Information Letter 28-4072-58 AA

UNICORN 5.10 AA

The information in this document is valid for the following software versions and systems:

UNICORN™ 5.10 AA

- ÄKTAdesign[™] systems:
 - ÄKTAexplorer[™] 100
 - ÄKTAexplorer 100Air
 - ÄKTAexplorer 10
 - ÄKTAexplorer 10S
 - ÄKTAexplorer 10 XT
 - ÄKTApurifier™ 10
 - ÄKTApurifier 10 XT
 - ÄKTApurifier 100
 - ÄKTAFPLC™
 - ÄKTAbasic™ 10
 - ÄKTAbasic 10 XT
 - ÄKTAbasic 100
 - ÄKTA™ Oligopilot™
 - ÄKTApilot™
 - ÄKTAxpress™
 - ÄKTAcrossflow™

- Ettan™ design systems:
 - Ettan LC
 - Ettan microLC
- BioProcess[™]
- UniFlux[™]
- OligoPilot
- OligoProcess[™]

UNICORN 5.10 AA is designed specifically to work under the Windows® 2000 and Windows XP operating system, with the PCI-900 or CU-950 Controller Cards.

Windows NT®, Novell™ Netware™, and the AT and CU-900 ISA Controller Boards are not supported.

The total functionality of UNICORN 5.10 AA is described in the UNICORN 5.1 User Reference Manual and the UNICORN 5.1 Administration and Technical Manual.



1. Cautionary Notes

1.1. UNICORN

General

- 1. Scenario: Using the online manuals or help files allows the user even in 'single application mode' to use the browser to access the computer hard disk.
 - **Action:** Restrict the operating system on user level using the Policy Editor, as described in the Administration and Technical Manual, section 5.5, Single application mode. Alternatively, choose not to install the online-manual.
- 2. Scenario: After performing backup to a removable disk, regardless of the amount of disks needed, the zip-utility will always ask to insert the first disk of the Multi-Volume set

Action: Click the OK-button and further disregard this message.

- **3. Scenario:** When naming Method and Result Files, remember that the characters \ / : *?" <> and | are not allowed in filenames in Windows.
- **4. Scenario:** After a drop-out of the local PC during a run, data will automatically be recovered when using the CU-950.

Action: Ignore the upcoming error-message in the audit trail on data recovery.

- **5. Scenario:** UNICORN will not be able to connect to the system(s) if you logoff from Windows, logon as a different user, and then start UNICORN.
 - **Action:** Do not logoff from Windows, just logoff and logon to UNICORN. If you have logged off from Windows, shut down and restart the computer, then start UNICORN and connect to the system(s).
- 6. Scenario: Systems settings and calibration values are reset when the strategy for a system is changed.
 Action: Check default values for System Settings before the strategy change and recalibrate all relevant modules afterwards.
- **7. Scenario:** Commas may not be used a separators for decimal values.

Action: Use decimal points as separators.

- 8. Scenario: UNICORN does not acknowledge a Pause/ Continue command made directly on the system. Action: Our recommendation is that the System Setting "Keyboard" always be set in KeyLocked mode (the default setting).
- Scenario: In some installations the HP DeskJet 895 will not print documents from UNICORN in landscape mode.
 Action: Do not use the printer driver for HP DeskJet 895 that is supplied with Windows. Select the printer driver for HP DeskJet 855C instead.
- 10. Scenario: UNICORN does not work properly after installing Microsoft's service pack 2 for Windows XP. Action: Follow instructions in the "Using UNICORN with Windows XP Service Pack 2 (SP2)" document found on

www.gehealthcare.com/unicorn. If OPC is used then also read the instructions in the document "Using OPC via DCOM with Windows XP Service Pack 2" found in the Tech Info/White Papers section at the OPC Foundation (www.opcfoundation.org). The file is called "Using OPC via DCOM with XP SP2.pdf".

11. Scenario: Problem when selecting a folder in browse folder dialogs.

Action: Select a folder by moving to its parent and select the preferred folder there. It is also possible to select the folder which contents is visible by selecting its "go to parent" icon.

12. Scenario: UNICORN requires permission for the operating system user to write in the windows registry. The permission is set according to document registryPermissions.ppt.

Action: Set permission for all operating system users that will be running UNICORN according to document registryPermissions.ppt.

13. Scenario: Error message "Unable to connect to system" is displayed when starting UNICORN directly after starting up the PC.

Action: Wait until the start up procedure of the PC is completed before starting UNICORN. UNICORN requires that some background processes is up running in order to connect to systems.

