sepmag®


# Qualitance

## Biomagnetic Separation Monitoring & Management Software

## User's Guide

Rev 20200127

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# Table of contents

# Welcome

**Qualitance** is software for monitoring biomagnetic separation processes using the electronics integrated in the **Sepmag** system and its inserts. The software and hardware combination enables lot tracing and early detection of potential quality issues.

In close cooperation of our IVD production customers, we have developed a tool not only for measuring, but also for managing the results, thus helping users validate their lots. **Qualitance** makes it possible to define multiple-step product processes. Each step is characterized and compared with standard separation curves. We hope these improvements and the built-in reports will be helpful and that they will simplify the validation and quality control of your biomagnetic separation process.

This manual is divided into four sections. The first is an introduction to the software, which explains how it works, the basic concepts and what's new.

The second section tells you how to install the software on your computer and how to connect the **Sepmag** hardware.

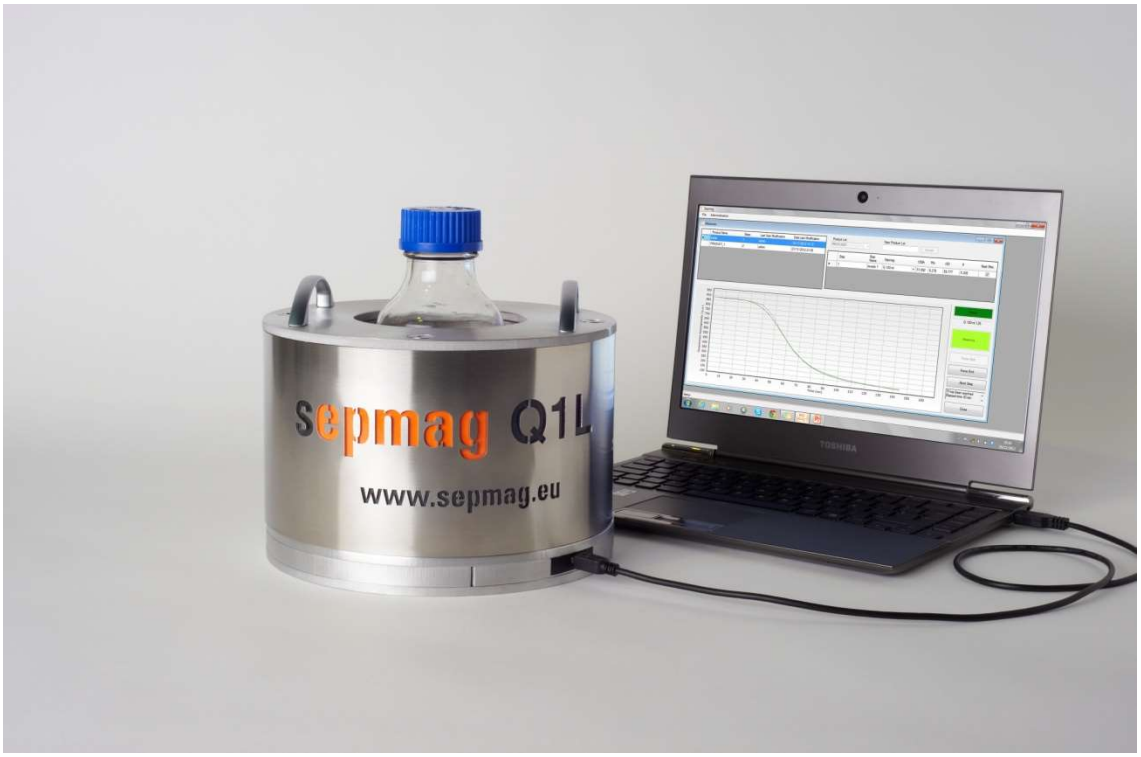


***Fig. 1**:* Sepmag® *Q1L with monitoring hardware, connected to a laptop running Qualitance software.*

The third section is a manual for users with standard privileges that explains how to measure, print reports and check historical data.

The fourth section of the manual covers functions that are accessible only to users with administrator privileges (managing users, processes and hardware). Administrators also have access to ‘standard’ functions, so we recommend you read sections three and four before using the software.

At the end of the document you will find a glossary with a brief definition of the key words and concepts used in this manual.

# 1. INTRODUCTION: BASIC CONCEPTS

**Qualitance** enables users to measure changes in the opacity of a suspension inside the **Sepmag** Biomagnetic Separation System and provides tools for tracing the entire production process of each lot.

## 1.1 What is QUALITANCE?

**Qualitance** is an electronic hardware, firmware and software system for measuring changes in opacity in a transparent/translucent vessel inserted in a **Sepmag**.

**Qualitance** has been developed to give technicians, researchers and managers a way of obtaining an objective measurement of the biomagnetic separation process. The goal is to replace the traditional determination of the separation time by visual estimation with a measurement of the opacity of the suspension throughout the **entire process**.

**Sepmag** monitoring hardware is composed of a cold lighting assembly of LEDs and an optical sensor. Both subsystems are controlled by an electronic card connected to a PC running Windows through a USB port. The software identifies the specific **Sepmag** unit or insert, records the data captured, generates printer-friendly reports, saves the historical data in a database and helps manage the steps in the biomagnetic separation process.

The hardware is normally supplied integrated into **Sepmag** Biomagnetic Separation Systems or its inserts.

## 1.2 Why should I monitor my biomagnetic separation processes?

The traditional way of checking whether a biomagnetic separation process is complete is by sight. The technician/researcher looks at the suspension. At the beginning of the process, the suspension is homogenous and opaque. When the separation process is complete, the magnetic beads are left on the walls of the vessel and the supernatant is transparent. When the suspension is ‘transparent’, the technician stops the process by extracting the supernatant, leaving the magnetic beads in the bottle. After repeating the same process several times, a separation time can be defined and used as a benchmark. With the traditional method, the only quality control record is the OK/Not OK signed by the person handling the vessel, with no supporting data. In the event of a quality issue with the product, it is not detected until a later stage, and there are no records to show whether the problem occurred before, during or after the biomagnetic separation.

In contrast, by continuously monitoring the opacity of the suspension while inside the **Sepmag**, technicians have a record of its evolution during the process. Changes in opacity inside a **Sepmag** Biomagnetic Separation System should be the same if the suspension is the same. Changes to the properties of the magnetic beads (diameter, magnetic charge), concentration and/or viscosity of the buffer lead to different opacity behaviors, so any deviations from the expected pattern serve as an early warning system.

The process is documented with printed reports for each stage. The acceptance parameter can be defined objectively, without subjective operator opinions. Moreover, the cumulative experimental data enables teams (technicians, researchers and managers) to audit, analyze, review and improve the biomagnetic separation steps in their manufacturing processes.

## 1.3 What does QUALITANCE measure?

**Qualitance** hardware measures changes in transmitted light through the bottle. Using cold light (LED), the vessel is illuminated and a sensor measures the transmitted light. At the start of the process, when the suspension is homogenous, opacity is at its maximum level. When separation is complete, the remaining suspension is clear and opacity is at its minimum level.

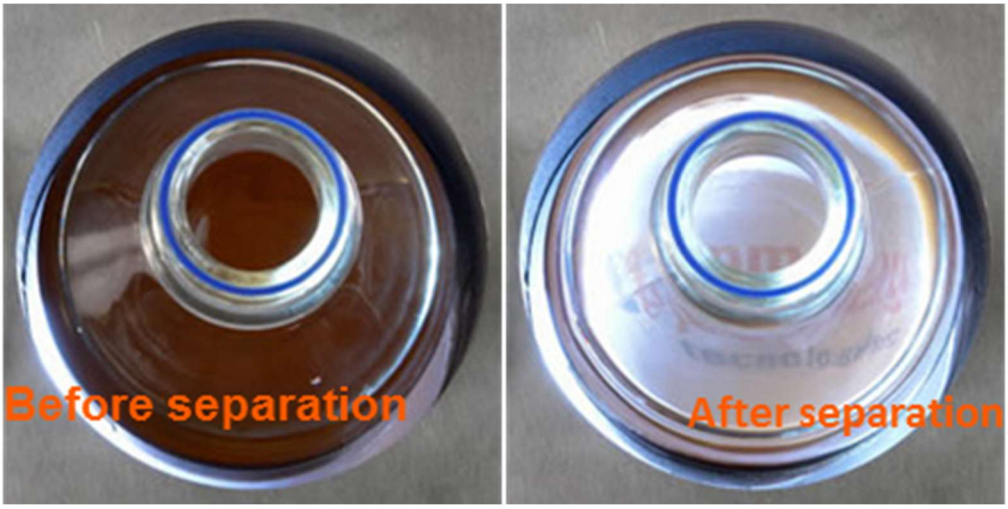


***Fig. 2**: Opacity of a typical magnetic bead suspension. Before separation, it is dark and homogenous. When separation is complete, the beads are left on the wall and the suspension becomes transparent.*

## 1.4 How does QUALITANCE characterize the biomagnetic separation process?

The typical biomagnetic separation curve has a sigmoidal shape. As discussed in several technical documents (www.sepmag.eu/resources/ebooks, www.sepmag.eu/blog), both the slope (defined by the dimensionless exponent *'p'*) and the separation time ($t_{50}$, expressed in seconds) depend on the buffer viscosity, the properties of the magnetic beads and the concentration. The biomagnetic separation conditions are homogenous and well known for each **Sepmag**, and both parameters can be used to define and validate each separation process.

