

The Deep Phenotyping Company

Met/DQ[™] Oxygen

User Manual



UM-MetIDQ-Oxygen-9

For Research Use Only. Not for use in diagnostic procedures.

Met*IDQ*[™] Oxygen

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Symbols

Symbol	Description
i	Note: pay attention to the user manual
	Manufacturer
	Attention
<u>1</u>	Comment
Į	Important information

1 Introduction

Biocrates[®] proprietary Met*IDQTM* 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/DQ[™] 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	 Microsoft[™] .NET Framework 4.5.2 or higher Microsoft[™] Visual C++ Redistributables: 2005, 2008, 2010, 2012, 2013 Oracle[®] Database (<i>either</i>) Oracle[®] Database 11g, 12c, or 18c Oracle[®] Database 18c Express Edition (installer provided with the kit) 			
requirements	• Oracle [®] Database 18c Express Edition needs a mini- mum of 10 GB additional free hard disk space			
	Microsoft [™] .NET Framework and Microsoft [™] Visual C++ Redistrib- utable installers are provided with the USB stick and will be installed during the first Met/DQ [™] installation.			



1.2 Software Information

Data is managed and graphically presented using Biocrates' proprietary Met/DQTM software. The Met/DQTM software is a desktop application with two components: Met/DQTM 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/DQTM 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/DQTM 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/DQTM database after data acquisition.



To avoid data loss, **perform regular Met/DQTM 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 O in Met/DQTM

the corresponding section or topic. Click the Help button \checkmark in Met/DQTM or use the links provided in this manual.

Comprehensive and up to date descriptions to $\ensuremath{\mathsf{Frequently}}\xspace{\ensuremath{\mathsf{Asked}}\xspace{\ensuremath{\mathsf{Questions}}\xspace{\ensuremath{\mathsf{can}}\xspace{\ensuremath{\mathsf{bh}}\xspace{\ensuremath{\mathsf{can}}\xspace{\ensuremath{\mathsf{$

Biocrates[®] FAQ section.



2 Install Met*IDQ*[™] and Oracle[®]

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

- 1) Installation of Met IDQ^{TM} and Oracle[®] (onto local PC)
- 2) Import of kit database into Oracle®
- 3) Installation of Windows[™] runtime components

To update Met/DQ[™] 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/DQ^{TM} 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 <u>not</u> already installed. <u>Note:</u> During the installation of Oracle[®] all existing Oracle[®] databases are <u>erased</u>!



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 <i>ID</i> Q [™] (incl. installation and setup)	Full support provided	vided	
Oracle [®] database	No support provided, except initial setup during Met <i>IDQ</i> ™ installation.	ļ	To avoid data loss, <u>perform</u> regular backups according to section 7.6.1.
Data backup and data loss	No support provided		Section 7.0.1.
Software of MS vendor, e.g. Analyst [®] , MassLynx [™] , Xcalibur [™] , MassHunter [™]	No support provided		

2.1 Install the Met/DQ[™] Software

Step	Instructions	Example		
Y	Full administrator privileges are required!			
<u> </u>	Met/DQ [™] and all installation files are provided with the kit USB stick, folder "MetIDQ and Oracle\Installation Files"			
	Met <i>ID</i> Q™ i	nstallation		
1	Run the Met <i>IDQTM</i> installation file with full ad- ministrator privileges. Follow the installation instructions on the screen and below.	MetIDQinstall-64bit.exe Open Image: Run as administrator Pin to Start		
2	A summary of the required software is shown. When Met/DQ [™] is installed for the first time, Oracle [®] XE, the kit database, and Windows [™] runtime components (e.g. C++ redistributable 2013) are installed.	Select Components Which components should be installed? Select the components you want to install; clear the components are ready to continue. Microsoft Visual C++ 2005 Redistributable Microsoft Visual C++ 2008 Redistributable Microsoft Visual C++ 2010 Redistributable Microsoft Visual C++ 2010 Redistributable Microsoft Visual C++ 2012 Redistributable Microsoft Visual C++ 2013 Redistributable		
	Oracle [®] XE installation			
3	A license agreement is displayed. Confirm the default installation directory.			



Step	Instructions	Example
4	Specify an Oralce® database password. <u>Note:</u> 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:	Oracle Database 18c Express Edition Oracle Database Information Specify the database password. This password will be used for SYS, SYSTEM and PDBADMIN accounts. Enter Database Password Confirm Database Password
5	Check the settings and start the installation.	
	Windows™ runtime co	mponents installation
6	Required Windows™ Visual C++ Redistribut- able runtime components are installed one by one.	Select Components should be installed? Microsoft Visual C++ 2005 Redistributable — Select the components should be installed? Microsoft Visual C++ 2005 Redistributable — Please read the following license agreement. Press the PA to see the rest of the agreement. Microsoft Image: Select the components with the set of the agreement. Image: Select the components with the set of the agreement. Image: Select the components with the set of the agreement. Image: Select the components with the set of the agreement. Image: Select the components with the set of the agreement. Image: Select the components with the set of the set

Step	Instructions	Example		
	Import of Kit Database			
7	Enter the Oracle [®] database password, speci- fied in step 4. Click "Next".	Database Configuration Import Kit Database Import Kit Database System User Password ••••••• Path to Oracle XE Installation c: \app\admin\product\18.0.0\dbhomexe		
8	An import dialog is opened. To confirm the set- tings type "y" and press Enter . The import process starts.	VOU ARE GOING TO IMPORT THE PROVIDED METKIT DUMP INTO YOUR DATABASE. ORACLE INSTALLATION DIRECTORY IS "c:\app\admin\product\18.0.0\dbhomexe" DRACLE SYSTEM USER PASSWORD IS "12345678" ARE THESE SETTINGS DKAY?[y/n]:>		
	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 - Production Version 18.4.0.0.0 Installation of database backup feature completed! Press any key to continue			
9	After the import process, close the window by pressing any key.			



2.2 Start the Met/DQ[™] Software

Step	Instructions	Example	
1	Start Met <i>ID</i> Q™.	~	
2	Check the database connection: Click on the connections button .	Iabadmin ENCERATES UF SCENCE The Deep Phenotyping Company Change Password After Login Remember Password Kit Database Image Connected!	
3	Select the "Kit Database". Click " Edit "	Database Connections Database Create Backup Stow Backups St Database	

Step	Instructions		Example		
		nection settings are shown. In local PC (default)	Setup Databa Connection Name:*		
	 Host: Service: Password: Other patting 	<i>localhost</i> or computer name <i>xepdb1</i> for Oracle [®] 18c XE <i>xe</i> for Oracle [®] 11g XE <i>metkit</i>	Service:* User Name:* Password:*		
	Oracle [®] installed o	s, see on right. on server freely selectable			
4	• Host:	IP or DNS name of server; Iocalhost, for local installation	Setup Databa Connection Name:	* <any name=""></any>	
	Service:	SID or service name <i>XE</i> , for local installation		 IP or name of server service ID or "XE" metkit 	
		default <i>metkit</i> default <i>metkit</i> default <i>1521</i>		* ••••• * 1521	
	If Oracle the PC of	local network administrator may b [®] is installed on another PC or a or server in the field "Host". [®] XE is not used, define the used	server, defin		





Step	Instructions	Example
	Connect with the Kit Database.	User Name: labadmin Password: ••••••
5	<u>Default log-in:</u> Username: <i>labadmin</i> Password: 12345678	Change Password After Login Remember Password Database: Kit Database Kit Database Kit Database
6	Request a Met/DQ [™] License. The first time you start Met/DQ [™] , please request a license. Enter your contact information. Mandatory fields are marked with a "*". Click Request License	License MetiDQ D:* #### Company Name:* ##### Street:* Post Code:* Post Code:*

Step		Instructions	Example
	<u>°</u>	A direct license request via internet may not be possible. Please send the license request file (.xml) by e-mail to us.	Do you want to send this request directly to <u>license@biocrates.com</u> ? If not, choose "No" to save the request to a file and send it manually Yes No
7		the license request file (.xml) and send it to se@biocrates.com.	Joint Save File Image: Desktop Image: Desktop
	ŗ	Licenses are generated manually <u>on work-ing days in Austria</u> (Central European Time). Please contact the Customer Support team for your personal licensing date.	File name: license_request.xml Save Files of type: Extensible Markup Language (*.xml) • Cancel
8	• A • Ir • S	I a Met <i>ID</i> Q [™] License. fter receiving a license, start Met <i>ID</i> Q [™] . In the <i>License Window</i> click on "Load License". elect the provided file " license.xml ". tore the license.	Look in: MetIDQ Lizenzen



Step	Instructions	Example	
	Apply a patch. ● Log-in to MetIDQ™		
	 Install a database patch, provided with the USB stick, folder "MetIDQ and Oracle\Patches". 		
9	 Install the appropriate patch for the kit and used cluded in the patch file name: MSmanufacturer_ 		
	<u>Example:</u>		
	Kit: p180		
	MS instrument: SCIEX		
	Patch: SCIEX_p180_DB108_Patch_18	30517.jar	
You	You Tube MetIDQ: User Interface, Barcodes and Patches		
10	For more details how to load database patches please refer to section 7.7.	.	
	Met/DQ [™] uses barcodes, which are provided by linked with samples and plates. The file " barcodes.x together with the license.	ů –	
	Import barcodes.		
11	Open the Settings.	🔀 🍃 🖉 General	
	Select tab General.		
12	Click "Import barcodes".	Import bar codes	

Step	Instructions	Example
13	Click No in the dialogue box.	Info MetIDQ Vour actual number of barcodes is 345: Do you want to retrieve a bulk of bar codes automatically over the Internet? If you press "No", you can load bar codes via a XML file obtained from support@biocrate.com. Yes No
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: <u>license@biocrates.com</u> . Barcodes may be available via internet, if not blocked by network security settings.	Info MetiDQ Your actual number of barcodes is 1,348: Do you want to retrieve a bulk of bar codes automatically over the Internet? If you press "No", you can load bar codes via a XML file obtained from support@biocrates.com. 2a Nein
	To check the number of available barcodes, go to Settings > General . The number of barcodes available is displayed, e.g. 346.	Number Of Available Bar Codes: 346
15	A Laboratory Administrator may create new user profiles and assign user roles based on the Met- IDQ^{TM} tasks the user will perform. Click on the User Admin icon in the Met/ DQ^{TM} toolbar.	82
16	To register a new user, click New in the User Ad- min window	User Admin User Admin Edit X Delete User Name First Name Last Name I labadmin labadmin



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- <i>IDQ</i> [™] .	New User User Name:* First Name:* Last Name:* E-Mail: Enter new password:* Repeat new password:* User Roles Vaboratory Administrator Scientist
	User Role	Tasks
	Laboratory Administrator	Can perform all Met <i>ID</i> Q™ tasks.
	Scientist	Restricted user rights.

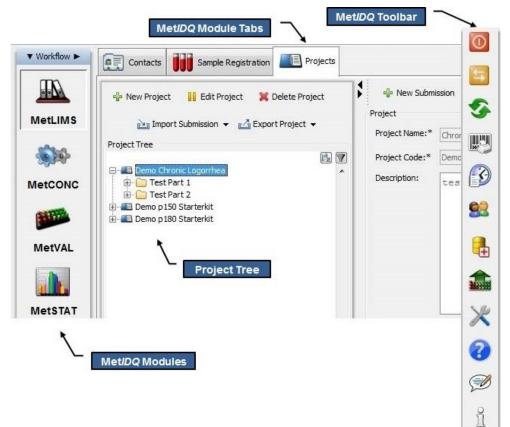
3 Using Met/DQ[™]

This chapter describes all essential and additional functions of Met/DQ™.



MetIDQ: User Interface, Barcodes and Patches

3.1 Met/DQ[™] 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. •	Project Tree
The Sort The Tree button sorts the project tree. Is 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.	Sort The Tree

Explanation	Example
Enlarge or minimize panels by using the black arrows.	Project Name:* Project Code:* Description:
Help button: Link to the Met IDQ^{TM} YouTube video tutorials.	?



4 Processing the Kit

The Met/ DQ^{TM} software supports you during the entire Kit workflow. The table below describes the Met/ DQ^{TM} elements used in order to prepare and to run a Kit.

Element	Short description	
MetLIMS	<u>Laboratory Information Management System</u> . 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 (<u>Operating Procedures</u>) are loaded by database patches (see section 7.7) into Met <i>IDQ</i> [™] 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 ac- quisition	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 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/DQ[™] 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 <i>Company Name</i> and <i>Contact</i> <i>Person.</i>	Contacts
2	Click New in the toolbar.	🐈 New 🔢 Edit 💢 Delete 🔏 Link State
3	The New Contact dialogue box will appear. It is up to you to keep the contact details sim- ple or more detailed. Mandatory fields are la- beled with an asterisk (*). Click OK when finished.	New Contact X Company Name:*

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.	Image: Contact Person Street Image: Contact Person Perso
5	Now you are ready to register a project. Choose the Projects tab. Click New Project in the toolbar.	Projects Project Edit Project Control Control Control Project Project Pr
6	You will see the New Project record win- dow. Choose the contact from the Unlinked Con- tact list. Move it to the Linked Contact list with the transfer bar.	New Project Project Contacts Available Contact Contact Person Street BIOCRATES Life Sciences, Mr. Smith Eduard-Bodem-C
<u>^</u>	The transfer bars (<i>add</i> or <i>remove</i>) are used in all Met <i>IDQTM</i> data transfer windows. To link e.g. a contact, select it by clicking on it. Then use the green arrow to link it.	> or <
7	Click on the Project Members tab to restrict access to the new project for selected Met- <i>IDQ</i> [™] users. By clicking the transfer bar, you can add us- ers to the Linked Project Members list. Note: If no members are linked, everybody will have access to the project.	New Project Project Contacts Project Members Available Project Member User Name First Name Last Name I labadmin labadmin



Step	Instructions	Example
8	Give the project a unique code and name, e.g. <i>Demo Metabolomics</i> . A <i>Description</i> is optional.	Project Code:* Demo Project Name:* Metabolomics
9	You will see the project listed in the MetLIMS project tree. With the Filter button you can filter your pro- jects and submissions.	♥ Filter Projects And Submissions Project Code: Project Name: Active: All Submission: All Plate Bar Code: Plate Bar Code: Matrial: Matrial: Run Time From: Run Time From: Image: Image: </td
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 .	+ New Submission
1	It is generally most convenient to use an indiv	vidual 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.	New Submission Submission Name:* Biocrates Kit Description:
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.	Demo Metabolomics

Step	Instructions	Example
2	Backup and data transfer: You can export and import projects or sub- missions 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/DQ™ Export files (.metidq). To import data, click Import Project / Im- port Submission . Then choose the .metidq file in your directory you want to import to Met/DQ [™] .	 Import Submission Export Project Import Submission Export Project Export Project Export Project Export Submission Export Project Export Submission Export Project Export Submission Export Vorbistion Export Submission Export Project Export Project
		netidq) irrespective of the exported type, <i>project</i> orted as submission and a project only as project.