Installation

- **1. Scenario:** It is not possible to install a PCI card along with a CU-950/960 Controller Unit on the same computer.
- 2. Scenario: Microsoft Windows XP/2000 contains a Digital Code Signing feature that helps to guarantee the authenticity and integrity of software running in Windows XP/2000. During installation of the drivers for CU-900PCI and CU-950, a warning message indicating the lack of such digital signature may pop up. Action: Consider this warning a precaution. The drivers for UNICORN controllers are completely safe to install. Click therefore the Yes button and proceed with the installation process.
- Scenario: The 16-bit unpacking programme used during installation of new templates cannot open folders with long names or names which contain symbols.
 Action: Change any folder names on drive c: if necessary before beginning installation.
- 4. Scenario: The error message "The dll snmpapi.dll could not be found in the specified path" may occur during installation. Snmpap.dll is a Microsoft dll and the name stands for "Simple Network Management Protocol API". This message likely indicates that the TCP/IP protocol isn't running on the target computer.

Action: Ignore the error message. It does not affect the installation.

5. Scenario: There is an error in the OPC Manual. The error affects customers that use OPC for communication

over a network. Depending on the network settings it is sometimes necessary to edit the 'Authentication Level'. **Action:** Set the 'Authentication Level' to 'None' instead of 'Default'. The COM server (alternatively the computer) must be restarted before OPC is used.

6. Scenario: There is an error in the OPC Manual. The error affects customers that use OPC for communication over a network. In Chapter 3 it says that "Change Identity from 'The interactive user' to 'The launching user'". It should be the other way around: "Change Identity from 'The launching user' to 'The interactive user'". The corresponding image shows the correct settings. Action: Change Identity from 'The launching user' to 'The interactive user'. The COM server (alternatively the computer) must be restarted before OPC is used.

UNICORN Manager

- **1. Scenario:** It is strongly recommended not to interrupt the backup process.
- 2. Scenario: It is not possible to restrict the ability to use the CONTINUE function in the System Control module. All users are able to use the CONTINUE function regardless of their access rights.
- **3. Scenario:** If for some reason log files appear in the UNICORN/DII directory they will be flagged as unknown in the list created by the Administration/Generate file list command.

Action: No action is needed. The files do not affect UNICORN in any way.

4. Scenario: Some of the sound files have deviant status (OK vs. runtime) in the list created by the Administration/ Generate file list command.

Action: No action is needed. The different status do not affect UNICORN in any way.

5. Scenario: The cu950.bin file in the UNICORN/bin directory is flagged as unknown file type in the list created by the Administration/Generate file list command.

Action: No action is needed. The type of this file does not affect UNICORN in any way.

Method Editor

 Scenario: When a name of a block is changed/replaced in text instructions-mode, a complete new block will be created, while the complete old block will be moved to (Unused).

Action: Rename the block by the Rename Block command in the Block drop down menu.

 Scenario: If you choose a column for a method made for a non-ÄKTAdesign system, there will be no question about Column Value Update as with ÄKTAdesign systems.

Action: Update the Column instructions in the method.

- **3. Scenario:** To be sure that the Gradient function works properly, we recommend that the entire gradient be structured in one block, where the End Block and Gradient length have the same value.
- **4. Scenario:** The list of techniques available for selection includes only techniques used for ÄKTAdesign systems. **Action:** Select technique Any for other systems.
- **5. Scenario:** Methods can not be opened in versions older than the one where they were created. I.e. UNICORN is not forward compatible.

Action: Create methods with the software that will run them.

System Control

- Scenario: When UNICORN is installed without Password protection, and a method containing a message instruction that needs to be authorized, this message dialogue can not be shut down and the instrument will run in infinite pause mode.
 - **Action:** Installation without password disables the confirm/sign function for a user. Therefore do not use Authorization when UNICORN is installed without password protection.
- 2. Scenario: UNICORN will not notify the user when free capacity of the storage disk is insufficient for saving the ongoing run. When possible, the data of the ongoing run will be stored in the Failed folder.
- 3. Scenario: When using a variable wavelength detector (Monitor UV-900) UV curves are automatically given names containing the UV wavelength. Names are displayed together with the curve in the System Control curve window. At the end of a run the curve name is changed back to its default name but the curve data from the run is still seen in the window and therefore the curve and curve name do not match.

Action: No action required. UV curves are always stored in the result file with the correct wavelength information in the curve name.

4. Scenario: If you execute the instructions Record_on and End_Timer or Pause_Timer simultaneously during manual runs, there is a possibility that the run will go on infinitely.

Action: Do not execute the instruction Record_on simultaneously with an End_Timer or Pause_Timer instruction.

5. Scenario: If you press the Continue button during PumpWash execution the Pump module may be set in End mode although the method continues to execute. This affects the whole method execution and will most probably give incorrect results.

Action: Never press Continue during PumpWash execution.

6. Scenario: Not possible to start batch run without having a result file open.

Action: Open any result file and choose batch run.

7. Scenario: No titles on report print-outs from Batch run-Evaluation procedure.

Action: Use the 'File-Report' command.

8. Scenario: The tuning dialog has disappeared from the System menu after upgrading from UNICORN 4.12 or earlier

Action: Get an updated strategy that includes feedback tuning settings or install UNICORN 4.12.

