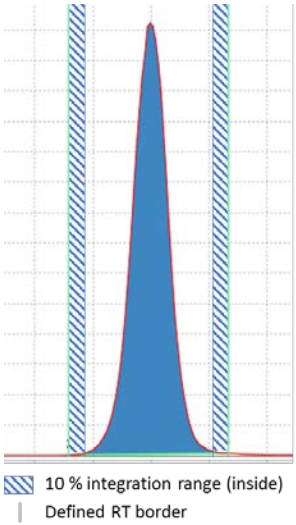
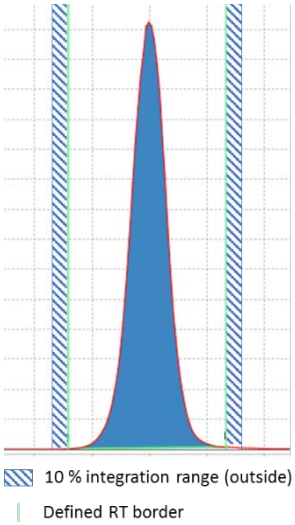
5.1 LC data quantitation – MxP® Quant 500 Kit

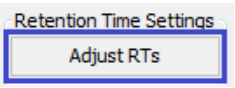
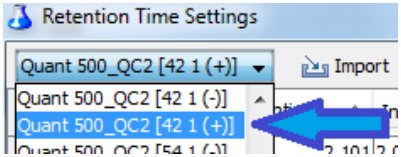
This feature is only available for MxP® Quant 500 Kit data.
LC data quantification of other Biocrates® Kits uses the corresponding MS software (Analyst®, Xcalibur™, or MassLynx™).

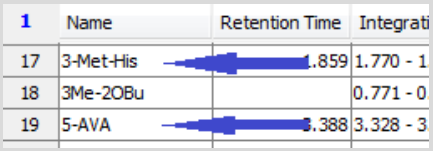
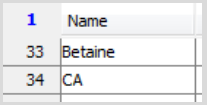
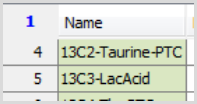
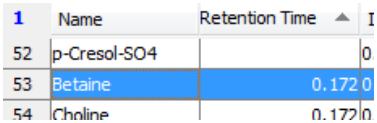
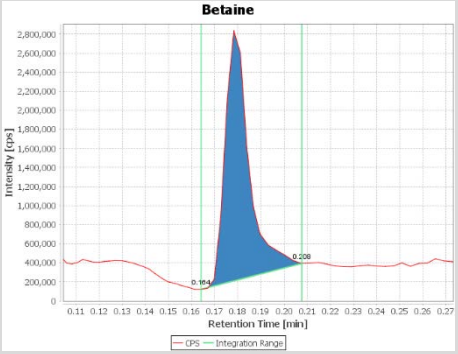
Step	Instructions	Example
⚠	For LC-MS data quantitation use, "." as decimal separator. Recommended: set number format in Windows™ control panel to "English (UK)" or "English (US).	
1	Go to the **MetCONC** module.	
2	Import LC-MS data (.wiff or .raw): Select the "Import Raw Files" tab.	
3	Click "Add Files" and Select the correct file type: Analyst® → Analyst® raw files (.wiff) MassLynx™ → Waters raw file [dir] (.raw) Xcalibur™ → Thermo raw files (.raw) Choose **all** LC data from one kit run. ℹ Load LC data files from LC1 and LC2 injections. - To load all files of one folder, press **Ctrl + A** - To load selected files, keep the **Ctrl** or **Shift** key pressed while clicking. Click **Open**.	

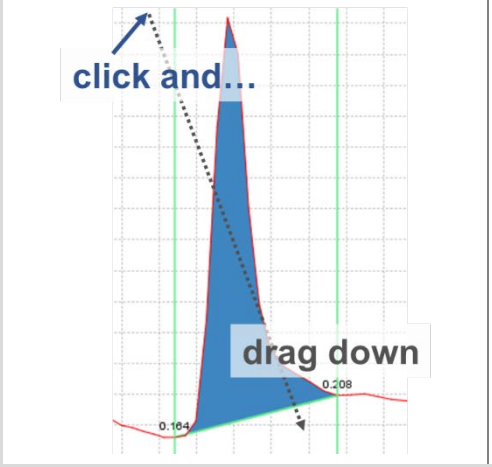
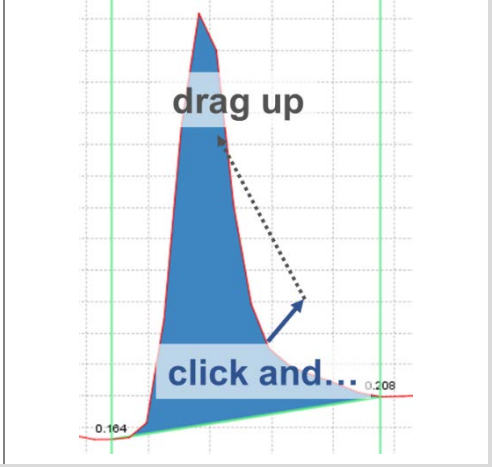
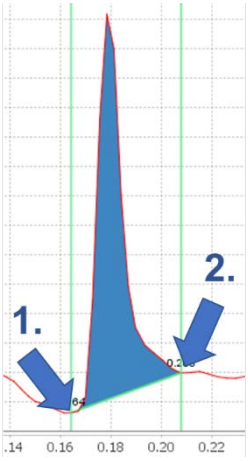
Step	Instructions	Example
4	Click **Yes**. Storing MS data in database: For a later re-integration or adjustment of integration parameter, it is recommended to store MS data in the database.	
5	After MS data is imported, a list of imported samples is shown in the "Job Table".	
6	Select the line with the plate barcode, here it's 1020341129-1.	
	Import Retention Times (RTs) – *optional*	
7	Import retention times (RTs): 1. Click **Adjust RTs** 2. Click **Import** 3. Select a .csv file containing RT ranges. *Information:* Met*IDQ*™ OPs are provided without RT windows. Default RT windows (integration ranges) can be imported from a *.csv* file, e.g. from USB stick: folder "SCIEX\Acquisition and Quantification Methods_Documents".	 Example RT ranges on USB Stick: LC_example_RT-ranges_for_MetIDQ.csv

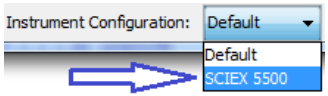
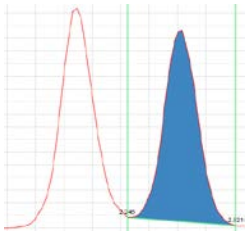
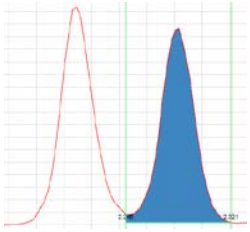
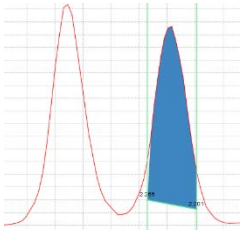
Step	Instructions	Example
	A .csv file containing integration ranges can be imported, e.g. with RT ranges that were previously exported from Met*IDQ*™. *Example:* "MxP500 LC_RT_2018-10-24.csv". To check the imported integration ranges or to set the ranges from scratch, continue with step 8.	
	Adjust Retention Times (RTs) and peak integration	
8	Before starting with the RT adjustment procedure, you may modify the Quantification Configuration. They are available from the Met*IDQ*™ Settings.  These options are available (see next page).	
	RTs are specific for an LC-MS instrument. If several instruments are in use, specific Instrument Setting can be defined for each LC-MS instrument, e.g. "SCIEX 5500".	

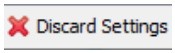
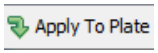
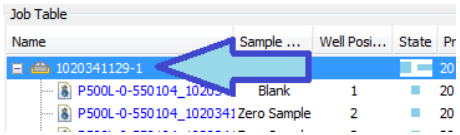
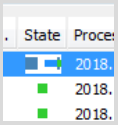
Option	*Definition*
Automatic Retention Time Border Optimization Auto-optimize RT borders: ☑ Auto-optimize RT borders within X% inside RT windows: 10 Auto-optimize RT borders within X% outside RT windows: 0	Automated peak detection and integration is performed for each peak within a defined range. This range can be defined, e.g. 10 % of RT window.
RT borders within 10% **inside** RT windows:  	RT borders within 10% **outside** RT windows: 
RT Overview Show Results of Blanks: ☐ Show Results of Zero Samples: ☐	Show or hide Blank and Zero samples in MetCONC Results.
Instrument Settings New Edit 1 Instrument Name 1 Default 2 SCIEX 5500	*If multiple LC-MS platforms are used:* Click New to create a new *Instrument Setting*. Later RTs can be saved specifically for each defined *Instrument Setting*, e.g. SCIEX 5500.

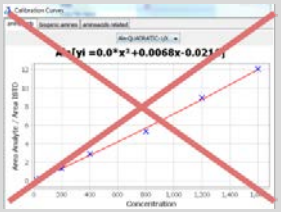
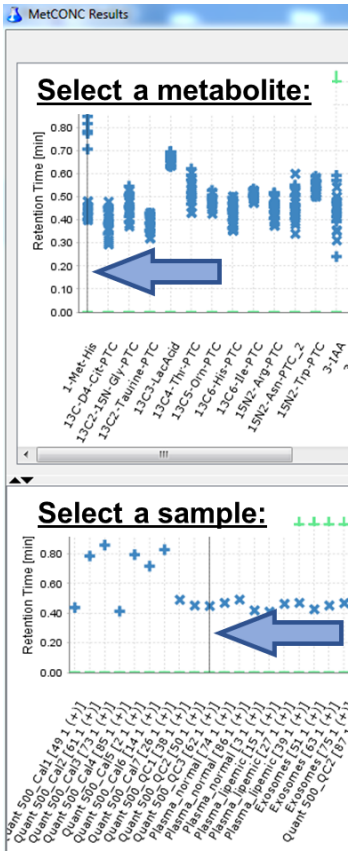
Step	Instructions	Example
9	Click **Adjust RTs**.	
	In the “Retention Time Settings” window, peak integration settings are defined. To check the integration settings, use QC level 2.	
10	For RT adjustment, use QC level 2. ➢ To adjust RTs of analytes analyzed with the LC1 method, select “Quant 500_QC2 [## Y (+)]. ➢ To adjust RTs of analytes analyzed with the LC2 method, select “Quant 500_QC2 [## Y (-)]. e.g. Quant 500_QC2 [42 1 (+)] and Quant 500_QC2 [42 1 (-)]	
	Two acquisition methods are used in LC part. Adjust the RTs of both injections. They can be identified by its name. LC1 run: Quant 500_QC2 [## Y (+)] LC2 run: Quant 500_QC2 [## Y (-)]	##: well position, e.g. 42 Y: injection number, e.g. 1

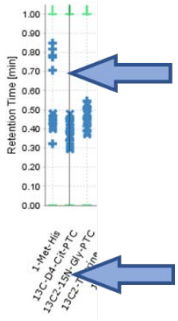
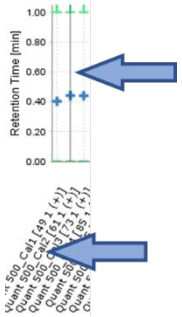
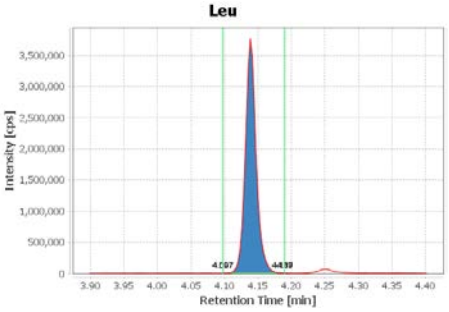
Step	Instructions	Example
	Only RTs of metabolites that were acquired with the selected LC data file are shown, e.g. 3-Met-His and 5-AVA with the Quant 500 LC1 run (positive mode). The RT of 3Me-2OBu is not shown, since the data selected was acquired with another acquisition method (negative mode). To adjust all RTs, a representative data file from both LC runs must be used.	
	Analytes are highlighted in white, e.g. Betaine and CA. Internal standards (ISTDs) are highlighted in green, e.g. 13C2-Taurine-PTC and 13C3-LacAcid.	*Metabolites*  *ISTDs* 
11	To adjust the RTs • sort the Retention Times by ascending order • select the 1st metabolite showing an RT, e.g. Betaine of the LC2 run (negative mode).	
	The corresponding chromatogram is shown, e.g. of Betaine.	

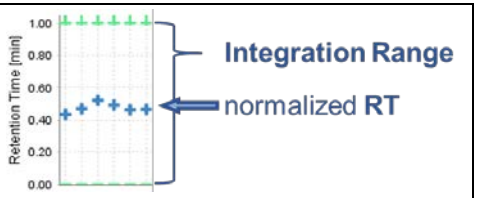
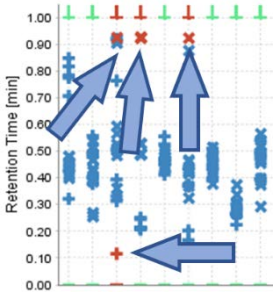
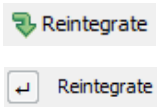
Step	Instructions	Example
	Zoom in  ***Zoom out*** 	
12	Define the RT range – use the mouse pointer. 1. Left click on the left side of the peak. 2. Left click on the right side of the peak. Redefine the RT range: - Left click close to the green vertical line. Move the RT range: - Use the left and right arrow keys. ! To identify the correct peak, refer to the section “Peak Identification” of the corresponding kit user manual.	

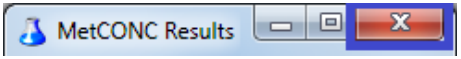
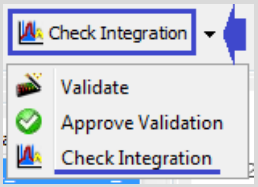
<table>
<tr><th>Step</th><th>Instructions</th><th>Example</th></tr>
<tr><td>13</td><td colspan="2">Define an Integration Type. These options are available.</td></tr>
<tr><td></td><td>Option</td><td>Definition</td></tr>
<tr><td></td><td>Instrument Configuration:</td><td>To save RT and integration settings for a specific LC-MS instrument, select an Instrument Configuration, e.g. SCIEX 5500.
This feature is only available, if an Instrument Configuration in addition to “Default” was defined. See page 71.</td><td> 
</td></tr>
<tr><td></td><td>Valley To Valley</td><td>Automated peak integration.
Integration base line from peak beginning to end.</td><td>  </td></tr>
<tr><td></td><td>Horizontal</td><td>Automated peak integration.
Horizontal integration base line used.</td><td>  </td></tr>
<tr><td></td><td>Manual</td><td>Manual peak integration.
User defined peak integration window and base line.</td><td>  </td></tr>
<tr><td>14</td><td colspan="2">Repeat steps 12 and 13 with all metabolites.</td></tr>
</table>

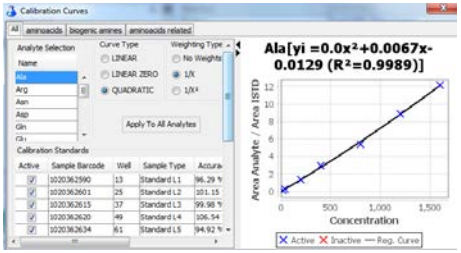
<table>
<tr><th>Step</th><th>Instructions</th><th>Example</th></tr>
<tr><td>15</td><td colspan="2">Save the adjusted RTs. These options are available.</td></tr>
<tr><td></td><td>Option</td><td>Definition</td></tr>
<tr><td></td><td>  </td><td>Discard all manual integration settings of the current sample.</td></tr>
<tr><td></td><td>  </td><td>Use defined RT and peak integration settings for all new integration procedures, for the currently selected Instrument Setting, see page 71.</td></tr>
<tr><td></td><td>  </td><td>Apply defined RT and peak integration settings to current plate.</td></tr>
<tr><td></td><td>  </td><td>Close window and apply defined RT and peak integration settings to current plate.</td></tr>
<tr><td>16</td><td colspan="2">Export the adjusted RTs and
close the window Retention Time Settings.</td><td>  </td></tr>
<tr><td></td><td colspan="3">Process LC Data</td></tr>
<tr><td>17</td><td colspan="2">Select the line with the barcode of an LC plate,
e.g. 1020341129-1.</td><td>  </td></tr>
<tr><td>18</td><td colspan="2">To begin with the quantification procedure, click Start.</td><td>  </td></tr>
<tr><td></td><td colspan="2">The peak integration and quantification process is completed when all samples status turned green. After this process two windows open:
➢ MetCONC Results and Calibration Curves</td><td>  </td></tr>
</table>

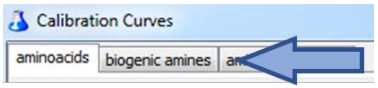
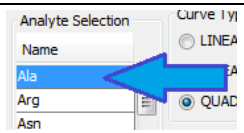
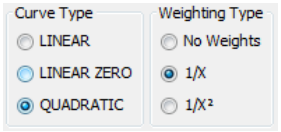
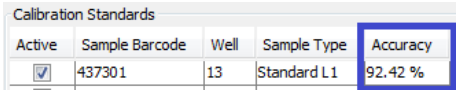
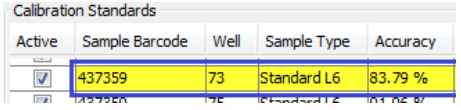
Step	Instructions	Example
	Integration check	
	To adjust peak integration settings in **MetCONC Results**, keep the **Calibration Curves** window closed.	
19	Check the peak integration in **MetCONC Results**. Here, the retention times (RTs) - of all metabolites - of all samples are shown normalize to a scale from 0 to 1. <u>Upper pane:</u> - RT ranges of all metabolites are shown. <u>Lower pane:</u> - RTs of a selected metabolite in all samples are shown. A summary of all features is given below.	

