Document Number:	**SASoM/METHOD/133.v1**
Title:	**Immunohistochemistry and immunofluorescence using Leica BOND RX autostainer**
Version:	**v1**
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Effective from:	10/03/2021
Valid to:	10/03/2023

SOP History		
Number	Date	Reason for Change
V1	10/03/2021	Original

1.0 Purpose –
This SOP describes the current procedure for performing immunohistochemistry and immunofluorescence using the Leica BOND RX autostainer in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM performing immunohistochemistry and immunofluorescence using the Leica BOND RX autostainer.

3.0 Responsibilities –
All staff performing immunohistochemistry and immunofluorescence using the Leica BOND RX autostainer are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

4.1 Bulk Reagent Preparation

1. The container for 'Dewaxing Solution' is filled with either (i) Leica 'Dewax Solution' (#AR9222), OR (ii) Lotoxane (Arrow Solutions, #C043). (**NOTE: Never put water or detergents in the dewax container).**
2. The container for 'Deinoised Water' is filled with up to 3L of with Elga water.
3. The container for 'Wash Buffer' is filled with Leica BOND (X10) Wash Buffer (#AR9590) after first diluting it 1:10 in Elga water. Alternatively, 0.1% 'ProClin 950' made up in TBS-T buffer can be used .
4. The container for 'Alcohol' is filled with absolute alcohol. **(NOTE: Never put water or detergents in the alcohol container.)**
5. The 'Non-hazardous Waste' container should be emptied before / after running the protocol. Non-hazardous waste can be poured down the sink with excess running tap water. The non-hazardous waste container can be cleaned using 0.5% bleach solution or industrial strength detergent before rinsing thoroughly with Elga water.
6. The 'Hazardous Waste' container should be disposed of in the Leica BOND waste container located next to the autostainer. This should then be stored in the external solvent store and disposed of through the official University Chemical Uplift procedures. The container can be cleaned using 0.5% bleach solution or industrial strength detergent and rinsed thoroughly with elga water.
7. ER1 container is filled up with Leica BOND ER1 Epitope Retrieval Solution 1 (#AR9961).
8. ER2 container is filled up with Leica BOND ER2 Epitope Retrieval Solution 2 (#AR9640).

4.2 Protocol set up

1. Pre-defined Leica biosystems protocols indicated with '*' (eg. '*IHC Protocol F' for immunohistochemistry) are available. They are editable, but other settings can be changed for user protocols.

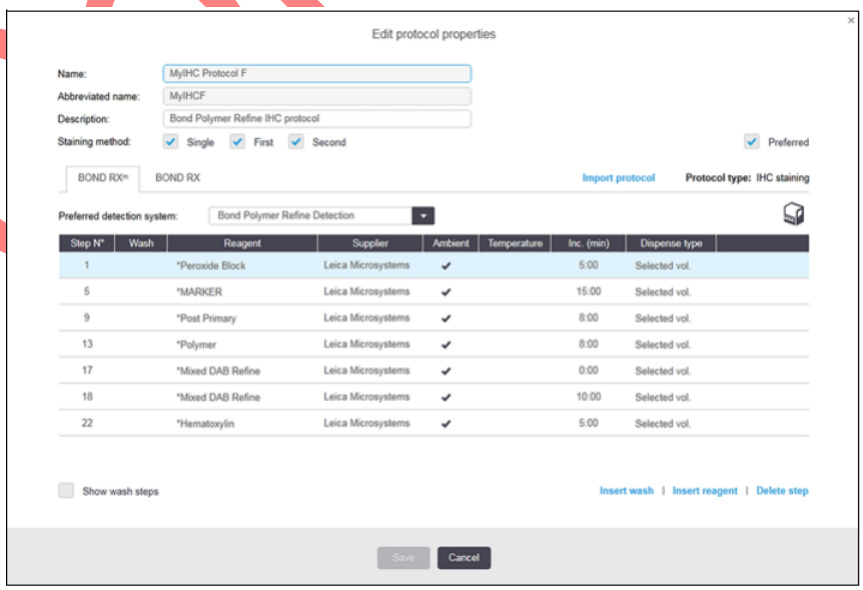


Figure 1 The Edit protocol properties dialog for a user protocol

2. Protocol rule - Protocol name and abbreviated name must be unique. All staining protocols must include at least one reagent from a Leica Biosystems BOND detection system or research reagent system. The last step of the staining protocol must be a wash step. Each step must be fully defined with a reagent, incubation time, dispense type and temperature (Figure 1).