Step	Instructions	Example			
Ĵ	 Import Worklist: In case you deleted an already measured project or submission by accident, you can regenerate the plate layout (Met/DQ[™] worklist) based on the MS raw files of the measured Kit plate. Select an existing Submission or create a new one Select Import Worklist In the dialog box select the folder containing Kit MS RAW files (e.g. all MS files of an p180 Kit measurement) Select Open >>> A worklist is generated 	 Demo Metabolomics Demo Metabolomics Import Worklists			
	 <i>Example:</i> Met/DQ[™] 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 <i>Import Worklist</i> feature to generate the worklist in Met/DQ[™]. Then import the corresponding MS data via MetCONC. <i>Note:</i> When using the <i>Import Worklist</i> feature, samples that are not available in Met/DQ[™] 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 <i>MetLIMS > Sample Registration</i> using the function "Import", see 4.1.3.1 <i>Import Sample Information</i>. 				

You Tube 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/ DQ^{TM} 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^{TM} 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 Regis- tration tab.	MetLIMS
2	 Find the example file <i>MetIDQsample_import_Example</i> (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 sam- ple information import file. You can import columns like <i>sample identification</i> and columns containing in- formation on groups and variables. The worksheet of the template (screenshot on the right) contains the columns sample identification <i>gender</i> containing the groups <i>male</i> and <i>female</i> age <u>Note:</u> If using a Microsoft [™] Excel [™] worksheet, add a column <i>test</i> after the last column you want to im- port. Put any entry in each row of the column <i>test</i> , e.g. 1.	ABCD1sample identificationgenderagetest2patient 1male4513patient 2male4814patient 3male4415patient 4female5316patient 5female4817patient 6female511

Step	Instructions	Example
3	If you want to import information on groups, have the category's name in the header, e.g. <i>gedner</i> . Type the exact name of each group into the column, e.g. <i>male</i> and <i>female</i> . <u>Note:</u> The fields are case sensitive so the group names must match exactly between the worksheet and Met/DQ TM . Save the worksheet as a .txt or .csv file. <u>Supported delimiter:</u> semicolon ";", tab and comma ","	B gender male male female female female
4	Each category and corresponding groups, as well as each kind of variable must be defined in Met <i>IDQ™</i> before you import your sample information. See MetIDQ section 7.3 Define Groups and Varia- bles in MetLIMS.	Groups Variables Categories I Name I gender gender I gender Gender I gende
5	Click Import to start the sample information import.	🏢 Print bar code 🛛 Export 🚵 Import
6	Choose the .csv or .txt file you want to import.	Image: Suchen jn: Image: MettDQ and Oracle Suchen jn: Image: MettDQ and Oracle Image: Patches Image: Patches Image: Dektop Image: MettDQ cample_import_Example.csv Dektop DateIname: MettDQ cample_import_Example.csv Offnen Dektop DateIname: MettDQ cample_import_Example.csv Dealerhype: Comma Separated Values (*.csv) Abbrechen
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 ","	Import Settings Delimiter ; ; Link colum Tab Available



Step	Instructions	Example
8	Met/DQ [™] will open the file in the Sample Importer window. Available columns are listed in the field File Columns .	Sample Importer Import Settings Delimiter Update existing samples Link Columns Sample Columns Available Columns Sample Identification Sample Identification Sample Identification Registration Date Immodel New New New New
9	Link the data from your spread sheet with the available columns in Met/ DQ^{TM} . 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 \bowtie button located between the two boxes.	Link Columns Sample Columns Available Columns File Columns Sample Columns Sample Run_Number Sample identification gender age test Gender Gender Gender TII ►
10	Linked entries appear in the Links table.	Links Field is Header 1 Pgender = gender
11	Click the Preview button.	66 Preview
12	This information box will appear when the data has been prepared for import.	Info MetIDQ

Step	Instructions	Example
13	The Preview table shows you the layout of the in- coming data. In this example, cohorts are separated into two gender columns.	Groups Variables Sample Identification age 1 male [Age] patient 1 45 2 male [Age] patient 2 48 3 male [Age] patient 3 44 4 female [Age] patient 4 53 5 female [Age] patient 5 48
	Now you can add other sample specific information. Choose them in the Additional Required Infor- mation box. In addition, you can link the samples to a pre-defined contact and submission. No Project : No Submission -	Additional Required Information (applied to all samples) Material: 0 (unspecified) Contact: BIOCRATES Life Sciences AG Submission: No Project : No Submission Species: human
14	You can also choose the sample material from the drop-down menu. <u>Note:</u> 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.	Additional Required Information (applied to all samples) Material: 0 (unspecified) Contact: 0 (unspecified) 1 (Blood) 1 Submission: 10 (blood) Species: 11 (blood spots) 12 (red blood cells) 20 (serum) 30 (plasma) 32 (cell culture supernatant)



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.	Import Import Summary 7 element(s) will be imported. Import Summary Import Summary Concerning Details (7) processed elements (7) imported elements (0) imported concentration columns (0) updated elements (0) not importable elements (0) not importable concentration columns (0) not importable concentration columns (0) not updateable elements (0) not updateable elements
	Existing samples in the Met/ DQ^{TM} 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 con- taining the Met/ DQ^{TM} Sample Bar Codes. In addition, link all columns containing information for the update process.	Import Settings Delimiter Uhy Columns Available Columns Available Columns Available Columns File Columns Sample Bar Code File Columns Sample Bar Code File Columns Sample Identification Preparation Date Sample Description Sample Identification File Column and file column Species Org. Info
16	You will see the samples listed in the Samples table. The samples are now registered and can be used to create a worklist.	1 Sample Bar Code Sample Identification 1 1001791768 patient 6 2 1001791773 patient 5 3 1001791769 patient 4 4 1001791754 patient 3 5 1001791740 patient 2 6 1001791735 patient 1

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.	Sample Registration
•	Click New Sample in the toolbar.	📲 New Sample 📕 Edit Sample 🗶 Delete
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. <i>Note:</i> For the fields <i>Org. Info</i> and <i>Sample Identification</i> you cannot use the following special characters: /\? * : " < >	New Sample 23 Contact: BLOCRATES Life Sciences AG - Mr. Smith Species: human Material:* 0 (unspecified) Org. Info: Sample Identification: Sample Description:
	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.	0 (unspecified) 20 (serum) 30 (plasma) 301 (spleen tissue cells)		
	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.		
	Sample Identification	Only this information will be shown in the plate report, worklist and as- say results table.		
	Sample Description	In this field you can add any information.		
Step	Instructions	Example		
4	If you are using the <i>Zebra TLP 2824 Plus</i> barcode printer you can print barcodes. Define the number of printed barcodes. The default number is 0.	Print bar code: 0 🗘 🖉 OK 🗶 Cancel		
5	Click OK when finished. New sample are shown in the Samples list. To add more samples, click New Sample again.			

Step	Instructions	Example
		▼ Filter Samples
		Project Code:
		Project Name:
	To search for samples, use the Filter – button in the upper right corner	Plate Bar Code:
		Sample Bar Code:
		Sample Description:
6		Material: All
Ū		Species: All 👻
		Org, Info: All 🔻
		User:
		Contact: All Registration Date From:
		Registration Date To:
		Variable: All
		Group:
		Not measured only:
		Clear Apply
10	After registering all samples for the assay, create a	project worklist and a plate report.
	See MetLIMS section 4.1.4 Generate Plate Layout	



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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.	Projects
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).	⊡■ Demo p 180 Starterkit ⊡
3	Right-click on a submission (e.g. "User Concentra- tion Data") and select Import Data .	E Demo p150 Starterkit Demo p180 Starterkit Demo p180 Starterkit Demo p180 Starterkit Degree Degree Paste Derived Worklist Delete Delete Import Worklists Import Data Export Submission
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".	Comma Separated Values (*.csv) Comma Separated Values (*.csv) Text file (*.txt)

Step	Instructions	Example
	 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. 	Sample Ingorter Prot Stitling Delane Xnalific Colores Xnalific Colores Xnalific Colores Protocolores Protocolore Sample Ingorter Sample Ingorter
5	 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"). 	Sample Importer Import Settings Deliniter Sample Cound Sample Cound Oncentration Columns I Name 1 Name 2 Material 3 Species 4 gender :F 5 gender:H 5 gender:H 6 TEST drivnic logorhea groups:desa 7 TEST drivnic logorhea groups:control I New I New I Name 1 Agender:F 5 gender:H 6 TEST drivnic logorhea groups:desa 7 TEST drivnic logorhea groups:control I New I New I New
ĺ	All concentration values are automatically imported w	rith the unit μM.
6	Select Preview and Import according to section 4.1.3.1. Analytes which are not part of the Met/ <i>D</i> Q [™] database are listed in the Import Summary in the section "not importable concentration columns".	Import Summary Import Summary 7 element(s) will be imported. Import Summary Import



Step	Instructions	Example
1	When importing analytes which are not part of the database you will be asked to submit them to the BI-OCRATES Support Team. We will generate a Met- IDQ^{TM} 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.	Biocrates: Customer Support Mal Some molecules are not registered in the database. Pose and the report to the support hom. We will end you a patch which loads the report to the support hom. We will end you a patch which loads the remove of free and extra power can be been deals of this report to a mail and send it to 'supportBloods.com' Bediessen Pease send me a patch for the following missing molecules: Example In Zwischensblage kopieren
7	User specific data are now loaded to a newly gener- ated worklist (OP: DATA-0-1) which belongs to the selected submission. Every sample is linked to an in- dividual well position.	Demo p 180 Starterkit Demo p 180 St
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 > Dis- play Data (see page 117).	1 Plate Bar Code Run Number Run Time OP Type OP 1 645993 1 5114.50.08.10:22.4 CMS DATA-0-1 Image: Copy Paste derived plate Delete Delete Show Validation Data Show Statistics Data

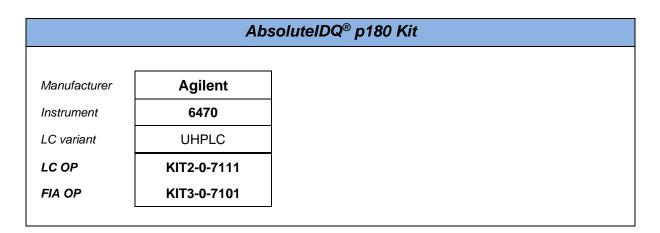
4.1.4 Generate Plate Layout and Worklist for MS run

You Tube MetIDQ Project Start: Worklist Generation

Step	Instructions	Example
	Open a project in the Project Tree. Choose the sub- mission (created in section 4.1.1).	*
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	Available Samples
	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.	3 105108 G I subject 4 30 (plasma) Unassigned 4 105111 G I subject 4 30 (plasma) Unassigned 5 105125 G I subject 5 30 (plasma) Unassigned 6 105139 G I subject 6 30 (plasma) Unassigned 7 105142 G I subject 7 30 (plasma) Unassigned 8 105156 G I subject 8 30 (plasma) Unassigned 4 105142 G I subject 7 30 (plasma) Unassigned 4 105156 G I subject 8 30 (plasma) Unassigned 4 105142 G I subject 8 30 (plasma) Unassigned 4 105156 G I subject 8 30 (plasma) Unassigned
<u>°</u>	To distinguish between samples that are already linked with a worklist and those that are not, use the filter option "Already in Worklist".	Filter Samples Already in Worklist: Sample Bar Code: All Sample Identification: Used
2	Click Generate Worklists.	Generate Worklists
3	You will see the Select OPs window first. Please refer to the lists below to select the appropri- ate OP for your Kit and MS instrument.	Select OPs Available OPs Available OPs I OP Type Short Name OP Code Material Version Number I LC type Kit and MS type Kit name 0 (unspecified) OP number



		AbsoluteID	Q [®] p180 Kit			
Manufacturer			SCIEX			
Instrument	4000 series	4500	series	5500 s	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	SCI	EX	SC	IEX		
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		Thern	no Fisher	
Instrument	TQ-S	TQ-S micro	TQ-XS	TSQ V	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 [®] 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		
					·		



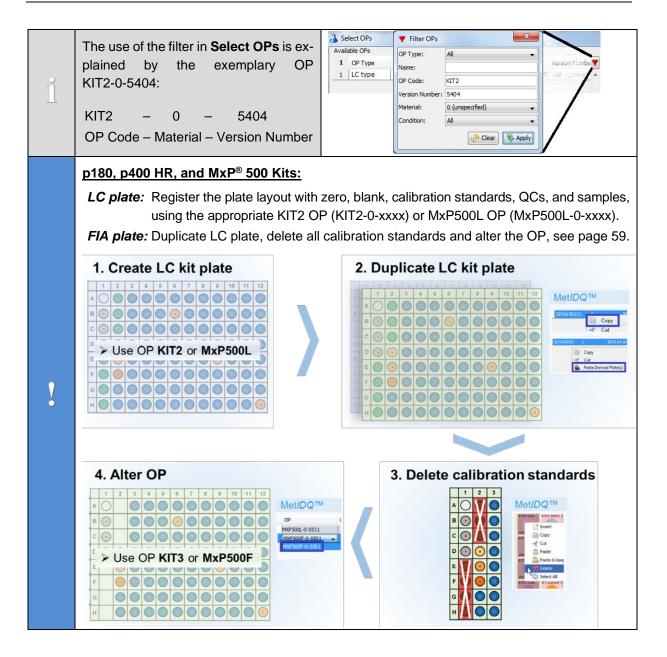
		MxP [®] Quant 500	Kit				
Manufacturer	SC	IEX		SC	IEX		
Instrument	5500 series			6500 series			
LC variant	variant UHPLC					HPLC	
LC OP	LC OP MxP500L-0-5511		M	MxP500L-0-5611 Mx		P500L-0-5601	
FIA OP	MxP500F-0-5501 MxP500F-0-5501		M	xP500F-0-5601	MxP500F-0-5601		
	SC	IEX		Waters			
	6500+	series		Xevo [®] TQ-	S		
	UHPLC	HPLC		UHPLC			
	MxP500L-0-5711	MxP500L-0-5701		MxP500L-0-8	111		
MxP500F-0-5701 MxP500F-0-5701		MxP500F-0-8101					