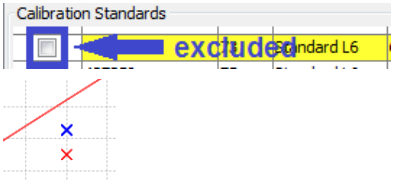
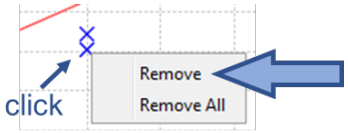
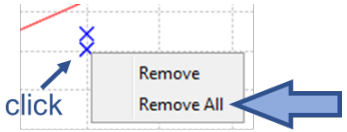
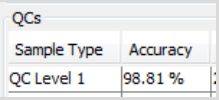
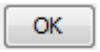
Choice	*Explanation*
Select a metabolite: in the upper pane, click on a metabolite name or click into the graph.	*Upper pane:* 
Select a sample: in the lower pane, click on a sample or click into the graph.	*Lower pane:* 
The corresponding chromatogram is shown.	

The lower pane shows the RTs and integration range of the selected metabolite.	
Check integration! Metabolites are highlighted in red, if their RT is outside or at the edge of the integration range.	
Adjust integration range: 1. Select a metabolite and sample. 2. Adjust the integration range. 3. Click **Reintegrate** or the **Enter** key.	
! To apply an adjusted integration range, click Reintegrate or press **Enter** for <u>each</u> peak.	

20	Once the procedure has been completed, close the window “MetCONC Results”.	
i	To re-open the window “MetCONC Results”, go to ***MetVAL > Validation*** and click **Check Integration**.	
	! For this feature, set the *Validation Settings* to *MANUALLY*. 	

	Check Calibration	
21	To check the calibration. Go to the window **Calibration Curves**. Here, all seven-point calibrated metabolites are shown in three separate tabs: amino acids, biogenic amines, and amino acids related. Check the performance of all metabolites in all calibration levels. If required, adjust the calibration parameters. A summary of all features is given below.	

Choice	***Explanation***
Select the metabolite class.	
Select a metabolite from the drop-down menu.	
You can change the “Curve Type” and “Weighting Type”. Default values: *Curve Type* Quadratic *Weighting Type* 1/X	
Accuracies are shown in the table “Calibration Standards”.	
Accuracies outside the acceptance range are highlighted in yellow.	

	Exclude calibration level(s) for **all** metabolites of currently selected metabolite class. ☞ Deselect in table “Calibration Standards” *Information:* excluded calibration level(s) are shown in red in the calibration curve window.	
	Exclude calibration level for selected metabolite. ☞ Click on corresponding calibration point and select “Remove”.	
	Exclude calibration level for **all** metabolites of currently selected metabolite class. ☞ *Click on corresponding calibration point and select “Remove All”.*	
	QC accuracies are shown in the table “QCs”.	
22	Once the procedure has been completed, click **OK**.	

5.2 LC data quantitation – Import Result Files – all kits

LC data quantitation can be performed using the MS software (MassLynx™, Analyst®, Xcalibur™), which is described in the corresponding kit user manual. The import of results is described below.

SCIEX – Analyst®

Sciex MS Measurement: Quantitation, Result File Import, Validation in MetIDQ

Waters – MassLynx™/TargetLynx™

Waters MS Measurement: Quantitation, Result File Import, Validation in MetIDQ

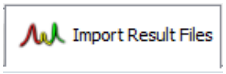
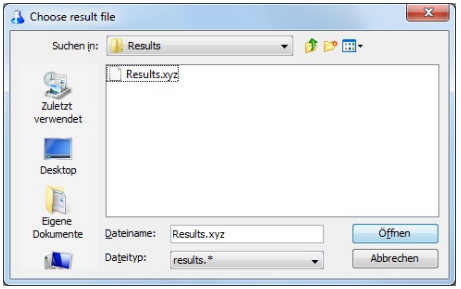
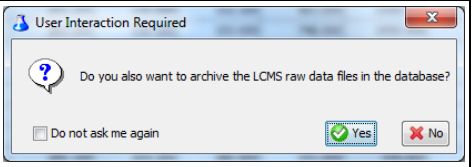
Thermo Scientific™ – Xcalibur™

Thermo MS Measurement: Quantitation, Result File Import, Validation in MetIDQ

This feature is available for all Biocrates® kits.

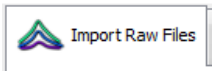
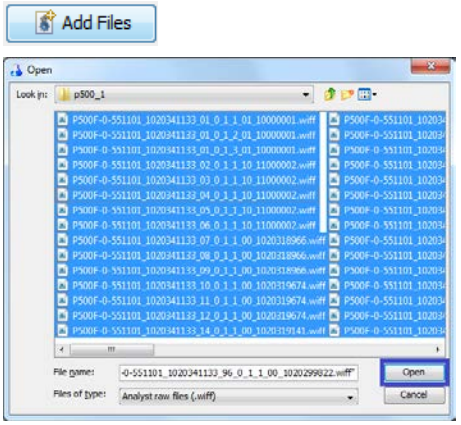
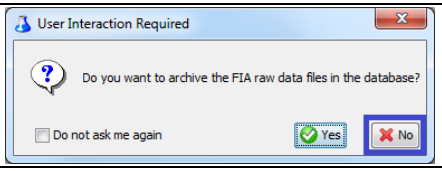
If used for the MxP® Quant 500 Kit, the integration and quantitation procedure described in section 5.1 must be performed in addition.

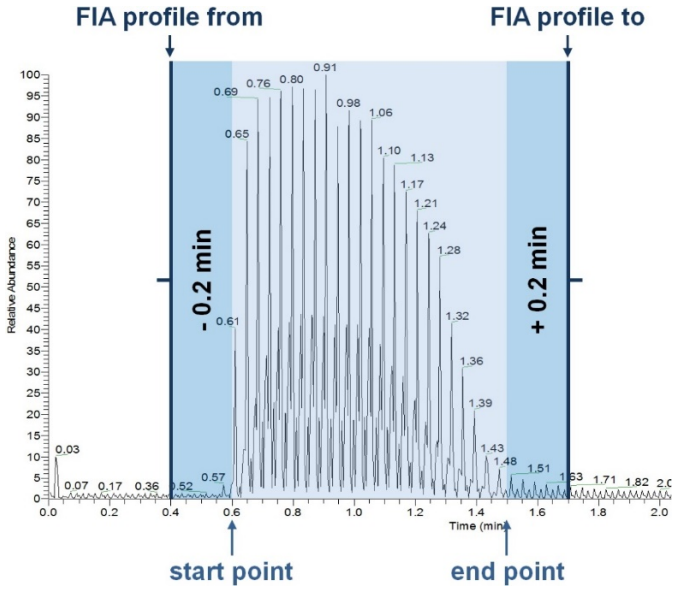
Step	Instructions	Example
⚠	For LC-MS data quantitation use "." as decimal separator. Recommendation: set number format in Windows™ control panel to "English (UK)" or "English (US).	
!	**Results from the Analyst® software can be imported from a text-based result file (.txt).** The import of Analyst® result files (.rdb) is no longer supported. ☞ For a description how to generate text-based result file, refer to section 7.13.	

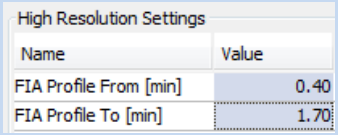
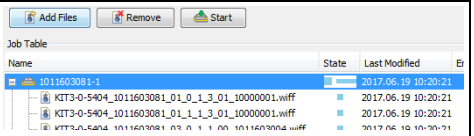
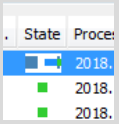
Step	Instructions	Example
1	Click on the **MetCONC** symbol.	
2	Import LC-MS results (.txt or .xls): go to “Import Result Files”.	
3	Click **Browse** and select the result file according to your instrument manufacturer: Analyst® → “sciex result files (*.txt)“ MassLynx™ → “waters result files (*.txt) Xcalibur™ → “thermo result files (*.xls)	
4	Click **No**. Storing MS data in database: Not required when during LC results import.	
5	After the import process, validate the plate, see section 5.4.	

5.3 FIA data – quantitation by Met*IDQ*™

FIA data is available using the MxP® Quant 500, p180, p400 HR, and p150 Kits.

Step	Instructions	Example
⚠	To import the FIA-MS data into Met*IDQ*™, it is recommended to set the decimal separator in the control panel of your operating system to "." (dot).	
1	Go to **MetCONC**.	
2	Import FIA-MS data (.wiff or .raw): go to "Import Raw Files".	
3	Click "Add Files" and select the correct file type: Analyst® → Analyst® raw files (.wiff) MassLynx™ → Waters raw file [dir] (.raw) Xcalibur™ → Thermo raw files (.raw) Choose **all** FIA data from one kit run. - To load all files of one folder, press **Ctrl + A** - To load selected files, keep the **Ctrl** or **Shift** key pressed while clicking. Click **Open**.	
4	Store raw data files: recommended for LC data. Storing MS data in database: Not required for FIA data.	

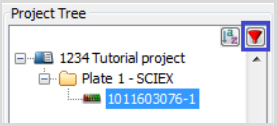
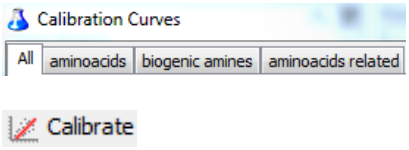
Step	Instructions	Example
5	**Define FIA profile width** – **required** for these kits**:** ➢ **Quant 500 Kit** ➢ **p400 HR Kit** ➢ **p180 Kit Agilent edition** Open example FIA MS files (.wiff or .raw) with the MS software, e.g. QC level 2 and an example sample. Identify the start and end point of the FIA profile as shown below. In this example: - start point 0.6 min - end point 1.5 min	

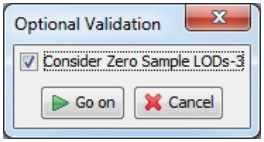
Step	Instructions	Example
	For a robust peak integration, subtract 0.2 min form the start point and add 0.2 min to the end point. - FIA Profile From [min] = 0.4 - FIA Profile To [min] = 1.7 Use these values for <u>every</u> FIA integration process.	
	If no times are defined (*Value* = 0.00) the FIA profile is automatically identified. Defining the FIA profile width makes the FIA integration process more robust by excluding small signals outside the FIA peak.	
6	Imported files are shown in the **Job-Table**.	
7	To begin the quantitation procedure click **Start**.	
	The quantitation process is performed by Met*IDQ*™.	
8	After the quantitation process, validate the plate, see section 5.4.	

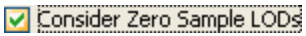
5.4 Validate the Kit Plate

The **MetVAL** module performs an automated quality assessment of the Kit data and checks the performance of blank, calibration standard, QC samples, and internal standards (ISTD). Status and condition of a plate is shown well by well.

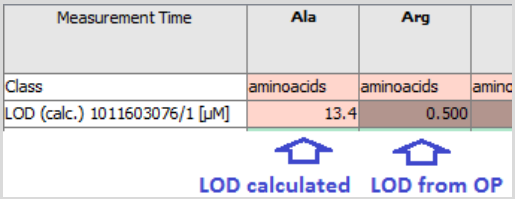
MetIDQ: Validation Features in Detail

Step	Instructions	Example
1	After each quantitation procedure, **MetVAL** module opens automatically.	
	In **MetVAL** the plate/s loaded last is/are shown. To show additional plates, change the filter settings.	
	The LOD for all metabolites is listed in the Analytical Specifications and is part of the Met*IDQ*™ database. Kit plate specific LODs can be calculated.	
2	**Only for Quant 500 Kit data:** Calibration: If not done previously, check and perform the calibration according to page 80.	
3	The validation process starts automatically. If the validation does not start or to re-validate a plate, click **Validate**.	

Step	Instructions	Example
4	Calculate the limit of detection (LOD): To calculate the LOD, use the **Optional Validation** box. A summary of features is given below.	

Choice	Result
Recommendation for at least **3 Zero replicates** per plate: 	The median value of all zero samples on a plate is calculated as approximation of the background noise. 3 x this value is the LOD for each analyte, which is shown in the **MetSTAT** results table. *LOD = 3 x median background noise*

LC data: If no concentration value for a metabolite is available in a LC result file, the LOD defined in the Met*IDQ*™ OP is used. If a calculated LOD or an LOD from the OP is used, this is indicated in ***MetSTAT*** as shown below.

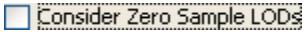


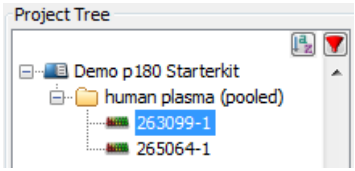
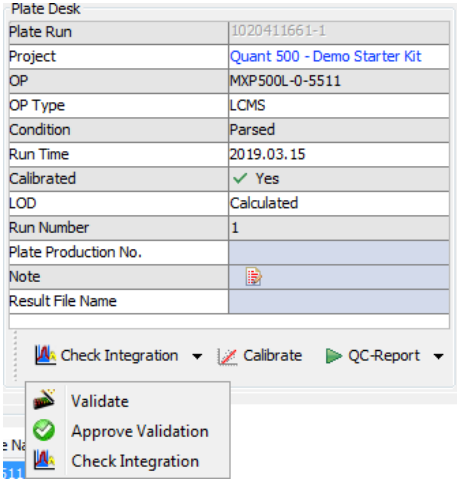
Measurement Time	Ala	Arg	
Class	aminoacids	aminoacids	aminc
LOD (calc.) 1011603076/1 [µM]	13.4	0.500	

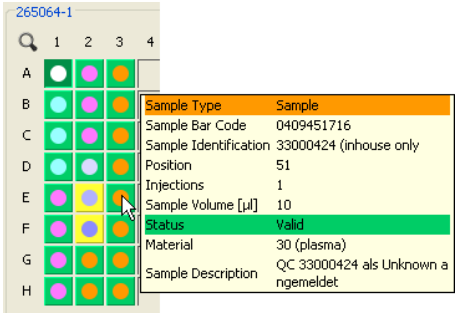
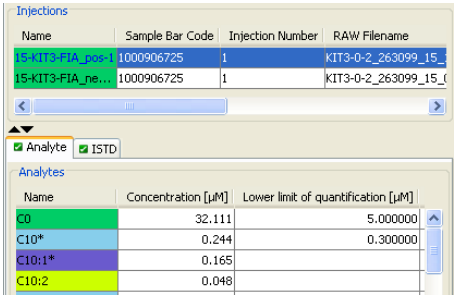
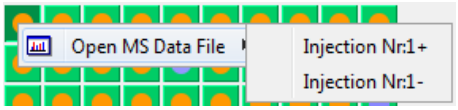
LOD calculated LOD from OP

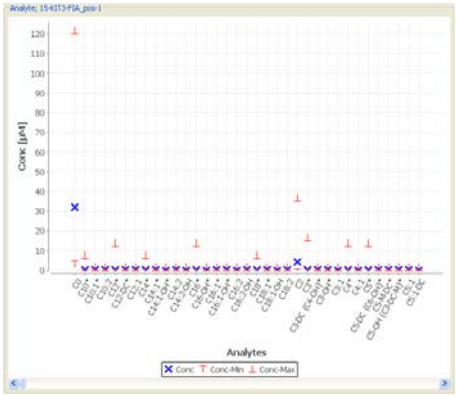
Why might a concentration value not be available for a metabolite of a zero sample?

The quantitation method may not detect peaks in zero samples in all analyte MRMs, due to low peak or signal intensities.

Recommendation for less than **3 zero replicates** per plate: 	The LOD values shown in the Analytical Specifications are used. These values are listed in the results table in **MetSTAT**.

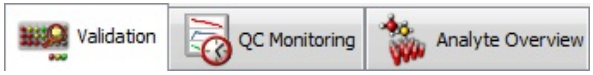
Step	Instructions	Example
	The validated Kit plate, e.g. plate barcode 263099, is highlighted in the Project Tree. Change the filter options to choose another Kit plate.	
5	The **Plate Desk** provides a detailed list of all plate information. Add notes to a plate: Double-click on the "Note" symbol. Notes are included in QC-Reports. Re-calibrate a plate: Click the **Calibrate** button. Show peak integrations: Click the **Check Integration** button. Available if validation was not approved. Generate a QC Report: Click the **QC-Report** button. A PDF is created. Go to MetSTAT: Click on the Plate Run number.	

Step	Instructions	Example
6	The plate graphic shows the contents and status of each well on the plate. The wells are color-coded to show the sample type and status. Here, the sample has the status *Valid,* indicated by a green background (all values are within the range set by the OP). If a well is highlighted in yellow this indicates that one or more values are out of range. The reason should be evaluated. Use the mouse and scroll over any well to open box with more information on the sample.	
7	Left click on a well to see the corresponding table with analytical details and the corresponding graph. Use the right-mouse button to open LC-MS/MS or FIA-MS data files (LC data are only available if they were imported in **MetCONC**, see section 5 *Quantitation*).	 * Concentrations isotope corrected (FIA part only) 

Step	Instructions	Example
	Different graphs are available in **MetVAL** to show the results for different sample types. Each of these graphs are explained and illustrated on the next pages.	

5.5 Explanation of MetVAL Graphs

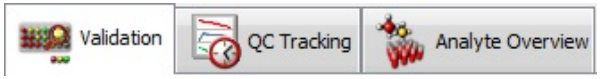
MetVAL categories: **Validation**, **QC Monitoring**, and **Analyte Overview**.