3. For staining protocols, select a detection system or research reagent system for use with the protocol (eg. BOND polymer detection system).

4. Add or remove protocol steps and change editable parameters in new and existing protocol steps by first double-clicking the parameter you want to change:

i) Select a reagent from the drop-down list. (NOTE: Select *Marker to indicate the step where the primary antibody is used in IHC protocols. Only *BOND wash solution or *Deinoised water can be used for wash steps.

ii) Set incubation time in minutes and seconds (mm:ss)

iii) Set temperature – If a temperature you want to set up is not ambient, first uncheck the Ambient parameter. Then, select the empty temperature parameter and enter the temperature in degress Celsius as a whole number.

iv) Set dispense type for staining protocols (150ul, Open or Intermediate).

4.3 Reagent set up

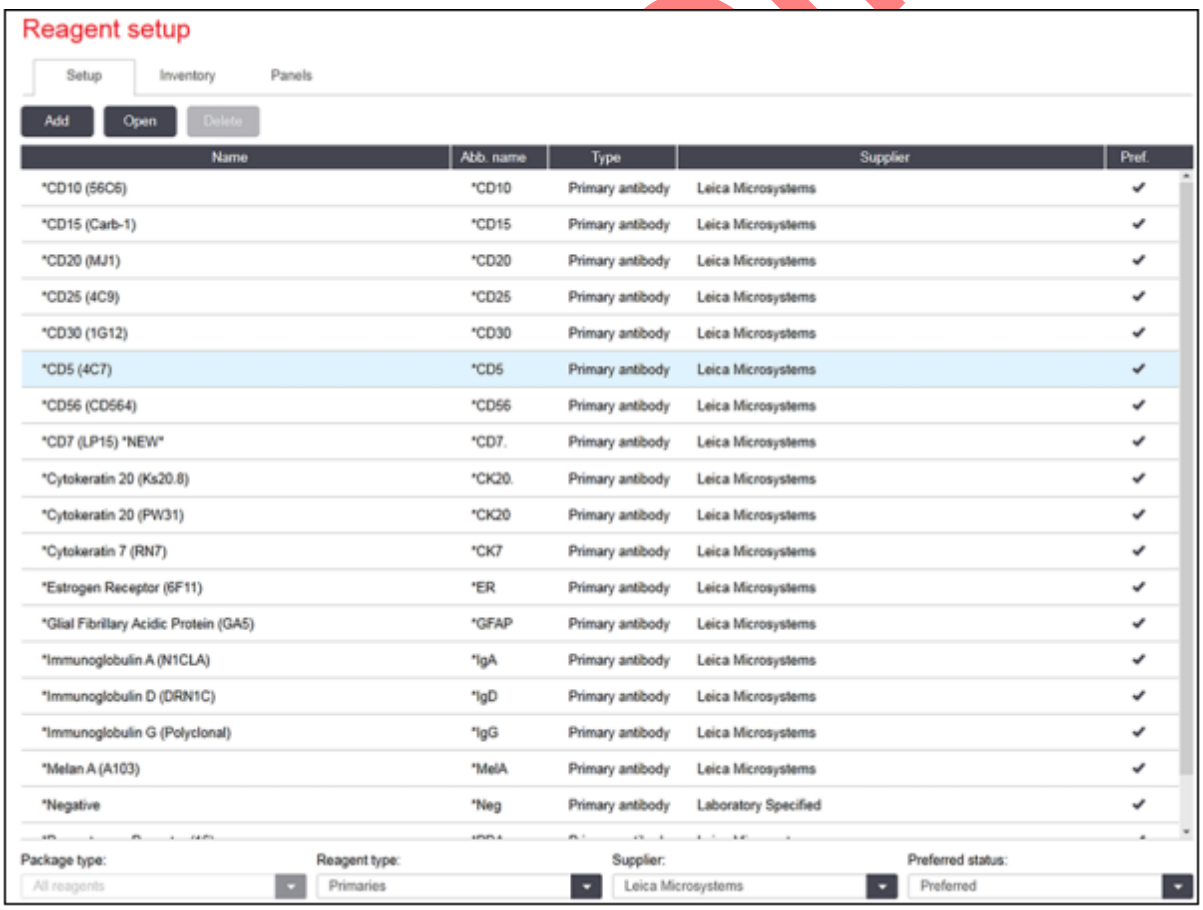


Figure 2 Reagent setup screen

1. Reagent setup can be used to add, edit or delete the reagents. The reagents setup screen (Figure 2) can display a complete list of all reagents known to the BOND RX system. The list does not include any pre-packaged reagent systems (eg. BOND detection systems)