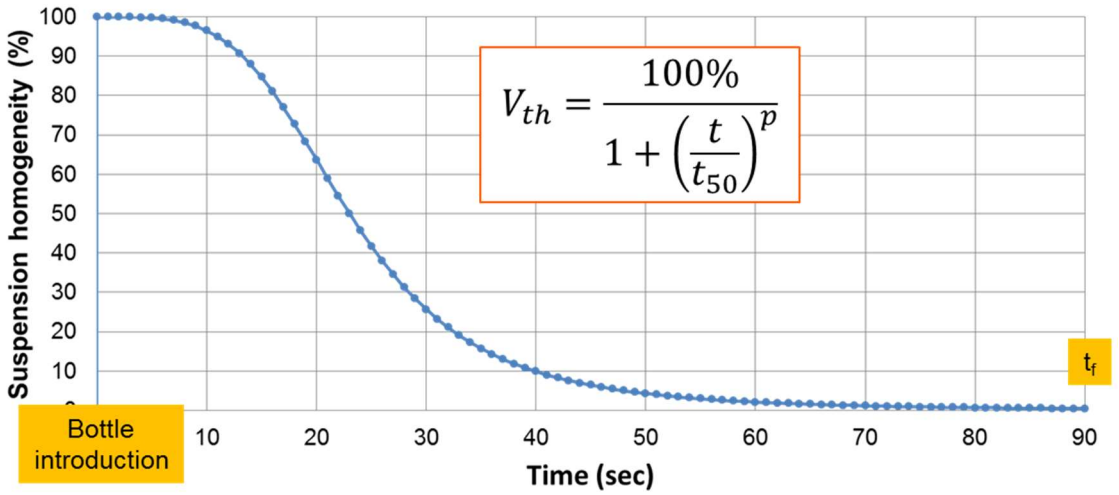


***Fig. 3**: Typical shape of biomagnetic separation curves measured using a **Sepmag**® System and **Qualitance** software.*

## 1.5 What happens while the bottle is in the SEPMAG?

In the **Qualitance** software, measurement starts automatically when the bottle is inserted into the device, taking readings while the bottle is inside the device and stopping automatically when the bottle is removed.

This means users do not need to define the length of the process in advance, allowing longer measurements when necessary.

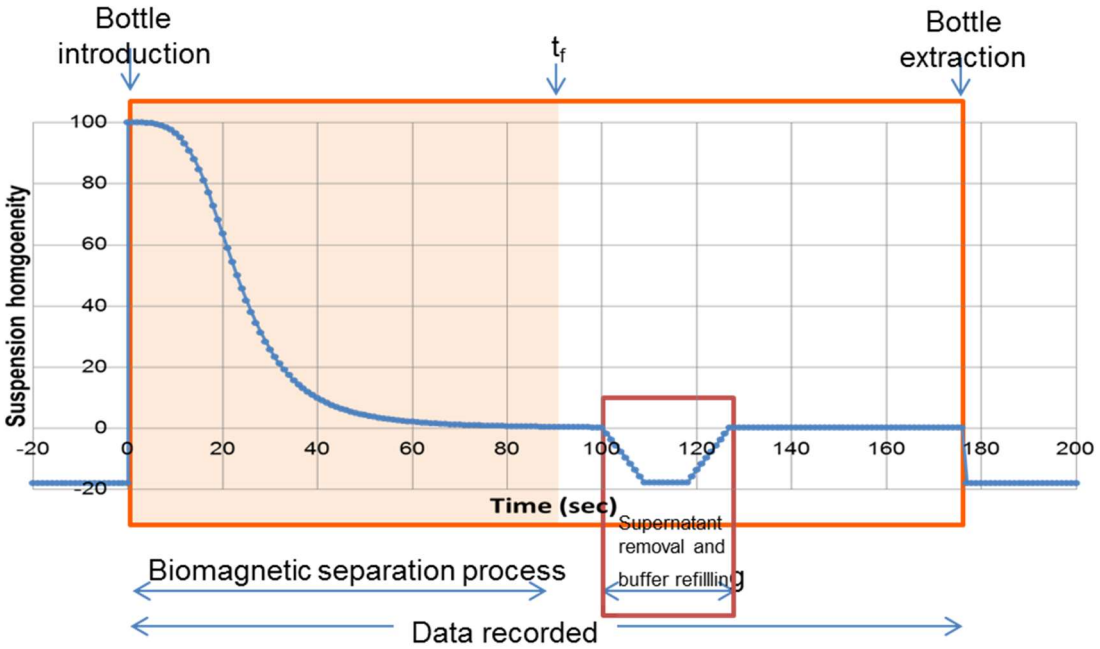


***Fig. 4**: Schematic representation of the curve while the bottle is inside the **Sepmag**®. Data is recorded while the bottle is inside the device, but only displayed between introducing the bottle and '$t_f$'*

The system then records three processes that typically occur while the bottle is inside the **Sepmag** ® System:

1. Biomagnetic separation takes place from the moment the bottle is inserted. This is shown by the sigmoidal-like curve described in the previous section.
2. Once separation is complete, the supernatant is extracted from the bottle, leaving the magnetic beads on the walls.
3. Clean buffer is usually added to the vessel while it is still inside the **Sepmag**.

A log of the entire process can be obtained using the Export option (see section *3.2, Access to recorded processes*). However, **Qualitance** only shows data from the biomagnetic separation process, between introducing the bottle and time *'$t_f$'*. Users with administrator privileges can modify the value of '$t_f$", which should always be a value between the end of the separation and the start of the supernatant extraction. Fittings to the sigmoidal-curve only use data recorded between inserting the bottle and $t_f$ to determine the values of $t_{50}$ and $p$. After refilling, the bottle is extracted and **Qualitance** stops taking measurements.

## 1.6 Multi-step Product Process

Product manufacturing usually requires several biomagnetic separation steps (for example, several washings between two coating processes). That is why the process macro for a product can include several steps (up to 15). All separation steps are recorded sequentially and the software is run in the order defined. The product process can be put on hold between two consecutive steps and the hardware and software can be used for a different process. This is useful if a suspension requires a long incubation period between two biomagnetic separation steps, because the **Sepmag** system can be used for a different product during the interval.

When you decide to resume the original process, **Qualitance** will remind you where you were in the process and will continue with the pre-set sequence.

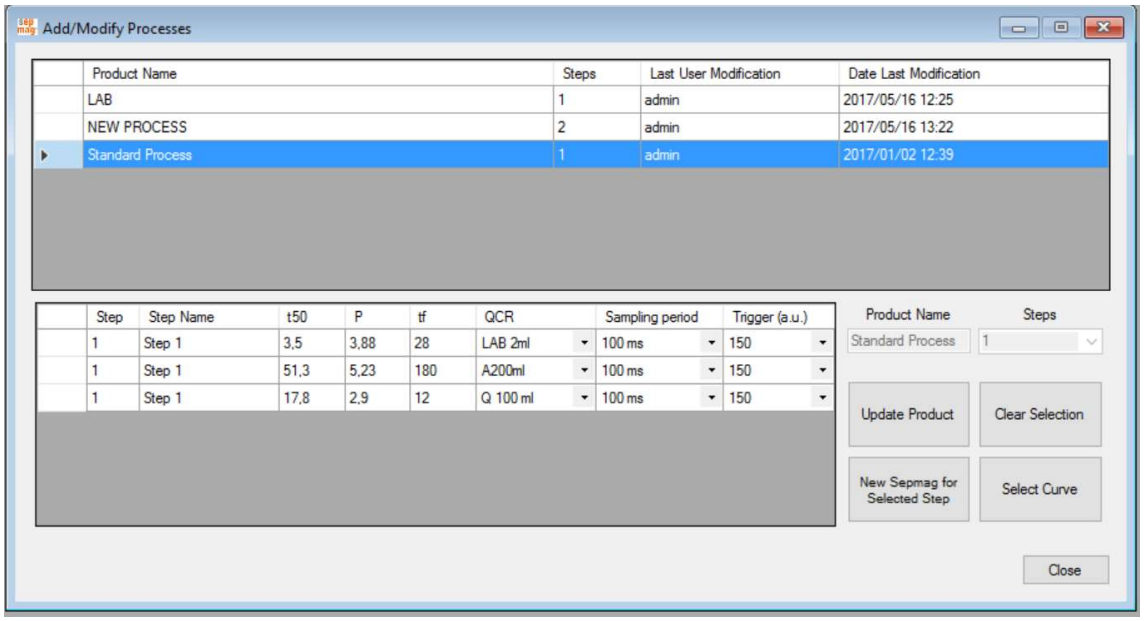


***Fig. 5**: Process macro window. Multi-step processes can be defined, using SEPMAG® devices with different volumes for the different steps. The software checks the ID of each individual SEPMAG® to confirm that the right one is used for the step.*

## 1.7 Individual SEPMAG identification

Each step of a product process is linked to a specific **Sepmag** ® device. To avoid confusion and/or mistakes, the software ensures that the **Sepmag** ® system is the one defined for the current step of the Product Process Macro.

If, for validation purposes or production needs, different **Sepmag** ® devices are suitable for a specific step, the same step can be doubled up for each system (although the value of *p* and $t_{50}$ will not be the same in different devices). Before taking a measurement, the software checks that you have connected the right equipment for the step selected.

## 1.8 Process parameter management

The **Qualitance** software fits the experimental results to a sigmoidal curve and determines the experimental biomagnetic separation parameters ($t_{50}$ and *p*).

This means that the software generates a historical database of the values of these two parameters, $t_{50h}$ and $p_{th}$ for each step. These two values can be used as references for comparison against future step measurements.

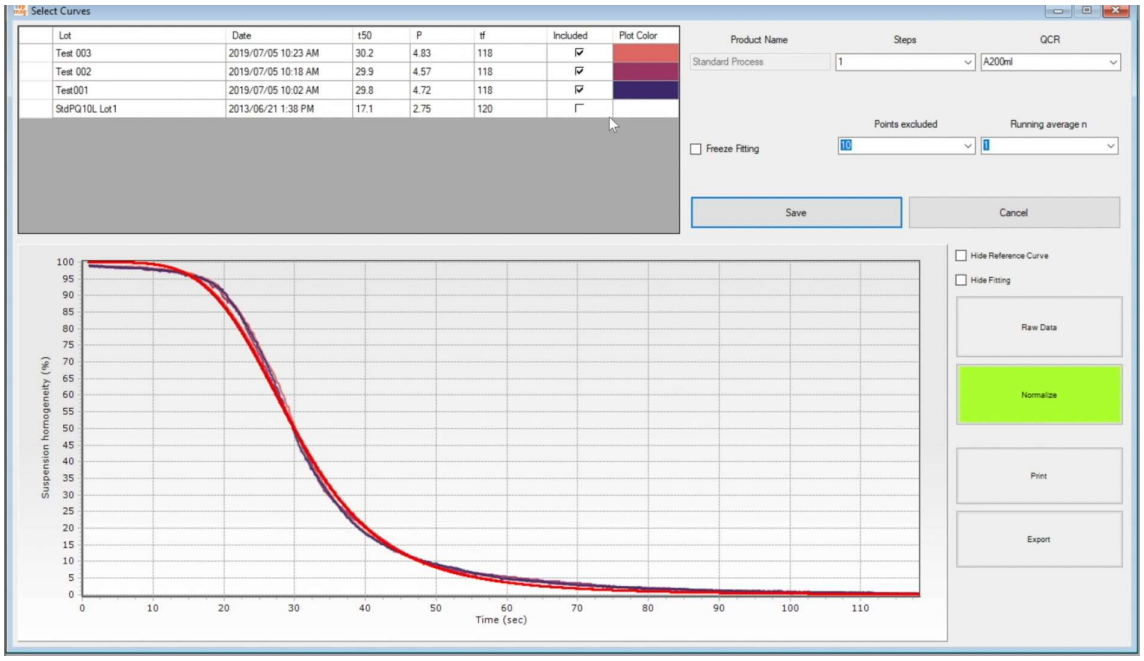


***Fig. 6**: Historical data of a product process step.*

The software manages historical data to generate reference curves automatically for each step of a product process. Only users with administrator privileges may exclude curves from the statistics, or 'freeze' the standard when enough data has been captured.