5.5.1 Presentation Options for all MetVAL Graphs

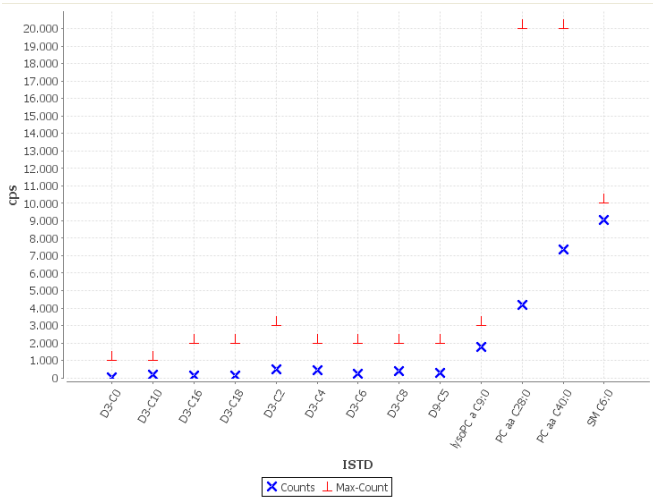
Presentation Option	***Instructions***
See the graph on full screen	Double-click on the graph to enter full-screen mode. Press **Esc** to return to normal size.
Save the graph as an image file	Right-click on the graph to show the context menu and choose “Save as” option. You can save the graphic as an image file for export.
Print the graph	Right-click on the graph to show the context menu and choose “Print” option. Choose the paper size and format as well as a printer.
Zoom in/Zoom out	Hold the left mouse button and drag it downwards to zoom in on the y-axis. Hold down the left mouse button and drag it upwards to zoom out.

5.5.2 MetVAL – Validation Graphs

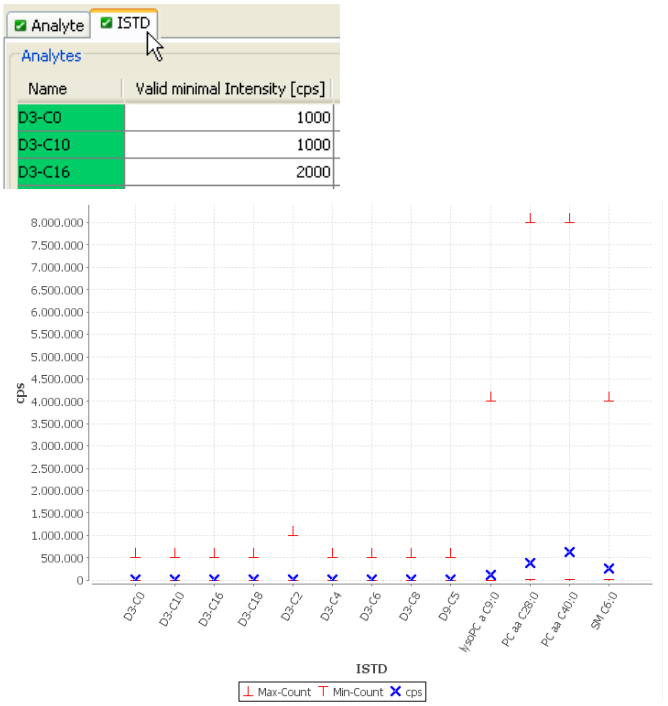


5.5.2.1 ISTD Graph (Blank Well)

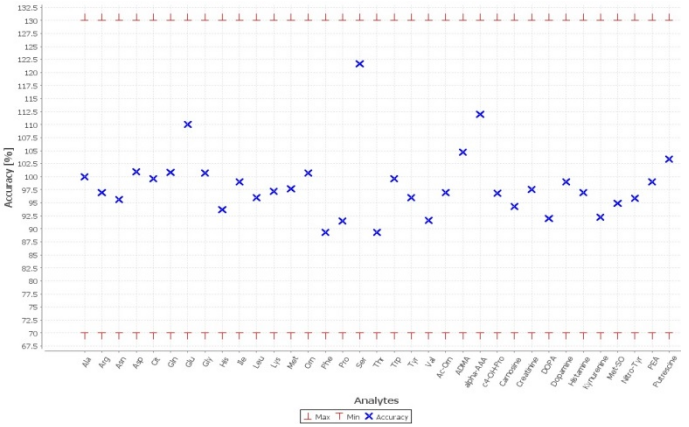
The ISTD graph for the blank well is different from the ISTD graph for the other wells.

Explanation	*Example*
The blank well is always on plate position A1. The graph shows the measured intensities (cps) of the internal standards (ISTD). The maximum cps are shown as red dashes. The blank does not contain any ISTD, consequently the cps values should be below the max. cps limits. If an ISTD value exceeds the max. cps: • The well will be yellow on the plate graphic. • The ISTD which is out of range will appear in yellow in the analyte list. • The corresponding analyte will also be highlighted in yellow in the results table in **MetSTAT**.	

5.5.2.2 ISTD Graph (other wells)

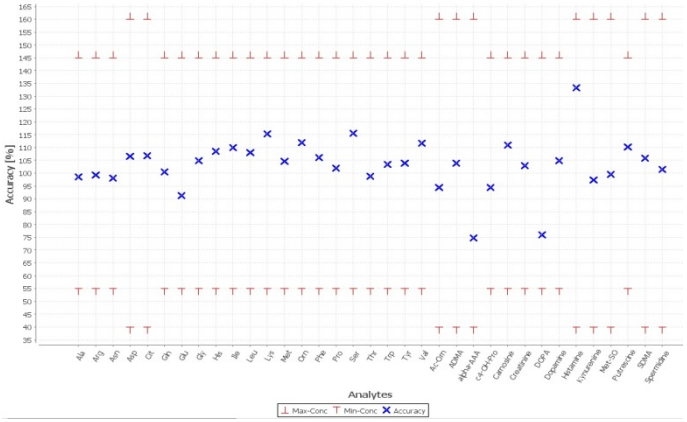
Explanation	*Example*
For all other samples, the ISTD graph shows upper and lower limits as well as the measured cps values. The ISTD graph will be shown when selecting the ISTD tab. If an ISTD is out of range: • The well will be yellow on the plate graphic. • The ISTD which is out of range appears in yellow in the ISTD list. • The corresponding analyte will also be highlighted in yellow in the results table in **MetSTAT**.	

5.5.2.3 Standards Graph

Explanation	*Example*
The calibration standards (STD) graph shows the accuracy of the Biocrates calibration standards. The accuracy is defined as: $\frac{measured\ concentration}{expected\ concentration}\ x\ 100$ The maximum and minimum tolerance values set by the OP are shown in red. If a standard is out of range: • The well will be yellow on the plate graphic. • The analyte which is out of range appears in yellow in the analyte list. • The analyte will be highlighted in yellow in the results table in **MetSTAT**. When you click on the analyte in the **Analytes** table, the analyte will be marked with a vertical line in the graph.	 STD ISTD Analytes

	Name	Tolerance [%]	Accuracy [%]	Concentration [µM]	Expected Concentration [µM]
1	Ala	30.000	100.000	1200.000	1200.000000
2	Arg	30.000	97.000	291.000	300.000000
3	Asn	30.000	95.667	287.000	300.000000
4	Asp	30.000	101.000	303.000	300.000000

5.5.2.4 QC Concentration Graph

Explanation	*Example*
This graph shows the accuracies of the quality control samples. The accuracy is defined as: $\frac{measured\ concentration}{expected\ concentration}\ x\ 100$ The maximum and minimum tolerance values set by the OP are shown in red. If an accuracy value is out of range: • The well will be yellow on the plate graphic. • The analyte which is out of range appears in yellow in the analyte list. • The analyte will be highlighted in yellow in the results table in **MetSTAT**. If you click on the analyte in the **Analytes** table, the analyte is marked in the graph.	 QC-Conc QC-Median ISTD Analytes

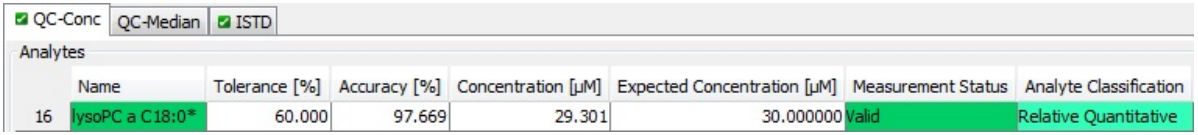
	Name	Tolerance [%]	Accuracy	Concentration [µM]	Expected Concentration [µM]
1	Ala	45.000	98.577	331.400	336.183006
2	Arg	45.000	99.387	92.499	93.070311
3	Asn	45.000	98.130	20.740	21.134742
4	Asp	60.000	106.683	10.783	10.107937

Whenever a QC was injected in replicates from the same well position (injection replicates), the first injection is evaluated in MetVAL.

Explanation	*Example*

QCs in FIA part – Quant 500, p180, p400 HR and p150 Kit:

Some analytes checked with the QCs may not be quantitative, e.g. *lysoPC a C18:0* in KIT3. Their Analyte Classification is “Relative Quantitative“ and the values in "Expected Concentration" for these analytes are in arbitrary units. However, their accuracy is checked.

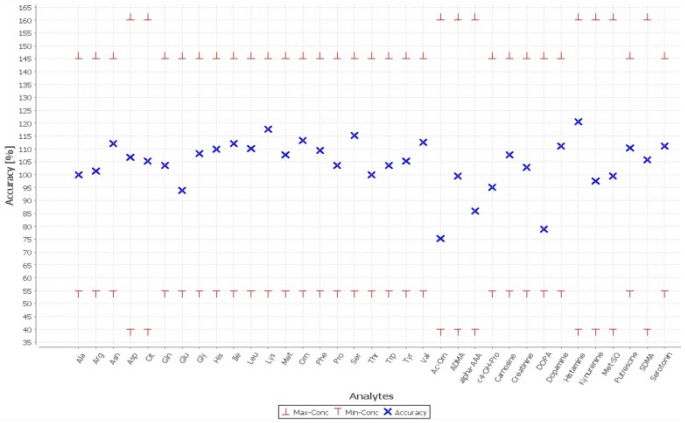

QC-Conc | QC-Median | ISTD

Analytes

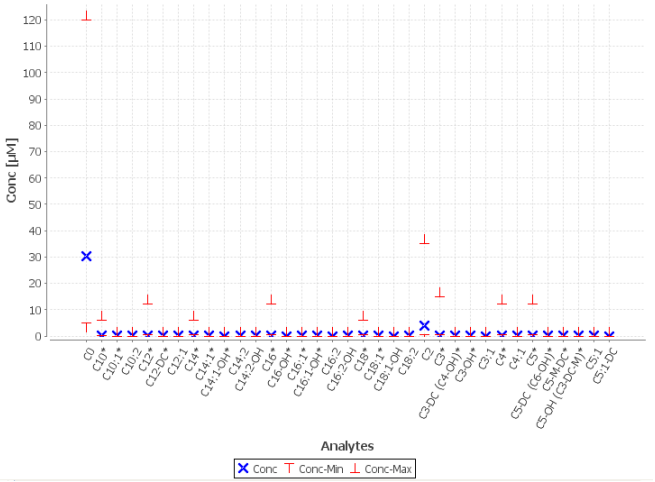
	Name	Tolerance [%]	Accuracy [%]	Concentration [µM]	Expected Concentration [µM]	Measurement Status	Analyte Classification
16	lysoPC a C18:0*	60.000	97.669	29.301	30.000000	Valid	Relative Quantitative

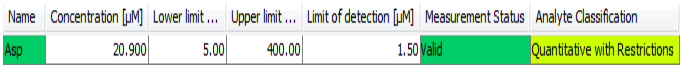
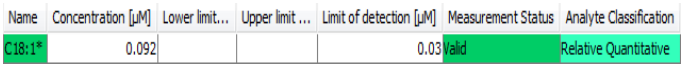
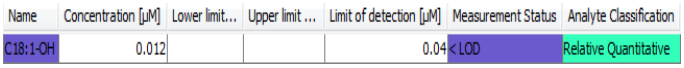
Reason: The absolute analyte concentration of “Relative Quantitative” analytes cannot be determined, as external standards or reference materials are not available. However, based on numerous analyses these analytes show good CVs and highly reproducible concentration values. Consequently, they can be analyzed precisely but maybe not accurately. The resulting analyte classification is “Relative Quantitative”. The accuracy given in Met*IDQ*™ is the deviation from the determined analyte target concentration.

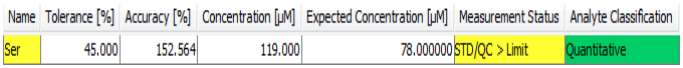
5.5.2.5 QC-Median Concentration Graph

Explanation	*Example*
When a QC level was measured in replicates over the Kit plate, this graph shows the median accuracies. If a median accuracy value is out of range: • The well will be yellow on the plate graphic. • The analyte which is out of range appears in yellow in the analyte list. • The analyte will be highlighted in yellow in the results table in MetSTAT. If you click on the analyte in the **Analytes** table, the analyte is marked in the graph.	 QC-Conc QC-Median ISTD Analytes Name Tolerance [%] Accuracy [%] Concentration [µM] Expected Concentration [µM] 1 Ala 45.000 99.979 336.111 336.183006 2 Arg 45.000 101.535 94.499 93.070311 3 Asn 45.000 112.051 23.682 21.134742 4 Asp 60.000 106.683 10.783 10.107937

5.5.2.6 Analyte Concentration Graph

Explanation	*Example*
The concentration graph of all analytes is shown for all samples. The lower limit of quantitation (LLOQ), and the upper limit of quantitation (ULOQ) are shown in red. These values are also listed in the Analytical Specifications. Information on all analytes is shown in the table left to the graph. The status is color-coded and explained on the next pages.	 Analyte ISTD Analytes Name | Concentration [µM] | Lower limit of quantification [µM] C0 | 30.157 | 5.000000 C10* | 0.111 | 0.300000 C10:1* | 0.054 | C10:2* | 0.032 | C12* | 0.035 | 0.400000 * Concentrations are isotope corrected (FIA part only)
The status of C10* is < *LLOQ*; name and measurement status are colored blue. The analyte classification is *Quantitative,* and the concentration value is below LLOQ, but above the limit of detection (LOD).	Name | Concentration [µM] | Lower limit... | Upper limit ... | Limit of detection [µM] | Measurement Status | Analyte Classification C10* | 0.157 | 0.30 | 6.00 | 0.15 | < LLOQ | Quantitative

Explanation	***Example***
The status of Asp is *Valid*; name and measurement status are colored green, which means the concentration value is within the quantitation range. The analyte classification is *Quantitative with Restrictions*: poorer precision expected (CV=15-30%)	 Name | Concentration [µM] | Lower limit ... | Upper limit ... | Limit of detection [µM] | Measurement Status | Analyte Classification Asp | 20.900 | 5.00 | 400.00 | 1.50 | Valid | Quantitative with Restrictions
The status of C18:1* is *Valid*; name and measurement status are colored green. The analyte classification is *Relative Quantitative,* and the concentration value is above the LOD. *Note*: LLOQ and ULOQ values are not defined for relative quantitative analytes.	 Name | Concentration [µM] | Lower limit... | Upper limit ... | Limit of detection [µM] | Measurement Status | Analyte Classification C18:1* | 0.092 | | | 0.03 | Valid | Relative Quantitative
The status of C18:1-OH is < *LOD*; name and measurement status are colored purple, which means that the concentration value is below LOD. The analyte classification is *Relative Quantitative*: precise (CV<15%) but accuracy not verified (FIA part only).	 Name | Concentration [µM] | Lower limit... | Upper limit ... | Limit of detection [µM] | Measurement Status | Analyte Classification C18:1-OH | 0.012 | | | 0.04 | < LOD | Relative Quantitative

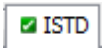
Explanation	*Example*
The status of Ser is *STD/QC > Limit*; name and measurement status are colored yellow. The analyte classification is *Quantitative,* but the accuracy of Ser is not within the acceptance range for Ser in this QC measurement. *Note*: *STD/QC > Limit* or *STD/QC < Limit* is only displayed for QC samples.	

Name	Tolerance [%]	Accuracy [%]	Concentration [µM]	Expected Concentration [µM]	Measurement Status	Analyte Classification
Ser	45.000	152.564	119.000	78.000000	STD/QC > Limit	Quantitative

* Concentrations are isotope corrected (FIA part only)

5.5.2.7 Measurement Status

A measurement status is given individually for each metabolite sample per sample. Analytes and internal standards (ISTD) are evaluated separately. Different *Measurement Status* values are available for Calibration Standards and Quality Controls or Samples and Zeros. According to their relevance, measurement statuses are prioritized. Only the *Measurement Status* of highest priority is shown in Met*IDQ*™.

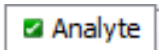


ISTD (internal standard) – all samples:

Measurement Status	*Sample*	*Description*
Valid	blank	background noise in range
Blank Out of Range	blank	background noise above limit
Valid	all except blank	ISTD intensity in range
ISTD Out of Range	all except blank	ISTD intensity not in range

STD , QC-Conc **STD (Calibration Standards) and QC (Quality Controls):**

Measurement Status	*Description*
Valid	accuracy in range
Smaller Zero	concentration below zero, e.g. -0.535 µM
< LOD	concentration below LOD
< LLOQ	concentration below LLOQ
> ULOQ	concentration above ULOQ
No Intercept	concentration cannot be calculated, also see FAQ
Missing Measurement	no measurement available
ISTD Out of Range	ISTD intensity not in range
STD/QC < Limit	accuracy below limit
STD/QC > Limit	accuracy above limit
Invalid	analyte excluded by user in Met*IDQ*™ or during quantitation
Incomplete	1. No measurement (concentration data) available for one well. 2. Accuracy cannot be evaluated.