AbsoluteIDQ [®] Stero17 Kit				
Manufacturer	SC	IEX	Water	s 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	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			rmo Fisher
Instrument	TQ-S	TQ-S micro	TSC	Q Vantage™
LC variant	UPLC	UPLC		UHPLC
OP	BA02-0-8112	BA02-0-8212	BA	02-0-9012





Step	Instructions	Example
4	Transfer the selected OP to the Linked OPs list with the transfer bar, e.g. KIT2-0-5404.	Select SOP: Available SOP: 1 0P Type: Short Name 0P Code: Metrial Version Number 1 LCMS p180 - AB SCIEX 4000 - IPAC: KI12 0 (unspecified) 5404 2 LOHS p180 - AB SCIEX 4500 - UPAC: KI12 0 (unspecified) 5404 3 LCMS p180 - AB SCIEX 5500 - HPAC: KIT2 0 (unspecified) 5504 4 LCMS p180 - AB SCIEX 5500 - HPAC: KIT2 0 (unspecified) 5514 5 LCMS p180 - AB SCIEX 6500 - HPAC: KIT2 0 (unspecified) 5504
5	Click Next.	C Next
6	You will see the second worklist wizard. Here, you can configure the plate layout.	Pipetting Mode Position Settings Protion All Image: Comparison of the set of the
7	The table overleaf explains the three options.	Pipetting Mode Horizontal Vertical Machine-made
	Choice	Explanation
	Horizontal	The plate will be pipetted horizontally.
	Vertical	The plate will be pipetted vertically, de- fault 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.	Position Settings Randomize Samples



Step	Instru	ctions	Example
9	corresponds to the num	The number in brackets ber of samples that are the table below for more	ij Blanks (1) 🔃 Zero Samples (3)
	Tab	Choice	Explanation
	ijj Blanks (1)	This is pre-configured and cannot be changed.	Well A1 on the Kit plate does not have internal standards. It is called <i>Blank</i> . This blank well is used to determine the background counts and to check your system for contaminations.
	🔀 Zero Samples (3)	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	Instru	ctions	Example
10	Calibration standards and QCs are already registered in MetLIMS .		1 Standards (0) 🛛 🔢 QC-lots (0) 🛛 🚻 Samples (26)
11	Click on the Standards tab.		🔣 Standards (0)
1	Standards are used for LC assay.	C-MS/MS analytics only. No	Standards will be linked in a FIA-MS/MS

Step	Instructions	Example
12	Select the set of calibration standards of your Kit in the Unlinked Standards list and then use the trans- fer bar to move the standards to the Linked Stand- ards list. Make sure that the sample barcodes of the selected standards match the barcodes on the standard vials included in the Kit!	IIII Blanks (1) IIII Zero Samples (3) IIII Standards (0) IIII QC-lots (0) Available Standards 1 Sample Bar Code Sample Type Sample Identification 1 ###### IIII Standard L1 KIT Cal1 2 ###### IIII Standard L2 KIT Cal2 3 ###### IIII Standard L3 KIT Cal3 4 ###### IIII Standard L4 KIT Cal4 5 ###### IIII Standard L5 KIT Cal5 6 ###### IIII Standard L6 KIT Cal6
13	Click on the QC lots tab.	🔃 QC-lots (0)
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!	Image: Blanks (1) Image: Zero Samples (3) Image: Standards (0) QC Pools Image: QC Pool Name Material 1 Name 30 (plasma) Image: QC Pool Name 2 Name 30 (plasma) Image: QC Pool Name 3 Name 30 (plasma) Image: QC Pool Name 3 Name 30 (plasma) Image: QC Pool Name 4 Mame 30 (plasma) Image: QC Pool Name 3 Name 30 (plasma) Image: QC Pool Name 4 Mame 30 (plasma) Image: QC Pool Name 5 QC Hots (0) Image: QC Pool Name Samples (0) Available QC Lots 1 Sample Bar Code Sample Type 1 Sample Bar Code Sample Type Sample Identification 1 ####### Image: QC Level 1 Kit name - QC1 2 ######## Image: QC Level 2 Kit name - QC2 3 ####### Image: QC Level 3 Kit name - QC3
15	Now QCs are linked to the submission. Distribute Replicates Recommendation: run a QC2 replicate after every 20 th sample. Define the number of replicates accord- ingly. 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.	Linked QC-Lots 1 Distribute Replicates Sample Bar Code Sample Type Sample Identification 1 1 1 annaea III QC Level 1 QC type - QC1 2 V H & statase IIII QC Level 2 CC type - QC2 3 1 1 Timmed. III QC Level 3 OC type - QC3 tes Replicates Sam 1 ### 1 ############################



Step	Instructions	Example	
	Example: Distribute QC replicates equally over a K	it plate	
	By activating the function "Distribute Replicates" the uted equally over all linked samples on one Kit plat licates over one Kit plate, please calculate the numb on the Kit plate (79 on the plate below). When link generate an additionally Kit plate automatically.	e. If you would like to distribute 4 QC rep- per of available positions for your samples	
	Please keep in mind:		
	 The number of QC replicates does not autoplate is generated. 	matically increase when more than one Kit	
	 The last well position is always a QC replicate, if "Distribute Replicates" is activated. 		
1	Bank p180 Cal5 G1 subject 3 G1 subject 1 G1 subject 3	t i G III subject G III subject G III subject G IV subject I G IV	
	PBs p180 Cale G I subject 4 G I subject 2 D IS0-MetaDe G II subject 3 13 14 15 18 17 18 15 11000002 2000006.6 155111 150274 493A61 15421 PB p160-Cele Coll Subject 1 5 Subject 2 105.74 493A61 154.21	1 G III subject G III subject G III subject G IV subject G IV subject 20 21 22 23 24 105501 105551 1055734 105814 1 G III subject G III subject G IV subject	
	25 27 28 29 30 31 105356 105356 105356 105356 105356 105356 105356 105356 105356 105356 105356 105511 985 9160-MaidaDi G 5 subject 6 G 5 subject 6 G 5 subject 1 G 10 subject 2 G 10 subject 9 G 10 subje	33 33 34 35 36 105515 105594 10564 105746 105821 1 G III subject G III subject G III subject G IV subject	
	37 38 35 40 41 42 42 11000002 403393 105130 105212 105361 105445 p100 Call epi80 Magded G is subject 7 G is subject 1 G is	41 45 41 67 45 105529 105607 105731 105835 1 6 GU Subject p180-Headbe G IV subject G IV subject G IV subject	
	49 50 51 52 53 54 55 20000611 493401 105142 10526 105305 105375 108499 p189 242 p189 1053142 10149 <td< th=""><th>56 57 58 59 60 105532 493491 105681 105765 105849 14 G III subject G IV subject G IV subject G IV subject 56 97 70 71 72</th></td<>	56 57 58 59 60 105532 493491 105681 105765 105849 14 G III subject G IV subject G IV subject G IV subject 56 97 70 71 72	
	20000612 492415 105156 105220 105313 105316 105462 p169 Ca3 G1 solject 1 G1 solject 9 G1 solject 1 G1 solject 3 G1 solject 1 G1 solject 3 G1 solject	105546 105616 105675 105779 105852 1 6 III subject G III subject G IV subject G IV subject G IV subject 100 81 82 82 83 10 10 10 10 10 10 10 10 10 10 10 10 10	
	20000613 105040 105140 105327 105372 105376 p180 C44 G1 subject 2 G1 subject 3 G1	105550 105620 105763 105782 105666 G G III wajser G III wajser G III wajser G III wajser 105782 105666 105582 105633 105777 105796 493408	

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.	Bamples (26)
17	All samples linked with a submission are shown. Fur- ther samples can be added. Injection replicates per sample can be defined. Choose the sample from the Linked Samples list. Double click on the <i>Replicates</i> 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 col- umn and enter the value	Linked Sample ba Sample Id Replicates Sample Vo Material 1 33000423 1 10 30 (plasma) 2 43000423 1 10 30 (plasma) 3 33000423 1 10 30 (plasma) 3 3 3000424 1 10 30 (plasma) 4 33000424 1 10 30 (plasma) 6 43000427 1 10 30 (plasma) 6 43000427 1 10 30 (plasma) 6 3000427 1 10 30 (plasma) 7 53000427 1 10 30 (plasma) 9 43000428 1 10 30 (plasma) 9 43000428 1 10 30 (plasma) 30 (plasma) 30 (plasma) 9 43000428 1 10 30 (plasma) 31 10 30 (plasma) 1 1 1 10 30 (plasma) 33 1 1 1 1 1 1 1 1 1 1 1 1 1
ĺ	The Sample Volume corresponds to the volume you pipette onto the Kit plate. This is pre-defined by the selected OP and normally 10 μ L. Based on this volume, the final metabolite concentrations will be calculated by Met/DQ TM . Before you change the pre-defined value, please contact our Customer Support.	Sample Volume [µ] Sample Volume [µ] Sample Volume [µ] 10 30 30 10 10
18	If you want to see the plate layout before you gener- ate the final worklist, click on the Positioning tab at the bottom of the window.	Selection Posit ring



Step	Instructions	Example
	The plate layout will be displayed based on your specifications. Specific colors are used for each sample type.	Image: Note of the second se
19	An example plate layout as overall positioning list is shown on the right. You can move samples from one well to another us- ing the standard drag-and-drop technique. The tab below illustrates this procedure.	Overall Positioning iiji 1 P1: 1: 10000001 () iiji 2 P1: 13: 11000002 (PBS) iiji 3 P1: 25: 11000002 (PBS) iiji 4 P1: 37: 11000002 (PBS) iiji 5 P1: 49: 20000611 (p180 Cal1) iiji 6 P1: 61: 20000612 (p180 Cal2) iiji 9 P1: 22: 20000614 (p180 Cal3) iiji 9 P1: 85: 20000614 (p180 Cal3) iiji 10 P1: 14: 20000615 (p180 Cal5) iiji 10 P1: 14: 20000615 (p180 Cal5) iiji 10 P1: 14: 20000615 (p180 Cal5) iiji 11 P1: 26: 20000617 (p180 Cal7) iiji 12 P1: 38: 493393 (p180-MetaDis QC1) iiji 15 P1: 74: 105086 (G I subject 1) iiji 16 P1: 86: 105090 (G I subject 1) iiji 16 P1: 81: 05108 (G I subject 3) iii 18 P1: 75: 105111 (G I metert 4)
	 To move a sample on the plate: Move the mouse pointer to the sample well you want to move. Hold down the left mouse button. Drag the sample to a new position on the plate. You will see the mouse pointer over the shape. Click Yes to make the change. 	G IV subject 38 105852 User Interaction Required Vou have moved a well on this plate to a new position. Do you want to save your changes? Do not ask me agan Ves X to
20	When you are satisfied with the plate layout, click Generate Worklist .	Senerate Worklist

Instructions 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.	Example
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	1 1002181016 1 2012.02.24 12;23 KIT2-0-2
	Well List Plate User 100214r016-1 Zoom %
Each plate has a plate production number attached (for example, P08265). Enter this number in the <i>Plate Production No.</i> field.	IOD I
You can also enter a note. Double click onto the Note symbol, type in your note and click OK .	Note
 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: Select a well with right mouse click. Choose Copy or Cut. Move the mouse to an empty well on the plate. Right click and choose Paste. The sample will be moved to the new position. 	G II subject - G III subject - G IIII subject - G IIIII subject - G IIII subject - G IIII subject - G IIII subject - G II
Usage of the Tissue Factor Tool: If you are measuring tissue samples, type in your μ L/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 μ L/mg factor that you used for tissue homogenization.	µL/mg Tissue Factor
	 Plate Production No. field. You can also enter a note. Double click onto the Note symbol, type in your note and click OK. 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: Select a well with right mouse click. Choose Copy or Cut. Move the mouse to an empty well on the plate. Right click and choose Paste. The sample will be moved to the new position. Usage of the Tissue Factor Tool: 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



Step	Instructions	Example
23	Usage of the Cell Normalization Tool: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.1For further information see section 7.11 Usage of	Cell Number Cell Extraction Volume [µl] 1300400 50,000 5700100 50,000 800600 50,000
24	<u>MxP[®] 500, p180 and p400 HR Kits:</u> now the worklist for the LC part was created. Copy the worklist to ob- tain the FIA worklist as described in the next step.	

4. Processing the Kit

Step

25

	-
Instructions MxP [®] 500, p180, and p400 HR Kits only – How to c	opy 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.	Plate Runs ■ Print Plate Bar Codes ▼ ■ Delete Me Plate Bar ▲ Run Number Run Ti 1 1024330456 © Copy <pre> Copy Cut X Delete </pre>
Right click somewhere below the LC plate and select Paste derived plate .	Plate Bar Run Number Run Time 1 1024330456 1 2019.05.28 16:37 Image: Copy of Cut Cut Image: Cut
 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. 	OP Type OP Plate Production LCMS KIT2-0-5514 Image: Comparison of the production LCMS BA02-0 Image: Comparison of the production KIT3-0-5504 KIT3-0-5504 KIT3-0-5504

🛒 Well List 🛛 🛲 Plate View

1 10000001

2

3

4

5

6

7 Sample Ba... Sample Iden... A Sample Type

p 180-UHPLC Cal 1 🔢 Stand

p180-UHPLC Cal2 🛛 🎁 Stand

p180-UHPLC Cal4 🛛 Stand

p 180-UHPLC Cal 5 🛛 🚻 Stand

ij Blank

p180-UHPLC Cal3 🔰 Stand. 📄 Copy

Refer to section 4.1.4, page 45 P

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.