2. When registering a new reagent, open 'Add' reagent dialog (Figure 3). A name, abbreviated name, type of reagents (primary antibody, probes, ancillaries, mixed reagents, and parallel double-stain primaries and probes), and supplier information can be added. If required, default staining protocol, epitope retrieval protocol (HIER) protocol and enzyme protocol can be added. (NOTE: In the Name field, a descriptive name should be added. New reagents cannot start with "*", which is reserved for Leica Biosystems reagents.)

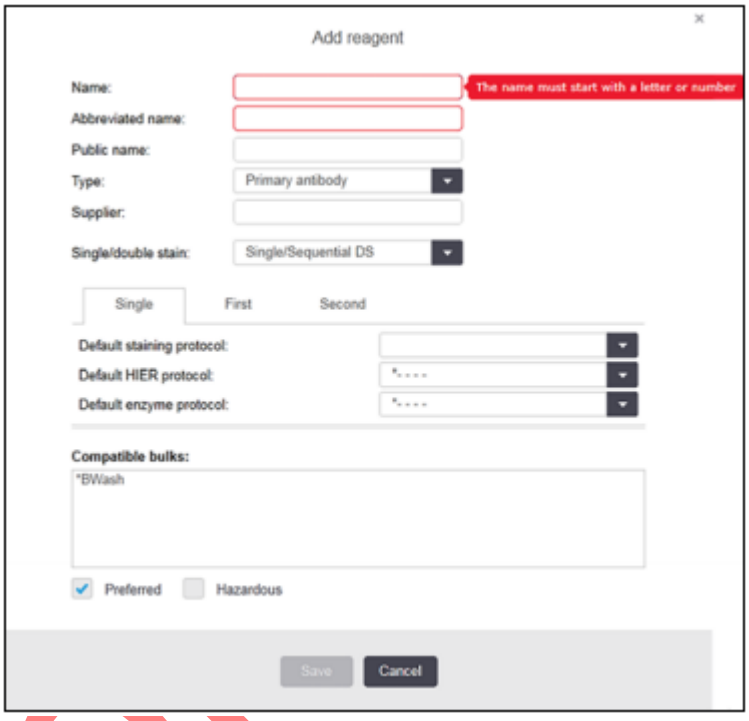


Figure 3 Add reagent dialog

3. If the reagents are hazardous, then 'Hazardous' should be ticked in this 'add reagent' dialog box (Figure 3).
4. To delete a reagent, select it from the list in the reagent setup screen (Figure 2) and click Delete. (NOTE: You cannot delete a reagent that is used by a registered research reagent system).
5. Assigning a reagent package (eg. Titration container,7ml or 30ml open container) to specific reagent names that already registered into BOND RX system. Once registered, the reagent package is locked to a particular reagent. (NOTE: Each reagent container is used for a total of 40ml of reagent. There's different maximum volume of reagents depending on the container size. The dead volume (eg. 300ul for titration container, 555ul for 7ml container and 1618ul for 30ml contrainer) should be also considered when making up reagents.)
6. Leica BOND Detection system must be included to operate any protocols.