Samples and Zero Samples:

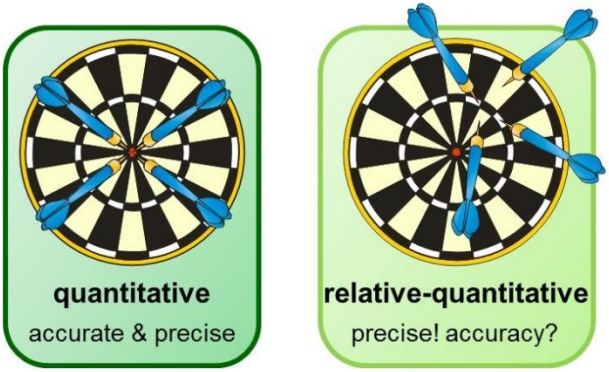
Measurement Status	***Description***
Valid	concentration between LLOQ and ULOQ
Smaller Zero	concentration below zero, e.g. -0.535 µM
< LOD	concentration below LOD
< LLOQ	concentration below LLOQ
> ULOQ	concentration above ULOQ
No Intercept	concentration cannot be calculated, also see FAQ
Missing Measurement	no measurement available
ISTD Out of Range	ISTD intensity not in range
Invalid	analyte excluded by user in Met*IDQ*™ or during quantitation
Incomplete	No measurement (concentration data) available for one well.

5.5.2.8 Analyte Classification

A validation status is given for each analyte. For detailed information refer to the Analytical Specifications document (AS_p180_#.pdf).

Analyte Classification	*Description*
Quantitative	Validation criteria fulfilled. LC-part: 7-point calibration. FIA-part: internal 1-point calibration.
Quantitative with Restrictions	Also “Quantitative”, but poorer precision expected (CV=15-30%) and/or poorer accuracy expected (accuracy=15-30%)
Relative Quantitative	FIA part only: precise (CV<15%) but accuracy not verified. Internal 1-point calibration.
Not Validated	Analyte not tested during validation.

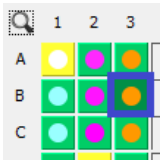
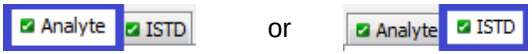
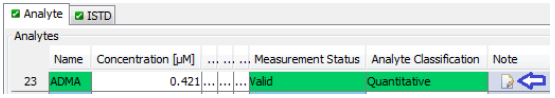
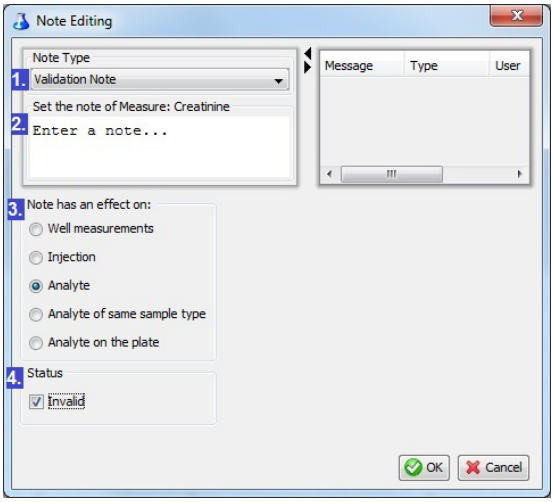
When is a metabolite classified as quantitative or relative-quantitative?

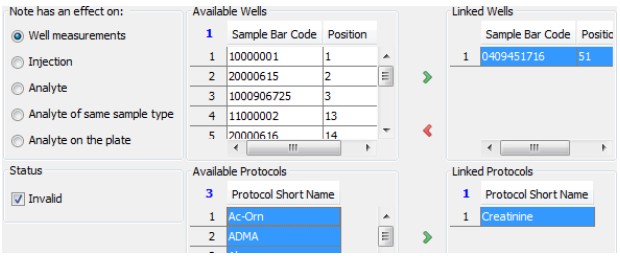
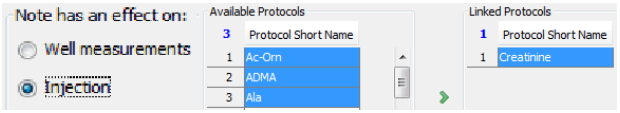
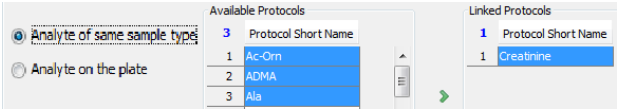
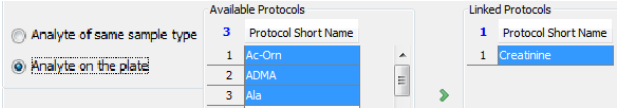
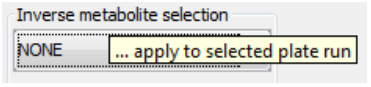


Quantitative:	verification of precision (CV) and accuracy according to validation criteria
Relative Quantitative:	verification of precision (CV) according to validation criteria verification of accuracy not possible, as no external standards available

5.5.2.9 Exclude Analytes or Wells from Validation

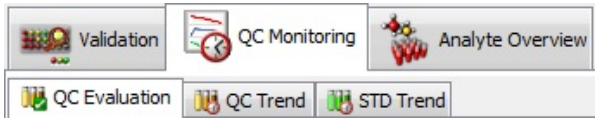
Analytes or wells can be excluded from validation. Their *Measurement Status* is Invalid. This may be required, e.g. when a sample was pipetted incorrectly onto one well position or the incorrect retention time (RT) was defined for one analyte.

Step	Instructions	Example
1	In MetVAL > Validation select a well.	
2	Select the tab *Analyte* or *ISTD*.	
3	Select an analyte (e.g. ADMA) and double-click on the note field.	
4	1. Select a *Note Type*. 2. Enter a note, e.g. the *Validation Note* “RT was defined incorrectly”. 3. Make a choice from which well or sample type analyte(s) should be excluded from validation. → Find a description below. 4. Activate the status *Invalid*. ***Note:*** If the status *Invalid* is not activated, only a note is saved.	

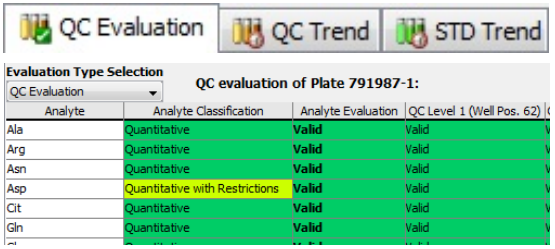
Choice	Result
	1. Make your choice of well(s). 2. Analyte(s) listed in the column Linked Protocols are excluded from validation of the selected well. ***Note:*** If injection replicates were done, this selection affects all injection replicates of the well.
	Analyte(s) listed in the column *Linked Protocols* are excluded from validation in the selected injection of a well.
	Chosen analyte of selected injection of a well is excluded.
	Analyte(s) listed in the column *Linked Protocols* are excluded from validation of the selected sample type, e.g. QC 1.
	Analytes listed in the column *Linked Protocols* are excluded from validation in all samples on the selected Kit plate.
	Inverse metabolite selection: 1. select a plate run 2. select metabolites that should <u>not</u> be excluded from validation 3. select the “Status” *Invalid* 4. click OK <u>*Example:*</u> If creatinine is selected, all further metabolites of a *plate run* get the status *Invalid*. At least two *plate runs* are required.

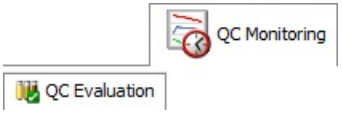
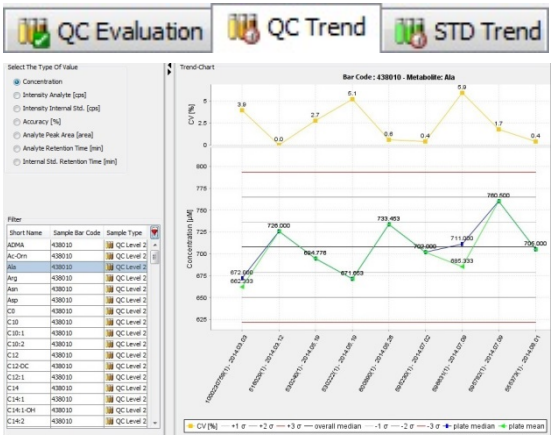
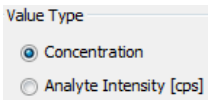
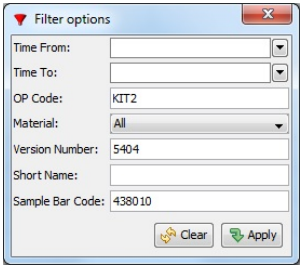
Step	Instructions	Example
5	Select *OK* in the “Note Editing” window.	
6	To apply all changes, click on *Validate* to re-validate the Kit plate.	
	While the validation status *Invalid* can be changed at any time, validation notes cannot be edited but deleted.	

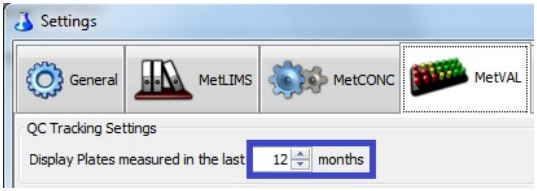
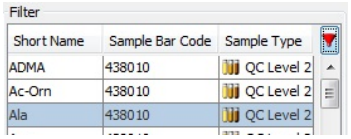
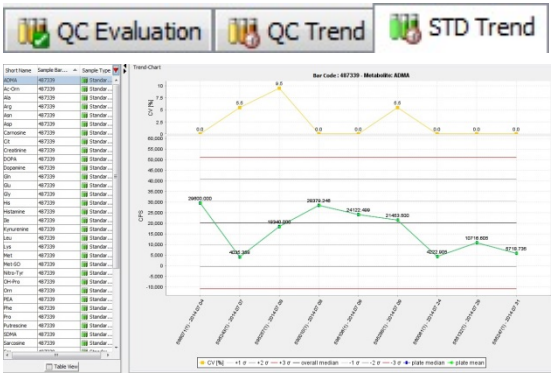
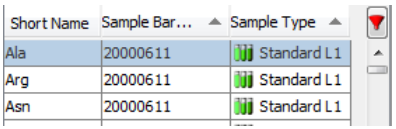
5.5.3 MetVAL – QC Monitoring Graphs



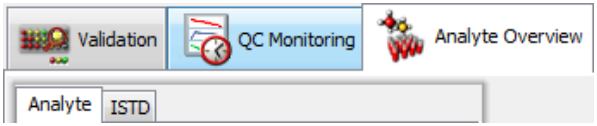
MetIDQ: QC and Calibration Standard Monitoring, Time-Dependent Quality Control

Step	Instructions	Example
1	In order to obtain a QC analyte evaluation based on the EMA guidelines select the **QC Evaluation** tab. The Kit validation from MetVAL is used to classify each QC analyte. It is *valid* if the accuracy is within the predefined ranges for a Kit. Otherwise the reason is displayed, e.g. *STD/QC > Limit*. If an analyte (see column *Analyte Evaluation*) meets the EMEA criteria it is highlighted in green (status: valid), otherwise it is marked in yellow (status: invalid). For further details please have a look at the EMA guidelines on bioanalytical method validation (European Medicines Agency, 2011).	

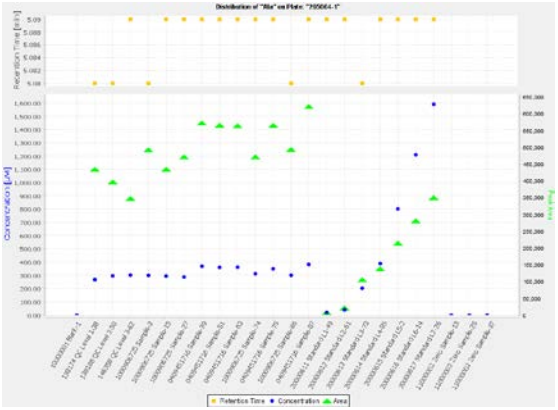
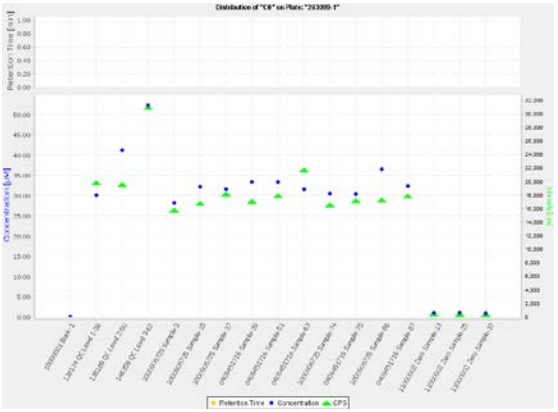
Step	Instructions	Example
	The QC Evaluation can be made for one Kit plate only. In **MetVAL > Validation**, select a Kit plate first and then switch to **QC Monitoring > QC Evaluation**.	1.  2. 
2	Select the **QC Trend** tab to obtain an overview of the inter-plate CVs of a selected QC analyte. This tool can be used to monitor the assay and instrument performance or reproducibility over a specified time period.	
3	In the top left panel **Value Type** define the type of value (e.g. concentration) you would like to monitor.	
4	Use the Filter  to monitor a certain QC level by the QC's bar code or use other filter options.	
5	Use the button **Table View** (bottom left) to show.	

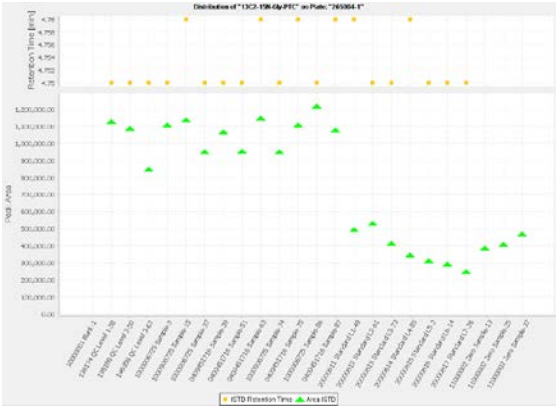
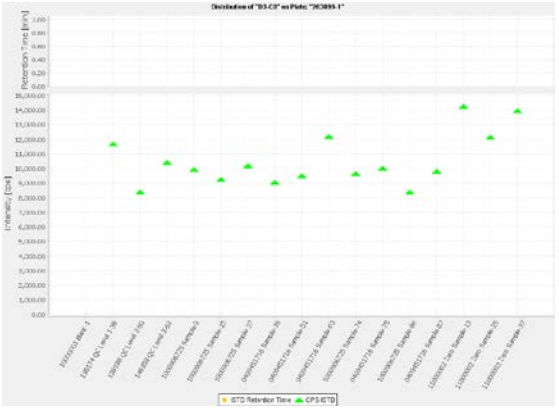
Step	Instructions	Example
	The time period is predefined to 12 months by default in the *Filter options* and can be changed in the Met*IDQ*™ settings (**Settings > MetVAL > QC Monitoring Settings**).	
6	In the **Filter** panel define the analyte and sample type you would like to monitor in the **Trend Chart** view. Graphs will be generated according to this selection.	
7	Select the **STD Trend** tab to obtain an overview of the inter-plate CVs and concentrations of a selected calibration standard level. This tool can be used to monitor the assay and instrument performance over a specified time period.	
8	According to step 5, use the Filter Option to specify a Standard level or set.	
9	In the panel **Filter** define the analyte and calibration standard level you would like to monitor in the **Trend Chart** view. Graphs will be generated according to this selection.	

5.5.4 MetVAL – Analyte Overview Graph



MetIDQ: QC and Calibration Standard Monitoring, Time-Dependent Quality Control

Step	Instructions	Example
1	Select the **Analyte Overview** tab and choose **Analyte** Analyte ISTD. Analyte per analyte the values • Concentration: blue dots • Retention Time: orange quadrangles • Peak Area (LC): green triangles • Intensity (FIA): green triangles are shown for the chosen Kit plate. ***Note:*** No retention times ( ) are available in FIA part.	LC part:  FIA part: 

Step	Instructions	Example
2	Select the **Analyte Overview** tab and choose **ISTD** Analyte ISTD. Analyte per analyte the values • Retention Time: orange quadrangles • Peak Area (LC): green triangles • Intensity (FIA): green triangles are shown for the chosen Kit plate. ***Note:*** No retention times ( ) are available in FIA part.	LC part:  FIA part: 
3	Use filter: choose analyte class or sample type.	

6 Export Results, Data Monitoring and Statistical Data Evaluation

In **MetSTAT** all Kit results can be compiled and displayed. From **MetSTAT** results can be saved directly as Microsoft™ Excel™ worksheet. It can also be saved in other file formats (e.g. .txt, .xml or .csv) for further data evaluation. For data transfer into *MetaboAnalyst* a specific export options are available. If you are using the statistics software "R", please read the Biocrates® user manual "RMetIDQ_1.pdf" which describes how to load the Kit data into "R".

In this section you will learn how to configure a report. First select a Report Context, then choose the samples you want to include and choose the report layout for your data. Finally, you can export the data.