9. Scenario: The communication between the PC and the "CU950 advanced mode" fails and then reestablishes by itself within 10 seconds when the PC goes into standby mode or hibernation mode.

Action: No data is lost so no action is needed. However, it is possible to switch off the standby and hibernation feature in the power options in the control panel of the PC.

Evaluation

- Scenario: When recalculating values manually, the obtained value will differ from the one calculated by UNICORN as UNICORN only shows the first few decimal digits of the values, while the software calculates with more decimal digits.
- 2. Scenario: When the Result Name is changed in the Start Protocol when starting a method, the Result Directory in the Result Name tab of the Documentation will reflect the Result Directory as defined in the Method Editor. The Result Name on the Result Information tab however will reflect the changes made in the Start Protocol.
- 3. Scenario: When a system is controlled remotely, and the result is saved locally, this Result file will not show up in the Recent Runs tab on the controlling computer.
 Action: Open the Result file on the remote computer using the UNICORN Manager or setup a network including the local disk of the remote computer.
- 4. Scenario: In the Results Information-tab in the Documentation window, time zones between 'Date of creation', 'Date of last modification' and 'Strategy date' may differ. 'Date of creation' and 'Strategy date' are based on the computer's settings, while 'Date of last modification' as based on the settings of the computer where the last modification is made.
- **5. Scenario:** When exporting curves to AIA-format using File-Export-Curves will overwrite any previously saved similar AIA curves without warning.

Action: Use the 'File-Export-Export Curve to AIA' command.

- **6. Scenario:** Only the curves belonging to the active chromatogram can be selected in the Report format.
- **7. Scenario:** The table in 'Operations-Fraction Histogram' does not exist.

Action: The Table has moved and has been extended. It is now found as a pool table in 'Operations-Pool'.

ÄKTAcrossflow

- **1. Scenario:** Inaccurate permeate volume readings for systems in cold environments.
 - **Action:** Increase the PPCV value in System settings -> Specials to at least 830. The optimal setting may vary between systems.
- 2. Scenario: In Define plot data in the Evaluation wizard: The numbers in the Marker position and in the table in the Curve name tab may differ. The reason is that the resolution may be higher in the result file than in the curve area on the screen.
- 3. Scenario: The retentate volume control is not accurate during diafiltration (Constant Retentate Mode) when having density differences between the sample and diafiltration buffer.

Action: Density differences during the diafiltration process have to be corrected manually according to information in Appendix 1.

4. Scenario: An empty gray area is visible in the Evaluation wizard -> Capacity plots -> Define plot data when plotting capacity versus external signal.
Action: The area is intentionally empty since there is no curve to show.

1.2. Wizard

1. Scenario: When making a new method that differs substantially from the latest made with the Method wizard, we recommend that you click on the Set Default button on the initial Method wizard dialogue.

1.3. Strategy

All 400/400B strategies

1. Scenario: Even when using the Pressure Flow Controlmode (in SystemPumpControlMode-Instruction) with a Pressure Level-parameter, an Alarm_Pressure at the column's maximal pressure should be initiated to prevent column damage.

1.4. Controller Unit

CU-950/960

- Scenario: If two local drivers on separate computers are configured to use the same CU-950 Advanced controller unit, the CU-950 Advanced will stop responding.
 Action: Avoid local drivers on separate computers to make use of the same CU-950 Advanced controller unit.
- 2. Scenario: When running method queues on several systems connected to the same CU-950 Advanced, it is recommended to avoid burdening the network as this might lead to a pause in the method gueues.
- Scenario: In rare cases, such as heavy network load, the system reports "Communication broken" when recovering data after a network failure.
 Action: No action needs to be taken. The data is fully recovered.

4. Scenario: The USB cable between the CU-950 and the PC has accidentally been unplugged.

Action: Wait until the PC-LED on the CU-950 starts to blink before reconnecting the USB cable.

5. Scenario: Loss of data after a communication error when running with an advanced CU-950 without the compact flash.

Action: Alter settings in System Control -> System -> Settings -> Control Unit from CONTINUE to PAUSE.

2. Deviations

2.1. UNICORN

General

- Scenario: The help button in the dialog window, asking whether data should be recovered, that is shown when starting UNICORN after restarting the computer with a CU-900 PCI controller unit following a shutdown during a run, displays the help information for another dialog window.
- 2. Scenario: When loosing contact with the server will the UNICORN Manager and Evaluation modules freeze for several minutes. When the end of the run is reached and contact is not restored, also the System Control module will stop responding. Processes that handle the Result file are running in the background and will not be affected. Action: UNICORN will behave normally when the server is up and running.
- **3. Scenario:** Depending on the operating system, it might happen that the time indication in the report format is missing.

Action: Change time-format in the operating system.

- 4. Scenario: When for some reason the computer needs to be restarted during the chromatography run, the first new chromatogram according to the instruction New_Chromatogram in the running Method will be named a default name. All other chromatograms will be stored correctly.
- 5. Scenario: The access rights are checked against the mapped drive, and not its subdirectories. Consequently, if the user has no read and write access directly on the parent directory, the user will not be able to open or store any data.