During measurement, once $t_{50th}$ is reached, the software shows a predictive curve using the reference values ($t_{50th}$, $p_{th}$). At the end of the measurement, it compares the experimental data with the 'standard' curve.

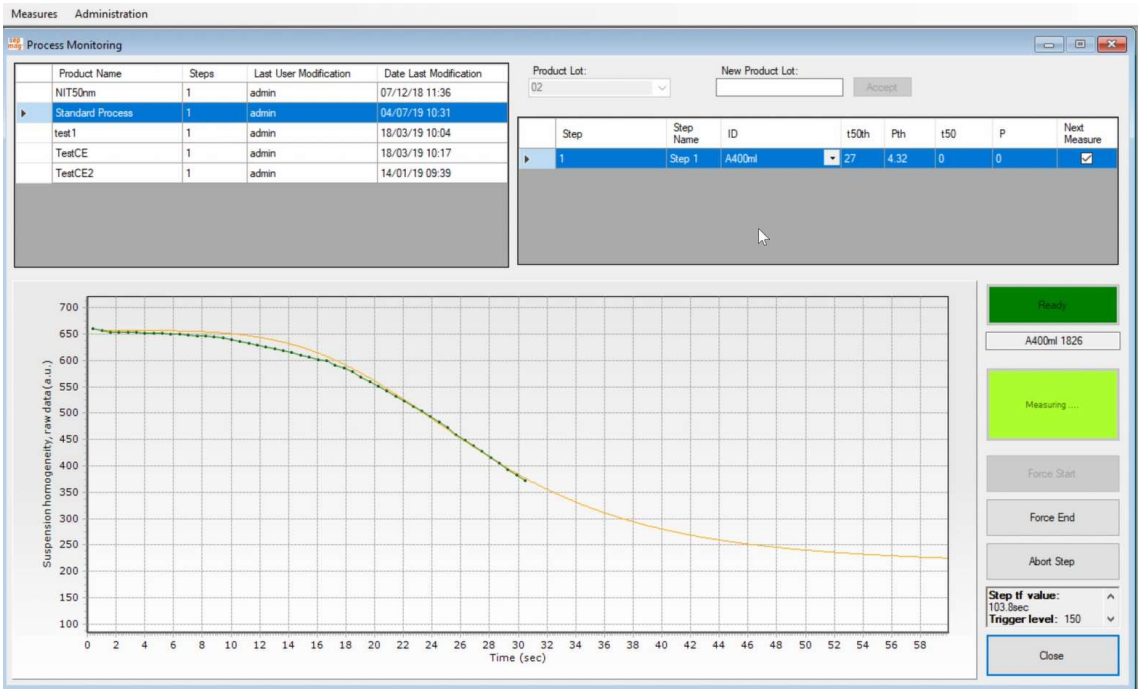


***Fig. 7**: Experimental curve (green) and predictive curve (orange) generated using the standard parameters ($t_{50th}$, $p_{th}$).*

## 1.9 User categories: standard and administrator

The program distinguishes between two different user categories:

i) Users with ***administrator*** privileges and

ii) ***Standard*** users.

**Standard** users have access to the **Measures** menu, which includes the options **Process Monitoring** (measuring) and **Retrieve Data** (consult data from older processes, generate and print reports, export measurements). Section 3 describes these functions in detail.

**Administrators** also have access to the 'Administration' menu. This menu includes the **Add/Modify Users** option (define new users, modify privileges), **Add/Modify Devices** (for including new **Sepmag** hardware), **Audit Trail** (a log of changes made) and **Add /Modify Processes**. The latter can be used to define new product processes, update

existing processes and select curves to define the standard for a specific step. Section 4 provides details on functions available to administrators only.

Changes made by the administrator are recorded in the audit trail. This log is also accessible in the administration menu, but it is not editable.

The following section explains how to install the hardware and software and the functions accessible to standard and administrator users.

# 2. INSTALLATION

The previous section described the **Qualitance** software features. This section describes how to set up your system. It indicates the computer requirements, how to install the software, the hardware supplied materials list and how to connect the **Sepmag** ® equipment.

## 2.1 System Requirements

A PC computer with an available USB port for connecting the **Sepmag** ® hardware is required. If a laptop computer is used, we recommended that the computer remain plugged into the power source while the **Qualitance** application is running to avoid a power failure that could lead to a loss of process information.

The **Qualitance** software is designed to run on **Windows 10** or later versions of Windows. The minimum requirements are a Core i5 or i7 processor, 8 GB RAM and an available USB port.

## 2.2 List of materials supplied with the SEPMAG

The **Sepmag** ® Biomagnetic Separation Systems consists of:

1. Monitoring hardware elements (already assembled in the **Sepmag** ® unit).
2. Cable to connect the **Sepmag** ® device to a computer via a USB port.
3. **Qualitance** data acquisition software (the software is supplied either as a hard copy or as a link to the downloadable file).

## 2.3 Unpacking, assembling and installation guidelines

To guarantee correct performance of the **Qualitance** system, please follow these steps:

1. Find the micro-USB port on the **Sepmag®** system.
2. Check that your computer meets the system requirements specified in Section 2.1 and is compatible with the **Qualitance** system.
3. Make sure the computer has a free USB port to connect to the **Sepmag®** and that the USB port is compatible.
4. Install the **Qualitance** software on your computer (see section 2.4)
5. Connect the hardware according to the instructions in section 2.5. The system is now ready to start operating (sections 3 and 4).

## 2.4 Software Installation

To run the software installation, execute the setup file (*Qualitance_v3.1.x.exe*) provided to you either as a hard copy or through an internet link.

The setup program will guide you through the steps required to install the software on your computer **(Fig. 8).** It will create a new directory containing all the necessary files in the selected location on your computer**.** When the installation process is complete, a shortcut to the program will automatically appear on the computer desktop.

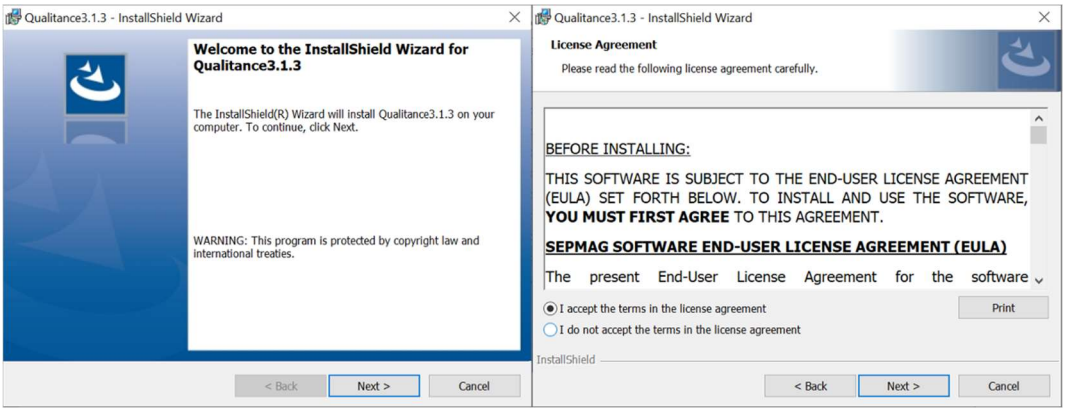


**Fig. 8**: Qualitance software installation process using the *Qualitance_v3.1* setup file.

## 2.5 Hardware – Software interface

Once you have installed the software and assembled the equipment, connect the **Sepmag**® to the computer using the USB cable provided **(Fig. 9):**


***Fig. 9**: Installation of the* **Sepmag**® *equipment and* ***Qualitance*** *software.*

A. Have the USB cable provided ready to connect the computer and the **Sepmag**® hardware for data acquisition.

B. Connect one end of the USB cable to the port on the **Sepmag**® device.

C. Connect the USB end of the cable to the USB port on the computer and start running the **Qualitance** software to monitor your process (See sections 3 and 4 for details).

## 2.6 Qualitance database backup

All processes saved by the different users are stored in the **Qualitance** database. Every time the user exits the program, a backup copy of the database is created and saved by the program in the folder file:///Volumes/C/Users/Public/Documents/Sepmag/QCR/.

The database (*SepmagDDBBv2_lastCopy*) is a backup copy of the program database (*SepmagDDBBv2*). It is important to keep a copy of this backup file in a safe place, because the original database will be lost if the program must be reinstalled on the computer.

***It is very important to keep a copy of the** SepMagDDBBv2 **back up file in a safe place.***

# 3. RUNNING THE QUALITANCE SOFTWARE AS A STANDARD USER

Start the application by double-clicking the shortcut represented by the **Sepmag®** logo, which appears on the computer desktop after the set-up process is complete and the software has been correctly installed on the computer.