- **For immunohistochemistry** the Leica biosystem BOND polymer refine detection system (#DS9800) is used.
- **For immunofluorescence**, the research kit which includes 3% Hydrogen peroxide, serum free protein block (Agilent, #X090930-2) or casein blocking buffer (Sigma, #B6429) and Hoechst 33342 (Thermo fisher, #H3570).

4.4 Slide setup

1. A study can be added by opening 'Add study' dialog (Figure 4) in slide setup screen. Study ID, study name, study comments, and researcher fields should be filled. (NOTE: Duplication of study is not allowed.)

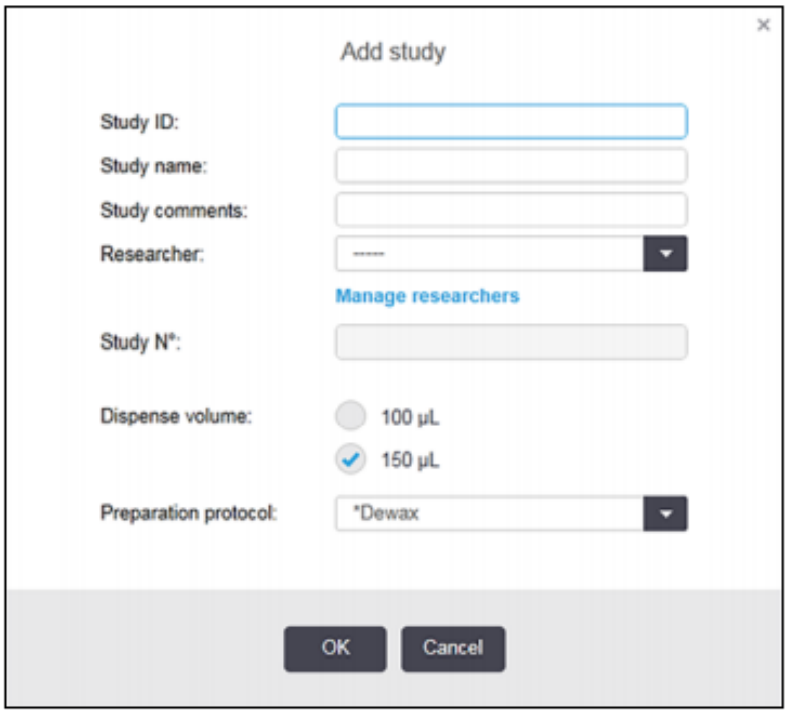


Figure 4 The Add study dialog

2. Under a study added, a new slide can be created by filling slide comments, tissue type, staining protocol and marker as seen in Figure 5.