Action: Allow the user to read and write on the parent directory containing the UNICORN software.

6. Scenario: If the user does not have full access to hidden files on the directory where the result files are stored then result files can become corrupted when they are modified and subsequently saved.

Action: Make sure the user has full access to hidden files on all directories where result files are stored.

Scenario: UNICORN will experience problems if a PDFwriter is used to print reports and if the destination folder is changed in the print dialog. **Action:** Avoid to alter the destination folder in the PDF-print dialog.

Installation

1. Scenario: When installing manuals, tracking the scrollbar to the end will not bring you down to the complete end of the list.

Action: Continue scrolling down using either mouse or arrows on keyboard.

2. Scenario: When moving back and forth in the Installation Program, the bitmap figures on the left side of the dialog windows may disappear.

UNICORN Manager

- Scenario: When double-clicking on a method in the UNICORN Manager, occasionally the default method is opened instead of the method that was intended.
 Action: Try to re-open the method once more.
- 2. Scenario: Occasionally, when the UNICORN Manager is left while performing backup to multiple disks, the UNICORN Manager will stall when it is opened again in response to a dialog window in the UNICORN Manager. Action: Do not activate another window while performing a backup. Alternatively, do the backup to a disk that has sufficient free space to contain the complete backup file.
- 3. Scenario: The names of imported old methods may have to be changed to have an mXX file extension.
 Action: Replace "XX" in the file extension with the number of the specific system the method is assigned to. If you only have one system connected, the file extension should be "m01"

Method Editor

- 1. Scenario: If Run1 is excluded in the Scouting tab of a method, and changes are made to the items that are shown when the method is started, as indicated in the Start Protocol-tab of the method, the first Result file will receive the serial number 001. Other Result files are named according to their position in the scouting table. Action: To avoid a Result file mistakenly being referred to as resultname 001, make no changes to the start protocol when starting the method. Alternatively make the first actual Run also the first one in the scouting table.
- Scenario: When a dialog window is opened in the Method Editor module, opening a Method file using the UNICORN Manager will cause the Method Editor module to stop responding.

Action: Close all dialog windows in the Method Editor module before opening another Method file.

 Scenario: Occasionally, the operating system settings will cause the Method Editor to fail to open automatically when double-clicking a method in the UNICORN Manager.

Action: Double-click once more on the method.

Alternatively, open the Method Editor manually by clicking on the Method Editor tab.

- 4. Scenario: Variables that are defined in blocks below level 10 in the call chain are always shown as 'unused variables', even if they are used. Also, variables in an infinite loop may be defined as 'unused' incorrectly. Action: Check 'Show unused variables' and 'Show details' to show all variables.
- 5. Scenario: When the Base of a block changes to a volume-dependent base, based on the samplepump with the command 'MethodBase Samplepump', the method will continue to calculate on the system pump's volume changes.

Action: Keep the system pump running at the same flow rate as the sample pump when changing MethodBase Samplepump.

6. Scenario: Sometimes variables may be missing from the Variables tab on the Run Setup.

Action: Click the Show details and Show unused variables checkboxes. This will display all variables.

7. Scenario: Sometimes infinite loops may be indicated when a text method is printed.

Action: The indications can be ignored if this hasn't been noted in the Method Editor text view or in the Run Setup.

- 8. Scenario: If you set a Gradient instruction before PumpWash (at the same breakpoint), the gradient will go to the target immediately (with length 0). However, the Gradient profile in the Method Editor will show the Gradient as if it followed the normal set length.

 Action: Place the Gradient instruction on another breakpoint after the PumpWash.
- 9. Scenario: In some cases the unit and tick marks on the x-axis do not match in the gradient diagram when switching to and from the gradient window. This is a visual effect only. It does not affect the method run in any way.
- 10. Scenario: A vertical line may appear at break point in the gradient diagram when switching to and from the gradient window. This is a visual effect only. It does not affect the method run in any way. The effect has only been found in editions earlier than 5.0.
- 11. Scenario: Syntax error in block after a block import. Blocks containing columns are not imported correctly. Action: Replace the (commented out) instruction containing the column with a new identical one.
- **12. Scenario:** An imported column with a long name is corrupt after the import.

Action: Delete the imported column and reimport it using a column name that has less than 44 characters.

13. Scenario: It takes longer time to start the method wizard and to open large methods than it did in earlier UNICORN versions.

Action: This does not affect any part of the method. No

action is needed.