7 iii Stand 104 Paste & I 8 🔰 Stand Delete 9 1020158015 p180 OC1 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.



Position

1

📫 Insert

of Cut

鶞 Paste

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

4.1.5 Export Worklist for Kit measurement

You Tube MetIDQ Project Start: Worklist Generation

Step	Instructions	Example
1	To export a worklist for Kit measurements, the Met- IDQ [™] worklist(s) "Condition" must be set to Ap- proved.	Project Tree Project Tree Plate 1 - SCIEX Worklist 0 (unspecified) 2015.02.09 16:13: Condition:* Approved Pending
2	Choose Export Worklist for MS , from the toolbar above the <i>Project Tree</i> .	New Project 📔 Edit Project 🔗 Overview

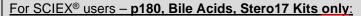




Step	Instructions	Example
3	You will see the Export Options dialogue box. Choose a folder for the <i>Worklist Export</i> . A detailed description is provided below.	Image: Storage Path C: Users WetIDQ Workist Export Settings File Path Style: default MS Type: SCIEX Use Autosampler: Aglent Order by Run number begins with: 1 Image: Sample Image: Number Degins with: 1 Image: Sample Triater Degins with: Image: Number Degins with: 1 Image: Number Degins with: Image: Number Degins with:

Item	Definition		
	Selected style of .csv sequence file name for Analyst [®] is shown, e.g. "default".		
Settings File Path Style: <u>default</u>	The style may be defined in the settings:		
Settings MS Type: SCIEX Use Autosampler: CTC Pal	Choose your MS type and autosampler in the drop-down menus.		
Run number begins with: 1	Leave the run number as 1 , except when you want to per- form 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.gwiff, .raw) are created.		
Order by Sample Acquisition method	Order by Sample is the default setting and recommended.		
Rack Positions	Select the plates you want to export/measure and select the rack position in the autosampler 1.		
I006062613-1 - KIT3-FIA_pos.meth 1 ☆	For Thermo autosampler 1:		
☑ Randomize Sample Injection Order	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.			





With Met/DQ[™], 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/DQ[™] 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 XcaliburTM 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.

		Subfolder							Definition	1	
ł	oarcode	S			Co	Contains the barcode label of each plate.					
(csv (SCIEX) txt (Waters, Thermo) plate report worklist Plate report				Contains the acquisition batch and must be imported i the MS software (MassLynx [™] , Analyst [®] , Xcalibur [™]) Contains the plate report and can be referenced during petting of the kit plate. Contains the sample list.						
t				the							
ł											
١				Co							
ł						Wo	orklist				
	Project Code: Demo Project Na Pate: 01005742 Plate code: 010100143500 Plate production on: P0205 Plate production on: P0205 Plate production on: P0205		.09 14:35:08			ect Code: E	Demo	Project Name: Meta	bolomics		
	1	2	з	4	5	6	Sub	mission Na	me: human plasma	Worklist ID: 317810	0000012630030
	A 993 (no sample to pipette)	20000615 Standard L5 93 (calibrators) KIT2 Cal5	105108 Sample 30 (plasma) G I subject 3	105187 Sample 30 (plasma) G I subject 11	105261 Sample 30 (plasma) G I subject 19	105344 Sample 30 (plasma) G II subject 7	Plate	1001806743	Plate date: 2012/01/09 14:35:08 Plate production no: P08205 Plate no:: 1	OP code KIT2 OP material 30 (plasma) OP version: 2	Position Order: Var
	11000002 Zero Sample 991 (water based zero samples) PBS	20000616 Standard L6 93 (calibrators) KIT2 Cal6	105111 Sample 30 (plasma) G I subject 4	a 105191 Sample 30 (plasma) G I subject 12	4 105274 Sample 30 (plasma) G I subject 20	s c 105358 Sample 30 (plasma) G II subject 8	Well 1 13 25 37 49	Barcode 10000001 11000002 11000002 11000002 20000011	Sample Identification PBS PBS PBS RT2 Cat1	Sample Type Dook Zero Sample Zero Sample Standard L1	Localization
	C B91 (water based zero samples) PBS	20000617 Standard L7 93 (calibrators) KIT2 Cal7	105125 Sample 30 (plasma) G I subject 5	15 105209 Sample 30 (plasma) G I subject 13 27	is 105288 Sample 30 (plasma) G II subject 1 is	17 18 105361 Sample 30 (plasma) G II subject 9 29 30	61 73 85 2 14	20000012 20000013 20000013 20000014 20000016	612 Ca2 612 Ca2 612 Ca3 612 Ca3 612 Ca3 612 Ca3 612 Ca3	Standard L2 Standard L3 Standard L4 Standard L4 Standard L5 Standard L6	-
	2ero Sample D 991 (water based zero samples)	290393 QC Level 1 30 (plasma) KIT2-MD01 QC1	105139 Sample 30 (plasma) G I subject 6	30 (plasma) G I subject 14	105291 Sample 30 (plasma) G II subject 2	105375 Sample 30 (plasma) G II subject 10	14 20 38 50	20000010 20000017 290393 290401	KIT2 Call KIT2 Call KIT2-MD01 QC1 KIT2-MD01 QC2	Standard L 6 Standard L 7 QC Level 1 QC Level 2	



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 (Mass-Lynx[™], 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.

<u>NEW Quant 500 Kit:</u> LC data quantitation can be performed by Met/DQ[™] (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/DQ[™] or the MS software (Analyst[®], MassLynx[™], Xcalibur[™]).



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1

5.1 LC data quantitation – MxP[®] Quant 500 Kit

This feature is only available for MxP[®] Quant 500 Kit data.

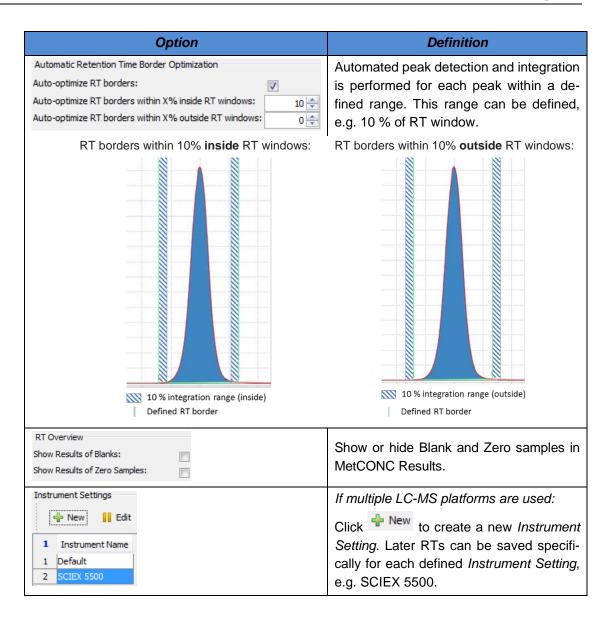
LC data quantification of other Biocrates[®] Kits uses the corresponding MS software (Analyst[®], Xcalibur[™], or MassLynx[™]).

Step	Instructions	Example			
	For LC-MS data quantitation use, "." as decimal separ				
	Recommended: set number format in Windows™ contro	ol panel to "English (UK)" or "English (US).			
1	Go to the MetCONC module.	MetCONC			
0	Import LC-MS data (.wiff or .raw):	A Interest Down Data Silan			
2	Select the "Import Raw Files" tab.	Minport Raw Data Files			
3	Click "Add Files" and Select the correct file type:Analyst® \rightarrow Analyst® raw files (.wiff)MassLynx™ \rightarrow Waters raw file [dir] (.raw)Xcalibur™ \rightarrow Thermo raw files (.raw)Choose all LC data from one kit run. $\stackrel{\circ}{l}$ Load LC data files from LC1 and LC2 injections.	Image: Solution of the second seco			
	 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. 	P 50010.550104 100231129.11_01_100 11002319674.wff P 50010.550104 1002031129.12_0_1_1_00_11002319674.wff P 50010.550104 1002031129.12_0_1_1_00_14020319674.wff P 50010.550104 10203 P P00010.550104 10020341129_13_0_1_1_0_2_437301.wff P 50010.550104 10203 P 50010.550104 10203 P P0010.550104 1020341129_96_0_1_1_0_0_1020299822.wfff P 50010.550104 10203 P 50010.550104 10203 File game: -0.550104_1020341129_96_0_1_1_0_0_1020299822.wfff P 50010.550104 10203 File s of type: Analyst raw files (.wff) Cancel			

Step	Instructions	Example			
4	Click Yes. Storing MS data in database: For a later re-integration or adjustment of in- tegration parameter, it is recommended to store MS data in the database.	User Interaction Required			
5	After MS data is imported, a list of imported samples is shown in the "Job Table".	Job Table Well Posi State Pr ■ 1020341129-1 ■ 20 ■ ₱ 500L-0-550104_1020341 Blank 1 ■ 20 ■ ₱ 500L-0-550104_1020341 Elank 1 ■ 20			
6	Select the line with the plate barcode, here it's 1020341129-1.	Job Table Well Posi State Pr ■ 1020341129-1 ■ 20 ■ P500L-0-550104_10203412ero Blank 1 ■ 20 ■ P500L-0-550104_10203412ero Sample 2 ■ 20			
	Import Retention Times (RTs) – optional				
7	 Import retention times (RTs): Click Adjust RTs Click Import Select a .csv file containing RT ranges. 	Retention Time Settings Adjust RTs Import			
7	<u>Information</u> : Met/DQ [™] OPs are provided without RT windows. Default RT windows (integration ranges) can be imported from a <i>.csv</i> file, e.g. from USB stick: folder "SCIEX\Acquisition and Quantification Methods_Documents".	Example RT ranges on USB Stick:			



Step	Instructions	Example					
l	A .csv file containing integration ranges can be imported, e.g. with RT ranges that were previously exported from Met/DQ [™] . <u>Example:</u> "MxP500 LC_RT_2018-10-24.csv". To check the imported integration ranges or to set the ranges from scratch, continue with step 8.	Select File					
	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/DQ [™] 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".						

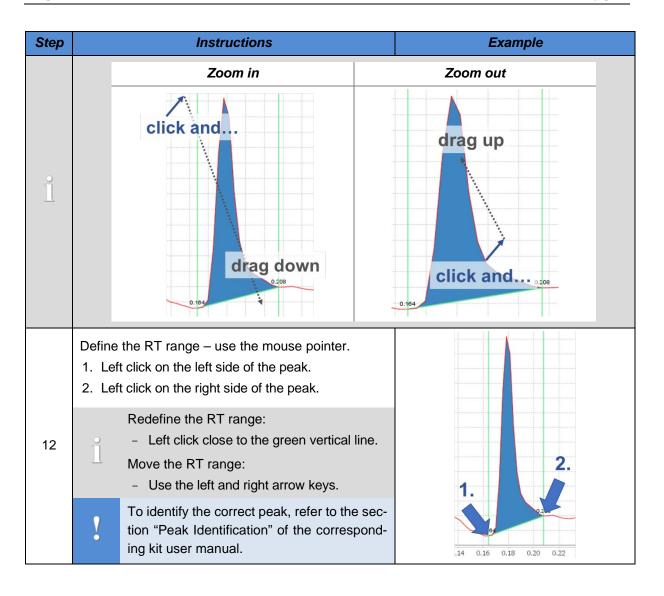




Step	Instructions	Example				
9	Click Adjust RTs .	Adjust RTs				
<u> </u>	In the "Retention Time Settings" window, peak integration settings are defined. To check the integration settings, use QC level 2.					
10	 For RT adjustment, use QC level 2. To adjust RTs of analytes analyzed with the LC1 method,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

Step	Instructions	Example
l	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.	1 Name Retention Time Integrati 17 3-Met-His
	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 1 Name 33 Betaine 34 CA
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). 	Name Retention Time I 52 p-Cresol-SO4 0. 53 Betaine 0.172 54 Choline 0.172
l	The corresponding chromatogram is shown, e.g. of Betaine.	Betaine 2,000,000 2,010,010 2,





5. Quantitation

Step	Instructions		Example
13	Define an Integration Type. These options are available.		
	Option	Definiti	on
	Instrument Configuration:	To save RT and integration settings for a cific LC-MS instrument, select an Instru Configuration, e.g. SCIEX 5500.	Instrument Configuration: Default
		This feature is only available, if a strument Configuration in addition "Default" was defined. See page 7	on to
	Valley To Valley 👻	Automated peak integration. Integration base line from peak beginni end.	ng to
	Horizontal 👻	Automated peak integration. Horizontal integration base line used.	
	Manual	Manual peak integration. User defined peak integration window base line.	and
14	Repeat steps 12 and 13 with all metabolites.		



Step		Instructions	Example	
15	Save the adjus	ted RTs. These options are available.		
	Option	Defi	Definition	
	💢 Discard Settings	Discard all manual integration setting	s of the current sample.	
	퀒 Apply As Default	Use defined RT and peak integration settings for all new integration procedures, for the currently selected <i>Instrument Setting</i> , see page 71.		
	<table-of-contents> Apply To Plate</table-of-contents>	Apply defined RT and peak integration settings to current plate.		
	×	Close window and apply defined RT and	d peak integration settings to current plate.	
16	Export the adjuct close the windo	usted RTs and ow Retention Time Settings.	<u>∎⊿</u> Export	
	Process LC	Data		
17	Select the line e.g. 102034112	with the barcode of an LC plate, 29-1.	Job Table Sample Well Posi State Pr ■ ● 1020341129-1 ■ 20 ■ ● \$5001-0-550104_10200 Blank 1 ■ 20 ■ ● > > \$5001-0-550104_10200 Blank 1 ■ 20 ■ ● >	
18	To begin with th	ne quantification procedure, click Start.	Import Raw Data Files >	
l	completed whe ter this process	gration and quantification process is en all samples status turned green. Af- two windows open: C Results and Calibration Curves	. State Proce: 2018. 2018. 2018.	