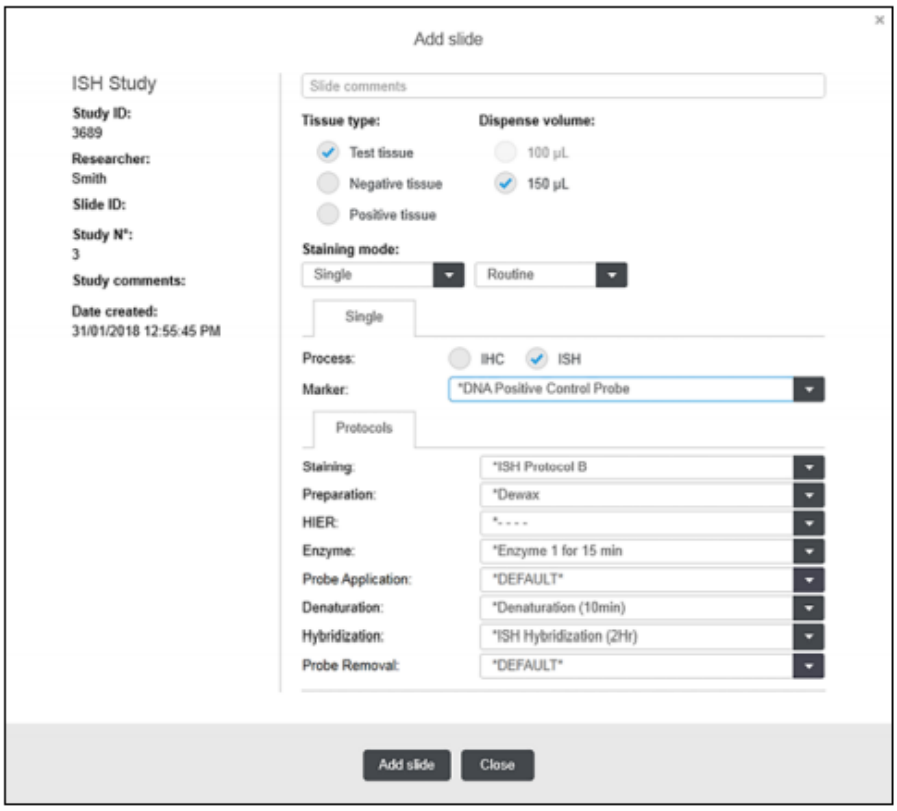


Figure 5 The ADD slide dialog

3. A Slide label MUST be printed as all slides that are stained on the BOND RX system must be labelled in order to be identified in the software.
4. Loading slides into the BOND RX as detailed in the BOND RX equipment SOP.

4.5 Running protocol

1. Once reagents and slides are loaded, the system status screen (Figure 6) will show if the run is ready or not. When all reagents required for the protocol is ready then the play button ▶ is activated. To start the run as soon as possible click ▶. When stopping the run, simply click ■. (NOTE: Runs with waxed slides can be scheduled to start at a specified future time (up to one week from the current time). To delay the run, right-click the slide tray and select ‘delayed start’ by setting date and time.)