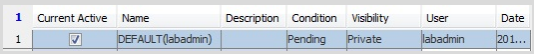
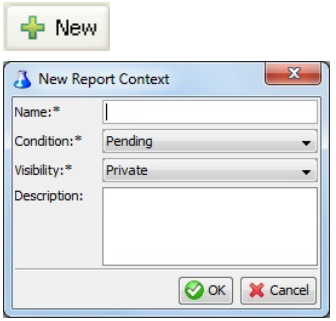
Report Context and Statistical Plots *(MetSTAT > Display Data > Plots)*

MetIDQ: Statistical Plots and Report Context in MetSTAT

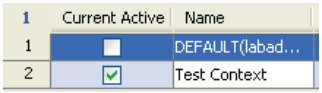
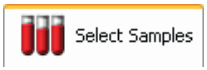
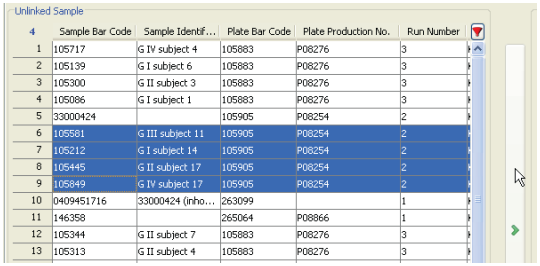
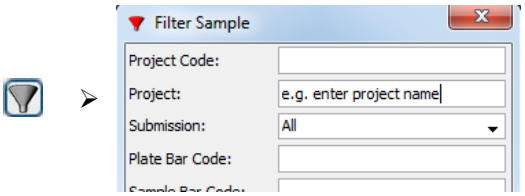
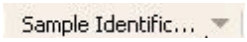
Report Context, Data Normalization and Ratio Explorer

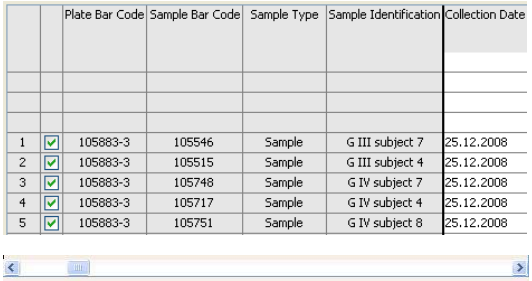
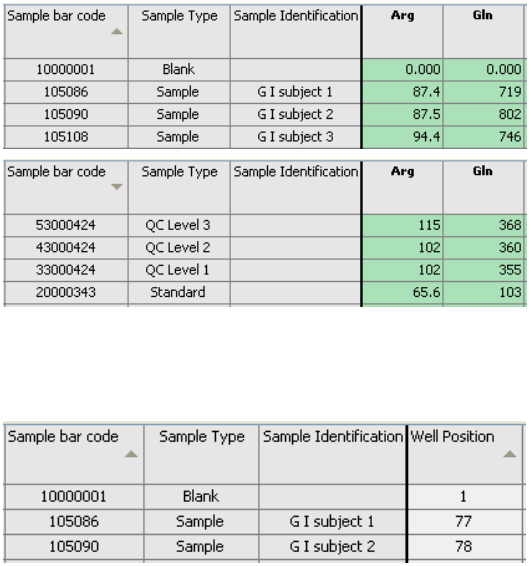
MetIDQ: Report Context, Data Normalization and Ratio Explorer

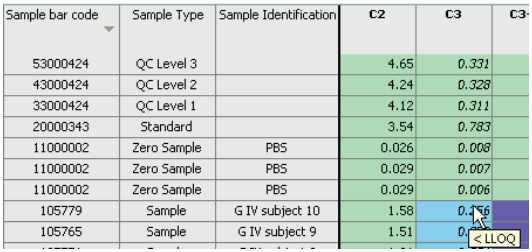
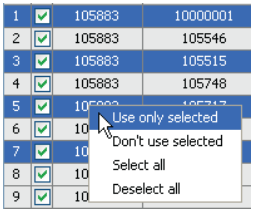
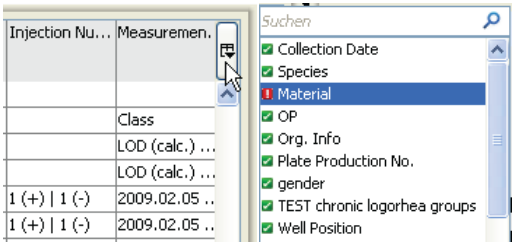
Step	Instructions	Example
1	Click the **MetSTAT** button.	
2	First you have to choose your **Report Context**. In a Report Context all linked samples and your **MetSTAT** settings (including StatPack) will be saved. You can either use the DEFAULT *Report Context* or create a new report context.	

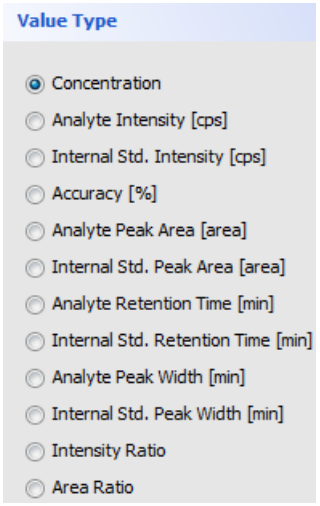
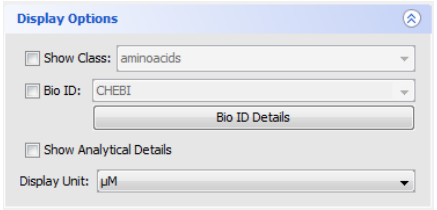
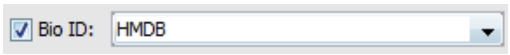
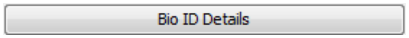
Step	Instructions	Example
	If you are not interested in using the **Report Context** feature, keep the Report Context "DEFAULT(labadmin)" active and continue with step 5 (Select Samples).	
3	To create a new *Report Context,* click **New** and enter a new name. Choose a "Condition" and the "Visibility" for the *Report Context*. The *Description* field is optional.	

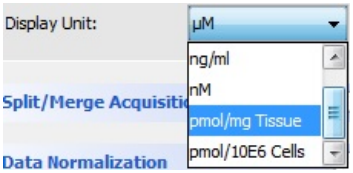
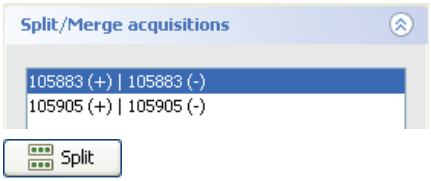
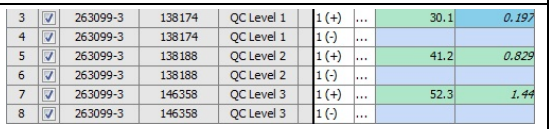
Choice	Explanation
Condition	If the condition is set to Pending, changes that are made will be saved in the *Report Context*. If the condition is set to Approved, changes will not be saved. ***Note:*** if the *Condition* is set to *Approved*, you cannot link or unlink samples in the **Select Samples** tab (steps 5-7). This is only possible when the Condition is set to *Pending*.
Visibility	The visibility can be set to Private or Global. *Private*: The *Report Context* is only visible for the Met*IDQ*™ user who creates it. *Global*: The *Report Context* is visible for all Met*IDQ*™ users.

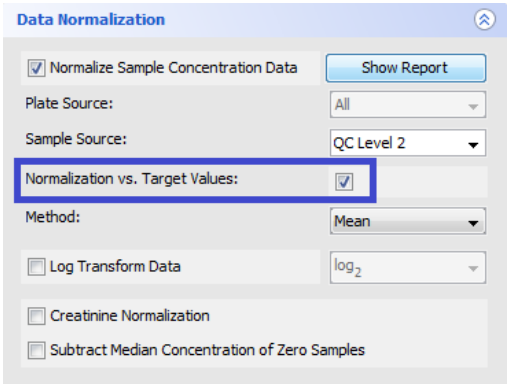
Step	Instructions	Example
4	Activate the checkbox of the Report Context that shall be used.	
5	Next, choose the **Select Samples** tab.	
6	Choose the samples for data evaluation and move them to the **Linked Sample** list using the transfer bar.	
7	You can use the filter before you choose samples. Use the **Filter** button in the upper right corner to open the filter tool. Click on the column header to sort any column. The direction of the grey triangle tells you the sorting order; either ascending (triangle is pointing up) or descending (triangle is pointing down).	  
8	Click on **Display Data** and select the **Data** tab.	

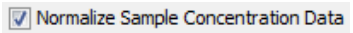
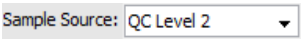
Step	Instructions	Example
9	You will see the sample table including all sample information and metabolite concentrations. Use the scroll bar at the bottom of the window to navigate to the right, showing all the columns in the table.	
10	Change the sort order in any column by clicking in the column header. Here, the column sort criteria for *Sample barcode* has been changed. ***Note:*** You may also sort data by more than one column. Hold the **Ctrl** key pressed and click on the column header which shall have the highest priority, for example *Sample barcode*. Click on the column header you also want to sort, for example *Well position*. The sample table is then sorted by *Sample barcode* then by *Well Position*.	

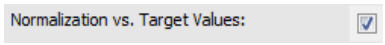
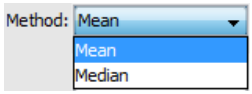
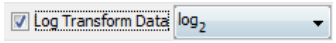
Step	Instructions	Example
	Each sample status has its own color. Drag the mouse cursor over a sample to see the status. Here, the blue color indicates that the value of 0.256 has the status < LLOQ (below Lower Limit of Quantification). Values written in *Italic* are isotope corrected (FIA only).	
11	The rows with active checkboxes will be exported. Press and hold the **Ctrl** key and click on the rows you want to select. Right click to see the context menu and select an option. To hide columns and exclude them from export, click on the column setup button (as shown on the right). Double-click on the column names in the list that you want to deselect. Double-click again will reselect the column.	 

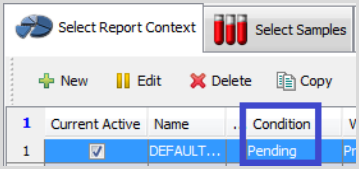
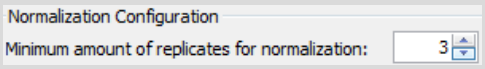
Step	Instructions	Example
12	Value Type Select the data type that is displayed in the main table. The default option is Concentration. Analyte Peak Area, Analyte Retention Time and Internal Std. Retention Time are not available for FIA data.	
13	Display Options Specify analyte class, Bio IDs, Analytical Details and the *Display Unit*.	
	We have implemented a direct link to public available databases like the Human Metabolome Database (HMDB) (http://www.hmdb.ca/) or LIPID MAPS (LMID) (http://www.lipidmaps.org/), which you can select as shown on the right. The Bio ID compound identifiers are then shown in the results table.	
	Available Bio IDs are shown in a table.	

Step	Instructions	Example
	Activate this option to show • LLOQ (Lower Limit of Quantitation) • ULOQ (Upper Limit of Quantitation) • OP (used OP for Kit measurement) in the report table.	
	You can choose between several different display units in the drop-down menu. • Using the Tissue option (see page 57) select “pmol/mg Tissue” • Using the Cells option (see page 58) select “pmol/10E6 Cells”	
14	Split/Merge Acquisitions You can split the positive and negative ion mode injections of each plate (if available, e.g. p180 Kit FIA part) into two rows for each sample in the results table. For this, click on the corresponding acquisition and click **Split**.	
15	Now the sample data is displayed in two rows: one for positive, one for negative ion mode results.	
16	To merge the rows again, select both injections and click **Merge**.	
	When you inactivate the checkbox, the FIA and LC run for each Kit plate will be split (if available, e.g. p180 Kit). You obtain two rows for each sample, one containing the FIA results and one containing the LC results.	

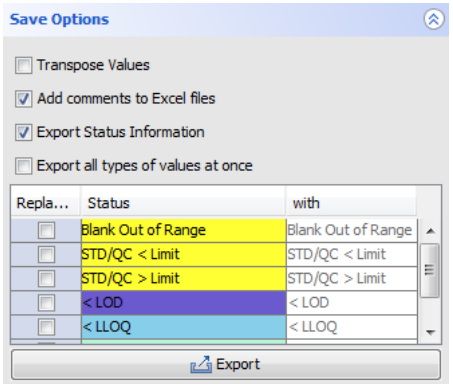
Step	Instructions	Example
!	**Comparability of results generated with different kits:** For best comparability of results generated with different kits, e.g. Quant 500 and p180 Kits, perform a ***Normalization versus Target Values*** of all datasets, using a **Biocrates® QC** as ***Sample Source***.	
17	***Data Normalization*** When measuring several Kit plates, slight inter-plate variations my occur. Therefore, an inter kit plate data normalization is recommended. To normalize a set of data 1. Link all samples (at least samples and QCs) from all Kit plates their data should be normalized to *Select Samples*. For example, create a new *Report Context* as described at the beginning of this section 6. 2. Go to ***Display Data > Data***. 3. Open the tab ***Data Normalization*** (shown on the right) and activate "Normalize Sample Concentration Data". Details to this feature are given below and in 7.1 *Data Normalization*, page 133.	

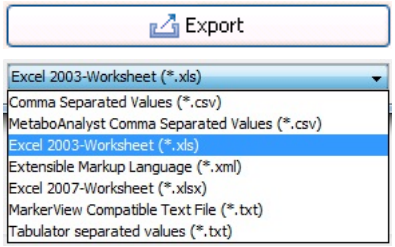
Choice	Explanation
	Activate to perform data normalization.
	Save normalization report as PDF document.
	For normalization select all plates or one specific plate as reference.
	Select a reference sample, e.g. QC Level 2.

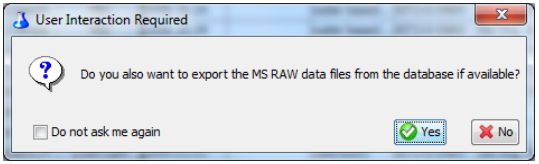
	To perform normalization across different QC batches, use this option, see Appendix 7.1.
Use **Normalization vs. Target Values** if QC from different batches were used.	
	For normalization concentrations form QC samples in replicates, e.g. QC Level 2, are averaged. Use “Mean” or “Median”.
	Perform a log-transformation.
	Used with p150 or p180 Kit for urine samples.

Step	Instructions	Example
	Requirements for Data Normalization: • The currently active Met*IDQ*™ “Report Context” condition has to be “Pending”. • The same sample type which is used for normalization (selected “Sample Source”) must be measured on all loaded Kit plates, e.g. QC level 2. ***Note:*** When **Normalization vs. Target Values** is used, QCs from the same batch are no longer required! • The used “Sample Source”, e.g. QC level 2, has to loaded together with the samples in **Display Data > Data**. • The “Sample Source” has to be measured at least in the number of replicates, that is defined in **Settings > MetSTAT** (see 7.4. Changing Settings in MetIDQ™ Software).	 

Step	Instructions	Example
	Creatinine Normalization: A creatinine normalization is recommended when urine samples were analyzed. For urine samples the Absolute*IDQ*® p180 Kit for Urine or the Absolute*IDQ*® p150 Kit for Urine are required. To perform a Creatinine normalization, link the samples in **MetSTAT > Select Samples**, go to **MetSTAT > Display Data** and activate the checkbox “Creatinine Normalization”. All metabolite concentration values will be divided by the creatinine concentration, sample per sample. Absolute*IDQ*® p180 Kit for Urine: Related samples from the FIA and LC part must be linked together (*MetSTAT > Select Samples*). ***Note:*** The checkbox can only be activated if creatinine was measured with all linked samples.	

Step	Instructions	Example
	Subtract Median Concentration of Zero Samples (recommended for FIA part only) When activating the checkbox, the median concentrations of the zero samples are subtracted from the concentrations of the samples (samples only, not QCs or Calibration Standards). This is an optional feature and should be selected if you have samples with very low metabolic concentration levels, like in cerebrospinal fluid (CSF). It is only active if you have selected the zero samples from the plate you are analyzing (see page 117).	
18	To export data as displayed in **MetSTAT**, use the settings as shown on the right. Two different example reports are given on page 129. Further formatting choices are explained in the table overleaf.	

	Choice	*Result*
	Transpose values	Transpose (interchange) rows and columns.
	Add comments to Excel™ files	Add *Status*, e.g. < LOD, as a comment. For large data exports do not use this option. Otherwise the exported file may not be compatible with old Excel versions.
	Export Status Information	Add *Status*, e.g. < LOD, to .csv or .txt files in an additional column per analyte.
	Export all types of values at once	Includes all "Value Types" (Concentration, Analyte Intensity, Accuarcy, etc.) in one export file.
	Replace values of	You can replace values of a certain status with any text or number for the report. For example, you can replace concentration values with the status < LOD with 0. Activate the checkbox and double click the field cell in the "with" column. Now you can type in a text of your choice. If no text is defined in the cell the concentration values will be replaced by the status (i. e. "< LOD").
19	When you are ready to export the data, click **Export**. You can save the data in different formats as shown on the right. Please find export examples in section 6.1 *Available Report* Formats, page 128.	

	Export of MS raw files: MS raw data files, stored in the Met*IDQ*™ database, can also be exported in this step (LC data are only available if they were imported, see section 5 *Quantitation*)	
	To change the decimal separator symbol, go to the **MetSTAT** settings. See 7.4 Changing Settings in MetIDQ™ Software (page 139).	

6.1 Available Report Formats

Met*IDQ*™ offers different export options (see step 14 above). The export contains all information (e.g. sample concentrations) as displayed in **MetIDQ > Display Data > Data**. Below you will find some export examples. A MetaboAnalyst compatible format is also available.