Step

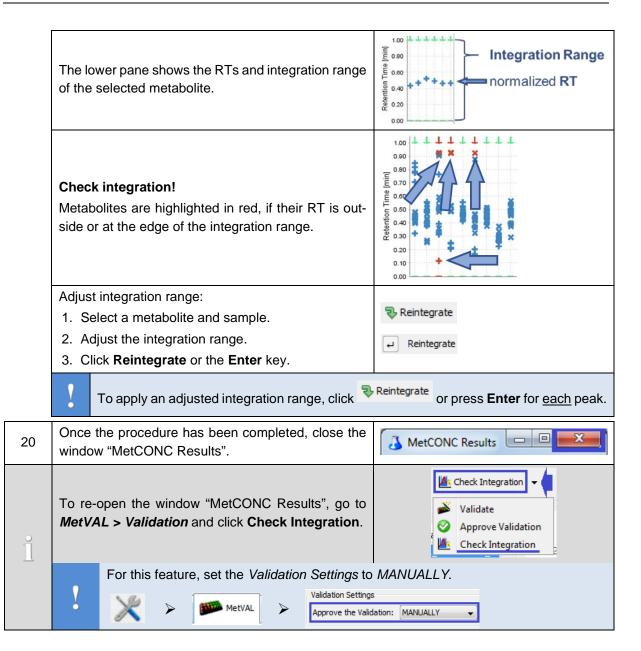
19

Instructions	Example	
Integration check		
To adjust peak integration settings in MetCONC Re- sults, keep the Calibration Curves window closed.	A cluster care Control of the control of the contr	
Check the peak integration in MetCONC Results .	Select a metabolite:	
 Here, the retention times (RTs) of all metabolites of all samples are shown normalize to a scale from 0 to 1. 	0.80 0.70 0.70 0.60 0.60 0.20 0.10 0.00	
<u>Upper pane:</u> - RT ranges of all metabolites are shown.	 12C 14 12C 16 12C 16	
 <u>Lower pane:</u> RTs of a selected metabolite in all samples are shown. 	Select a sample:	
A summary of all features is given below.		



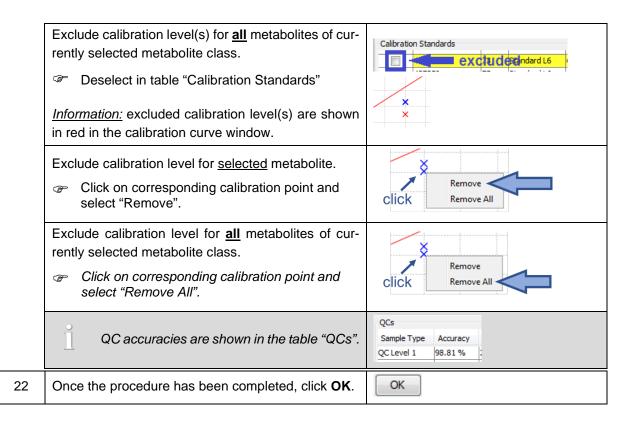
Choice	Explanation
Select a metabolite: in the upper pane, click on a me- tabolite name or click into the graph.	Upper pane:
Select a sample: in the lower pane, click on a sample or click into the graph.	Lower pane:
The corresponding chromatogram is shown.	Leu 3,500,000 3,000,000 52,500,000 1,000,000 0 3,000,000 0 3,000,000 0 3,000,000

Met*IDQ*[™] Oxygen





	Check Calibration	
21	To check the calibration. Go to the window Calibra- tion Curves . Here, all seven-point calibrated metabolites are shown in three separate tabs: amino acids, biogenic amines, and amino acids related. Check the perfor- mance of all metabolites in all calibration levels. If re- quired, adjust the calibration parameters. A summary of all features is given below.	Coloradio Correst Correction Correction<
	Choice	Explanation
	Select the metabolite class.	Calibration Curves aminoacids biogenic amines am
	Select a metabolite from the drop-down menu.	Analyte Selection Curve 1 y
	You can change the "Curve Type" and "Weighting Type".	Curve Type Weighting Type Curve Type No Weights
	Default values: Curve Type Quadratic Weighting Type 1/X	 LINEAR ZERO 1/X QUADRATIC 1/X²
	Accuracies are shown in the table "Calibration Standards".	Calibration Standards Active Sample Barcode Well Sample Type Accuracy Image: Weight of the standard light
	Accuracies outside the acceptance range are high- lighted in yellow.	Calibration Standards Active Sample Barcode Well Sample Type Accuracy Image: Accuracy 437359 73 Standard L6 83.79 % Image: Accuracy 75 Standard L6 83.79 %





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.

<u>SCIEX – Analyst®</u>

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



<u>Waters – MassLynx™/TargetLynx™</u>

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.	
•		
4	For a description how to generate text-based	result file, refer to section 7.13.

5 2 I

Met*IDQ*[™] Oxygen

5. Quantitation

Step	Instructions	Example
1	Click on the MetCONC symbol.	MetCONC
2	Import LC-MS results (.txt or .xls): go to "Import Result Files".	M Import Result Files
3	Click Browse and select the result file according to your instrument manufacturer: Analyst [®] \rightarrow "sciex result files (*.txt)" MassLynx TM \rightarrow "waters result files (*.txt) Xcalibur TM \rightarrow "thermo result files (*.xls)	Choose result file
4	Click No . ¹ ³ ³ ⁵	User Interaction Required
5	After the import process, validate the plate, see section 5.4.	MetVAL

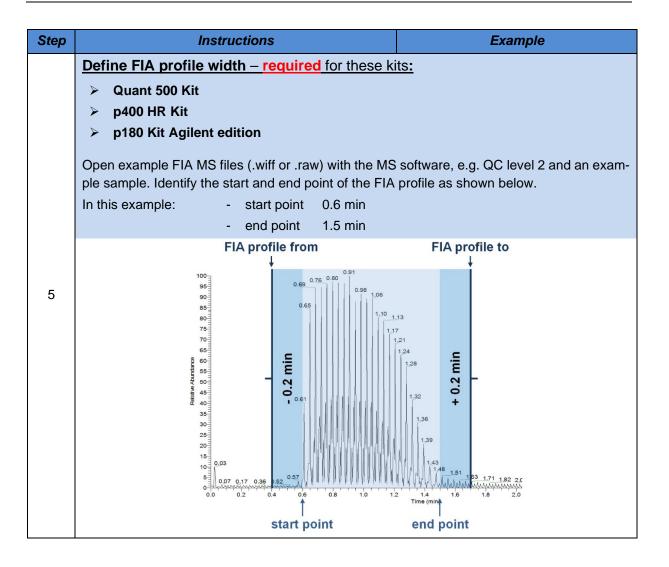


5.3 FIA data – quantitation by Met*IDQ*[™]

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 <i>IDQ</i> [™] , it is recome the control panel of your operating system to "." (dot).	
1	Go to MetCONC .	MetCONC
2	Import FIA-MS data (.wiff or .raw): go to "Import Raw Files".	Import Raw Files
	Click "Add Files" and select the correct file type:	Add Files
	Analyst [®] \rightarrow Analyst [®] raw files (.wiff)	_} Open
	MassLynx [™] → Waters raw file [dir] (.raw)	 PS00F-0-551101 1020341133 01 0 1 1 01 10000001.wff PS00F-0-551101 102034 PS00F-0-551101 1020341133 01 0 1 2 01 10000001.wff PS00F-0-551101 102034
3	Xcalibur [™] → Thermo raw files (.raw)	Poste 6 - 05101 (20234133 (01) 1 (01) (20000) wm P (000 + 0.51101 (2023)) Poste 6 - 051101 (20234133 (01) 1 (10) (20000) wm P (000 + 0.51101 (2023)) Poste 6 - 051101 (20234133 (01) (1) (20100002) wm P (000 + 0.51101 (2023)) Poste 6 - 051101 (20234133 (01) 1 (1) (2010002) wm P (000 + 0.51101 (2023)) Poste 6 - 051101 (20234133 (01) 1 (1) (1010002) wm P (000 + 0.51101 (2023))
0	Choose <u>all</u> FIA data from one kit run.	■ PSOF 0 551101_020341133_06_0_1_1_10_3100002_wiff ■ PSOF 0.551101_02034 ■ PSOF 0 551101_020341133_07_0_1_1_00_1020318866wiff ■ PSOF 0 551101_02034 ■ PSOF 0 551101_020341133_07_0_1_1_00_1_1020344
	- To load all files of one folder, press Ctrl + A	 P500F-0-551101_1020341133_09_0_1_1_00_1020318866.wiff P500F-0-551101_10203- P500F-0-551101_1020341133_10_0_1_1_00_1020319674.wiff P500F-0_551101_102034
	- To load selected files, keep the Ctrl or Shift key	 PS00F-0-551101_020241133_11_0_1_1_00_1020118674.wiff PS00F-0-551101_10203 PS00F-0-551101_1020341133_12_0_1_1_00_1020319674.wiff PS00F-0-551101_102034 PS00F-0-551101_002041133_14_0_1_1_00_0020319141.wiff PS00F-0-551101_102034
	pressed while clicking.	4 m 1 File game: -0-551101_020341133_96_0_1_1_00_1020299822.wiff
	Click Open .	Files of type: Analyst raw files (.wiff)
	Store raw data files: recommended for LC data.	User Interaction Required
4	Storing MS data in database:	Do you want to archive the FIA raw data files in the database?
	Not required for FIA data.	Do not ask me again

5. Quantitation





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 every FIA integration process. 	High Resolution Settings Name Value FIA Profile From [min] 0.40 FIA Profile To [min] 1.70
	 If no times are defined (<i>Value</i> = 0.00) the FIA protine FIA profile width makes the FIA integration profile signals outside the FIA peak. 	
6	Imported files are shown in the Job-Table .	Add Files Remove Start Job Table Start E Nome State Last Modfied E a <
7	To begin the quantitation procedure click Start .	Remove Start
	The quantitation process is performed by Met <i>ID</i> Q [™] .	State Proces ■ 2018. ■ 2018. ■ 2018.
8	After the quantitation process, validate the plate, see section 5.4.	MetVAL

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.	MetVAL
°]	In MetVAL the plate/s loaded last is/are shown. To show additional plates, change the filter settings T .	Project Tree
	The LOD for all metabolites is listed in the Analytical Specifications and is part of the Met IDQ^{TM} 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.	Calibration Curves All aminoacids biogenic amines aminoacids related Mile Calibrate
3	The validation process starts automatically. If the validation does not start or to re-validate a plate, click Validate .	📸 Validate



Step	Instructions	Example
4	<u>Calculate the limit of detection (LOD):</u> To calculate the LOD, use the Optional Validation box. A summary of features is given below.	Optional Validation
	Choice	Result
	Recommendation for at least 3 Zero replicates per plate:	The median value of all zero samples on a plate is calculated as approxima- tion 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 f the LOD defined in the Met/DQ™ OP is used. If a calculated LOD or an LOD from OP is used, this is indicated in MetSTAT as shown below. Measurement Time Ala Arg Class aminoacids aminoacids amino LOD (calc.) 1011603076/1 [µM] 13.4 0.500 0.500 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 MRN	
	Recommendation for less than 3 zero replicates per plate:	The LOD values shown in the Analyti- cal Specifications are used. These val-
	Consider Zero Sample LODs	ues are listed in the results table in MetSTAT .

5. Quantitation

Step	Instructions	Exa	ample
° 	The validated Kit plate, e.g. plate barcode 263099, is highlighted in the Project Tree. Change the filter options to choose another Kit plate.	Project Tree	
	The Plate Desk provides a detailed list of all plate information.		
	Add notes to a plate:	Plate Desk	
		Plate Run	1020411661-1
	Double-click on the "Note" symbol. Notes are in-	Project	Quant 500 - Demo Starter Kit
	cluded in QC-Reports.	OP	MXP500L-0-5511
	•	OP Type	LCMS
	Po colibrato o plato:	Condition	Parsed
	Re-calibrate a plate:	Run Time Calibrated	2019.03.15
	Click the Calibrate button.	LOD	Calculated
_		Run Number	1
5	Show peak integrations:	Plate Production No.	
		Note	
	Click the Check Integration button.	Result File Name	
	Åvailable if validation was <u>not</u> approved.	🔼 Check Integration 👻	Z Calibrate D QC-Report V
		💕 Validate	
	Generate a QC Report:	Approve Validation	
	Click the QC-Report button. A PDF is created.	Check Integration	
	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 <i>Valid,</i> 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 sam- ple.	265064-1 Q 1 2 3 4 A A Sample Type Sample B A Sample Bar Code 0409451716 C A Sample Bar Code 0409451716 Sample Identification 33000424 (inhouse only D Position 51 Injections 1 F A G Sample Volume [ul] Sample Description QC 33000424 als Unknown a ngemeldet
7	Left click on a well to see the corresponding table with analytical details and the corresponding graph.	Injections Name Sample Bar Code Injection Number RAW Filename IsktTs-FIA_ne 1000906725 1 KIT3-0-2_263099_15 IsktTs-FIA_ne 1000906725 1 KIT3-0-2_263099_15 IsktTs-FIA_ne 1000906725 1 KIT3-0-2_263099_15 Analyte ISTD Analyte ISTD Analytes ISTD 0 32.111 5.000000 C10* 0.244 0.300000 0 C101* 0.165 0 0 C10:2 0.0448 0 0 * Concentrations isotope corrected (FIA part only)
	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 <i>Quantitation</i>).	Open MS Data File Injection Nr:1+ Injection Nr:1-

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.	Kodyte: 1 120 1 120 1 100 0 00 0 00 0 100 1 100





5.5 Explanation of MetVAL Graphs

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

Validation	QC Monitoring	Analyte Overview
Validation		page 93
QC Monitoring		page 106
Analyte Overview		page 112

5.5.1 Presentation Options for all MetVAL Graphs

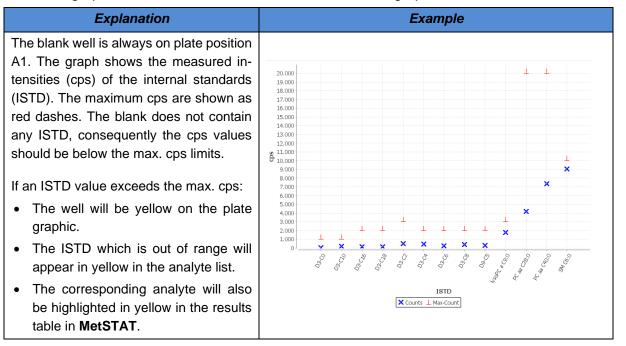
Presentation Option	Instructions
See the graph on full screen	Double-click on the graph to enter full-screen mode. Press Esc to return to normal size.
Save the graph as an image file	Right-click on the graph to show the context menu and choose "Save as" option. You can save the graphic as an image file 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

Validation	QC Tracking	Analyte Overview
ISTD Graph (blank well)	page 93
ISTD Graph (other wells)		page 94
Standards Graph		page 95
QC Concentration Graph		page 96
Analyte Concentration Graph		page 99

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.