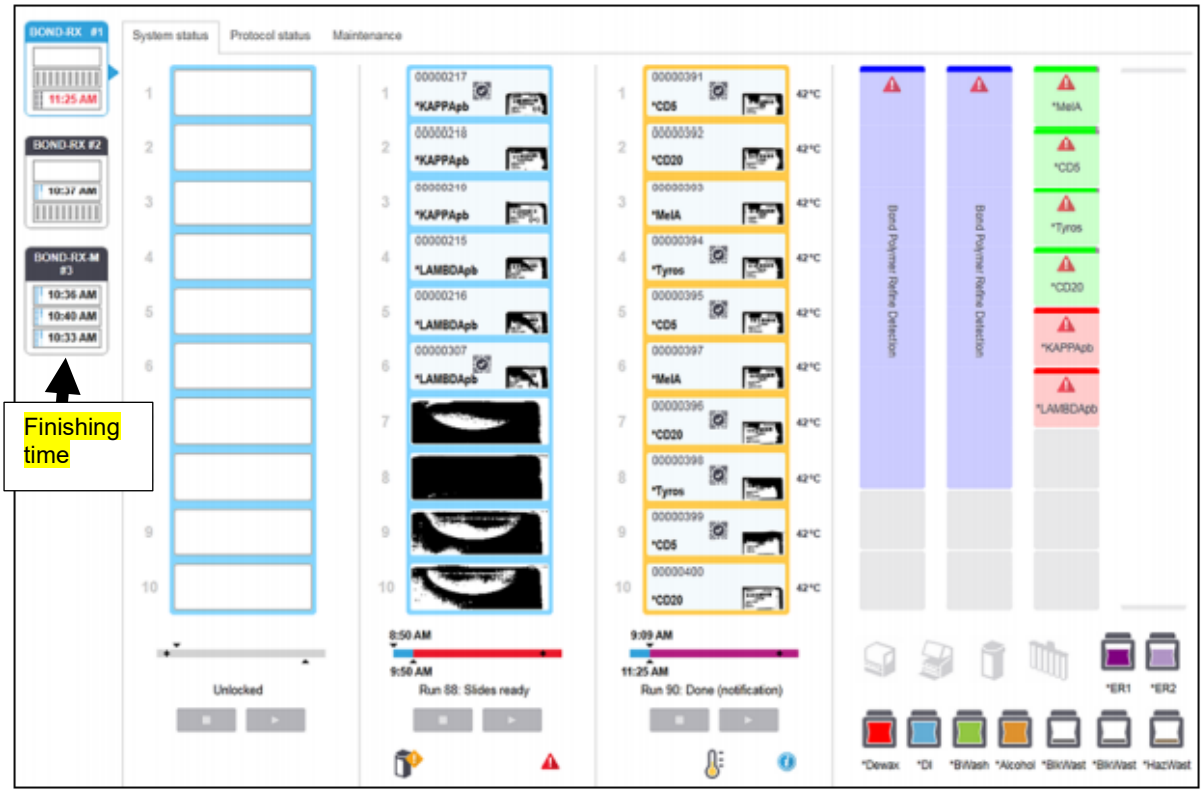


Figure 6 The System status screen for a BOND RX instrument

2. If warning symbols (Figure 8) are shown, either replenish or empty the bulk containers and the reagents.
3. Once the run started, its finishing time is displayed (Figure 6).
4. After the run, there are three different status indicators seen in Figure7.

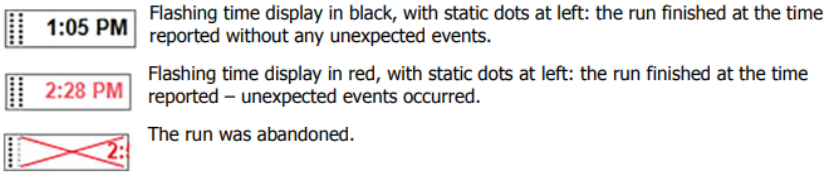


Figure 7 Examples indicator of after the run

Bulk Containers	Indicates
	Container is full.
	Container is more than 1/2 full.
	Container is less than 1/2 full.
	Container is nearly empty or empty.
	Appears if the following occurs: • waste is nearly full and needs to be emptied immediately • reagent is running low and needs to be filled immediately • container is missing • insufficient volume to start a run See 12.2.2 Replenishing or Emptying Bulk Containers.
 or	Appears if a run has been paused because one of the following occurs: • waste is full and needs to be emptied urgently (warning) • reagent is low and needs to be filled urgently (warning) • container is missing and needed for processing (alarm) See 12.2.2 Replenishing or Emptying Bulk Containers.

Figure 8 Warning symbols

5. Run events will show the report of each slide which had an issue during the run.

4.6 Completion of IHC and IF

1. For immunohistochemistry run, remove the covertiles and dehydrate, clear and mount the slides according to the SOP (SASoM/METHOD/025.v6-'Immunohistochemsitry').
2. For immunofluorescence run, remove the covertiles. Mount slides with prolong gold antifade mountant (Thermo fisher, P36930) with coverslip.