When you start the program, a *Login* dialog window pops up. Users must enter a User Name and Password to start a session **(Fig. 10).**

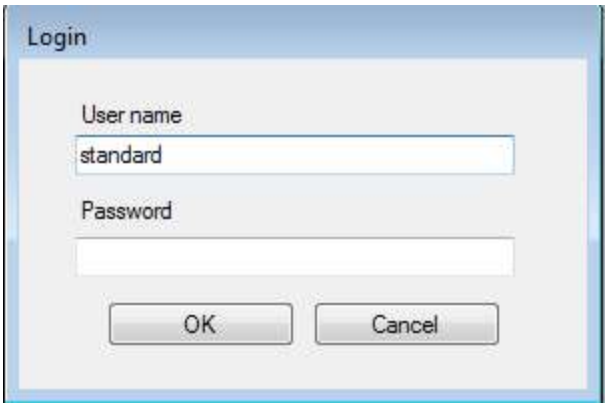


***Fig. 10**: Initial dialog window for user identification. Users must log in to start a session. The program requires a user name and password.*

**When the program is used for the first time**, the default user name is *standard* and the default password is *standard.* This default configuration has *standard* privileges.

A user with standard privileges has access to the *Measures* menu, to execute the defined product process through *Process Monitoring* (section 3.1), as well as access to previous measurements through *Retrieve Data* (section 3.2).

The *Logout* option takes you back to the *Login* menu, allowing a different user to log in without exiting the application.

*Exit* closes the program and returns to Windows. You can also exit the program by closing the main menu window (by clicking the X in the top right-hand corner).


***Fig. 11**: User interface for the main menu of the program. To exit the program, either select Exit in the Measures menu or simply close the main menu window.*

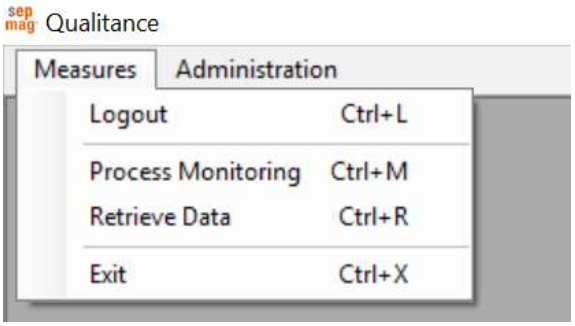


***Fig. 12**: Initial dialog window for users with standard privileges. The Process Monitoring option gives access to the measurement menu and Retrieve Data enables you to consult existing measurements.*

## 3.1 Measures: Process Monitoring

Click the Process Monitoring option to access the Measures window.

If no **Sepmag** ® system is connected, the message at the top right is red and displays the message '***Waiting for the Qualitance connection'***.

If a **Sepmag** ® system is connected, the message is green and reads **'*Ready'***.

Once the system is **'*Ready*'**, select a process from the top left-hand table (a 'Standard Process' is included by default).

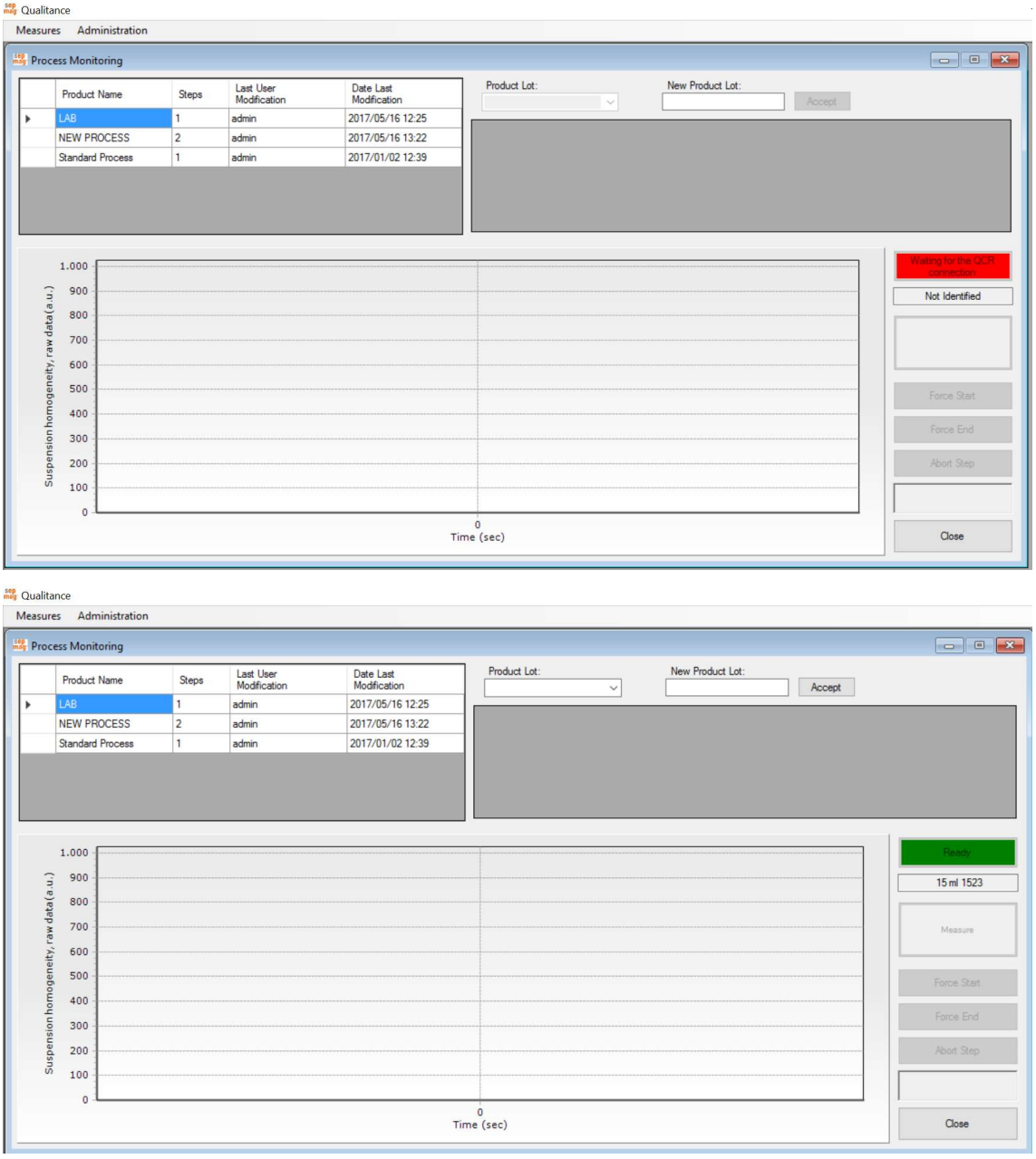


***Fig. 13**: Measures window (Process Monitoring) when no* **Sepmag** ® *is detected (above) and when a* **Sepmag** ® *is correctly connected via USB port to the computer running* ***Qualitance*** *software (below).*

Next, enter the lot name:

- If it is a new lot, the name must be entered manually in **New Product Lot**. The software checks the database and issues a warning if the lot name already exists. Duplicate names are not allowed.
- If the lot is already in progress (i.e., some steps have already been executed and the sequence is on hold), the lot name must be selected from the options in **Product Lot** (click to open the list)

The table on the right shows the list of steps. The tick in the **'Next Measurement'** column shows which step will be executed based on the current Product Process. If more than one **Sepmag®** system is allowed for the current step, you must select one of them. When ready, click **Start Step**. A pop-up window will open asking for confirmation.

Once the data entry sequence is complete, a ***'Waiting'*** message flashes on an orange background. **<u>This means that the system is ready and waiting for the bottle to be inserted.</u>**

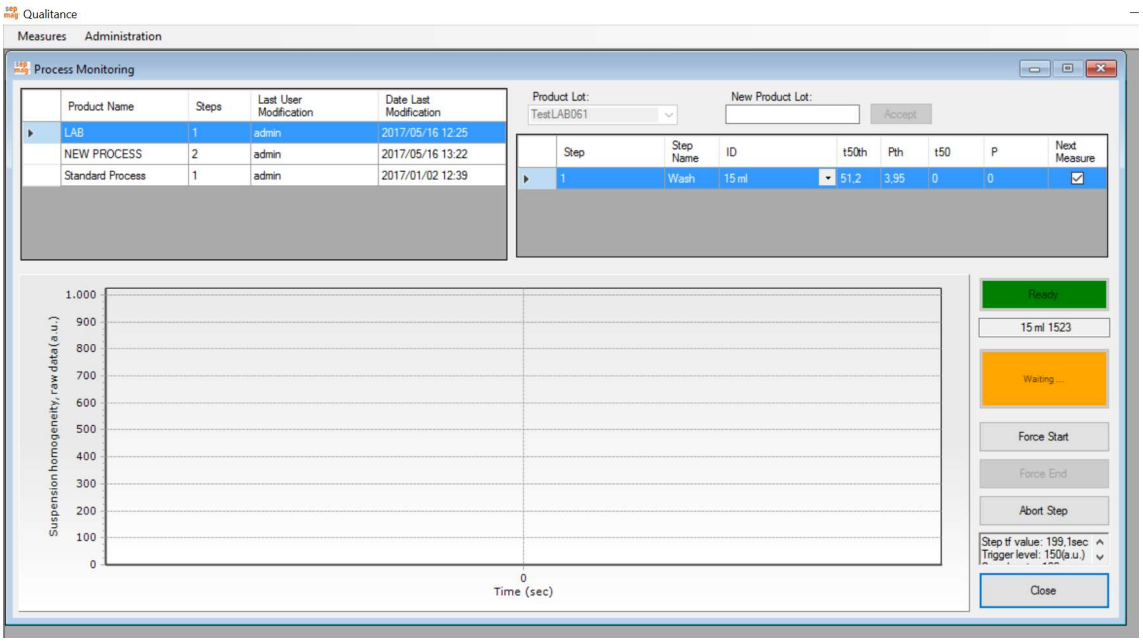


***Fig. 14**: System ready to take a new measurement. The Waiting message indicates that **Qualitance** is waiting for the bottle to be introduced.*

Place the bottle in the **Sepmag®** system and the measurement will start automatically (**Force Start** can be used in rare cases in which the trigger fails to activate the measurement). The message will change to ***Measuring*** with a light-green background.

Once the measurement reaches $t_{50th}$, the program will display a predictive curve with an approximation of the expected behavior for the current step. For obvious reasons (no historical data exists), the predictive curve does not appear the first time a process is run.

When the measurement reaches the set 'final time', $t_f$, you will hear a beep. No more information is displayed on the screen, but the data is recorded until the bottle is extracted or you click **Force End**. The additional data is accessible via **Export** (see section 3.2).