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. 	Analyte ISTD Analytes Name Valid minimal Intensity [cps] D3-C0 1000 D3-C10 1000 D3-C16 2000 8.000.000 7.500.000 6.000.000 5.500.0000 5.500.0000 5.500.0000 5.500.0000 5.500.0000 5.500.00000 5.500.00000 5.500.00000 5.500.00000000
	⊥ Max-Count ⊤ Min-Count ★ cps

5.5.2.3 Standards Graph

Explanation The calibration standards (STD) graph shows the accuracy of the Biocrates calibration standards. The accuracy is defined as:

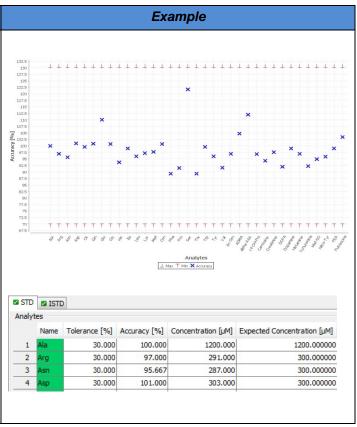
 $\frac{measured\ concentration}{expected\ concentration}\ x\ 100$

The maximum and minimum tolerance values set by the OP are shown in red.

If a standard is out of range:

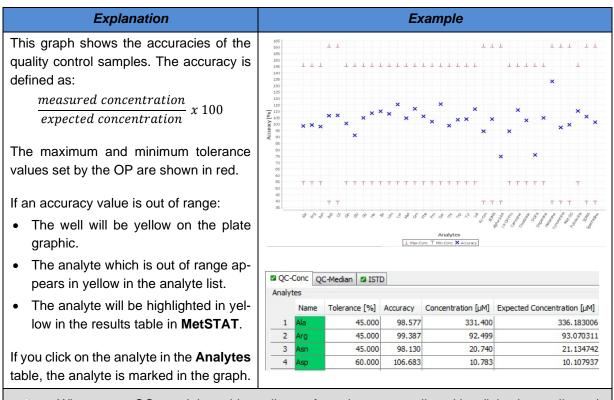
- The well will be yellow on the plate graphic.
- The analyte which is out of range appears in yellow in the analyte list.
- The analyte will be highlighted in yellow in the results table in **MetSTAT**.

When you click on the analyte in the **Analytes** table, the analyte will be marked with a vertical line in the graph.





5.5.2.4 QC Concentration Graph



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

Explanation				Exa	mple	
QCs in FIA part	- Quant 50	<u>)0, p180, j</u>	p400 HR and	p150 Kit:		
Some analytes checked with the QCs may not be quantitative, e.g. <i>lysoPC a C18:0</i> in KIT3. Their						
Analyte Classifica	ation is "Re	lative Qua	antitative" and	the values in "Expe	cted Concentr	ation" for these
analytes are in ar	bitrary unit	s. Howeve	er, their accura	acy 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	30.00000	Valid	Relative Quantitative
as external standa these analytes sh	ards or refe low good C	erence mat	terials are not ghly reproduc	ative Quantitative" ar available. However, ible concentration va	based on num	erous analyses uently, they can



5.5.2.5 QC-Median Concentration Graph

Explanation	Example
When a QC level was measured in repli- cates over the Kit plate, this graph shows the median accuracies.	
If a median accuracy value is out of range:	X X X X X X X X X X X X X X X X X X X
• The well will be yellow on the plate graphic.	66 68 58 TTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
• The analyte which is out of range appears in yellow in the analyte list.	≈
• The analyte will be highlighted in yel- low in the results table in MetSTAT.	Analytes
If you click on the analyte in the Analytes	Name Tolerance [%] Accuracy [%] Concentration [µM] Expected Concentration [µM] 1 Ala 45.000 99.979 336.111 336.183006
table, the analyte is marked in the graph.	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 <u>lower limit of guantitation (LLOQ)</u> , and the <u>upper limit of guantitation</u> (ULOQ) are shown in red. These values are also listed in the Analytical Specifica- tions.	120 110 100 90 90 90 90 90 90 90 90 90
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 Concentration [µM] Lower limit of quantification [µM] C0 30.157 5.000000 C10* 0.111 0.300000 C10:1* 0.054 0.032 C12* 0.035 0.400000 * Concentrations are isotope corrected (FIA part only)
The status of C10* is < <i>LLOQ</i> ; name and measurement status are colored blue. The analyte classification is <i>Quantitative</i> , and the concentration value is below LLOQ, but above the <u>limit of detection</u> (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 CLOQ Quantitative



Explanation	Example
The status of Asp is <i>Valid</i> ; name and measurement status are colored green, which means the concentration value is within the quantitation range. The analyte classification is <i>Quantitative with Restrictions</i> : 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 <i>Valid</i> ; name and measurement status are colored green. The analyte classification is <i>Relative Quantitative</i> , and the concentration value is above the LOD. <u>Note</u> : 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 < <i>LOD</i> ; name and measurement status are colored pur- ple, which means that the concentration value is below LOD. The analyte classification is <i>Relative</i> <i>Quantitative</i> : precise (CV<15%) but ac- curacy 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

Explanation	Example
The status of Ser is <i>STD/QC</i> > <i>Limit</i> ; name and measurement status are colored yellow.	
The analyte classification is <i>Quantitative</i> , but the accuracy of Ser is not within the acceptance range for Ser in this QC measurement. <u>Note</u> : <i>STD/QC > Limit</i> or <i>STD/QC < Limit</i> 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/ DQ^{TM} .

υ.

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.g0.535 µM	
< LOD	concentration below LOD	
< LLOQ	concentration below LLOQ	
> ULOQ	concentration above ULOQ	
No Intercept	concentration cannot be calculated, also see	
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 <i>ID</i> Q [™] or during quantitation	
Incomplete	 No measurement (concentration data) available for one well. Accuracy cannot be evaluated. 	



Analyte Samples and Zero Samples:		
Measurement Status	Description	
Valid	concentration between LLOQ and ULOQ	
Smaller Zero	concentration below zero, e.g0.535 μ M	
< LOD	concentration below LOD	
<ltod< td=""><td>concentration below LLOQ</td></ltod<>	concentration below LLOQ	
> ULOQ	concentration above ULOQ	
No Intercept	concentration cannot be calculated, also see	
Missing Measurement	no measurement available	
ISTD Out of Range	ISTD intensity not in range	
Invalid	analyte excluded by user in Met <i>ID</i> Q [™] 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: in- ternal 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.

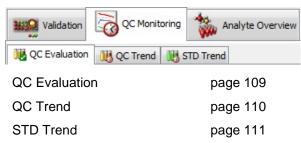
Step	Instructions	Example
1	In MetVAL > Validation select a well.	1 2 3 A O O B O O C O O
2	Select the tab Analyte or ISTD.	Analyte ISTD Or Analyte ISTD
3	Select an analyte (e.g. ADMA) and double- click on the note field.	Øi Analyte Øi ISTD Analytes Name Name Concentration [µM] 23 ADMA 0.421
4	 Select a Note Type. Enter a note, e.g. the Validation Note "RT was defined incorrectly". Make a choice from which well or sample type analyte(s) should be excluded from validation. → Find a description below. Activate the status Invalid. Note: If the status Invalid is not activated, only a note is saved.	Note Editing Note Type Validation Note Set the note of Measure: Creatinine Enter a note Note Type User User Solution Note has an effect on: Well measurements Injection Analyte Analyte of same sample type Analyte of the plate Status V invalid COK Cancel

Choice	Result
Note has an effect on: Available Wells Linked Wells Well measurements 1 Sample Bar Code Position Injection 1 10000001 1 Sample Bar Code Position Analyte 3 1000906725 3 Image: Code Position 1 0409451716 51 Analyte of same sample type 4 110000002 13 Image: Code Image	 Make your choice of well(s). 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 repli- cates of the well.
Note has an effect on: Available Protocols Linked Protocols Image: Well measurements 3 Protocol Short Name 1 Image: Accorn 2 ApMA Image: Accorn 3 Als	Analyte(s) listed in the column <i>Linked</i> <i>Protocols</i> are excluded from validation in the selected injection of a well.
Analyte	Chosen analyte of selected injection of a well is excluded.
Available Protocols Available Protocols Available Protocols Available Protocols Available Protocols Available Protocols Linked Protocols I Protocol Short Name Ac-Orn Analyte on the plate Ac-Orn Ac-Orn	Analyte(s) listed in the column <i>Linked</i> <i>Protocols</i> are excluded from validation of the selected sample type, e.g. QC 1.
Analyte of same sample type 3 Protocol Short Name Analyte on the plate 1 Ac-Orn 2 ADMA 3 Ala	Analytes listed in the column <i>Linked</i> <i>Protocols</i> are excluded from validation in all samples on the selected Kit plate.
Inverse metabolite selection NONE apply to selected plate run	 Inverse metabolite selection: 1. select a plate run 2. select metabolites that should <u>not</u> be excluded from validation 3. select the "Status" <i>Invalid</i> 4. click OK <u>Example:</u> If creatinine is selected, all further metabolites of a <i>plate run</i> get the status <i>Invalid</i>.
	$\hat{1}$ At least two <i>plate runs</i> are required.



Step	Instructions	Example	
5	Select OK in the "Note Editing" window.	<u>ок</u>	
6	To apply all changes, click on <i>Validate</i> to re- validate the Kit plate.	Validate	
	While the validation status <i>Invalid</i> 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 <i>valid</i> if the accuracy is within the prede- fined ranges for a Kit. Otherwise the reason is displayed, e.g. <i>STD/QC > Limit</i> . If an analyte (see column <i>Analyte Evalua- tion</i>) 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 <u>EMA guide- lines on bioanalytical method validation</u> (Eu- ropean Medicines Agency, 2011).	Valuation Evaluation QC Evaluation of Plate 791987-1: QC Evaluation Plate 791987-1: QC Evaluation Analyte Classification Analyte Evaluation IQC Level 1 (Well Pos. 62) (Ala Quantitative Valid Valid



Step	Instructions	Example
<u>1</u>	The QC Evaluation can be made for one Kit plate only. In MetVAL > Validation , select a Kit plate first and then switch to QC Monitor- ing > QC Evaluation .	1. Validation 2. QC Monitoring
2	Select the QC Trend tab to obtain an over- view of the inter-plate CVs of a selected QC analyte. This tool can be used to monitor the assay and instrument performance or repro- ducibility 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.	Value Type © Concentration © Analyte Intensity [cps]
4	Use the Filter 🔽 to monitor a certain QC level by the QC's bar code or use other filter options.	▼ Filter options Time From: Time To: OP Code: KIT2 Material: All Version Number: Short Name: Sample Bar Code: 438010 @ Clear
5	Use the button Table View (bottom left) to show.	Table View

Step	Instructions	Example
<u>°</u>	The time period is predefined to 12 months by default in the <i>Filter options</i> and can be changed in the Met/ DQ^{TM} settings (Settings > MetVAL > QC Monitoring Settings).	Settings General MetLIMS MetCONC MetVAL QC Tracking Settings Display Plates measured in the last 12 months
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.	Filter Short Name Sample Bar Code Sample Type ADMA 438010 III QC Level 2 Ac-Orn 438010 III QC Level 2 Ala 438010 III QC Level 2
7	Select the STD Trend tab to obtain an over- view of the inter-plate CVs and concentra- tions 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 cal- ibration standard level you would like to monitor in the Trend Chart view. Graphs will be generated according to this selection.	Short Name Sample Bar Sample Type Image: Comparison of the type Ala 20000611 Ijjj Standard L1 Image: Comparison of type Image: Comparison of type Arg 20000611 Ijjj Standard L1 Image: Comparison of type Image: Comparison of type Arg 20000611 Ijjj Standard L1 Image: Comparison of type Image: Comparison of type Asn 20000611 Ijjj Standard L1 Image: Comparison of type Image: Comparison of type



5.5.4 MetVAL – Analyte Overview Graph

Validation	QC Monitoring	🐝 Analy	te Overview
Analyte ISTD]



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

Select the Analyte Overview tab and choose Analyte Analyte Ism. Analyte per ana- lyte the values Concentration: blue dots • Retention Time: orange quadran- gles • 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.	<figure><section-header></section-header></figure>



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

Met*IDQ*[™] Oxygen

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.	MetStat
2	First you have to choose your Report Con- text . In a Report Context all linked samples and your MetSTAT settings (including Stat- Pack) will be saved. You can either use the DEFAULT <i>Report Con-</i> <i>text</i> or create a new report context.	Select Report Context



Step	Instructions	Example
<u>_</u>	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).	1 Current Active Name Description Condition Visibility User Date 1 Image:
3	To create a new <i>Report Context,</i> click New and enter a new name. Choose a "Condition" and the "Visibility" for the <i>Report Context</i> . The <i>Description</i> field is optional.	Image: Pending Visibility: * Private Description: Image: Pending Image: Private
	Choice	Explanation
	Condition	If the condition is set to Pending, changes that are made will be saved in the <i>Report</i> <i>Context</i> . If the condition is set to Approved, changes will not be saved. <u>Note:</u> if the <i>Condition</i> is set to <i>Approved</i> , you cannot link or unlink samples in the Select Samples tab (steps 5-7). This is only possi- ble when the Condition is set to <i>Pending</i> .
	Visibility	The visibility can be set to Private or Global. <i>Private</i> : The <i>Report Context</i> is only visible for the Met/DQ [™] user who creates it. <i>Global</i> : The <i>Report Context</i> is visible for all Met/DQ [™] users.

Step	Instructions	Example
4	Activate the checkbox of the Report Context that shall be used.	Current Active Name 1 DEFAULT(labad 2 Image: Test Context
5	Next, choose the Select Samples tab.	Select Samples
6	Choose the samples for data evaluation and move them to the Linked Sample list using the transfer bar.	Unlinked Sample Sample Rec Code Sample Rec Code Sample Rec Code Sample Rec Code Plate Bar Code Plate Production No. Run Number 1 105717 G IV subject 4 105883 P08276 3 2 105300 G II subject 3 105883 P08276 3 3 105300 G III subject 3 105883 P08276 3 4 105096 G III subject 1 105883 P08276 3 5 3300424 105983 P08276 3 5 3300424 105963 P08276 3 6 105965 P08276 3 105905 6 105961 G III subject 11 105905 P08276 2 9 105445 G II subject 17 105905 P08254 2 9 05834 G III subject 17 105905 P08254 2 10 1040451716 33000424 (nho 263094 1 1 11 1405583 105905
7	You can use the filter before you choose sam- ples. Use the Filter button in the upper right corner to open the filter tool. Click on the column header to sort any col- umn. The direction of the grey triangle tells you the sorting order; either ascending (trian- gle is pointing up) or descending (triangle is pointing down).	Filter Sample Project Code: Project: e.g. enter project name! Submission: All Plate Bar Code: Comple Rer Code: Sample Identific Sample Identific
8	Click on Display Data and select the Data tab.	Display Data Data Plots





Step	Instructions	Example
9	You will see the sample table including all sample information and metabolite concentra- tions. Use the scroll bar at the bottom of the window to navigate to the right, showing all the columns in the table.	Plate Bar Code Sample Bar Code Sample Type Sample Identification Collection Date 1 ✓ 105883-3 105546 Sample G III subject 7 25.12.2008 2 ✓ 105883-3 105515 Sample G III subject 4 25.12.2008 3 ✓ 105883-3 105718 Sample G IV subject 7 25.12.2008 4 ✓ 105883-3 105717 Sample G IV subject 4 25.12.2008 5 ✓ 105883-3 105751 Sample G IV subject 4 25.12.2008
10	Change the sort order in any column by click- ing in the column header. Here, the column sort criteria for <i>Sample bar- code</i> has been changed.	Sample bar code Sample Type Sample Identification Arg Gin 10000001 Blank 0.000 0.000 105086 Sample G I subject 1 87.4 719 105090 Sample G I subject 2 87.5 802 105108 Sample G I subject 3 94.4 746 Sample bar code Sample Type Sample Identification Arg Gin 53000424 QC Level 3 115 368 33000424 QC Level 1 102 355 20000343 Standard 65.6 103 103
	<i>Note:</i> 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 <i>Sample bar- code</i> . Click on the column header you also want to sort, for example <i>Well position</i> . The sample table is then sorted by <i>Sample barcode</i> then by <i>Well Position</i> .	Sample bar code Sample Type Sample Identification Well Position 10000001 Blank 1 105086 Sample G I subject 1 77 105090 Sample G I subject 2 78

Step	Instructions	Example
l	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 <i>Italic</i> are isotope corrected (FIA only).	Sample bar code Sample Type Sample Identification C2 C3 C3 53000424 QC Level 3 4.65 0.331 0.328 43000424 QC Level 2 4.24 0.328 33000424 QC Level 1 4.12 0.311 20000343 Standard 3.54 0.783 11000002 Zero Sample PB5 0.026 0.008 11000002 Zero Sample PB5 0.029 0.007 11000002 Zero Sample PB5 0.029 0.006 105779 Sample G IV subject 10 1.58 0.756 1057765 Sample G IV subject 9 1.51 0
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 col-	1 ✓ 105883 10000001 2 ✓ 105883 105546 3 ✓ 105883 105748 5 ✓ 105883 105748 6 ✓ 10 Use only selected 7 ✓ 10 Dont use selected 8 ✓ 10 Deselect all 9 ✓ 10 Deselect all 9 ✓ 10 Deselect all ✓ Collection Date ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓
	umn names in the list that you want to dese- lect. Double-click again will reselect the col- umn.	LOD (calc.) Image: Color C





Step	Instructions	Example
	Value Type Select the data type that is displayed in the main table. The default option is Concentra- tion.	Value Type Concentration Analyte Intensity [cps] Internal Std. Intensity [cps] Accuracy [%]
12	Analyte Peak Area, Analyte Retention Time and Internal Std. Retention Time are not available for FIA data.	 Analyte Peak Area [area] Internal Std. Peak Area [area] Analyte Retention Time [min] Internal Std. Retention Time [min] Analyte Peak Width [min] Internal Std. Peak Width [min] Internal Std. Peak Width [min] Area Ratio
13	Display Options Specify analyte class, Bio IDs, Analytical De- tails and the <i>Display Unit</i> .	Display Options Image: Constraint of the second s
l	We have implemented a direct link to public available databases like the Human Metabo- lome Database (HMDB) (http://www.hmdb.ca/) or LIPID MAPS (LMID) (http://www.lipidmaps.org/), which you can se- lect as shown on the right. The Bio ID com- pound identifiers are then shown in the results table.	▼ Bio ID: HMDB
<u> </u>	Available Bio IDs are shown in a table.	Bio ID Details

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. 	Show Analytical Details
	 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" 	Display Unit: ng/ml Split/Merge Acquisitic pmol/mg Tissue 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 sam- ple in the results table. For this, click on the corresponding acquisition and click Split .	Split/Merge acquisitions Image: Constraint of the system of
15	Now the sample data is displayed in two rows: one for positive, one for negative ion mode re- sults.	3 V 263099-3 138174 QC Level 1 1 (+) 30.1 0.197 4 V 263099-3 138174 QC Level 1 1 (-) - 5 V 263099-3 138174 QC Level 2 1 (+) 41.2 0.829 6 V 263099-3 138188 QC Level 2 1 (+) 41.2 0.829 7 V 263099-3 146358 QC Level 3 1 (+) 52.3 1.44 8 V 263099-3 146358 QC Level 3 1 (-)
16	To merge the rows again, select both injections and click Merge .	m Merge
1	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 re- sults and one containing the LC results.	Merge: based on Sample and Position



Step	Instructions	Example
ļ	Comparability of results generated with different For best comparability of results generated with different a Normalization versus Target Values of all data	ferent kits, e.g. Quant 500 and p180 Kits, perform
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 <u>and</u> QCs) from all Kit plates their data should be normalized to <i>Select Samples</i>. For example, create a new <i>Report Context</i> as described at the beginning of this section 6. 2. Go to <i>Display Data > Data</i>. 3. Open the tab <i>Data Normalization</i> (shown on the right) and activate "Normalize Sample Concentration Data". Details to this feature are given below and in 7.1 <i>Data Normalization</i>, page 133. 	Data Normalization Image: Normalize Sample Concentration Data Plate Source: All Sample Source: QC Level 2 Normalization vs. Target Values: Method: Method: Method: Creatinine Normalization Subtract Median Concentration of Zero Samples
	Choice	Explanation
	Vormalize Sample Concentration Data	Activate to perform data normalization.
	Show Report	Save normalization report as PDF document.
	Plate Source:	For normalization select all plates or one spe- cific plate as reference.
	Sample Source: QC Level 2	Select a reference sample, e.g. QC Level 2.

	Normalization vs. Target Values:	To perform normalization across different QC batches, use this option, see Appendix 7.1.
	Use Normalization vs. Target Values if C	QC from different batches were used.
	Method: Mean	For normalization concentrations form QC samples in replicates, e.g. QC Level 2, are averaged. Use "Mean" or "Median".
	✓ Log Transform Data log2	Perform a log-transformation.
	Creatinine Normalization	Used with p150 or p180 Kit for urine samples.
Step	Instructions	Example
	 Requirements for Data Normalization: The currently active Met/DQ[™] "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. <u>Note:</u> 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. 	Select Report Context Provide Select Samples Provide Select Select Samples Provide Select Select Samples Provide Select Select Samples Provide Select Sel



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

Step	Instructions	Example
	Subtract Median Concentration of Zero Sam- ples (recommended for FIA part only) When activating the checkbox, the median concentrations of the zero samples are sub- tracted from the concentrations of the samples (samples only, not QCs or Calibration Stand- ards). This is an optional feature and should be selected if you have samples with very low metabolic concentration levels, like in cerebro- spinal fluid (CSF). It is only active if you have selected the zero samples from the plate you are analyzing (see page 117).	Subtract Median Concentration of Zero Samples
18	To export data as displayed in MetSTAT , use the settings as shown on the right. Two differ- ent example reports are given on page 129. Further formatting choices are explained in the table overleaf.	Save Options Transpose Values Add comments to Excel files Export Status Information Export all types of values at once Repla Status Blank Out of Range Blank Out of Range STD/QC < Limit



	Choice	Result				
	Transpose values	Transpose (interchange) rows and columns.				
	Add comments to Excel™ files	Add <i>Status</i> , 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 <i>Status</i> , 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 ex- port file.				
	Replace values of	You can replace values of a certain status with any text or number for the report. For ex- ample, 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 <i>Available Report</i> Formats, page 128.	Excel 2003-Worksheet (*.xis)				

	Export of MS raw files:	User Interaction Required
1	MS raw data files, stored in the Met/ DQ^{TM} da- tabase, can also be exported in this step (LC data are only available if they were imported, see section 5 <i>Quantitation</i>)	Do you also want to export the MS RAW data files from the database if available? Do not ask me again Or not ask me again
l	To change the decimal separator symbol, go to the MetSTAT settings. See 7.4 Changing Settings in MetIDQ [™] Soft- ware (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)

	А	В	С	D	E	F	G	н	1
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	1000001	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)

	А	В	С	D	E	F	G	Н		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	acylcarniti	acylcarniti	acylcarniti	acylcarniti	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 subject	2,0	10,0	3,0	29,78405	0,106306	0,057208	0,019279	0,03512
8	105883-3	105515	Sample	G III subject	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 subject	14,0	10,0	3,0	30,15686	0,110722	0,054459	0,032469	0,035022
14	105883-3	105532	Sample	G III subject	15,0	10,0	3,0	31,56528	0,122227	0,046707	0,025052	0,03722
15	105883-3	105563	Sample	G III subject	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

Туре	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)

- 24	A	В	С	D	E	F	G	Н		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	acylcarniti	acylcarniti	acylcarniti	acylcarniti	acylcarniti
4							LOD (calc.) 108	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 subject	2,0	10,0	3,0	29,78405	0,106306	0,057208	0,019279	0,03512
8	105883-3	105515	Sample	G III subject	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 subject	14,0	10,0	3,0	30,15686	0,110722	0,054459	0,032469	0,035022
14	105883-3	105532	Sample	G III subject	15,0	10,0	3,0	31,56528	0,122227	0,046707	0,025052	0,03722
15	105883-3	105563	Sample	G III subject	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)

	Α	В	С	D	E	F	G	Н	1	J	K	L	M
1	Concentrat	tion 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 Ba	ar Code			10000001	105546	105515	105748	105717	105751	11000002	105529	105532
4	Sample Ty	pe			Blank	Sample	Sample	Sample	Sample	Sample	Zero Samp	Sample	Sample
5	5 Sample Identification					G III subject	G III subject	G IV subje	G IV subje	G IV subje	PBS	G III subje	G III subjec
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 Positi	ion			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	2009.02.0	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.

You Tube 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. <u>Information:</u> Select a time window (e.g. 2015/01/01 till 2015/12/31) to monitor the Kit measurements stability.	Filter Sample Project Code: Project Name Submission: All Plate Bar Code: Sample Bar Code: V
2	Click on Display Data and select the Plots tab.	Display Data
3	LOD, Blank, Zero, Calibration Standard, QC, and Unknown Sample concentrations are dis- played 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).	Control Ala-KIT2-0-8014 Provi Courts Social State Social State Social State Social State

St

Step	Instructions	Example
4	 Use the table Metabolites to page through all available analytes. Concentration values of all samples per Kit plate (blue arrow) are visualized in the form of box plots in the Chart Key pane. 	Metabolites SOP ADMA KIT2-0-5404 Ac-Crn KIT2-0-5404 Arg KIT2-0-5404 Asn KIT2-0-5404 Carmosine KIT2-0-5404 Cit KIT2-0-5404 Creathinic KIT2-0-5404 Cit KIT2-0-5404
5	Use the Export Charts button to create a graphical report in PDF format.	Export Charts Export Charts
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) 	Print Image: Copies Print: Image: Copies Pages to Phat Advanced Pages to Phat Image: Copies Pages to Phat Image: Comments & forms Page to Poster Image: Comments Page of the Image: Comments 85 x 11 Inches Page of the Image: Comments Image: Comments Image: Comments Image: Comments <t< td=""></t<>





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 <u>Biocrates[®] QC</u> 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/DQTM:

- 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 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 C_{b1} . In detail, $C1_{b1} = A1_{b1}/TV_{b1}$ is the correction factor for an analyte on Kit plate 1, $C2_{b1} = A2_{b1}/TV_{b1}$ 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 C_{b1} , e.g. c(analyte, plate1)_{normalized} = c(analyte, plate1) / $C1_{b1}$ (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 <u>or</u> 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/DQ[™]:

- 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(analyte, plate1)_{normalized} = c(analyte, plate1) / C1(analyte).