4.6 Maintenance

1. Daily – clean covertiles using alcohol clean (Figure 9)

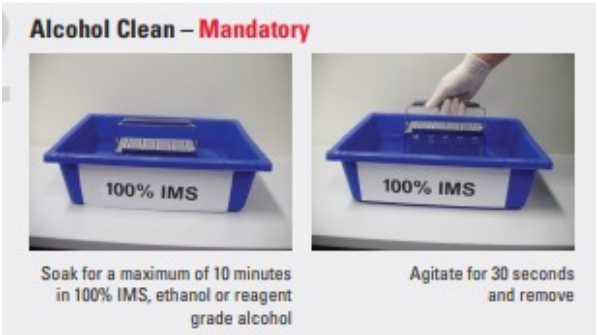
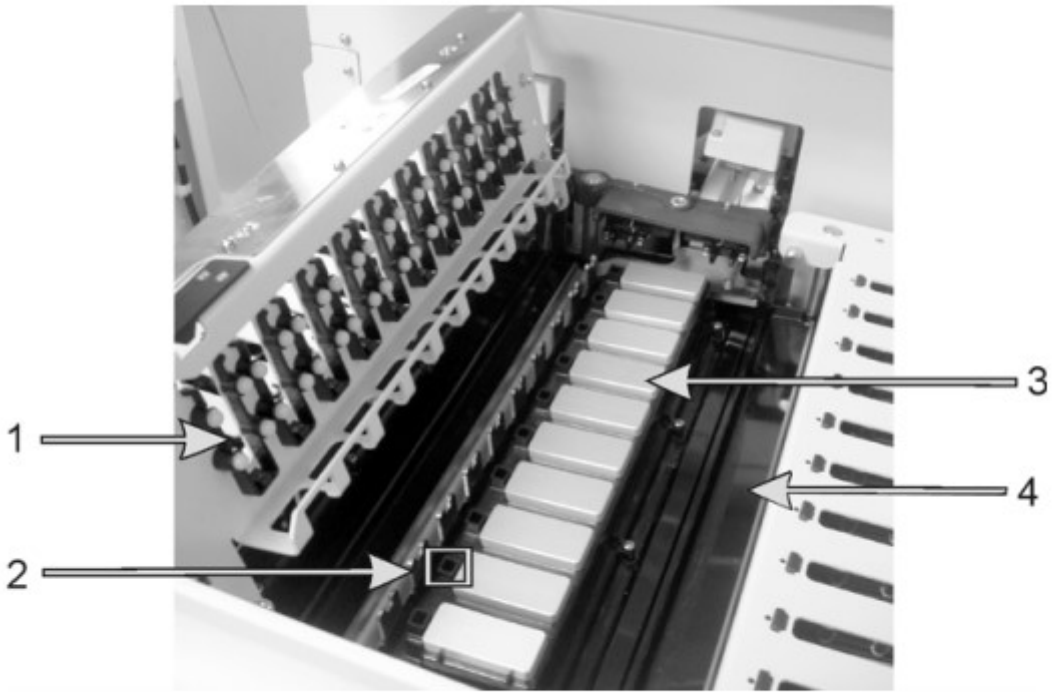


Figure 9 Covertile cleaning -alcohol clean

2. Weekly cleaning (Figure 10)
- clean covers, doors and lid with lint free cloth
- clean slide staining assemblies (heater pads, drainage ports and wicking posts and the drip tray) with a lint-free cloth moistened with 70% alcohol (as little as possible)
- check covertile clamps



igure 126: Slide staining assembly with top plate open, showing Covertile clamps (1), drainage port and wicking posts (2), heater pads (3) and drip tray (4)

Figure 10 Slide staining assembly

3. Cleaning aspirating probe – every 300 slides, the aspirating probe should be cleaned using BOND aspirating probe cleaning kit (CS9100).

5.0 Personal protection -
A Howie laboratory coat and lab gloves must be worn at all times.

6.0 Spillages -
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with 70% ethanol.

7.0 Training -
Training is mandatory.

8.0 Related documents –

8.1 Risk assessments –
RA22386-Use of the Leica Bond Rx Autosampler.

8.2 SOPs -
SASoM/METHOD/025.v6-Immunohistochemsitry.
SASoE/EQUIP/106.v1-Use and Maintenance of Leica BOND RX autostainer.

9.0 Approval and sign off –

Author:		
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Signature:		Date:
QA release by:		
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Position:	QA Manager	
Signature:		Date: