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1.0 Purpose

This procedure describes the standardization of the pH monitor for the AKTA Pilot 600R Chromatography System, and the cleaning procedure for the system. SOP 14142 is also applicable for AKTA Pilot 600R standardization and cleaning.

2.0 Scope

This SOP applies to BDP personnel who perform standardization of the pH monitor and cleaning of the AKTA Pilot 600R Chromatography System. The AKTA Pilot 600R conductivity monitor calibration is part of AKTA Pilot 600R PM service performed by Cytiva Engineers.

3.0 Authority and Responsibility

3.1 Manager, Late Process Sciences, Biopharmaceutical Development Program (BDP)

- Defines this procedure.

3.2 BDP personnel

- Implements this procedure

3.3 Biopharmaceutical Quality Assurance (BQA)

- Provides quality oversight of this procedure.

4.0 Equipment and Materials

4.1 WFI Quality Water.

4.2 Wipes, Sterile, 12x12 BDP PN 20315, or BDP approved equivalent.

4.3 70% Isopropyl Alcohol-Sterile, Decon-ahol, Sterile, (70% IPA) BDP PN 30129, or BDP approved equivalent.

4.4 0.5 N NaOH, BDP PN 46109CL, or BDP approved equivalent.

4.5 20% Ethanol, BDP PN 46202CL, or BDP approved equivalent.

4.6 pH Strips, BDP PN 20473, or BDP approved equivalent.

4.7 PBS, BDP PN 47350, or BDP approved equivalent.

4.8 Micro Alpha Swab, BDP PN 21674 or BDP approved equivalent.

4.9 15 mL polypropylene screw cap centrifuge tube, BDP PN 20006, or BDP approved equivalent.

4.10 TOC vials,40 mL BDP PN 20442 or BDP approved equivalent.

4.11 T-connector, 5/16-24 AKTAPILOT, BDP PN 21875 or BDP approved equivalent.

4.12 pH 4 buffer, BDP PN 30107, or BDP approved equivalent.

4.13 pH 7 buffer, BDP PN 30108, or BDP approved equivalent

4.14 pH 10 buffer, BDP PN 30109, or BDP approved equivalent.

5.0 Standardization of pH Monitor

5.1 Standardization of the pH Monitor

Note: Standardization of the pH monitor is required before every run. You can also follow the procedure mentioned in SOP 14142 for pH standardization. Both procedures are applicable to AKTA Pilot 600R system.

5.1.1 Attach a new pH probe to the AKTA Pilot.

5.1.2 Turn on the AKTA Pilot and computer, if not already on.

5.1.3 Log into the Unicorn software.

5.1.4 Go to the system control menu > system > calibrate.

5.1.5 From the pull-down menu, select pH.

5.1.6 For the buffer 1, enter the pH value of first calibration solution.

5.1.7 Insert a syringe filled with a 20ml WFI to the manual injection port on pH monitor. Make sure that the syringe is securely attached.

5.1.8 Open the port, slowly inject everything, and close the port. Don’t open the port without a syringe attached.

5.1.9 Remove the water syringe and insert a 20ml of the first calibration solution into the flow cell and close the port.

5.1.10 When the displayed current value is stable, press calibrate.

5.1.11 Repeat step 5.1.7 and 5.1.8 to wash the pH electrode.

5.1.12 When the display changes to pH for buffer 2, enter the pH value of second calibration solution.

5.1.13 Remove the water syringe, and insert a syringe filled with 20ml of second calibration solution.

5.1.14 Open the port, slowly inject everything and close the port.

5.1.15 When the displayed current value is stable, press calibrate.

5.1.16 Repeat step 5.1.7 and 5.1.8 to wash the pH electrode. Remove the water syringe and keep the port closed.

5.1.17 Document the standardization on Form 14155-03.

6.0 Cleaning Procedure

6.1 Using a cleanroom wipe and WFI followed by 70% IPA, wipe the exterior of the AKTA Pilot 600R Chromatography System. Visually inspect the AKTA Chromatography System exterior and verify that it is clean.

6.2 Connect the AKTA Pilot 600R Chromatography System solvent lines (A1-A6 and B1-B3) to WFI at ambient temperature using the AKTA Pilot 600R inlet valve.

Note: AKTA Pilot 600R can be bought with different configurations which may have more valves or sample pump.

6.3 Connect the Outlet valve lines (F2-F6), W1-W2 line, and bubble trap purge line in an appropriately sized waste container.

6.4 Determine the appropriate cleaning required (see 6.4.1-6.4.4) and document on forms 14155-01,14155-02 and any associated forms from **SOP 21529, *Equipment Interproduct Cleaning and Clearance***. The cleaning procedure remains the same, differing only in the post-cleaning sample requirements.

6.4.1 For in-process cleaning, no QC testing is required.

6.4.2 For interbatch cleaning, an LAL rinse sample from the Outlet valve line is required.

6.4.3 For post-maintenance cleaning, an LAL rinse sample of each affected pathway is required.

6.4.4 For release cleaning (inter-product), QC testing is required per **SOP 21529.**

6.5 Either manually or using the AKTA Pilot 600R System Flush program, flush each of the AKTA Pilot 600R Chromatography System Outlet and Waste lines (F2-F6 and W1- W2) with WFI through each of the solvent lines (A1-A6 and B1-B3) and column 1 and 2 positions. Verify that each line is flushed at a minimum flow rate of 100 mL/min for at least 2 minute.

Note: If using a manual flush, a suggested procedure that may be employed throughout this section is as follows:

- Flush solvent line A1 to waste line W2 with column 1 down, and column 2 up.
- Flush solvent line A1 to waste line W2 with column 1 up, and column 2 down.

- Flush solvent line A2 to waste line W2 with column 1 bypassed, and column 2 bypassed.
- Flush solvent line A3 to waste line W2 with column 1 bypassed, and column 2 bypassed.
- Flush solvent line A1 to outlet line W1 with bubble trap online, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A1 to outlet line F2 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A2 to outlet line F3 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A3 to outlet line F4 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A1 to outlet line F5 with bubble trap offline, column 1 bypassed, and column 2 bypassed
- Flush solvent line A1 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed
- Flush solvent line A4 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A5 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A6 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line B1 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line B2 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line B3 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.

6.6 Connect the AKTA Pilot 600R Inlet valve lines to 0.5N NaOH at ambient temperature.

6.7 Either manually or using the AKTA Pilot 600R System Flush program, flush each of the AKTA Pilot 600R Chromatography System Outlet and Waste lines (F1-F6 and W1-W2) with 0.5N NaOH through each of the solvent lines (A1-A6 and B1-B3) and column 1 and 2 positions. Verify that the pH of the eluate from each of these lines is greater than or equal to 13 using pH strips. If the pH does not meet specification, continue flushing until the desired pH is achieved.

6.8 Allow the AKTA Pilot 600R Chromatography System to be exposed to 0.5 N NaOH for ≥ 60 minutes. Exposure begins when the desired pH is achieved in section 6.7.

6.9 Connect the AKTA Pilot 600R inlet valve to WFI at ambient temperature.

6.10 Either manually or using the AKTA Pilot 600R System Flush program, flush each of the AKTA Pilot 600R Chromatography System Outlet and Waste lines (F1-F6 and W1-W2) with WFI through each of the solvent lines (A1-A6 and B1-B3) and column 1 and 2 positions. Verify that the conductivity of the eluate from each of the lines is less than or equal to 1 mS/cm. If the conductivity does not meet the specification, continue flushing until the desired conductivity is achieved.

6.11 Disconnect the tubing connections if required for sampling.

6.12 The AKTA Pilot System may be stored in WFI if the system is going to be used with 24 hours.

6.12.1 Disconnect the Inlet valve tubing's from the WFI and seal the open end with end cap.

6.12.2 Seal the open end of the Outlet valves tubing's by endcap or hemostat.

6.12.3 Label the equipment per ***SOP 14150, Labeling of cGMP Purification Equipment for Cleaning Status***.

6.13 If storing for greater than 24 hours, connect the AKTA Pilot 600R Inlet valve lines to 20% Ethanol at ambient temperature.

6.13.1 Either manually or using the AKTA Pilot 600R System Flush program, flush each of the AKTA Pilot 600R Chromatography System Outlet and Waste lines (F1-F6 and W1-W2) with 20% Ethanol through each of the sample lines (1-4) and solvent lines (A1-A4 and B1-B4) and column 1 and 2 positions. Verify that each line is flushed at a minimum flow rate of 100 mL/min for at least 2 minutes.

6.13.2 Disconnect the Inlet valve tubing's from the 20% Ethanol and seal the open end with end cap.

6.13.3 Seal the open end of the Outlet valves tubing's by endcap or hemostat.

6.13.4 Label the equipment per ***SOP 14150, Labeling of cGMP Purification Equipment for Cleaning Status.***

6.14 At the start of each purification lot all pathways of the AKTA Pilot 600R Chromatography System will be flushed with WFI until the conductivity of the effluent from all pathways is ≤ 5 μS/cm. Document in BPR (if applicable).

6.15 Document all cleaning activities on Form 14155-02 and indicate cleaning performed in the system logbook Form 14155-01.

7.0 References and Related Documents

SOP 21529, ***Equipment Interproduct Cleaning and Clearance***

SOP 14142, *Standardization and Cleaning of the AKTA Pilot Chromatography System*

SOP 14150, Labeling of cGMP Purification Equipment for Cleaning Status

AKTA Pilot 600R Instruction Manual

Form 14155-01 AKTA Pilot Chromatography System Use Log

Form 14155-02 Cleaning of the AKTA Pilot Chromatography System

Form 14155-03 Standardization of the AKTA Pilot pH Monitor

8.0 Change Summary