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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.	Sample Registration
2	Look at the lower panel in the Sample Regis- tration window. Click on Groups and Config- ure .	Groups Variables O Configure Categories Name Description
3	You will see the Groups Configuration win- dow.	Groups Configuration
4	Click New in the Categories panel.	💠 New
5	You will see the New Category window. Name the category. Here it is <i>gender</i> . The Description field is optional. Click OK when finished.	Name:* gender Description:



Step	Instructions	Example
6	You will see the new category listed. Click New in the Groups panel.	∂ Groups Configuration Categories ↓ New ↓ Edit ↓ Delete ↓ New ↓ Edit ↓ Name 1 TEST chronic logorhea groups 2 gender ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓
7	In the New Group window, add a group that be- longs to the selected category. Here, <i>female</i> is added as a group in the <i>gender</i> category. Click OK when finished. Repeat the process to add more groups, e.g. <i>male</i> .	New Group
8	This category now has two groups. Category: <i>gender</i> Groups:female, male Click Close . Note: there is no limit on the number of groups which can be added to a category.	Groups Configuration

Step	Instructions	Example
9	When the categories and groups are defined, you can link them to samples. Select the sam- ples 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). <u>Note:</u> 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 <i>male</i> and <i>female</i> to the same sample, but you can link <i>male</i> from the category <i>gender</i> and for example <i>diseased</i> from another category to the same sample.	Contigure Contigure Categories 1 Name A Description 1 IEST dronic logorites gro disease-related chars 2 pender M I Iest International Contegority (Contegority) 2 pender M III International Contegority (Contegority) 2 pender M IIII International Contegority (Contegority) 2 pender M IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
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 .	Sroups Variables
11	This will open the Variables Configuration window. Click New .	Variables Configuration
12	You will see the New Variable dialogue. Type in a name for the variable. The field <i>Unit</i> is optional.	New Variable Name:* Unit: Datatype:* Number Image:* Image:* </td



Step	Instructions	Example
13	Choose a data type from the drop-down menu.	Number Number Text Date
14	Here, the new variable is <i>age</i> . The <i>Unit</i> is <i>years</i> , and of datatype is <i>Number</i> .	New Variable Name:* age Unit: years Datatype:* Number Image Image
15	Next, link the variable to the samples as de- scribed in step 9 on page 137. Click on the sam- ple name in the Linked Variables list.	2 Sample Bar Code Sample Identification Material Species Registration I 1 105086 G I subject 1 30 (plasma) unassigned 2099.02.02 1 2 105090 G I subject 2 30 (plasma) unassigned 2009.02.02 1 2 05090 G I subject 2 30 (plasma) unassigned 2009.02.02 1 2 0 Coups Variables unassigned 2009.02.02 1 Configure Available Variables Unit Datatype 1 1 Age Year Number Se 2 Age Year Number Add
16	The variable <i>age</i> is now linked to the samples <i>105086</i> and <i>105090</i> .	Linked Variables Unit Datatype 1 Sample Bar Code Sample Identification Name Value Unit Datatype 1 105086 G I subject 1 Age 0.000 Year Number 2 105090 G I subject 2 Age 0.000 Vear Number
17	You can change the Value by double clicking the cell and typing in another value. Here <i>65,000</i> is specified as the new value.	Value Value 0,000 y 65,000 y

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

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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 <i>ID</i> Q [™] toolbar.	×
2	Click on the MetSTAT tab.	MetStat
3	Select the decimal separator "." or "," from the drop-down menu. The number of decimal places displayed in MetSTAT can be defined blow.	Settings Image: Settings Image: Settings MetSTAT Settings Separator Decimal Separator Decimal Separator Settings Number Of Decimal Places Set the number of decimal places Set the number of decimal places Image: than 0.1: Image: than 100 (exclusive): Image: than 100 (exclusive): Image: than 100 (exclusive): Image: than 100 (exclusive): Image: selection Configuration Daplay Samples measured in the last Image: selection Configuration Minimum amount of replicates for normalization: Image: Reset Image: Cancel



Choice	Explanation
- smaller than 0.1:	0.081
- between 0.1 and 1 (exclusive): 3	0.331
- between 1 and 10 (exclusive): 2 🜩	8.23
- between 10 and 100 (exclusive): 1 📚	11.0
- larger than 100:	893
Normalization Configuration Minimum amount of replicates for normalization:	Defines the minimum of replicates on every Kit plate for normalization.
StatPack Graphs Don't show empty combinations in box and scatter plots	If you activate this checkbox, analytes with missing concentration values (empty com- binations) will not be shown in StatPack graphs.
Statistics Environment Configuration File path of 'RScript.exe': C\\MetIDQ\tools\r4metidq\bin\Rscript.exe	Defines the path where the "Rscript.exe" is located.

7.5 Met/DQ[™] Software Update

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

C	2	
ì	1	
J.	L,	

>

Version Info

e.g.

Version Info	Legal Disclaimer	License Agreement
VERSION	7.	11.2-DB108-Nitrogen-2831
BUILD NR	28	31

Step	Instructions	Example
1	Double click on the Met/DQ [™] install file on the USB stick and follow the installation instructions below.	MetIDQinstall.exe
2	Select "Yes, update the existing installation". Follow the instructions of the Install Wizard.	Welcome to the Biocrates MetIDQ™ Setup Wizard This will install Biocrates MetIDQ™ on your computer. A previous installation has been detected. Do you wish to update that installation?
3	Your current Met <i>IDQ</i> [™] version will be un-in- stalled and the new version installed. It is recommended to "Keep all currently stored MetIDQ [™] user preferences".	Installing Please wait while Setup installs Biocrates MetIDQ™ on your computer. Preparing to install Setup Setup Keep all currently stored MetIDQ™ user preferences? Yes No



Step	Instructions	Example
4	Start Met/DQ™, connect to the kit database(default: "Kit Database") and log in as userwith administrative rights, e.g.Username:labadminPassword:12345678	User Name: Iabadmin BIOCRATES Password: Change Password After Login Change Password After Login Remember Password Database: Kt Database Connected!
1	If a database update is required, do steps 5 - 8.	Update confirmation Database Kit Customers Database is running on server Vit2 Nas to be updated from version n to version n+1. Please confirm the update by entering the update password specified in the user manual. Password: Concellent
	A <u>database backup</u> is <u>highly recommended</u> as described in section 7.6.1 <i>Database Backup / Export</i> , page 143, before starting the database update process.	
5	The password <i>metkit</i> is required for the update process.	Update confirmation Database Kit Customers Database is running on server Please confirm the update by entering the update pass Password: metkit
6	The update process may take several minutes.	Database-Updater
	Never abort the update process!	Regent die Oddobe Stability
7	After the update process close the Database - Updater window and restart Met/DQ [™] .	

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, **<u>perform regular backups</u>**. 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 <i>ID</i> Q [™] and click on the database connec - tions 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).	Database Connections Database Kit Database Create Backup Show Backups Kit Database Create Backup Create Ba	
	This process can take several minutes. When the process has finished, a backup report win- dow opens. The path where the backup file (*.dmp) is located can be found at the end of the backup report window.		
1	Master table "BIOCRATESBACKUPADMIN"."EXPORTMETKIT" successfully loaded/unloaded ***********************************		



Step		Instructions	Example
3	is: C:\or The o metk Each trans	default backup file location for Oracle [®] XE aclexe\app\oracle\admin\XE\dpdump default dumpfile name is: it_YYYY-MM-DD_hh-mm-ss.dmp backup file can be used for a database fer to a second PC (see: 7.6.4 Transfer a DQ™ 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.	
l	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").		Database Connections Database Connections Database Connections Create Backup Show Backups Kit Database Create Backup OK Cancel Backup Manager Restore Rest
4	To delete a database backup: Select a database backup and click Delete.		Backup Manager

7.6.2 Database Import

Met/ DQ^{TM} databases can be imported from a local, removable or network drive to the PC where Met/ DQ^{TM} is installed.

Step	Instructions	Example
1	Open the Backup Manager.	
2	Choose the Import button.	Backup Manager Backup M
3	Select the Met <i>ID</i> Q™ backup file (*.dmp). Click Open .	Import database export file. Suchen jn: dpdump METXT_2014 08 04_18:03-58:DMP Zulett Dateiname: METXT_2014 08 04_18:03-58:DMP Netzwerk Dateiname: METXT_2014 08 04_18:03-58:DMP Offnen Abbrechen
႞	You will see the imported backup in the Backup Manager list.	Backup Manager Coalhost DIRECTORY: C:\pradexe lapp\prade\u00e4nin\ve \u00e4pdump\ Scheme Flemame Created backup file path MetRitt metSit_2014-08-04_18-03-58 2014-08-04 18:04:1



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 current	ly 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.	Backup Manager		
2	When the User Interaction window appears, click Yes .	User Interaction Required		
3	Enter the database password " <i>metkit</i> ". Then, the restore process starts (this may take sev- eral minutes).	Enter Password Database Password: OK X Cancel		

7.6.4 Transfer a Met/DQ[™] Database to a Second PC

This option can be used to move Met/DQ[™] and all Kit data to a second PC.

Step	Instructions	Example
1	Install Oracle [®] and Met <i>IDQ</i> [™] 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 \rightarrow 7.6.2 Database Import, page 145	
4	Restore the backup at your new PC \rightarrow 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 ad- ministrator. The latest version is provided by the Customer Support.	Run as administrator		
2	Type " y " (yes) to update BIO- CATES BACKUP ADMIN.	Administrator: Metkit Import YOUR ARE GOING TO IMPORT THE PROVIDED METKIT DUMP INTO YOUR DATABASE. ENTER THE ORACLE SYSTEM USER PASSWORD1: 1/2345678 ORACLE INSTALLATION DIRECTORY IS "C:\oraclexe\app\oracle\product\11.2.0\server\ ORACLE SYSTEM USER PASSWORD IS "12345678" ARE THESE SETTINGS OKAYY[y/n]:>y I Datei(en) kopiert. 1 Datei(en) kopiert. BIOCRATES BACKUP ADMIN already exists! DO YOU WANT TO UPDATE YOUR BIOCRATES BACKUP ADMIN? [y/n]:>y		
3	When the update process is completed, press any key. The Backup Admin is now updated.			
<u> </u>	The update process does not affe	ct any concentration data in the Met <i>ID</i> Q [™] 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/DQ [™] toolbar click Apply Database Patch.	-		
2	Choose the desired database patch. Met/DQ [™] will load it automatically into the Met/DQ [™] database. Kit patch's name example: MSmanufacture_KIT_DB###_Patch_YYMMDD.jar	Sicher Jrach File Sucher Jru Patches WSmanufacture,XTL,DB###_Patch,YVMMDD.jar Zuletti Werwendet Deteromer: ABSCIEV, p.130, D997, Patch_140331,jar Delgetop: MettQ Patch File (jar)		
3	Click OK to complete the operation.	Patch successfully executed!		
$\underline{\hat{\mathbf{I}}}$	Refer to the kit user manual to look up the required Met <i>IDQ</i> [™] 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 but- ton in the toolbar.	
2	Use the Filter to select one or more Kit plates.	Alter Plate Runs To New OP Project Filter Projects Froject: Submission: Bar code: 105883 Clear SAPPY
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.	Alter Plate Runs To New OP
4	Click Alter.	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 μ L extraction solvent.

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

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)
$$NR = \frac{TF + 1 \,\mu L \,tissue}{1 \,\mu L \,tissue} = \frac{TF + 1}{1}$$

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/ DQ^{TM} , 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 " μ L/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 " μ L/mg Tissue Factor".

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

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

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).

cnormalized(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 " μ L/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.

	Exa	You	see in MetIDQ™	
Sample	Cell Number	Cell Extraction Volume [µL]	Cell Number	Cell Extraction Volume [µl]
001	1 300 400	50	1300400	50.000
002	5 700 100	50	5700100	50.000
003	800 600	50	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 [μ L]".

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

For sample #001 and the exemplary $c_{\text{analyte}} = 1.23 \,\mu\text{M}$ 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 10⁶ cells.

$$\begin{split} & \text{c}_{cells}(Sample \ 001) = 47.29 \ \frac{10^{-18} \ mol}{cell} = 47.29 \ \frac{pmol}{10^6 \ cell} \\ & \text{c}_{cells}(Sample \ 002) = 10.79 \ \frac{10^{-18} \ mol}{cell} = 10.79 \ \frac{pmol}{10^6 \ cell} \\ & \text{c}_{cells}(Sample \ 003) = 76.81 \ \frac{10^{-18} \ mol}{cell} = 76.81 \ \frac{pmol}{10^6 \ cell} \end{split}$$



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^{TM} 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.

3 Settings						
Gene	eral MetLIMS					
CSV File Se	ttings					
Append the	e following information automatically to Wiff-file names:					
Activate sir	multaneous acquisition and quantitation in Analyst:					
Specify the	e path to the acquisition method files (Thermo only):					
Settings for	r Cell Cultures					
Normalize o	concentration to the following amount of cells: 10E 6 🚔					
	Reset OK X Cancel					
Display Unit: pmol/10E6 Cells 🚽						
μM						
Enlit/Morea	ng/ml					
plit/Merge nM						
	pmol/mg Tissue					

7.12 Database connection troubleshooting

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

User Name:	labadmin	Change Password After Login		
Password:	•••••	Remember Password		
Database:	Your Database 🔹	Connection failed!		

If the connection failed, perform troubleshooting.

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

Port 1521 is used. If Met/DQ[™] 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".
- O 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;



- Restart Met/DQ[™] and check the database connection.

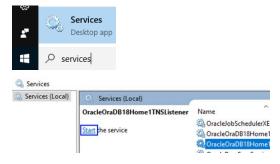


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3. Oracle® Listener service was not started

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

- Press the Windows key, type in "Services" and press Enter.
- Select "OracleOraDB18Home1TNSListener" and click Start.



3. Close and restart Met*IDQ*[™].

Y

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/DQ[™] 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 .
	ENTER THE ORACLE SYSTEM USER PASSWORD!
4	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:
ľ	"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/DQ™.

1

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 <i>ID</i> Q [™] , export the results:	1. Summary Analyte
1. Right-click anywhere in the table and select Full .	File Edit View Icols Window Script [™] New Ctrl+N Ctrl+N Ctrl+N [™] Open Ctrl+O Ctrl+D
2. Select File > Export	2. Close Open Workspace Save Workspace
3. Export the results as "Text Files (*.txt).	Save Workspace As Close Wo <u>r</u> kspace
This .txt file will be imported into Met IDQ^{TM} (refer to Met IDQ^{TM} user manual).	Save Ctrl+S Save As
	3. Text Files (*.txt)



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		

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Website:

BIOCRATES

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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