System Control

- 1. Scenario: When a method using BufferPrep failed to start because of a compilation error in the method, the command 'BufferPrep ON 1' will appear as the first line in the Logbook as soon as a new method is started, regardless whether or not this new method contains a BufferPrep command. After this notification in the Logbook, the base is reset and the normal logbook for the new method is displayed.
- 2. Scenario: When running 4 systems from the same computer over the network, and all 4 systems end simultaneously, creating very large Result files, some of these files may be stored in the \Failed-directory.
- 3. Scenario: If an End or Pause Timer is activated manually before a Method is started, the timer will still be active, although it is not registered in the Logbook.
 Action: When an End or Pause Timer is activated manually, the manual run should first be ended before the next method is started.
- 4. Scenario: The base of a Watch-function will be used in the logbook as soon as the condition occurs, regardless of the base of the block that is ongoing at that time. If a Hold_Until command is used at that point, the base that is recorded in the logbook will then be the base that is used in the Watch-function when the condition occurs, even if this is not the base for the Hold-Until function. This discrepancy does however only apply to the logbook.
- **5. Scenario:** The hatches in a chromatogram, created by the Hatch-function on the Curve Style and Color tab are missing when printing.
- **6. Scenario:** System name and Strategy information is not registered in Documentation when performing a manual run

Action: Document the system name and strategy information in the Notes tab of the Documentation.

- 7. Scenario: System Control will stop responding if the user edits the tube number-variable, belonging to the 'Start At' parameter for the Frac-950 instructions 'Fractionation' and 'Peak_Fractionation', on the Scouting-tab of the documentation at the same time as the run ends.
 Action: Avoid editing these values during runs if possible.
- Scenario: The manual SetMark and SnapShot instructions cannot be executed through UNICORN OPC Server.
- 9. Scenario: If a method compilation error appears when trying to run a BufferPrep mode method, the method does not start but the BufferPrep recipe is still downloaded to the Pump instrument module. An error will then occur as soon as another BufferPrep recipe is downloaded, e.g. if you try to run a BufferPrep method again. This error will also occur when a BufferPrep

recipe is selected manually before a method running in BufferPrep mode is started and when running a method which includes BufferPrep.

Action: To "remove" the downloaded BufferPrep recipe from the Pump module, execute any instruction, e.g. a Flow instruction, and then press the End button. (The dummy instruction must be executed for the END button to be enabled.) This action applies to all situations where you want to abort BufferPrep mode.

- 10. Scenario: If an ALARM event occurs during PumpWash, the system is Paused as intended but is Continued when PumpWash has ended. The same applies to the SystemWash instruction. Normally, the system will remain in Pause after an ALARM.
- **11. Scenario:** When pausing all ÄKTAxpress systems that are running manual runs, the Continue all and End all buttons are inactive.

Action: Continue or End every system separately.

- **12. Scenario:** No result file is generated when running scouting and method queue simultaneously. **Action:** Do not run method queues and scoutings simultaneously.
- **13. Scenario:** Error when running evaluation procedure in the first scouting run. If the evaluation procedure contains an export command then an error may occur after the first run in a scouting sequence.

Action: Run the evaluation procedure from the procedure editor instead.

14. Scenario: Decimals are missing on the x- and y-axis in graphs.

Action: No action is needed. The problem is a display problem only. The graphs and the axis are correct in other respects.

15. Scenario: The result file may be missing after a long (e.g. a 24-hour) manual run.

Action: It is recommended to run a method instead in these cases.

16. Scenario: Run errors that occur when no UNICORN application gui is open are not shown and are not possible to clear.

Action: Clear the error by restarting the CU and the PC. Note: in some systems the CU is integrated in the PC. The error can then be found in the system audit trail in the UNICORN Manager application.

17. Scenario: The max, min and average readings of pressure values from the marker in the run curves become wrong when the unit is changed from e.g. bar to psi

Action: Double check the values and if necessary convert them before using them.

18. Scenario: The filter area in the evaluation report for a normalized water flux (NWF) shows the default area (45 cm2) regardless of the area given in the method.
Action: The displayed area is never used in calculations

in NWF. No action needed.

Evaluation

- **1. Scenario:** The function 'Adjust retention zero to injection number' is not functional in report format.
- 2. Scenario: When new items are added to an existing report, and the 'exit' function is used to close and save the modified report, an error message may appear, stating 'The format could not be saved. The report layout file has been modified by another user. You can save this layout with a new name'.

Action: Save the modified report using 'Save as' under the same name as the original report.

3. Scenario: In rare occasions, the Result file opened in the Evaluation window is indicated as filename.rez in the title

Action: No action required, the Result file will be treated as usual.

- 4. Scenario: Depending on the length of the Result files' names, the amount of Result files selected and the computer's capacity, selecting a large amount of Results when browsing for Result files in the 'Open Curves to Compare'-dialog window will cause an application error. Action: First open a smaller amount of curves to compare, and then repeat this command until all curves required are displayed.
- **5. Scenario:** Neither the X-axis, nor the Y-axes will be updated when zooming in on the curve in the Evaluation Multifile Peak Compare wizard on the Peak Selection page.
- 6. Scenario: The window value for the 'Reference peak for relative retention' in the Identification Settings dialogue, which is reached from the Define Components dialog when creating or editing a Quantification Table in the Analysis Module, is not used when a check is made for overlapping retention windows when the Define Components dialog is closed. This can result in an error message. The actual quantification however is performed with the correct values.

Action: Temporarily switch to Absolute retention in the Identification Settings dialog and edit the retention window for the reference peak in the Define Components dialog to a value that does not overlap with the retention windows for the other components. Then change back to Relative Retention in the Identification Settings dialog and set the window for the reference peak.

7. Scenario: When no printer is installed, the Evaluation module will stop responding when a Preview is requested in the Print Chromatograms window.

Action: Install a printer.

- **8. Scenario:** The Help text for the Copy to Clipboard command is incorrect.
- **9. Scenario:** Attempts to apply the Operations-Pool command on a fraction curve containing only one fraction mark will cause the program to shut down.

10. Scenario: On the Peak Selection page in the Multifile Peak Compare Wizard, the peak selection is removed if Set peak name command is used while the corresponding checkbox is not checked.

Action: Check any of the checkboxes before using the Set peak name command.

11. Scenario: The units for Vol. and Target vol. are missing in the ÄKTAxpress Pool Table.

Action: The unit for Vol. and Target vol. is ml.

12. Scenario: When several chromatograms have been imported into a single window the order in which they appear on a print-out may not be as required, e.g. a comparison of several different runs at different pH may not be displayed in a consecutive numerical order of pH values

Action: Use the Report Generator to place the chromatograms in the desired positions.

13. Scenario: If one or several chromatograms, but not all, are selected for a standard report layout, the number of actually printed pages may exceed what was selected as the desired number of pages.

Action: Select Active Chromatogram, All Chromatograms, or select all chromatograms individually, alternatively Select a Custom report format.

- **14. Scenario:** The bases Y, Z and Q are reported incorrectly in the synthesis data of the OligoPilot and OligoProcess result files. This concerns both DNA and RNA and both thiolated and oxidated bases. The bases are reported as follows: Y is reported as Q; Z is reported as Y; Q is reported as Z.
- **15. Scenario:** When opening a curve to compare into an empty chromatogram the application shows two curves with the same name.

Action: Assure that you have at least two shiftable curves for each unit among the selected curves. Use File->Open->Curves to open a single curve.

16. Scenario: A baseline becomes associated with the wrong curve after editing of the baseline provided that the baseline calculation does not belong to curve 01.
Action: Avoid to edit baselines belonging to other curves than curve 01.

17. Scenario: Synthesis data in oligo systems crashes when printing if the algorithms have given non-physical values with too many digits.

Action: Do not print the synthesis data via UNICORN in those cases.

18. Scenario: If You run a method that has two Evaluate instructions on the same time/volume then curves may disappear from the result. Also curve names can be wrong (curves in chromatogram "15" can be named "result:14_UV" instead of "result:15_UV".

Action: Avoid to use two evaluate instructions on the same time/volume.

ÄKTAcrossflow

1. Scenario: Oscillation on the feed flow when performing the EmptyReservoir instruction.

Action: The oscillations can normally be neglected. However, if the following 2 rules are followed then there will be no oscillations: 1. Assure that the reservoir never contains a small amount of liquid. 2. Never change the default value of the MaxFeedFlow parameter.

- Scenario: The air sensor gives false alarms.
 Action: Assure that there is liquid at the air sensor when activating the air sensor for the first time after strategy change.
- **3. Scenario:** Retentate volume decreases slightly at transitions between product steps when using the method wizard.

Action: If the decrease of volume cannot be neglected then it is possible to stop the permeate pump at the end of each product step by adding a control mode stop (TMP or flux control) in the method.

4. Scenario: Air gets into the retention loop when using the method wizard with "Load all sample" and is considered being part of the sample and thus generating false retentate volume.

Action: Reduce the flow rate for sample transfers.

- **5. Scenario:** In Define plot data in the Evaluation wizard: The marker bar is not repositioned when zooming.
- 6. Scenario: Problems when renaming curves in Evaluation wizard -> Any vs. any. The new names may get lost if the wizard's back button is used to change data in previous wizard pages.

Action: Rename the curves again.

- 7. Scenario: The filter area in the evaluation report for a normalized water flux (NWF) shows the default area (45 cm2) regardless of the area given in the method.
 Action: The displayed area is never used in calculations in NWF. No action needed
- 8. Scenario: The serial numbers obtained from the maintenance manager in system control are not correct. Action: Use the serial numbers from the system certificate or from the loggbook instead.

2.2. Strategy

All 400/400B strategies

- Scenario: When a 'Continue'-command is given less than 2 seconds before a 'PumpWash'-command, will the pump not respond to the 'PumpWash'-command. Action: Add a small delay before the 'PumpWash'command.
- Scenario: the maximal pressure for P950 and P960 pumps is 1 resp. 2 Mpa, though the instruction Watch_Samp_Pres can be set to 2.5 MPa.

Action: Do not set the Watch_Samp_Pres (P950) higher than 1 Mpa or Watch_P960_Press (P960) higher than 2 Mpa.

ECA_400/ECAF400/ECA_400B/ECAF400B/

 Scenario: Air sensor 1 and air sensor 2 are default selected in the System Settings Component list. When starting a run on the ÄKTAexplorer, an error message stating "Air cell 2 is not connected" will be shown.
 Action: Select the air sensor P-960 in the Components list and uncheck air sensor 2.

Pilo 100/111

1. Scenario: A Warning message "Flow ignored" should be displayed if the instruction SampleFlow is active in manual mode and the user executes the instruction Flow. Sometimes this Warning message is not issued when SampleFlow is running, and the instruction Flow will be idle. If the user manually sets 0 ml/min as SampleFlow during this, the Flow will start.

Action: Do not run Flow when SampleFlow is active.

Multipurpose

 Scenario: If the flow rate is changed during execution of the instruction Superloop™, the time for filling or emptying Superloop is not adjusted to the new flow rate.
 Action: Do not change the flow rate when filling or emptying Superloop.

2.3. Controller Unit

CU-950/960

- 1. Scenario: When the CU-950 Data Recovery Settings is set to OFF for CU-950 Advanced, or CU950 USB is used and the Control Unit loses power due to a power failure, this power failure will not be indicated in the logbook. It will however be indicated that data could not be collected from the control unit.
- 2. Scenario: When restarting a computer with the CU-950 USB running on Windows 2000, an error dialog window indicating Unsafe Removal of Device will pop up. Action: When restarting a computer, the CU-950 USB should also be restarted simultaneously.
- **3. Scenario:** If data is stored locally on a nearly full or very fragmented hard disk, the CU-950 controller unit may in rare cases loose and regain contact with the computer when saving backup files or Result files.

Action: Either remove obsolete files from the hard disk, alternatively defragment the hard disk.

 Scenario: Negative values are rounded incorrectly in alarm blocks when they are sent for display from the CU-950 to UNICORN.

Action: No action is needed. The alarm is trigged correctly. It is a display problem.

5. Scenario: CU indicates memory card full in CU even if not full. When the Compact Flash memory on a CU-950/960 has once been full, the CU will indicate that the memory card is full when a lost communication with the CU is restablished, even if the memory card is not full.
Action: Shut down the CU-950/960 by unplugging the power cable and then start it again

2.4. System

ÄKTAdesign

1. Scenario: When pressure has been calibrated and the flow re-started the pressure values on screen in System Control show zero pressure.

Action: Press End. Restart Flow.

2. Scenario: Calibrations made directly on ÄKTAdesign system modules are logged only in the System Control logbook and not in the Calibration page of Documentation or in the Audit Trail. This means that Calibration page and Audit Trail information might not be up-to-date.

Action: Always calibrate ÄKTAdesign system modules by using the UNICORN calibration functions in System Control

- 3. Scenario: The P-900 pump will not change its mode when the system changes mode (e.g. from Pause to Run after a PumpWash) immediately before an Airsensor alarm even if UNICORN changes its mode to Pause. The pump will continue to operate.
 - **Action:** Deactivate the Airsensor alarm and click Continue, or finish the run with the End command.
- 4. Scenario: When using the UV-900 monitor the peak fractionation automatically ends after a certain time, which is related to the MinPeakWidth setting. A MinPeakWidth setting of 0.15 will result in max 7.5 min peak fractionation, a MinPeakWidth setting of 0.25 will result in max 12.5 min peak fractionation, and a MinPeakWidth setting of 0.30 will result in max 15 min peak fractionation, etc. The maximum fraction volume is 50 times the MinPeakWidth.
- **5. Scenario:** The system will not hold when InjectionPickup is performed with the A-900. This problem does not occur if the Wizard is used.
 - **Action:** Perform a Hold command immediately after the InjectionPickup command, either manually or in the method or use the Wizard.
- 6. Scenario: The following sequence will cause the outlet valve positions to be wrong when used with 10 and 100 system strategies and a Frac-900: Insert a flow rate and fractionation command; Switch Outlet Valve to F1; Switch back Outlet Valve to F2. This will cause the Outlet Valve to be switched to F2, but it will immediately switch back to F1. This is not noted in the logbook. Any other position than F2 for the Outlet Valve works well. The problem occurs both if the sequence is used manually or in a method.

Action: Give the F2 command again or choose another outlet position.

- 7. Scenario: Instant runs cannot be preformed using the following ÄKTA 3D Kit templates: Affinity Blank Run; Column CIP; Metal Ion Charging; Metal Ion Stripping. Action: Save the templates as methods and run the saved template methods.
- 8. Scenario: Peak fractionation will not work using the Frac-900 if the peaks are too close. The fractionation is cancelled and the fraction numbering is reset when this happens. The error occurs when there is approximately 1 s between the end of one peak and the beginning of the next. It is caused by the automatic valve adjustment of the Outlet Valve.
- **9. Scenario:** Some 18 mm tubes may not fit for the Frac-950 18 mm rack.

Action: Following tubes are recommended for the Frac-950 18 mm rack: Falcon 15 ml 17x120 mm, Sarstedt 10 ml 16x100 mm, Sarstedt 15 ml 17x120 mm, Borex 16x100 mm, Elkay 15 ml, TPP 15 ml, Cerbo 17x100 mm, LP-Italiano 16x100 mm.

10. Scenario: When using pump P-960 with module software version 01.01.00, the message "CAN ID (Manual at Pump P-960 [0])" will repeatedly appear in the logbook. This message is just a logbook entry and has no further function.

BioProcess

- 1. Scenario: When the template "Empty" has been selected, the unit for the column volume in the Base instruction in the text window is incorrectly shown as ml. The real unit is litre.
 - **Action:** Mark the Base instruction and click on Replace in the instruction dialogue box to change the unit to litre.
- 2. Scenario: Systems with a continuous sound at alarm may not sound the subsequent alarm if the Continue command is used before the first alarm is acknowledged. Action: Click Acknowledge before Continue.

Appendix 1

Cautionary notes, ÄKTAcrossflow

Retentate volume accuracy when running Diafiltration with density differences between sample and DF buffer

Scenario: The Retentate volume control is not accurate during Diafiltration (Constant Retentate Mode) when having density differences between the sample and diafiltration buffer.

Problem description: When running in Constant Retentate Mode, the weight of the liquid in the reservoir is kept constant by help for the reservoir level sensor, which is actually a pressure sensor. Density differences during the diafiltration process have to be corrected manually by setting a new retentate volume (RetVol) after the diafiltration step.

Solution: The exact density of the retentate at the end of the diafiltration step ρ_{Target} can be calculated as function of the number of diavolumes (DV):

$$\rho_{taraet} = \rho_{sample} \cdot e^{\text{(-DV)}} + \rho_{DF \text{ Buffer}} \cdot \text{(1- } e^{\text{(-DV)}}\text{)}$$

As a rule of thumb, the density of the retentate equals the density of the diafiltration buffer if more than 4 diavolumes have been applied. The correct retentate volume that has to be set at the end of the diafiltration step is calculated from the volume in the reservoir only, because it is only the volume in the reservoir that is monitored by the pressure (level) sensor:

$$\mathsf{RetVol}_{\mathsf{Target}} = \rho_{\mathsf{Sample}} / \rho_{\mathsf{Target}} \cdot \mathsf{V}_{\mathsf{Reservoir, initial}} + \mathsf{V}_{\mathsf{holdup}} + \mathsf{V}_{\mathsf{Filter}}$$

 $V_{\mbox{\tiny Reservoir, initial}}$ Volume in reservoir at start of DF step

 V_{holdup} System holdup volume V_{Filter} Filter holdup volume

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Amersham Biosciences AB, a General Electric company going to market as GE Healthcare.

GE Healthcare Amersham Biosciences AB Björkgatan 30, 751 84 Uppsala, Sweden

GE Healthcare Amersham Biosciences Europe GmbH Munzinger Strasse 9, D-79111 Freiburg, Germany

GE Healthcare Amersham Biosciences UK Ltd Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Amersham Biosciences Corp 800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Amersham Biosciences KK Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

Asia Pacific Tel: +852 2811 8693 Fax +852 2811 5251 • Australasia Tel: +61 2 9899 0999 Fax +61 2 9899 7511 • Austria Tel: 01/57606-1619 Fax 01/57606-1627 • Belgium Tel: 0800 73 888 Fax 03 272 1637 • Canada Tel: 800 463 5800 Fax 800 567 1008 • Central, East, & South East Europe Tel: +43 1 982 3826 Fax: +43 1 982 3826 Fax: +43 1 985 8327 • Denmark Tel: 45 16 2400 Fax: 45 16 2424 • Finland & Baltics Tel: +358 (10)9 512 39 439 • France Tel: 01 69 35 67 00 Fax: 01 69 149 677 • Grand Tel: 07 6761/4903-490 Fax: 761/4903-490 Fax: 551 13 933 7306 • Middle East & Africa 14: 30 210 9600 687 Fax: +30 210 9600 693 • Netherlands Tel: 0165 580 410 Fax: 0165 580 401 • Norway Tel: 815 65 555 Fax: 815 65 666 • Portugal Tel: 21 417 7035 Fax: 21 417 3184 • Russia & other C.I.S. & N.I.S Tel: 7 (095) 230 0250 Fax: 7 (095) 230 6377 • South East Asia Tel: 60 3 8024 2080 Fax: 60 3 8024 2090 • Spain Tel: 93 594 49 50 Fax: 93 594 49 55 • Sweden Tel: 018 612 1900 Fax: 018 612 1910 • Switzerland Tel: 0848 8028 12 Fax: 0848 8028 13 • UK Tel: 0800 616928 Fax: 0800 616927 • USA Tel: 800 526 3593 Fax: 87 7295 8102