The software then asks whether to save the measurement (if the answer is 'no', it asks you to confirm your answer) and whether you want to print a Product Process Step report.

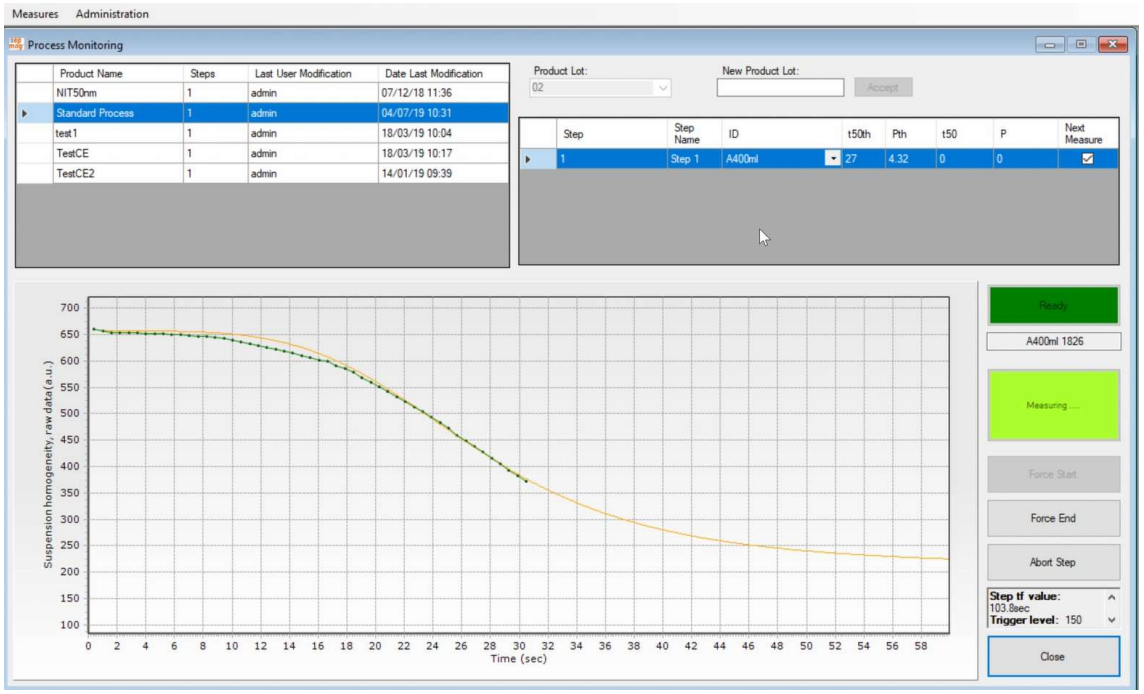


***Fig. 15**: Experimental curve (green dot) and predictive curve (orange line) generated using the standard parameters ($t_{50th}$, $p_{th}$).*

You can then go on to the next step in the current product process, select a new product process or close the measures window.

As explained in the following section, all data is accessible in the *Retrieve Data* window, through the Measures/Retrieve Data menu.


***Fig. 16:*** *Measurement complete. The green dot curve is the experimental curve, the orange line is the fitting to a sigmoidal curve and the red line is the reference curve for this specific step.*

**Important:** When you run the Product Process Step for first time, the 'end time', $t_f$, is not yet defined. In this case, stop the measurement using ***Force End*** when the Biomagnetic Separation process is complete and before starting the supernatant extraction. The program will use this time as the default $t_f$. The value can be edited by a user with 'administrator' privileges in the **Process Macro** menu.

## 3.2 Access to recorded data: Retrieve Data

The main **Measures** menu gives Standard users access to existing measurements. It prints Lot reports for the complete Product Process (all steps in sequential order) and checks and compares individual steps.

To access these features, click the **Retrieve Data** option.

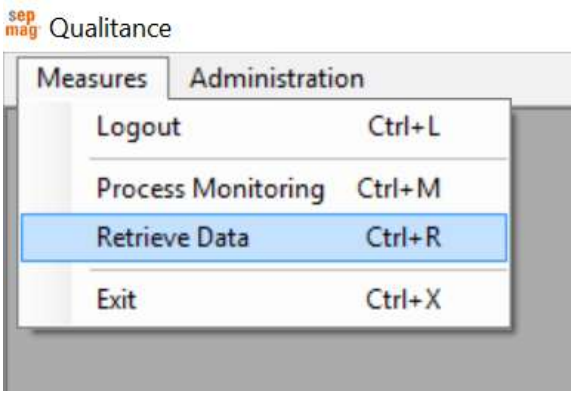


***Fig. 17:*** *Measures menu. The Retrieve Data option displays existing measurements.*

The Processes window displays all the measurement processes in the top left table, in measurement date order. Select one to see details of each step in the top right table.

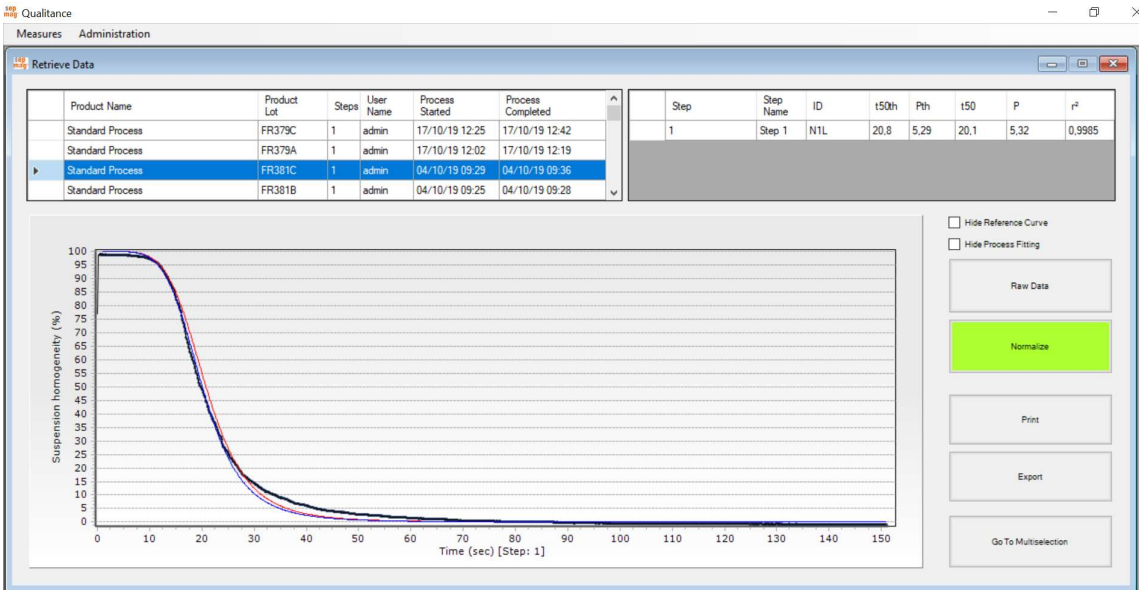


***Fig. 18:*** *Process window. All steps in a Product Process Lot are displayed together.* ***Print*** *generates a printer-friendly report and* ***Export*** *gives the user access to the raw measurement data.*

When you select a specific Lot, the data corresponding to the different steps are plotted in a single graph by clicking **View Lot**. Reference curves can be hidden by clicking the corresponding box. Zoom in on graphs by holding down the left mouse button and clicking.

The data can be shown as **Normalized** (0-100% according the sigmoidal fitting) or as **Raw Data** (values as obtained during the measurement') by clicking the appropriate buttons.

**Print** generates a standard printer-friendly report (a new window pops up with the document).

**Export** generates a data file with all the recorded data. The file can be in *.xls (Excel) or *.txt format. **Note**: the exported data also includes the values between 'tf' and the bottle extraction –or Force End–.

## Curves Multi Selection

The final option in the Process window is **Curves Multi Selection**. Click this button to open a new window.

**Curves Multi Selection** enables you to compare individual steps freely, regardless of the Product Process.

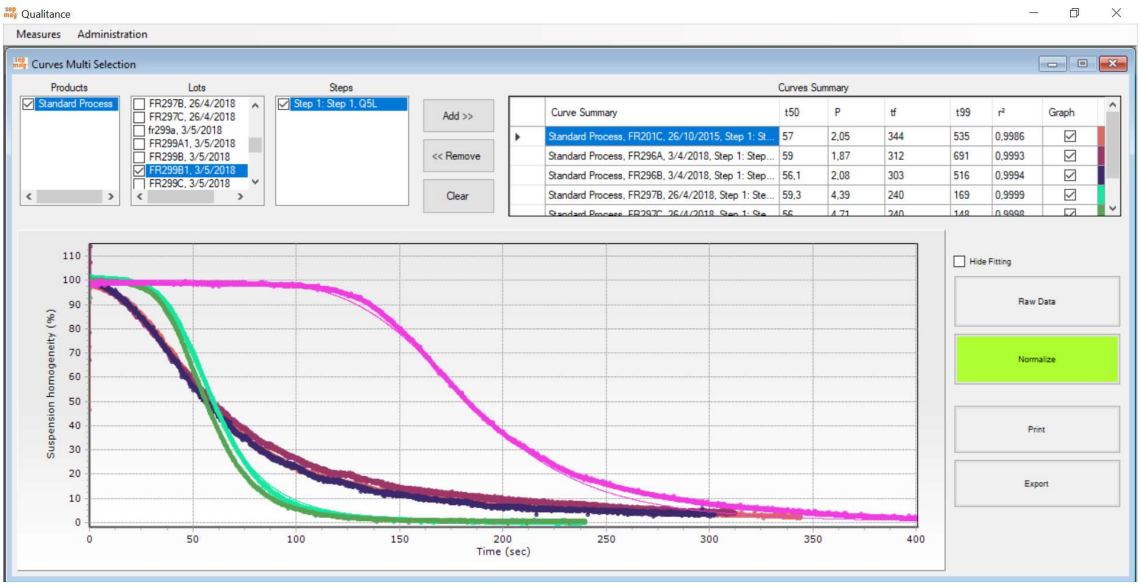


***Fig. 19:*** *Curves Multi Selection window. The different steps can be selected with the boxes in the top left. The Curves with 'graph' ON are shown when 'View' is clicked.*

First select the **Product Name**, **Lot** and **Step**, and then click on **Add**. The process will then appear in the **Curves Summary** table in the top right. Several **Steps** can be added to the table.