Standard Report (.csv or .txt)

	A	B	C	D	E	F	G	H	I
1	Concentration µM								
2	Plate Bar Code	Sample Bar Code	Sample Type	Sample Identification	Material	Well Position	C0	C10	
3						Class	acylcarnitines	acylcarnitines	
4						LOD (calc.) 105	3.52	0.096	
5						LOD (calc.) 105	3.31	0.106	
6	105883-3	10000001	Blank		no sample to pipette	1	0.000	Blank Out of Range	0.000
7	105883-3	105546	Sample	G III subject 7	plasma	2	29.8	Blank Out of Range	0.106
8	105883-3	105515	Sample	G III subject 4	plasma	3	29.6	Blank Out of Range	0.127
9	105883-3	105748	Sample	G IV subject 7	plasma	4	24.3	Blank Out of Range	0.059
10	105883-3	105717	Sample	G IV subject 4	plasma	5	24.1	Blank Out of Range	0.075
11	105883-3	105751	Sample	G IV subject 8	plasma	6	25.4	Blank Out of Range	0.061
12	105883-3	11000002	Zero Sample	PBS	water based zero samples	13	1.17	Blank Out of Range	0.032
13	105883-3	105529	Sample	G III subject 5	plasma	14	30.2	Blank Out of Range	0.111
14	105883-3	105532	Sample	G III subject 6	plasma	15	31.6	Blank Out of Range	0.122
15	105883-3	105563	Sample	G III subject 9	plasma	16	32.5	Blank Out of Range	0.112
16	105883-3	105681	Sample	G IV subject 1	plasma	17	23.9	Blank Out of Range	0.077
17	105883-3	105765	Sample	G IV subject 9	plasma	18	22.3	Blank Out of Range	0.055

Microsoft™ Excel™ Report (.xlsx or .xls)

	A	B	C	D	E	F	G	H	I	J	K	L
1	Concentration µM											
2	Plate Bar Code	Sample Bar Code	Sample Type	Sample Ide	Well Position	Sample Volun	Run Number	C0	C10	C10:1	C10:2	C12
3							Class	acylcarnitii	acylcarnitii	acylcarnitii	acylcarnitii	acylcarnitii
4							LOD (calc.) 105	3.52	0.096	0.106	0.017	0.051
5							LOD (calc.) 105	3.31	0.106	0.104	0.033	0.043
6	105883-3	10000001	Blank		1,0	10,0	3,0	0	0	0	0	0
7	105883-3	105546	Sample	G III subjec	2,0	10,0	3,0	29,78405	0,106306	0,057208	0,019279	0,03512
8	105883-3	105515	Sample	G III subjec	3,0	10,0	3,0	29,57153	0,126929	0,046397	0,02038	0,03016
9	105883-3	105748	Sample	G IV subje	4,0	10,0	3,0	24,29767	0,058934	0,045786	0,021144	0,02758
10	105883-3	105717	Sample	G IV subje	5,0	10,0	3,0	24,14804	0,075189	0,046633	0,022291	0,023944
11	105883-3	105751	Sample	G IV subje	6,0	10,0	3,0	25,36758	0,061359	0,043492	0,017171	0,027298
12	105883-3	11000002	Zero Sample	PBS	13,0	10,0	3,0	1,17298	0,032099	0,031342	0,004928	0,015571
13	105883-3	105529	Sample	G III subjec	14,0	10,0	3,0	30,15686	0,110722	0,054459	0,032469	0,035022
14	105883-3	105532	Sample	G III subjec	15,0	10,0	3,0	31,56528	0,122227	0,046707	0,025052	0,03722
15	105883-3	105563	Sample	G III subjec	16,0	10,0	3,0	32,53328	0,111655	0,044359	0,01751	0,036067
16	105883-3	105681	Sample	G IV subje	17,0	10,0	3,0	23,92288	0,077378	0,039763	0,023048	0,025108
17	105883-3	105765	Sample	G IV subje	18,0	10,0	3,0	22,2856	0,054676	0,030346	0,01902	0,023319

Table Formatting Examples

Type	*Description*
Excel™	The color coding as given in **MetSTAT** is shown in the worksheet as well.
.csv .txt	**MetSTAT** color coding is not retained. Additional columns are added to show the status.

Transposed Values Report Examples

Non Transposed (Excel™ Report)

	A	B	C	D	E	F	G	H	I	J	K	L
1	Concentration µM											
2	Plate Bar Code	Sample Bar Code	Sample Type	Sample Id	Well Position	Sample Volun	Run Number	C0	C10	C10:1	C10:2	C12
3							Class	acylcarnitii	acylcarnitii	acylcarnitii	acylcarnitii	acylcarnitii
4							LOD (calc.) 10	3.52	0.096	0.106	0.017	0.051
5							LOD (calc.) 10	3.31	0.106	0.104	0.033	0.043
6	105883-3	10000001	Blank		1,0	10,0	3,0	0	0	0	0	0
7	105883-3	105546	Sample	G III subje	2,0	10,0	3,0	29,78405	0,106306	0,057208	0,019279	0,03512
8	105883-3	105515	Sample	G III subje	3,0	10,0	3,0	29,57153	0,126929	0,046397	0,02038	0,03016
9	105883-3	105748	Sample	G IV subje	4,0	10,0	3,0	24,29767	0,058934	0,045786	0,021144	0,02758
10	105883-3	105717	Sample	G IV subje	5,0	10,0	3,0	24,14804	0,075189	0,046633	0,022291	0,023944
11	105883-3	105751	Sample	G IV subje	6,0	10,0	3,0	25,36758	0,061359	0,043492	0,017171	0,027298
12	105883-3	11000002	Zero Sample	PBS	13,0	10,0	3,0	1,17298	0,032099	0,031342	0,004928	0,015571
13	105883-3	105529	Sample	G III subje	14,0	10,0	3,0	30,15686	0,110722	0,054459	0,032469	0,035022
14	105883-3	105532	Sample	G III subje	15,0	10,0	3,0	31,56528	0,122227	0,046707	0,025052	0,03722
15	105883-3	105563	Sample	G III subje	16,0	10,0	3,0	32,53328	0,111655	0,044359	0,01751	0,036067
16	105883-3	105681	Sample	G IV subje	17,0	10,0	3,0	23,92288	0,077378	0,039763	0,023048	0,025108
17	105883-3	105765	Sample	G IV subje	18,0	10,0	3,0	22,2856	0,054676	0,030346	0,01902	0,023319

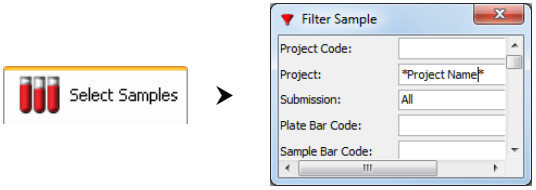
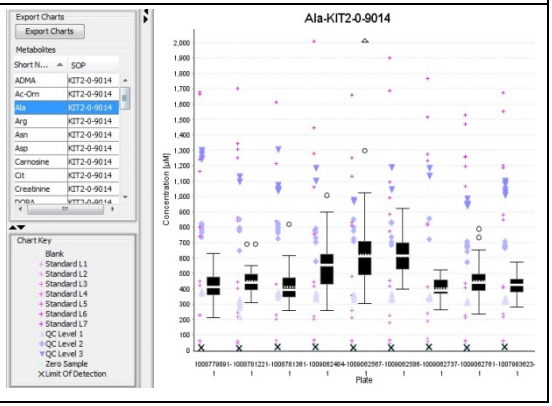
Transposed Values Report (Excel™ Report)

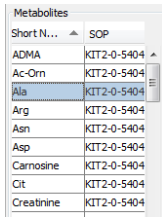
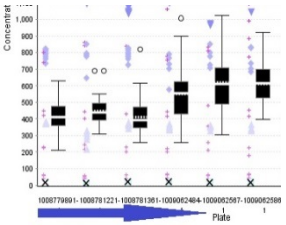
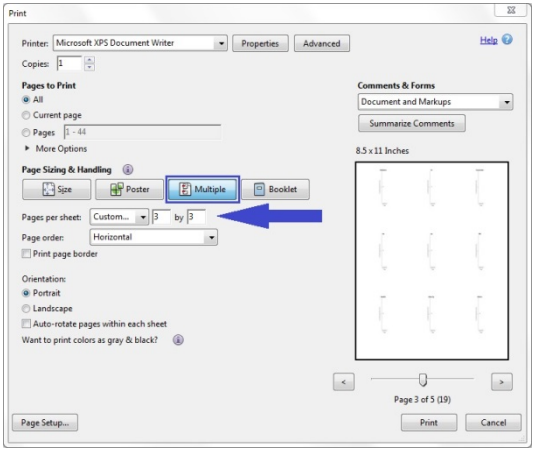
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Concentration µM												
2	Plate Bar Code				105883-3	105883-3	105883-3	105883-3	105883-3	105883-3	105883-3	105883-3	105883-3
3	Sample Bar Code				10000001	105546	105515	105748	105717	105751	11000002	105529	105532
4	Sample Type				Blank	Sample	Sample	Sample	Sample	Sample	Zero Samp	Sample	Sample
5	Sample Identification					G III subje	G III subje	G IV subje	G IV subje	G IV subje	PBS	G III subje	G III subje
6	Material				no sample	plasma	plasma	plasma	plasma	plasma	water base	plasma	plasma
7	OP				KIT1-30-5	KIT1-30-5	KIT1-30-5	KIT1-30-5	KIT1-30-5	KIT1-30-5	KIT1-30-5	KIT1-30-5	KIT1-30-5
8	Well Position				1,0	2,0	3,0	4,0	5,0	6,0	13,0	14,0	15,0
9	Measurem	Class	LOD (calc.	LOD (calc.	2009.02.05	2009.02.05	2009.02.05	2009.02.05	2009.02.05	2009.02.05	2009.02.05	2009.02.05	2009.02.05
10	C0	acylcarnitines	3.52	3.31	0	29,78405	29,57153	24,29767	24,14804	25,36758	1,17298	30,15686	31,56528
11	C10	acylcarnitines	0.096	0.106	0	0,106306	0,126929	0,058934	0,075189	0,061359	0,032099	0,110722	0,122227
12	C10:1	acylcarnitines	0.106	0.104	0	0,057208	0,046397	0,045786	0,046633	0,043492	0,031342	0,054459	0,046707
13	C10:2	acylcarnitines	0.017	0.033	0	0,019279	0,02038	0,021144	0,022291	0,017171	0,004928	0,032469	0,025052
14	C12	acylcarnitines	0.051	0.043	0	0,03512	0,03016	0,02758	0,023944	0,027298	0,015571	0,035022	0,03722
15	C12-DC	acylcarnitines	0.157	0.180	0	0,061962	0,043682	0,065563	0,074902	0,065309	0,047301	0,042651	0,048841
16	C12:1	acylcarnitines	0.204	0.208	0	0,082528	0,07784	0,084575	0,080405	0,071507	0,057443	0,088131	0,068213
17	C14	acylcarnitines	0.032	0.037	0	0,037351	0,044705	0,030699	0,038323	0,040041	0,011276	0,035729	0,042527

6.2 Inter-Kit measurement monitoring

In ***MetSTAT > Display Data > Plots*** the Kit measurements can be monitored over time in order to compare the results from Kit to Kit. For example, plate effects that can be caused by the instrument, Kit preparation, or sample matrix can be visualized here. LOD, Blank, Zero, Calibration Standard, QC, and Real Sample concentrations are displayed analyte by analyte for every chosen Kit plate. Finally, a graphical summary can be created.

YouTube MetIDQ: Statistical Plots and Report Context in MetSTAT

Step	Instructions	Example
1	Go to **Select Samples** and use the filter option to select the Kit runs you want to monitor. ***Information:*** Select a time window (e.g. 2015/01/01 till 2015/12/31) to monitor the Kit measurements stability.	
2	Click on **Display Data** and select the **Plots** tab.	
3	LOD, Blank, Zero, Calibration Standard, QC, and Unknown Sample concentrations are displayed analyte by analyte for every chosen Kit plate. The Unknown Sample concentrations are displayed as black boxplots. The symbols in the graph are explained in the **Chart Key** (lower left side).	

Step	Instructions	Example
4	1 Use the table **Metabolites** to page through all available analytes. 2 Concentration values of all samples per Kit plate (blue arrow) are visualized in the form of box plots in the **Chart Key** pane.	1  2 
5	Use the **Export Charts** button to create a graphical report in PDF format.	
6	The PDF report consists of one graph per page for every analyte. For an overview, it might be useful to print multiple graphs (i.e. multiple analytes) per page. Print multiple graphs: • Open the report with the Adobe® Reader® (version 10 or higher recommended) • File > Print… (Ctrl + P) • Select the number of pages you would like to print per sheet (see screenshot)	

7 Appendix

7.1 Data Normalization – Based on QC Target Values

For data normalization of unknown samples, Biocrates QCs can be used. In order to guarantee a robust data normalization, we recommend analyzing BIOCRATES QC level 2 in replicates, at least of 4. If QCs were run in less replicates than 3, please refer to page 140 to adapt MetSTAT settings. To perform a data normalization, select and link all BIOCRATES QC and unknown samples of the Kit plates in ***MetSTAT > Select Samples***. Be aware that data normalization will change the displayed concentration values. Data normalization can be undone by deactivating the checkbox.

Note: Biocrates® QCs from different production batches can be used.

1. Select a **Plate Source** for normalization.
   All: average values of all linked plates are used for normalization.
   Specific plate: values of the selected plate are used as reference for normalization.
2. Select a Biocrates® QC in the **Sample Source** field.
3. Activate the option Normalize vs. Target Values.
4. For average concentration calculations of replicates, select the algorithm *median* or *mean* for data normalization in the **Method** field. We recommend using the *median*.

- **Data Normalization** does not overwrite original Kit plate concentration data.
- To use non-BIOCRATES QC samples, refer to 7.2.

The following steps describe how the data normalization is carried out by Met*IDQ*™:

a. Analyte by analyte and for every Kit plate, the median or mean values (if above LOD) of the selected sample source are calculated, hereinafter referred to as **A**. For plate 1 the value **A1** is calculated, for plate 2 the value **A2,** and so on.

b. Per analyte and per QC level batch, one concentration *T*arget *V*alue (**TV**) is available in the Met*IDQ*™ database, e. g. TV_{b1} for QC batch 1.

c. The ratio $\mathbf{A_{b1}/TV_{b1}}$ is calculated and represents the correction factor for each Kit plate where QC batch 1 was used, hereinafter referred to as $\mathbf{C_{b1}}$. In detail, $\mathbf{C1_{b1} = A1_{b1}/TV_{b1}}$ is the correction factor for an analyte on Kit plate 1, $\mathbf{C2_{b1} = A2_{b1}/TV_{b1}}$ is the correction factor for an analyte on Kit plate 2, and so on.

The metabolite concentrations are finally normalized by dividing each concentration value by the correction factor $\mathbf{C_{b1}}$, e.g. $c(\text{analyte, plate1})_{\text{normalized}} = c(\text{analyte, plate1}) / \mathbf{C1_{b1}}(\text{analyte})$.

7.2 Data Normalization – Based on Reference Sample

For data normalization of unknown samples, results of a QC sample can be used as reference. In order to guarantee a robust data normalization, we recommend analyzing BIOCRATES QC level 2 or your own QC sample in replicates, at least of 4. If QCs were run in less replicates than 3 please refer to page 140. To perform a data normalization, select and link all QC and unknown samples of the Kit plates in ***MetSTAT > Select Samples***. Be careful as the data normalization will change the displayed concentration values. Data normalization can be undone by deactivating the corresponding checkbox.

1. Select a **Plate Source** for normalization.
   All: average values of all linked plates are used for normalization.
   Specific plate: values of the selected plate are used as reference for normalization.
2. Select a Biocrates® QC or any of your samples in the **Sample Source** field.
3. Deactivate the option Normalize vs. Target Values.
4. For average concentration calculations of replicates, select the algorithm *median* or *mean* for data normalization in the **Method** field. We recommend using the *median*.

- **Data Normalization** does not overwrite original Kit plate concentration data.
- Every sample that was measured with all linked Kit plates (MetSTAT > Select Samples) can be used as sample source for data normalization. This offers the flexibility to use own QC samples.

The following steps describe how the data normalization is carried out by Met*IDQ*™:

a. Analyte by analyte and for every Kit plate, the median or mean values (if above LOD) of the selected sample source is calculated, hereinafter referred to as **A**. For plate 1 the value **A1** is calculated, for plate 2 the value **A2**, and so on. If the selected sample source has been pipetted only once onto a Kit plate, there is only one value per analyte.

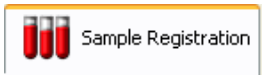
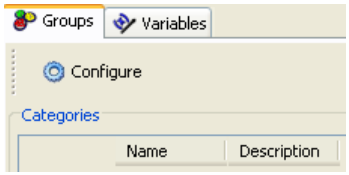
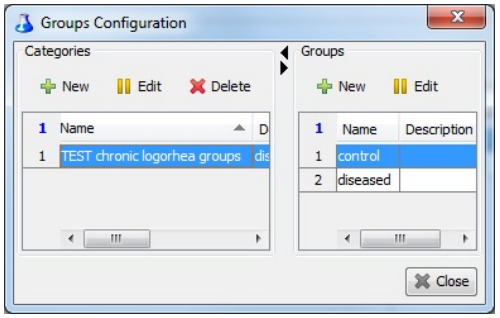
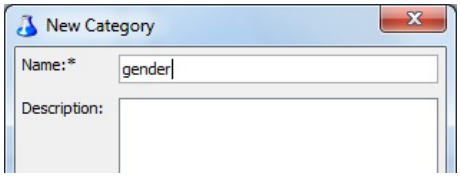
b. Analyte by analyte, the overall median or mean value across all selected plates is calculated, hereinafter referred to as **B**.

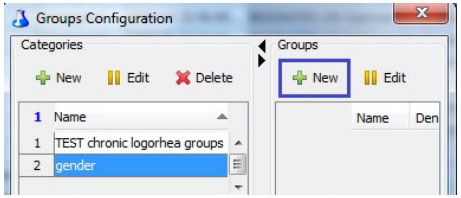
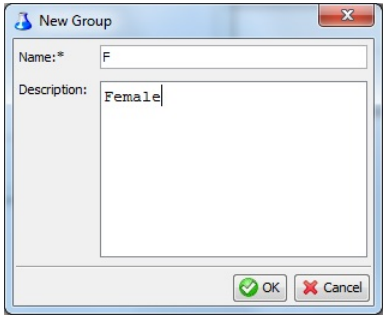
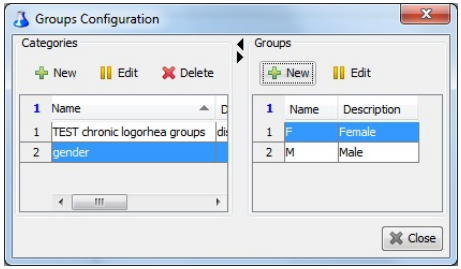
c. The ratio **A/B** is calculated and represents the correction factor for each Kit plate, hereinafter referred to as **C**. In detail, **C1 = A1/B** is the correction factor for an analyte on Kit plate 1, **C2 = A2/B** is the correction factor for an analyte on Kit plate 2, and so on.

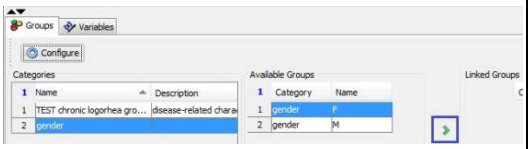
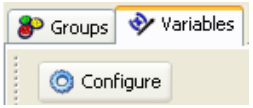
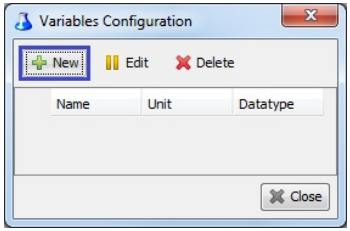
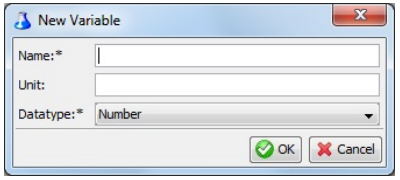
d. The metabolite concentrations are finally normalized by dividing each concentration value by the correction factor **C**, e.g. $c(\text{analyte, plate1})_{\text{normalized}}$ = c(analyte, plate1) / **C1**(analyte).

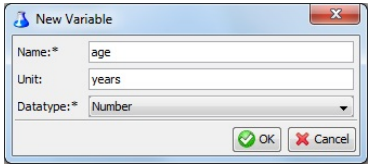
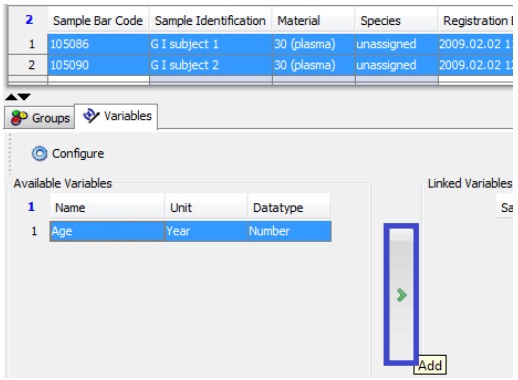
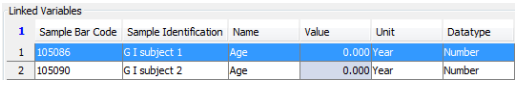
7.3 Define Groups and Variables in MetLIMS

[MetIDQ Project Start: Sample Registration, Groups, Variables]

Step	Instructions	Example
1	Choose the Sample Registration tab in MetLIMS.	
2	Look at the lower panel in the **Sample Registration** window. Click on **Groups** and **Configure**.	
3	You will see the **Groups Configuration** window.	
4	Click **New** in the **Categories** panel.	
5	You will see the **New Category** window. Name the category. Here it is *gender*. The **Description** field is optional. Click **OK** when finished.	

Step	Instructions	Example
6	You will see the new category listed. Click **New** in the **Groups** panel.	
7	In the **New Group** window, add a group that belongs to the selected category. Here, *female* is added as a group in the *gender* category. Click **OK** when finished. Repeat the process to add more groups, e.g. *male*.	
8	This category now has two groups. Category: *gender* Groups:female, male Click **Close**. ***Note:*** there is no limit on the number of groups which can be added to a category.	

Step	Instructions	Example
9	When the categories and groups are defined, you can link them to samples. Select the samples in the sample table and link a group to the samples using the transfer bar. You can also import samples and add group information in one step as described in section 4.1.3.3 (page 42). ***Note:*** there is no limit on the number of groups that can be linked to a sample. However, only one group of each category can be added to a sample. For example, you cannot add *male* and *female* to the same sample, but you can link *male* from the category *gender* and for example *diseased* from another category to the same sample.	
10	To add numeric information (age, amount of treatment etc.) to the samples, define a new variable. Click on the **Variables** tab and on **Configure**.	
11	This will open the **Variables Configuration** window. Click **New**.	
12	You will see the **New Variable** dialogue. Type in a name for the variable. The field *Unit* is optional.	

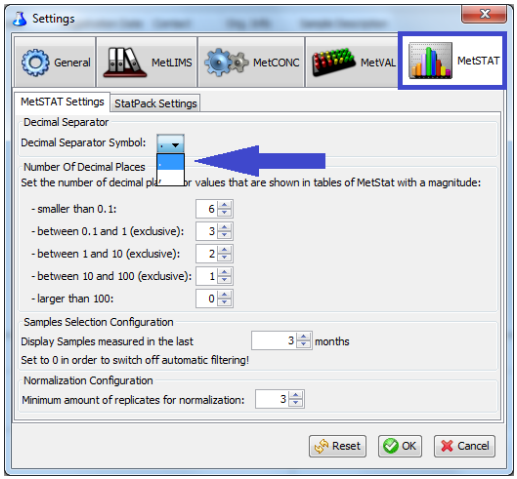
Step	Instructions	Example
13	Choose a data type from the drop-down menu.	
14	Here, the new variable is *age*. The *Unit* is *years*, and of datatype is *Number*.	
15	Next, link the variable to the samples as described in step 9 on page 137. Click on the sample name in the **Linked Variables** list.	
16	The variable *age* is now linked to the samples *105086* and *105090*.	
17	You can change the **Value** by double clicking the cell and typing in another value. Here *65,000* is specified as the new value.	

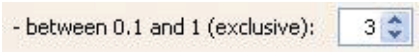
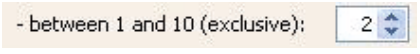
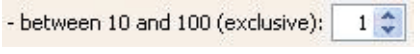
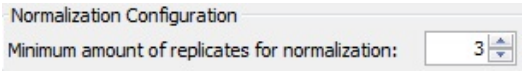
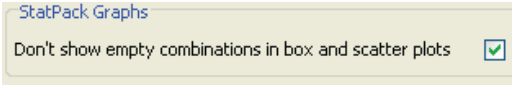
For sample registration: Refer to section 4.1.3, page 33.

7.4 Changing Settings in Met*IDQ*™ Software

For more information watch the following video tutorial:

MetIDQ Toolbar and General MetIDQ Settings

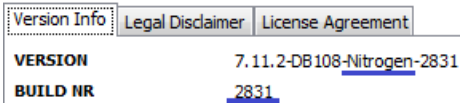
Step	Instructions	Example
1	Click on the **settings** button in the Met*IDQ*™ toolbar.	
2	Click on the **MetSTAT** tab.	
3	Select the decimal separator "**.**" or "**,**" from the drop-down menu. The number of decimal places displayed in **MetSTAT** can be defined blow.	

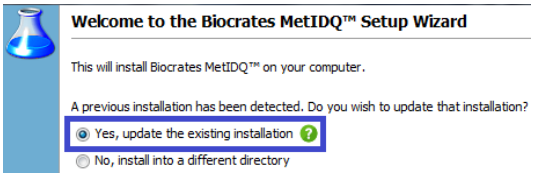
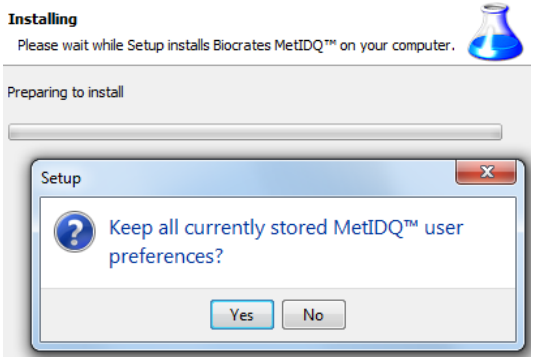
Choice	*Explanation*
	0.081
	0.331
	8.23
	11.0
	893
	Defines the minimum of replicates on every Kit plate for normalization.
	If you activate this checkbox, analytes with missing concentration values (empty combinations) will not be shown in StatPack graphs.
	Defines the path where the “Rscript.exe” is located.

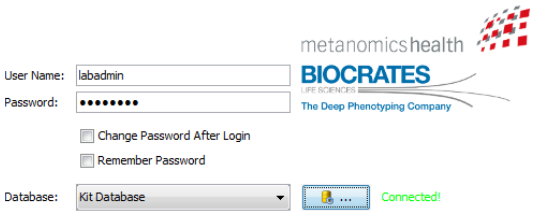
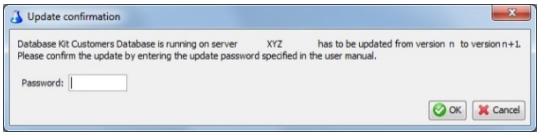
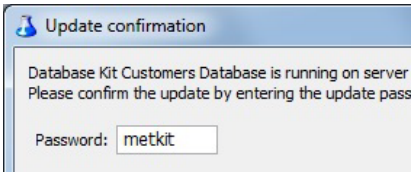
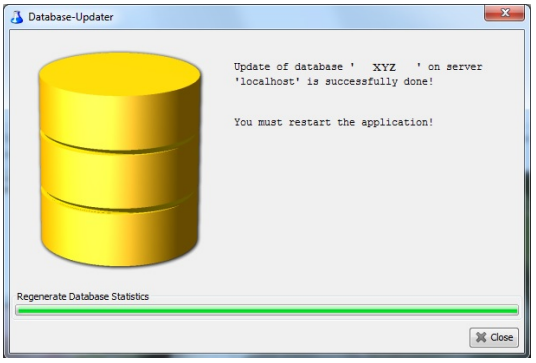
7.5 Met*IDQ*™ Software Update

A Met*IDQ*™ update will be provided with a USB stick shipped with a kit or by our Customer Support. During the update process the current Met*IDQ*™ version will be replaced. To check the current Met*IDQ*™ version and build number click

➤ *Version Info* e.g.



Step	Instructions	Example
1	Double click on the Met*IDQ*™ install file on the USB stick and follow the installation instructions below.	MetIDQ-…-install.exe
2	Select “Yes, update the existing installation”. Follow the instructions of the Install Wizard.	
3	Your current Met*IDQ*™ version will be un-installed and the new version installed. It is recommended to “Keep all currently stored MetIDQ™ user preferences”.	

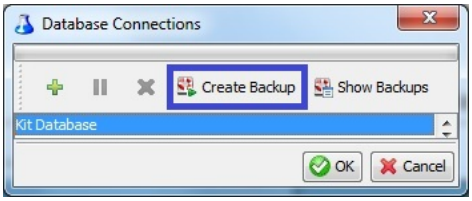
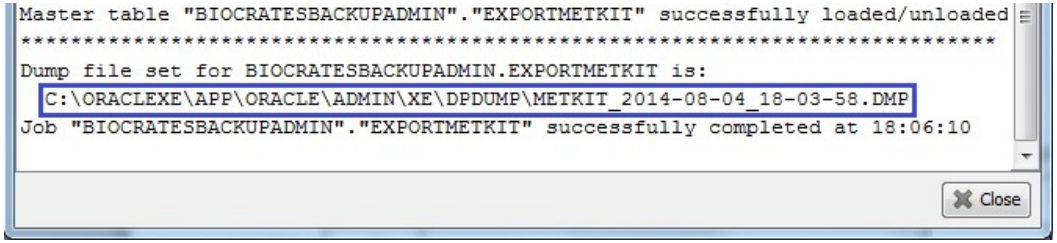
Step	Instructions	Example
4	Start Met*IDQ*™, connect to the kit database (default: “Kit Database”) and log in as user with administrative rights, e.g. Username: *labadmin* Password: *12345678*	
ℹ	If a database update is required, do steps 5 - 8.	
⚠	A database backup is highly recommended as described in section 7.6.1 *Database Backup / Export*, page 143, before starting the database update process.	
5	The password *metkit* is required for the update process.	
6	The update process may take several minutes. ⚠ Never abort the update process!	
7	After the update process close the **Database-Updater** window and restart Met*IDQ*™.	

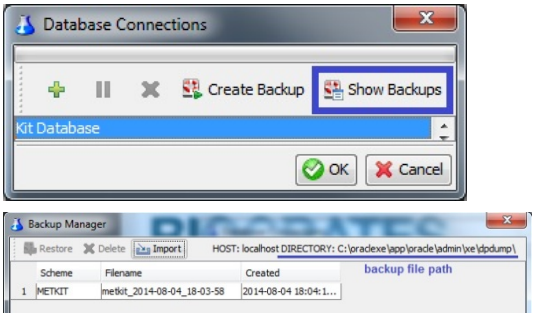
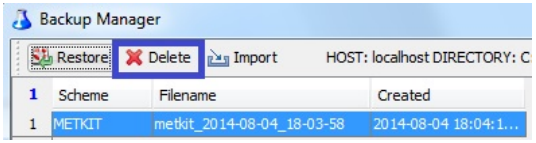
7.6 Backup, Restore, Transfer, Import or Update the Met*IDQ*™ Database

All Met*IDQ*™ data are stored in an Oracle® database. Perform regular backups.

To avoid data loss, **perform regular backups**. Save the backup file (.dmp) at a secure place (network drive, external hard drive, USB stick) physically separated from the PC on which the database is installed.

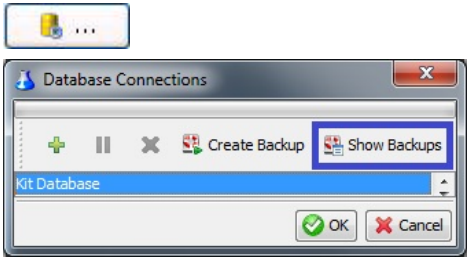
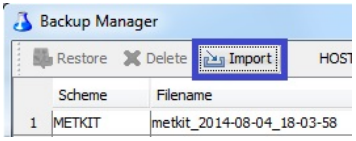
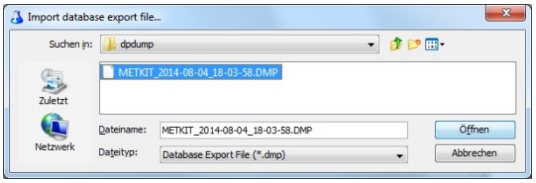
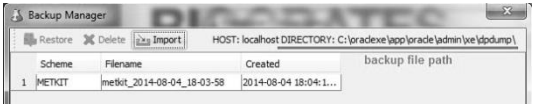
7.6.1 Database Backup / Export

Step	*Instructions*	*Example*
1	To generate a database backup, start Met*IDQ*™ and click on the **database connections** button.	
2	Click the **Create Backup** button. When an User Interaction Window appears, click **Yes**. The backup will be written into an Oracle® dumpfile (*.dmp).	
	This process can take several minutes. When the process has finished, a backup report window opens. The path where the backup file (*.dmp) is located can be found at the end of the backup report window.	
		

Step	Instructions	Example
3	The default backup file location for Oracle® XE is: C:\oraclexe\app\oracle\admin\XE\dpdump The default dumpfile name is: metkit_YYYY-MM-DD_hh-mm-ss.dmp Each backup file can be used for a database transfer to a second PC (see: 7.6.4 Transfer a MetIDQ™ Database to a Second PC) ! For a secure back up copy and **store this file at a secure and physically separated place** from the PC on which the database is installed.	
i	The backup file directory and a list of all back-ups can be found in the **Backup Manager** (open the **Database Connections** window and select “Show Backups”).	
4	To delete a database backup: Select a database backup and click **Delete.**	

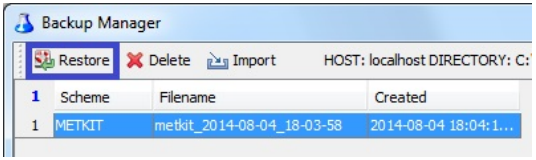
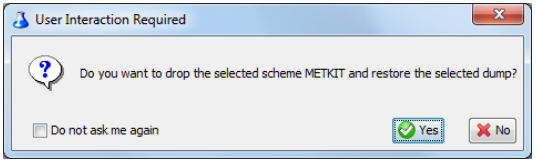
7.6.2 Database Import

Met*IDQ*™ databases can be imported from a local, removable or network drive to the PC where Met*IDQ*™ is installed.

Step	Instructions	Example
1	Open the Backup Manager.	
2	Choose the **Import** button.	
3	Select the Met*IDQ*™ backup file (*.dmp). Click **Open**.	
	You will see the imported backup in the Backup Manager list.	

7.6.3 Restore a Database

This step is only necessary in case data has been lost, corrupted, or if you want to transfer a Met*IDQ*™ database to a second PC (see 7.6.4). You can load the backup file you have generated in previous steps to restore the original database.

Step	*Instructions*	*Example*
⚠	The following procedure will erase your currently used MetIDQ™ database permanently!	
1	To restore a database from a backup, select a database in the **Backup Manager** and select **Restore**. How to import a data base back up from an external source, see section 7.6.2 Database Import.	
2	When the **User Interaction** window appears, click **Yes**.	
3	Enter the database password “*metkit*”. Then, the restore process starts (this may take several minutes).	

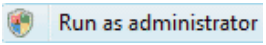
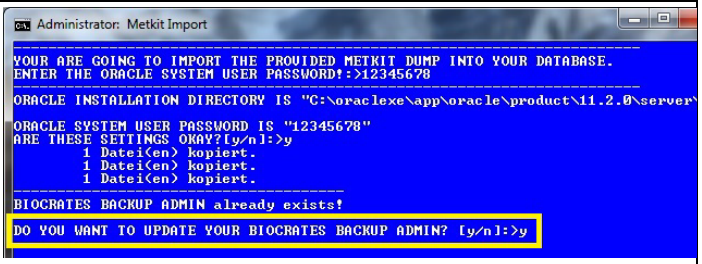
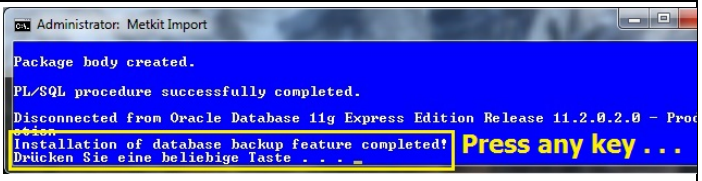
7.6.4 Transfer a Met*IDQ*™ Database to a Second PC

This option can be used to move Met*IDQ*™ and all Kit data to a second PC.

Step	*Instructions*	*Example*
1	Install Oracle® and Met*IDQ*™ on the second PC according to section 2 Install MetIDQ™, page 11.	
2	Perform a database export → 7.6.1 Database Backup / Export, page 143	
3	Import the database on the second PC → 7.6.2 Database Import, page 145	
4	Restore the backup at your new PC → 7.6.3 Restore a Database, page 146	

7.6.5 Update the METKIT Backup Admin

The “Backup Admin” is used to perform METKIT database backups and imports. To keep the “Backup Admin” updated, an update may be necessary from time to time.

Step	*Instructions*	*Example*
1	**Run** “import_metkit.bat”. **as administrator**. The latest version is provided by the Customer Support.	 
2	Type “**y**” (yes) to update BIOCATES BACKUP ADMIN.	
3	When the update process is completed, press any key. The Backup Admin is now updated.	
	The update process does not affect any concentration data in the Met*IDQ*™ database.	

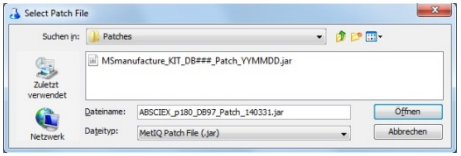
7.7 Import the metkit Database into Oracle® by DBAs

Optional and for advanced users only: If an Oracle® database is already installed, following these instructions. A database administrator (DBA) may be required.

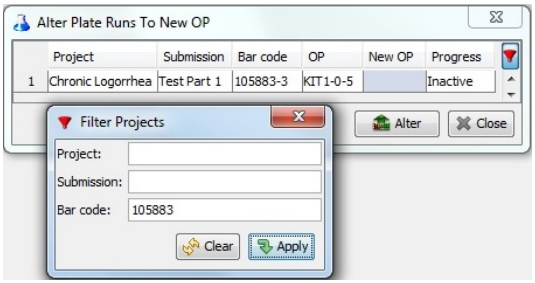
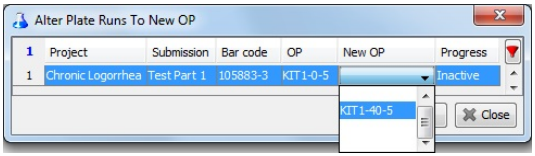
Step	*Instructions*
1	Find the **metkit.dmp** file in the USB stick folder “MetIDQ and Oracle\Installation Files”. This file contains the pre-configured “metkit” schema in tablespace “users”. The default password for the user METKIT is “metkit”.
2	Copy the dumpfile metkit.dmp to the “datapump” directory. e.g. $ORACLE_BASE\admin\$ORACLE_SID\dpdump
3	Import the dumpfile using Oracle® datapump. impdp system/password directory=DATA_PUMP_DIR dumpfile=metkit.dmp schemas=metkit remap_tablespace= users:***Your_Tablespace***

7.8 Installing Database Patches

After installing Met*IDQ*™, database patches can be applied. Follow the steps below:

Step	*Instructions*	*Example*
1	In the Met*IDQ*™ toolbar click **Apply Database Patch.**	
2	Choose the desired database patch. Met*IDQ*™ will load it automatically into the Met*IDQ*™ database. Kit patch’s name example: MSmanufacture_KIT_DB###_Patch_YYMMDD.jar	
3	Click **OK** to complete the operation.	
	Refer to the kit user manual to look up the required Met*IDQ*™ patch(es).	

7.9 Alter Plate Runs to New OP (FIA Only)

Step	Instructions	Example
1	Click on the **Alter plate runs to new OP** button in the toolbar.	
2	Use the **Filter** to select one or more Kit plates.	
3	In the **Alter Plate Runs To New OP** window, double click the cell **New OP** of the plate you want to alter and choose the OP you want to apply.	
4	Click **Alter**.	

7.10 Usage of the Tissue Factor Tool

The Tissue Factor (TF) is defined as the ratio of extraction solvent volume and tissue volume (see equation 1). For this the following assumption is made: 1 mg tissue = 1 µL extraction solvent.

(1) $$\mathrm{TF} = \frac{\mu\mathrm{L\ extraction\ solvent}}{\mu\mathrm{L\ tissue}}$$

In order to calculate the Normalization Ratio (NR) the total extraction volume is taken into account. The Normalization Ratio (NR) is calculated according to equation 2.

(2) $$\mathrm{NR} = \frac{\mathrm{TF} + 1\ \mu\mathrm{L\ tissue}}{1\ \mu\mathrm{L\ tissue}} = \frac{\mathrm{TF} + 1}{1}$$

According to equation 3, the Normalization Ratios are used to calculate the normalized analyte concentration (c).

(3) $c_{normalized}$(analyte) = NR x c(analyte)

Normalized concentrations are displayed in MetSTAT. Navigate to **MetSTAT > Display Data > Display Options > Display Unit** and choose the option “pmol/mg Tissue”. The Tissue Factor normalization tool, which is part of Met*IDQ*™, is described in more detail below.

Example for normalization of tissue extracts:

Select your “Worklist” in the “Project Tree” of **MetLIMS > Projects**. In the section “Well List” you will find the column „µL/mg Tissue Factor“. Value “0” is entered by default and no normalization will be executed. Using diluted samples, please enter a value apart from “0”. For example, if you have added the 4-fold volume of extraction solvent to your tissue, enter “4” into the column “µL/mg Tissue Factor“.

1	Sample Bar Code	Sample Identifi...	Sample Type	Position	Material	Injections	Sample Volume [µl]	µL/mg Tissue Factor
1	10000001		Blank	1	993 (no sample to pipette)	3	10	0.000
2	105086	G I subject 1	Sample	74	30 (plasma)	1	10	4.000
3	105090	G I subject 2	Sample	86	30 (plasma)	1	10	4.000
4	105108	G I subject 3	Sample	3	30 (plasma)	1	10	4.000
5	105111	G I subject 4	Sample	15	30 (plasma)	1	10	4.000

As the tissue volume must be taken into account, the Normalization Ratio (NR) is calculated according to equation (2).

$$\mathrm{NR} = \frac{\mathrm{TF}+1}{1} = \frac{4+1}{1} = 5$$

According to equation 3, all analyte concentrations form sample “G I subject 1” will be multiplied by 5 when selecting the display option „pmol/mg Tissue“ in MetSTAT (see below).

$c_{normalized}$(analyte) = NR x 5

Subsequent data normalization by Tissue Factors:

For subsequent data normalization, please select the corresponding plate in **MetLIMS**, change the “Condition” form “Approved” to “Pending” and click “Delete Measurements”. Afterwards enter the dilution factor values in the column “µL/mg Tissue Factor“. Then set the “Condition” back from “Pending” to “Approved” and go to **MetCONC**. Import your corresponding data files (FIA part) and import the result file (LC part) again. For further assistance please refer to chapter 5.

7.11 Usage of the Cell Normalization Tool

When using cell lysis extracts the analyte concentrations can be normalized to the cell number and the used extraction solvent volume. For this the used number of cells and extraction volume can be defined for each sample on one Kit plate. The functionality of the Cell Normalization Tool is described by an example.

Example			***You see in MetIDQ™***
Sample	Cell Number	Cell Extraction Volume [µL]	
001	1 300 400	50	
002	5 700 100	50	
003	800 600	50	

Cell Number	Cell Extraction Volume [µl]
1300400	50.000
5700100	50.000
800600	50.000

Cell Number and Cell Extraction Volume can be defined in ***MetLIMS > Projects > Well List*** in the corresponding columns. In this manner, each sample extract's analyte concentration ($c_{analyte}$) can be normalized by the Cell Extraction Volume (V_{extr}) and the Cell Number (N_{cells}) according to equation 1. To deactivate this feature please enter "1" in both columns, "Cell Number" and " Cell Extraction Volume [µL]".

(1) $$c_{cells} = \frac{c_{analyte} \cdot V_{extr}}{N_{cells}}$$

For sample #001 and the exemplary $c_{analyte}$ = 1.23 µM this will be:

$$c_{cells}(Sample\ 001) = \frac{1.23\ \mu M \cdot 50\ \mu L}{1300400\ cells} = 47.29\ \frac{10^{-18}\ mol}{cell}$$

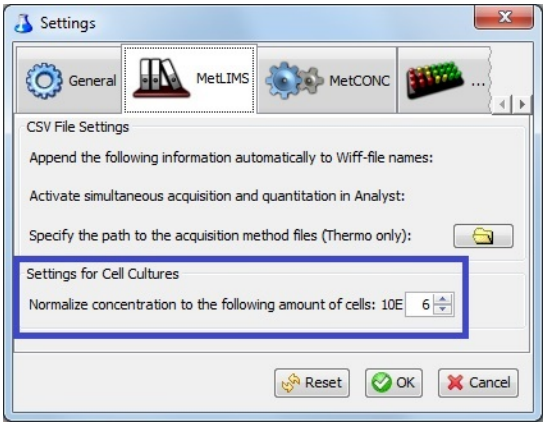
The cell number of the normalized amount of substance (n_{cells}) can be considered, e.g. n_{cells} per 10^6 cells.

$$c_{cells}(Sample\ 001) = 47.29\ \frac{10^{-18}\ mol}{cell} = 47.29\ \frac{pmol}{10^6 cell}$$

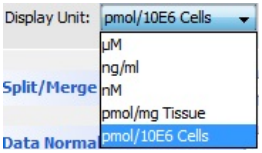
$$c_{cells}(Sample\ 002) = 10.79\ \frac{10^{-18}\ mol}{cell} = 10.79\ \frac{pmol}{10^6 cell}$$

$$c_{cells}(Sample\ 003) = 76.81\ \frac{10^{-18}\ mol}{cell} = 76.81\ \frac{pmol}{10^6 cell}$$

The number of cells to which the amount of substance (n_{cells}) should be normalized can be chosen in **Settings > MetLIMS** (see blue frame on the right).



The Cell Normalization Tool does not affect the analyte concentration unit in the Met*IDQ*™ database (which is µmol/L by default).



Subsequent use of the Cell Normalization Tool:

For the subsequent use of the Cell Normalization Tool, please select the corresponding plate in **MetLIMS**, change the “Condition” form “Approved” to “Pending” and click “Delete Measurements”. Afterwards define the number of cells and extraction volume. Then set the “Condition” back from “Pending” to “Approved” and go to **MetCONC**. Import your corresponding data files (FIA part) and import the result file (LC part) again. For further assistance please refer to chapter 5.

7.12 Database connection troubleshooting

Met*IDQ*™ requires a connection to an Oracle® database containing the user *METKIT*. An inactive connection is displayed in the login screen, as shown below.

If the connection failed, perform troubleshooting.

1. The port used by the Oracle® database is blocked

Port 1521 is used. If Met*IDQ*™ and the database are installed on different computers, please verify that this port is open.

In order to open a port in Windows 10, follow these steps:

1. Using Windows 10, navigate to *Control Panel* > *System* > *Security* > *Windows Firewall*.
2. Click *Advanced settings* and select “Inbound Rules” in the left pane.
3. Right click “Inbound Rules” and select “New Rule”.
4. Add port 1521. Click “Next”.
5. Define rules for port 1521 and the protocols TCP/UDP in the next window. Click “Next”.
6. Select “Allow the connection“ in the next window. Click “Next”.
7. Select the relevant network type(s) and click “Next”.
8. Name the rule and click “Finish”.

↻ Repeat steps 1 – 8 for “Outbound Rules”.

ⓘ Ports may be controlled by another firewall.

2. *METKIT* password expired

After some time, the *METKIT* password may expire. Unlock the database using SQL commands.

- Run the *SQL Command Line*: in Windows go to ***All Programs > Oracle database ##g Express edition > Run SQL Command Line***.
- In the *SQL Command Line*, type the following commands:

  `connect;`

  username: `system`

  password: It is the password which was selected during Oracle® XE installation (see section 2.1 *Install the MetIDQ™ Software*).

  press ENTER

```
SQL> connect
Enter user-name: system
Enter password:
Connected.
```

- You are connected to the Oracle® database. To unlock the database, use the following SQL commands:

```
alter profile DEFAULT limit PASSWORD_LIFE_TIME unlimited;

alter user METKIT identified by metkit;

alter user METKIT account unlock;

commit;
```

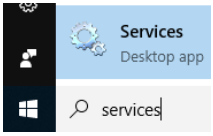
```
User altered.

SQL> _
```

- Restart Met*IDQ*™ and check the database connection.

3. *Oracle® Listener service was not started*

The Windows® service Oracle® Listener may not have been started automatically. To start this service manually, follow these steps:

1. Press the *Windows* key, type in „Services“ and press *Enter*.



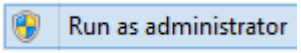
2. Select “OracleOraDB18Home1TNSListener” and click *Start*.

3. Close and restart Met*IDQ*™.

> If the Oracle® Listener was not started during the Met*IDQ*™ installation, the required import of the “metkit” database may have failed. Please proceed with the next issue.

4. *Configure Oracle® for MetIDQ™ - run “import_metkit.bat”*

If the Oracle® Listener was not started during the Met*IDQ*™ installation, the required import of the “metkit” database may have failed. To perform the import, please do the following steps.

Step	*Do this*
1	Find the “**import_metkit.bat**” file on the USB stick. ☞ folder “MetIDQ and Oracle\Installation Files”  import_metkit
2	Right click on the “import_metkit.bat” file and select Run as administrator.  Run as administrator

Step	Do this
3	The database import window opens: `ENTER THE ORACLE INSTALLATION DIRECTORY!` Chose the Oracle® installation directory. By default, this is “C:\Oraclexe”. If Oracle is installed in another directory, enter the correct Oracle installation directory. Press **Enter**.
4	`ENTER THE ORACLE SYSTEM USER PASSWORD!` Enter the Oracle password you chose in section 2.1. Press **Enter**.

5	`YOUR SETTINGS`
	`ORACLE INSTALLATION DIRECTORY IS "c:\oraclexe"`
	`ORACLE SYSTEM USER PASSWORD IS "metkit"`
	`ARE THESE SETTINGS OKAY?[y/n]:>`
	To confirm the settings type “y.
	Press **Enter**.

6	The import process will begin.

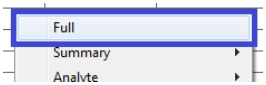
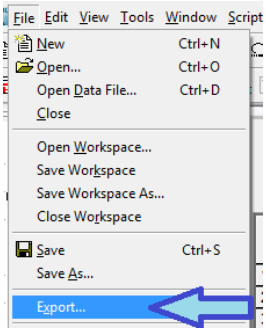
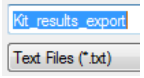
!	The import process may take several minutes. Wait until this message is displayed: “**Installation of database backup feature completed! Press any key . . .**“!
	`Procedure created.`
	`PL/SQL procedure successfully completed.`
	`Disconnected from Oracle Database 18c Express Edition Release 18.0.0.0.0 - Product`
	`Version 18.4.0.0.0`
	`Installation of database backup feature completed!`
	`Press any key to continue . . .`

7	When the import is complete, to close the window press any key.
8	Close and restart Met*IDQ*™.

7.13 Create Analyst® Results of LC Data

This issue applies only for clients using the Analyst® software.
The described procedure replaces the export of LC results as an Analyst® result file (.rdb), described in the corresponding kit user manual.

The LC data quantification procedure is described in the corresponding MxP® Quant 500, p180, Bile Acids, or Stero17 Kit user manual. After LC data quantification using the Analyst® software, export the results in a text-based file (.txt).

Instructions	*Examples*
For results transfer to Met*IDQ*™, export the results: 1. Right-click anywhere in the table and select **Full**. 2. Select **File > Export…** 3. Export the results as “Text Files (*.txt). This .txt file will be imported into Met*IDQ*™ (refer to Met*IDQ*™ user manual).	1.  2.  3. 

7.14 Abbreviations

CSF	cerebrospinal fluid
EMA/EMEA	European Medicines Agency
FIA	Flow Injection Analysis
ISTD	Internal Standard
LC	Liquid Chromatography
LIMS	Laboratory Information Management System
LLOQ	Lower Limit of Quantitation
LOD	Limit of Detection
MS	Mass Spectrometer
OP	Operation Procedure
PBS	Phosphate Buffered Saline
PDF	Portable Document Format
QC	Quality Control
STD	Standard
ULOQ	Upper Limit of Quantitation

7.15 Index

Ordering and Technical Support

Order Absolute*IDQ*® or Biocrates® Kits:

E-Mail:	*Phone:*
sales@biocrates.com	+43 512 579 823

Technical assistance:

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	Dr. Manuel Kratzke	+43 676 848 434 105
	Stephen Dearth, PhD	+1 704 421 6512 (North America)

Website:

http://www.biocrates.com

Video Tutorials:

https://www.youtube.com/playlist?list=PLGETE8vMY-Plp_gSz4eMaSLG1QKB_mdFpk

Frequently Asked Questions (FAQ):

https://support.biocrates.com/tiki-index.php

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