The **Graph** check boxes show which curves are displayed when you click **View**. The curves added by default are visible. Click the **Graph** check box again to deselect it from the graph, but not from the table.

To remove a step from the **Curves Summary** table, click the step and then click **Remove**. **Clear** removes all the steps from the table.

Click **View** to display all selected graph curves in the table. Zoom by holding down the left mouse button and moving it from the top left-hand corner to magnify the selected area and moving from the top righthand corner to decrease the magnification. The fitting curves can be hidden by clicking the box.

The **Print** option generates a printer-friendly document.

In addition to the standard function, **Qualitance** has functions accessible only to users with administration privileges. The fourth section of the manual describes these additional features.

# 4. RUNNING THE QUALITANCE SOFTWARE AS AN ADMINISTRATOR USER

The **Qualitance** software has two user categories. Standard users can use the program to take measurements, print reports and consult previous measurements. All these functions are accessible through the Measures menu. Administrators have access to both the **Measures** and **Administration** menus, where they can set up new **Sepmag®** hardware, add users and generate/modify new product process macros.

The Administration menu functions can also be executed when no **Sepmag®** hardware is connected to the computer.

This section describes the functions available only to **Administrators**. For Standard functions, please see Section 3 (*Running the Qualitance software as a Standard User*).

Start the application by double-clicking the shortcut represented by the **Sepmag®** logo, which appears on the computer desktop after the set-up process is complete and the software has been correctly installed on the computer.

When you launch the program, a **Login** dialog window pops up and asks you for your **User Name** and **Password** to start a session (Fig. 20).

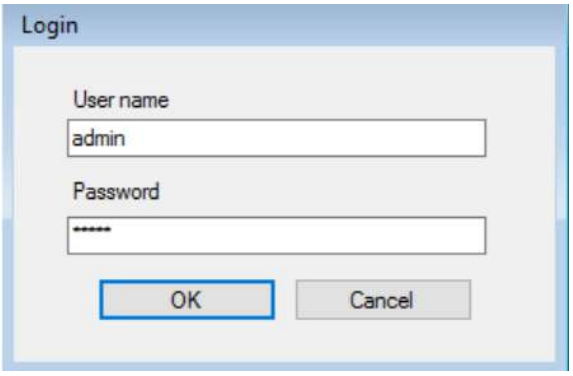


***Fig. 20:*** *Initial dialog window for user identification. Users must log in to start a session. The program asks for a user name and password.*

When you use the program for the first time, the default **User Name** is *admin* and the default **Password** is *admin*. This default configuration has administrator privileges. (NOTE: Because the admin user name and password are published in the manual, it is strongly recommended that you change the password. See section 4.1 for instructions).

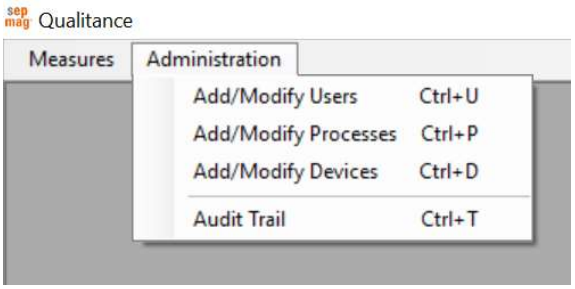


***Fig. 21:*** *Administration menu.*

In addition to the **Measures** menu, the **Administration** menu appears in the top left-hand part of the window. Click to see the four options:

- **Add/Modify Users:** This is where you create and edit user accounts and privileges.
- **Add/Modify Processes**: This is where you define and edit product processes and generate the reference curve for each step in the process.
- **Add/Modify Devices**: This is where the **Qualitance** software assigns a name and ID to each **Sepmag®** device.
- **Audit Trail**: This logs the changes made in the Administration menu.

Any changes to **Users**, **Process Macro** and **Devices** are recorded by the system.

The following sections provide details on these four options.

## 4.1 Create or edit users: Add/Modify Users menu

When you click **Add/Modify Users** in the **Administration** menu, the user administration window opens. The table at the top lists the **User Name**, **Password** and whether the user has **Administrator** privileges.

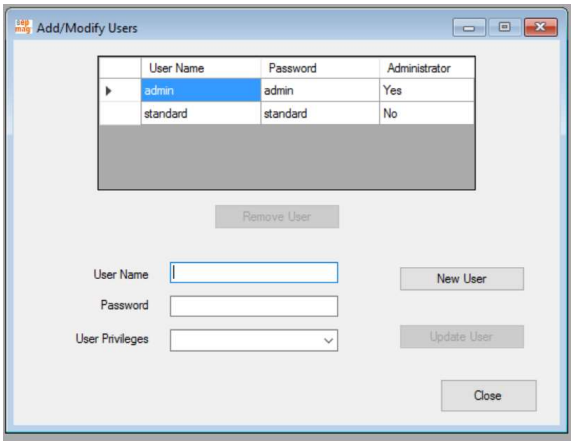


***Fig. 22**: Access the User Administration window by clicking Add/Modify Users in the Administration menu.*

To create a New User, enter the User Name, Password and User Privileges and click **New User**.

To edit/delete an existing user, click the table row with the user you want to modify. The **Remove User** and **Update User** options are displayed.

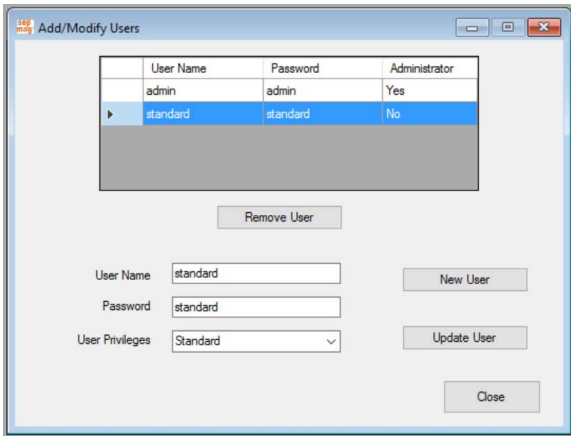


***Fig. 23**: User administration window.*

The User Name, Password and/or User Privileges can be then edited. Click **Update User**. A pop-up window will be displayed asking you to confirm the changes.

Click **Remove User** to delete the account (a warning message appears and asks for confirmation before deleting the user). There must always be at least one user with administrator privileges. The software does not allow you to delete the last administrator.

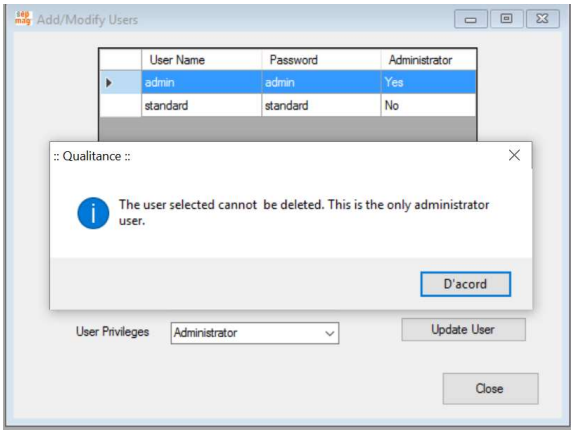


***Fig. 24**: User administration window. This warning is displayed if you attempt to remove the last administrator user.*

To return to the **Administration** menu, click **Close** or close the User Administration window.

## 4.2 Connecting new SEPMAG devices to the computer: Add/Modify Devices

When a new **Sepmag** ® system is received, a custom **Qualitance** software installer is provided. The table shows the name of the SEPMAG® and the identification number of the monitoring hardware.

When you purchase additional **Sepmag** ® Biomagnetic Separation systems and their corresponding monitoring options, you will need to add the device names and IDs. This enables the **Qualitance** software to identify the equipment connected to the computer and prevents you from using a Product Process defined for one specific volume device with a different one.

To add new **Sepmag** ® hardware, simply enter the new and ID number (provided with the Technical Data) and click **New Pair**.

If necessary, the values can be edited by selecting a row in the table (the Update Pair button is displayed), modify the values and click **Update Pair**.

To return to the Administration menu, click **Close** or close the User Administration window.

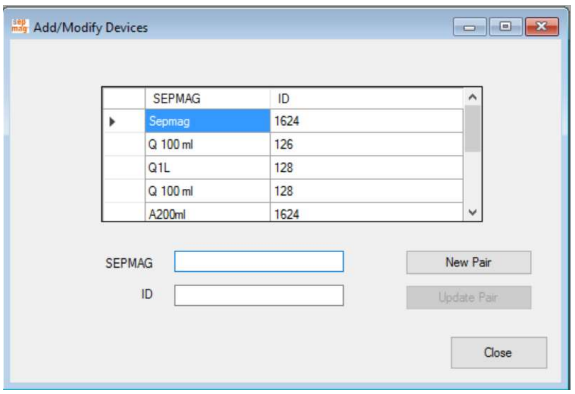


***Fig. 25**: Add/Modify Devices window. This shows the name of the SEPMAG® Biomagnetic Separation Systems that can be connected and the ID number of the monitoring hardware.*

## 4.3 Defining New Product Processes: Add/Modify Processes

Users with administrator privileges can access **Add/Modify Processes** by clicking this option on the menu.

As mentioned in the introduction, a process is made up of several steps (i.e. washing 1, washing 2, etc.) that must be executed in the sequence defined.

To define a **New Product Process,** enter the **Product Name** and define the number of steps in the boxes under the main table (see figure 26)

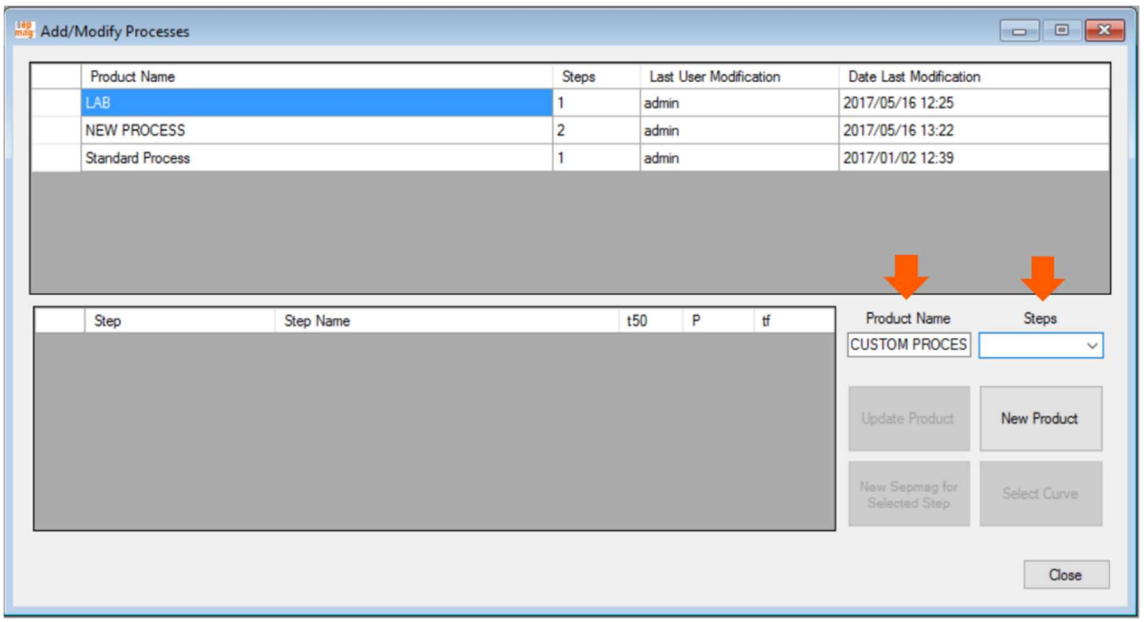


***Fig. 26**: Process Macro window. Arrows indicate where to enter the Product Name and the number of steps when a new process is generated.*

One row for each defined **Step** is shown in the bottom left-hand part of the table. Assign a **Step Name** to the individual steps, and associate a Sepmag device to each one (you may add additional Sepmag devices later; see section 4.5). When the row is complete, click **New Product**.

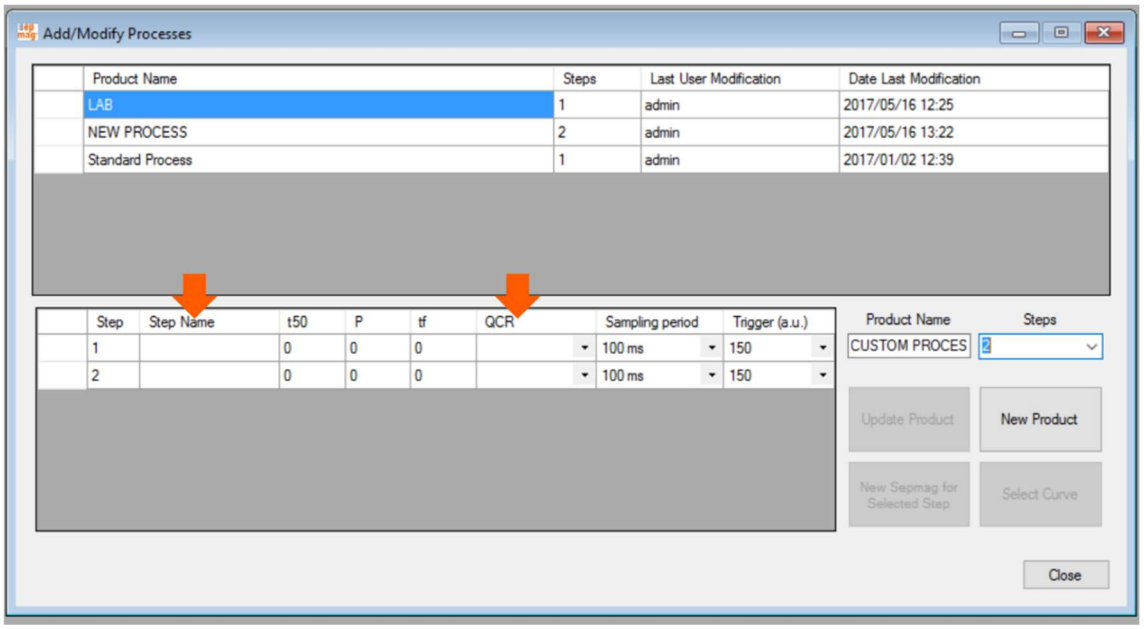


***Fig. 27**: Process Macro window. The arrows show where to enter the Step Name and where to select the Sepmag associated with each Step.*

The new process appears in the table at the top with the **Product Name** and the number of **Steps**. The name of the **User** who made the last modification and details of the changes are displayed. The entire change history can be traced through the **Audit Trail** option.

The new **Product Process** is then ready to be used. Access and use it with the **Measures/Process Monitoring** menu (see section 3.1). When a product process step is run for first time, the 'final time', $t_f$, is not yet defined. In this case, stop the measurement using **Force End** when the biomagnetic separation process is complete and before starting the supernatant extraction. The program uses this time as the default $t_f$. The value can be edited by users with **Administrator** privileges in the **Add/Modify Processes** menu, as explained in section 4.5.

The **Sampling period** is 100 ms, but for long separation processes (large volumes, small particles and/or viscous buffer), the value can be changed to up to 30 s. The **Trigger** is set at 150, but can be reduced if the concentration is low (i.e. low contrast) or increased if the ambient lighting causes false starts.

When a step involves several measurements, it is important to check that the automatic reference curve is correctly generated. The next section explains how.

## 4.4 Selecting/Deselecting curves for generating the reference curve

After defining a Product Process Macro as explained in the previous section, any user (Standard or Administrator) may start measurements, following the established sequence of steps (see section 3.1 for details).

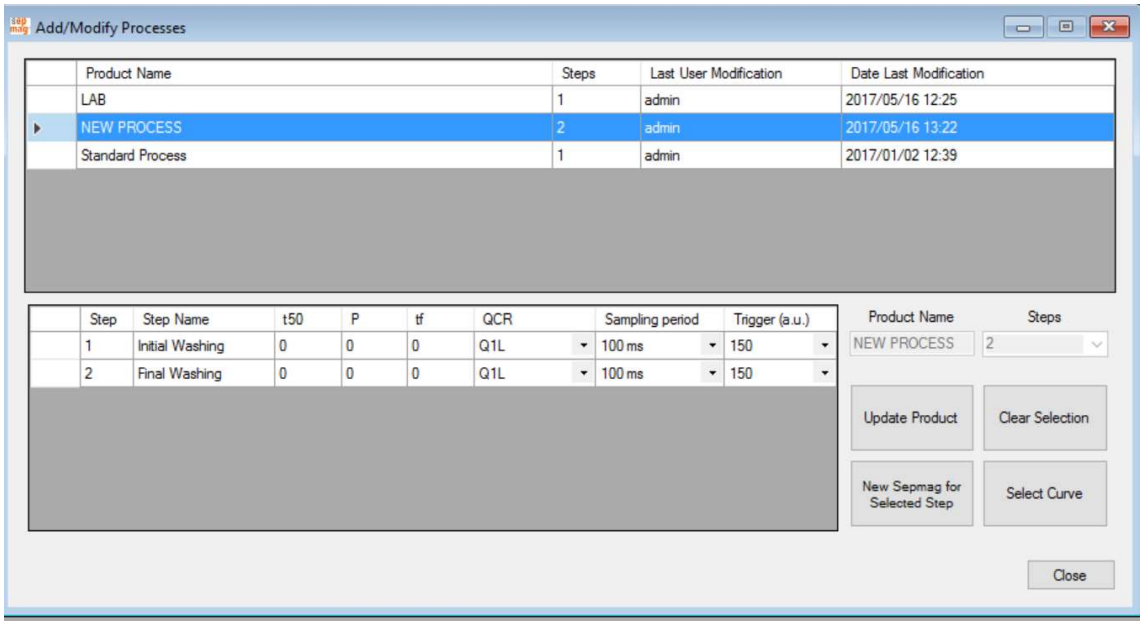


***Fig. 28**: Process Macro window. For each step in the selected product process, the average values of $t_{50}$ and p for each step are shown at the bottom. The **Select Curve** option is in the bottom right-hand area, right above the **Close** button.*

When the step is complete, the experimental data is fitted to a sigmoidal curve and the $t_{50}$ (the time at which opacity falls to 50% of the original value) and the exponent *p* are obtained. The fitting uses data gathered between '0' and the '$t_f$'.

For each new measurement of the same step, the software will calculate the 'theoretical' $t_{50}$ and *p* for each process, taking the average of the values from all the experiments. Administrators can remove curves (if the measurements were wrong) by clicking **Select Curve** on the Processes Macro screen.

When you select a product in the top table and click **Select Curves**, a new window pops up. You can then select the specific step and the Sepmag used.

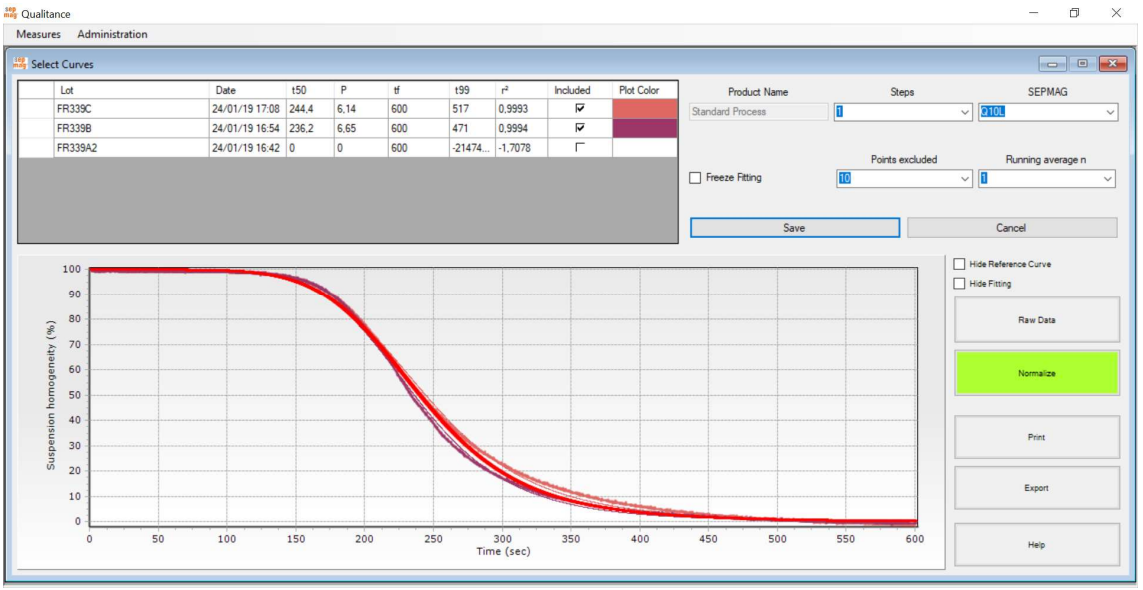


***Figure 29.*** *Selected curves window. The reference values of $t_{50}$ and p for each step are defined by the average of the 'Included'. The red curve is the reference generated with the 'Included' curves.*

The top table shows all the measurements performed for this step, displaying the Lot name and date, as well as the *'$t_f$'* value used for the fitting and the resultants $t_{50}$ and *p*. All the measurements are included in the statistics by default, as well as all the curves displayed on the graph. However, curves can be added/removed by clicking the **Included** box on the corresponding row.

Once you have enough statistics, the results can be 'frozen' by clicking the **Freeze** box. After that, no data from new curves will be added to the average value.

In some special cases, the fitting algorithm may have problems converging. The problem normally involves some initial values captured before the vessel reaches the final position, or some data at the end of the measurement because the bottle is extracted before 'tf'. Or simply because the measurement is too noisy (low bead concentration, translucent plastic bottles). To avoid the first two causes, the software excludes some points at the start and at the end of the measurement. The default number of excluded points is 10 (using a 100 ms sample rate, it means the first and last second are not used for the fitting). The value can be modified by the Administrator in the **Points excluded** box. For noisy measurements, it is also possible to use the **Running average** option to smooth the curve. By default, the value is 1 (no running average: just the central value is used), but up to 21 data points can be averaged (up to 10 data points before the central value, the central value itself and up to 10 values after the central value).

These modifications are applied to all the selected curves, the new $t_{50}$ and P values are displayed in the table and the new curves is displayed in the Graph window.

**Important**: **the modifications are recorded only when you click Save.**
*After a few measurements, we recommend you define $t_f$ in the template. This will mean that all the curves will use the same period for calculating the parameters.*

To edit '$t_f$', close the **Select Curves** window and return to the **Add/Modify Processes** window. Then select the Product (top table), and enter a new '$t_f$' value in the Step. Click **Update Process**; you will be asked for confirmation. If any of the measurements are not long enough to use the new '$t_f$', a warning will be displayed. The selected curves that stop before the new '$t_f$' will use the old '$t_f$' for the fitting. The '$t_f$' value used for fitting each Lot is displayed in the **Select Curves** table.

## 4.5 Adding a new SEPMAG device to an existing process

When you define a **Product Process** macro, a specific **Sepmag**® device is assigned to each step.

With time, it may be necessary to manufacture with a different **Sepmag**® (because the Lot volume needs to be increased due to production requirements or because smaller volume Lots are required for validation purposes).

When additional **Sepmag**® Systems are needed for a specific step, select the **Product Name** in the top table and the step in the bottom left-hand table. Then click **New Sepmag for Selected Step**. A duplicate of the Step is displayed. Select the required SEPMAG® devices and then click **Update Product** on the new row.

Next time you select the product process, the step will be duplicated, with the associated **Sepmag**® devices. Note that '$t_f$', '$t_{50}$' and '$p$' will be different. Even when the magnetic force is the same for each **Sepmag**® device, the distance travelled by the beads changes with different bottles. (Constant magnetic force leads to constant separation speed).

## 4.6 Audit Trail

Changes made by Administrators affect measurement records directly. Defining and editing *Product Processes*, adding new *users* and *Sepmag devices* or modifying the parameters affecting the *Reference Curve* for a Step, has an impact on process reporting.

Because this, all changes made in the Administration menu are logged in the **Audit Trail**. This provides a record of the changes for future Quality Audits.

To access to the Audit records, go to the **Administration** menu and click **Audit Trail**. Select the start and end dates to display all changes made during that period.

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# GLOSSARY

**Add/Modify Devices: Qualitance** menu for managing the **Sepmag®** devices connected to the computer.

**Add/Modify Processes: Qualitance** menu for managing the product processes, including the reference curves**.**

**Add/Modify Users: Qualitance** menu for managing the users and their privileges.

**Administrator (or administration user):** User who, in addition to the functions accessible to a *standard user*, can set up new **Sepmag®** hardware, add users, generate/modify new Product Processes.

**Audit Trail:** Log file that records all changes made by the administrator in the Add/Modify Users, Processes or Devices menus.

**Experimental curve:** Results of the acquisition of the optical opacity (to cold white light) while the vessel is in the **Sepmag®**.

**Fitted curve:** Sigmoidal curve showing the best approximation to the experimental curve using the less square methods. The parameters $t_{50}$ and p are obtained from this fitting.

**Lot (or Product Lot):** Each individual execution of a Product Process.

**Measurement:** Acquisition of optical opacity (to cold white light) while the vessel is in the **Sepmag ®**.

**Monitoring hardware:** Electronic hardware and firmware that measures changes in opacity of a transparent/translucid vessel inserted in a **Sepmag®**.

**Monitoring Process: Qualitance** menu for taking new measurements.
**p:** Exponent of the Sigmoidal curve
**$p_{th}$**: For each step and **Sepmag®**, the average value of the Sigmoidal curve exponent for the selected curves.

**Points excluded:** Number of data points not used for the fitting at the beginning and the end of the measurement to eliminate the noise of the introduction/extraction of the bottle. The default value is 10.

**Predictive curve:** Sigmoidal curve generated during a measurement by using $t_{50th}$, $p_{th}$ and the opacity values at t=0 and t= $t_{50th}$. The result shows a crude approximation of the expected Biomagnetic Separation behavior for the selected Step and **Sepmag®**.

**Product Process**: Set of defined successive biomagnetic separation steps necessary to successfully complete a Product Lot. Between two successive steps, operations that are not related with **Sepmag®** operation may be necessary (incubation, buffer conditions modification, etc.).

**Qualitance:** Software for measuring the biomagnetic separation process executed in **Sepmag®** Systems and managing the results.

**Reference curve:** Theoretical sigmoidal curve generated using the $t_{50th}$ and $p_{th}$ of a specific step and **Sepmag®**.

**Retrieve Data: Qualitance** menu for retrieving measurements, printing reports and comparing curves.

**Running average n:** The number of data points used for the calculated the running average (smooth/filter noisy data). The average is calculated using the (n-1)/2 data before and after the central value. The default value is 1.

**Sampling period:** Time between two consecutive opacity measurements. The default value is 100 ms.

**Sepmag®:** Advanced Biomagnetic Separation System characterized by generating homogenous radial magnetic force in the bore.

**Sigmoidal curve**: Mathematical function defined as

$$V(t) = V_{min} + \frac{V_{max} - V_{min}}{1 + \left(\frac{t}{t_{50}}\right)^{p}}$$

**Standard user**: User authorized to perform measurements, print reports or consult earlier measurements.

**Step:** Part of the process that occurs between introducing the vessel in a specific **Sepmag®** System and its removal. It always includes biomagnetic separation of the magnetic beads, but it can also include supernatant extraction and the addition of a new buffer.

**$t_{50}$**: Time, expressed in seconds, at which the measured opacity reaches 50% of the value between the maximum and minimum of the fitted curve.

**$t_{50th}$**: For each step and **Sepmag®**, the average value of $t_{50}$ for the selected curves.

**$t_f$**: User defined time, expressed in seconds, indicating when the biomagnetic separation process is complete. It defines the final time value used for fitting the experimental curve and displaying the measurement.

**Trigger:** Minimum difference between two consecutives opacity measurements that makes the **Qualitance** software start the data acquisition. The same value, but with the opposite sign, is used to stop the measurement after $t_f$ is reached. The default value is 150.

**User:** Person using a **Sepmag®** system equipped with the monitoring hardware and **Qualitance** software**.**

# Annex: Sigmoidal curve fitting troubleshooting

**If the determination coefficient $r^2$<0.99, the obtained fitting values ($t_{50}$, $p$, $V_0$, $V_{max}$, $t_{99}$) should be critically revised.**

The optical changes of the suspension when it is inside the SEPMAG (i.e. when a constant magnetic force is applied) can usually be fitted to a sigmoidal curve. To test the quality of the fitting, the software provides the coefficient of determination $r^2$, issuing a warning if its value is less than 0.99.

A bad fitting can be caused by a bad convergence of the algorithm or because the separation curve doesn't have a sigmoidal behavior.

If you want to improve the fitting, you can:

- **Change the number of points that the algorithm doesn't take into account for the fitting**

  The firsts/lasts points of the measurements can be affected by the introduction or removal of the bottle. These points out of the expected sigmoidal behavior may mislead the algorithm. Removing the $N_p$ firsts and lasts values will help the convergence. By default, $N_p$ =10.

- **Filter the noise using the running average**

  Very diluted samples and/or translucid bottles may generate a low contrast, then the experimental values will be 'noisy'. Using a running average, the curve will be smoothed and will improve the determination coefficient. By default, $N_{av}$ =1.

- **Modify the final time, $t_f$**

  Having too much data once the curve near its minimum value may overweight the fitting algorithm, losing detail on the initial part of it. Having not enough data after $t_{50}$, by contrast, will produce a loss of adjustment in the final part. We recommend a $t_f$ between $t_{99}$ and 3*$t_{99}$. For high '$p$' values, $t_f$ can be close to $t_{99}$. For low '$p$' values, $t_f$ can be close to $3*t_{99}$. By default, $t_f$ is fixed as the time when you press Force End in the first measurement of a new process.

In QUALITANCE, you may modify these fitting parameters at the Administration/Add/Modify Processes/Select Curves window. Once you change and save the new parameters value, they would be applied to ALL the curves of the step of the Process and the change would be recorded in the Audit Trail.

*For additional information, questions and technical support, please contact your local sepmag® representative or email*

contact@sepmag.eu

For additional resources on biomagnetic separation (free eBooks, technical Posts, etc.), visit our website www.sepmag.eu