USER MANUAL

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hybriSpot12 PCR AUTO User Manual V2_EN (01/04/2019)
This manual can also be used for the hybriSoft's version 2.2.0 R00. For compatibility with other versions, please contact the manufacturer / supplier

CONTENTS:

1. DISCLAIMER

Our company disclaims all responsibility for any damages that can be caused by any kind of modifications made on the hardware or software due to connections to other instruments that are not conducted by our qualified personnel or previously authorized by our company.

If the device is damaged, do not turn it on until it is repaired by our specialized technician. Any kind of electrical installation required to install or fix the machine must be conducted by our personnel. Please, do not proceed in any other way.

To guarantee that the results obtained are equivalent to those described in the technical specifications, the machine must be used under the environmental and safety conditions according to the regulations specified in the sections 2 and 3 of the following chapter.

Use this device after reading this manual only.

2. SAFETY PRECAUTIONS

Although the hybriSpot 12 PCR AUTO (henceforth HS12a) does not pose any risk to the operator, it must be only used by trained personnel and exclusively for its designated use. The instructions for use must be simultaneously applied with the risk prevention and environment protection standards in force in the country in which the instrument has been installed.

- HS12a must be installed in a firm and clean surface
- It is recommended to follow the next steps during the instrument installation: in accordance with the Isolation and Safety Standards for Electronic Instruments (IEC 1010-2-2), a safety area of 300 mm around the instrument must be foreseen.
- The safety area must be free from dangerous substances while the machine is running.
- According to the international standard EN 61010-2-101, the electric system must be equipped with an Emergency Power Off (EPO) system.
- HS12a must not be used in areas with explosion risks.
- HS12a must not be used in the presence of:
    - Explosive materials
    - Materials that, when in contact with others, can cause the release of big amounts of energy.
- The user must handle the samples according to the laboratory protocols described for biological materials containing pathogens.
- It is strictly prohibited to use non-original accessories and spares.
- The safe functioning of the instrument HS12a and its reliability are only guaranteed if the HS12a is used in full accordance with the operational guidelines.
- All verification and repair must be carried out by the personnel authorized by the manufacturer.

- The electrical installation of the instrument location must meet the ICE standards.
- This IVD equipment meets the requirements for immunity and emission of IEC 61326-2-6:2006. This equipment has been designed and tested according to the CISPR 11 Class A. In a domestic environment, it can cause radio interference, in which case it may be necessary to take action to minimize these interferences. The magnetic environment must be assessed before using the equipment. Do not use this equipment near sources with strong electromagnetic radiations (e.g.: RF sources without protection) as it may interfere with the correct functioning.

WARNING: *In the case of non-compliance of the previous indications, the warranty will be declared void.*

3. SPACE AVAILABILITY AND ELECTRICAL CONNECTION

The dimensions of the instrument and the required space for its installation are indicated in the technical specifications.

- Make sure there is enough ventilation and that the voltage, the frequency, and the fuse value are correct (read information on the tag).
- The instrument HS12a must be connected to a standard shockproof plug through the cable provided by in the shipment kit.

SUPPLY	100-240 V, 50/60 Hz
DISTRIBUTION	1 fuse, neutral and ground
FUSE	5x 20 mm “T” type 3.15 A 5 x 20 mm “T” type 10 A

4. SYMBOL TABLE

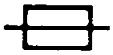
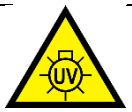
NUMBER	SYMBOL	DESCRIPTION
1		Alternating current
2		Earth terminal
3		On
4		Off
5		Fuse
6		Warning: risk of electric shock
7		Warning: refer to attached documentation
8		Biological hazard
9		Warning: hot surface
10		Warning: moving parts
11		Warning: ultraviolet light
12		CE marking
13		In Vitro Diagnosis

Table 1: Symbols

5. GENERAL DESCRIPTION

5.1 Introduction

The HS12a equipment is a new platform capable of fully automating the amplification, denaturation and hybridization processes, bringing together all the technical specifications that are necessary to facilitate the work to the customer and obtain high performance and efficiency.

The platform is based on the DNA-FLOW technology offering a diagnostic system for rapid and simultaneous analyses of multiple biomarkers, DNA as well as proteins through reverse dot blot on macroarrays. This technology allows the target molecules to cross the chip through vertical flow to hybridize to its complementary probes immobilized in a porous matrix in a three-dimensional environment, as opposed to the hybridization on a conventional surface.

Figure 1 HybriSpot 12 PCR AUTO.

5.2 Technical Specifications

MODEL	hybriSpot 12 PCR AUTO
CODE	VIT-HS12A
MAXIMUM NUMBER OF SAMPLES	12
MEASUREMENTS (Width x Length x Height)	VIT-HS12A (Hood closed): 71.1 x 52 x 66.2 cm
	VIT-HS12A (Hood open): 71.1 x 52 x 100.8 cm
	VIT-HS12A (Hood closed) & PC: 140 x 52 x 66.2 cm
	SAI: 11.2 x 38.2 x 30.1 cm
WEIGHT	50 Kg
POWER SUPPLY VOLTAGE	110 – 240 VAC, 50 – 60 Hz
HYBRIDIZATION	Temperature range: 25 – 51 °C
	Max. heating speed: 4.5 °C/min
	Max. cooling speed: 2 °C/min
	Accuracy: ± 2 °C
	Uniformity: ± 1.5 °C
THERMOCYCLER	Temperature range: 4 – 100 °C
	Max. heating speed: 5 °C/s
	Max. cooling speed: 3 °C/s
	Accuracy: ± 0.1 °C
	Uniformity: ± 0.5 °C
COMMUNICATION INTERFACE	General control: USB 2.0 Full Speed 12Mbps
	Image capture: USB 2.0 High Speed 480Mbps
RECOMMENDED ENVIRONMENTAL WORKING CONDITIONS	Temperature range: 18°C – 30°C
	Maximum relative humidity: 80%

Table 2: Technical specifications.

6. HS12a INSTALLATION

The installation of the HS12a must be carried out by a qualified technical department according to the instructions included in the Technical Support Manual of the HS12a.

6.1 Surface

The equipment must be installed on a plain surface.

6.2 Required free space

There must be a free space of at least 30 cm around the equipment.

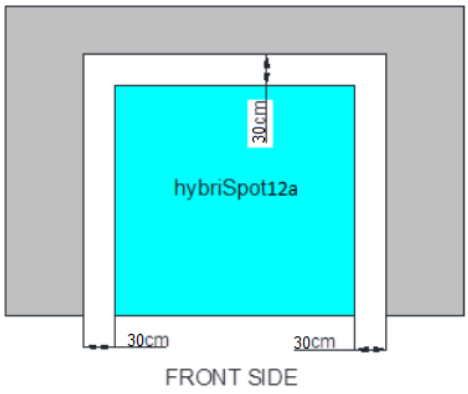


Figure 2. Required space.

6.3 USB connection

The equipment must be connected to a computer through a USB cable (included with the equipment).

6.4 Bar-code reader installation

A bar-code reader is needed. In this case, you have to make sure that the bar-code reader has the appropriate PC USB keyboard configuration. It is necessary to add a CR (carriage return) suffix, for the appropriate functioning.

Refer to the manufacturer's specific bar-code reader documentation to obtain further information on how to set it up.

7. HYBRISOFT INSTALLATION

HybriSoft HSHS is the sample management software of the hybriSpot platforms. It must be installed by the Technical Support, according to the established installation procedures. The compatible versions of HSHS with HS12a are 2.2.0 and higher.

8. MANAGEMENT OF HS12a WITH HYBRISOFT

The instrument HS12a needs the hybriSoft support to carry out the processing of the samples.

8.1 HS Start-up

The device has two switches, one on one side and another on the front side. After plugging the device in, press the side switch to allow the current to pass through.

To turn the instrument on, press the front switch for 2 seconds. **Use this switch to turn off the machine in case of emergency.**

Figure 3. Front switch. HS12a on/off panel.

Next to the front switch, there are two padlock-shaped led indicators, one closed and one opened.

- If the device is off, both indicators will be in grayish.
- If the device is on:
  - Open padlock in green→ The device can be used for any action that may be carried out from its inside.
  - Closed padlock in red→ The device is running.

    ⚠ Do not access the inside of the device while the closed padlock is red.
  - Closed and open padlock with blinking red and green lights→ It indicates that there is an error. Errors will be shown in Hybrisoft's pop-up windows.

The instrument will start once it is on and "Hybrisoft" has detected it. The instrument will proceed to position the robotic arm, the diluter and the thermocycler cover automatically (process we will call "homing").

8.2 HybriSoft start-up

Before launching hybriSoft, make sure that the HS12a equipment is connected to the computer through a USB port 2.0. In order to launch hybriSoft, click twice on the hybriSoft icon, fill in the user and password fields and press the green key to log in.

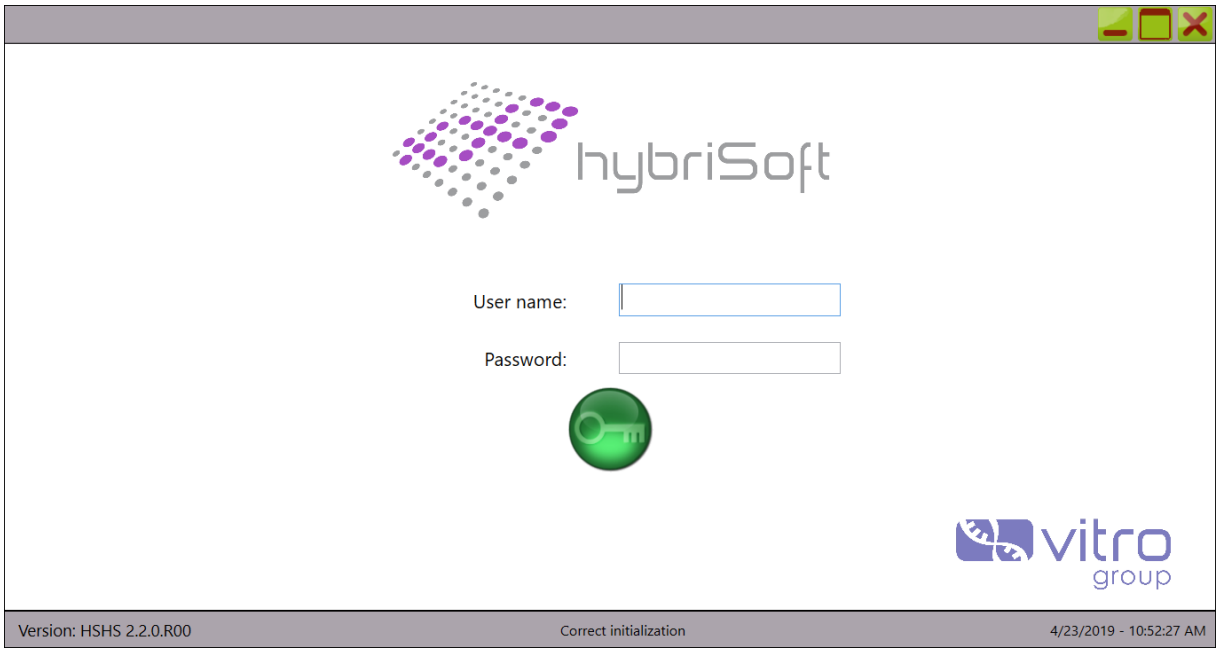


Figure 4. Home screen.

In the application, the "administrator" user has the highest permissions. To gain access with this user, you must contact the distributor.

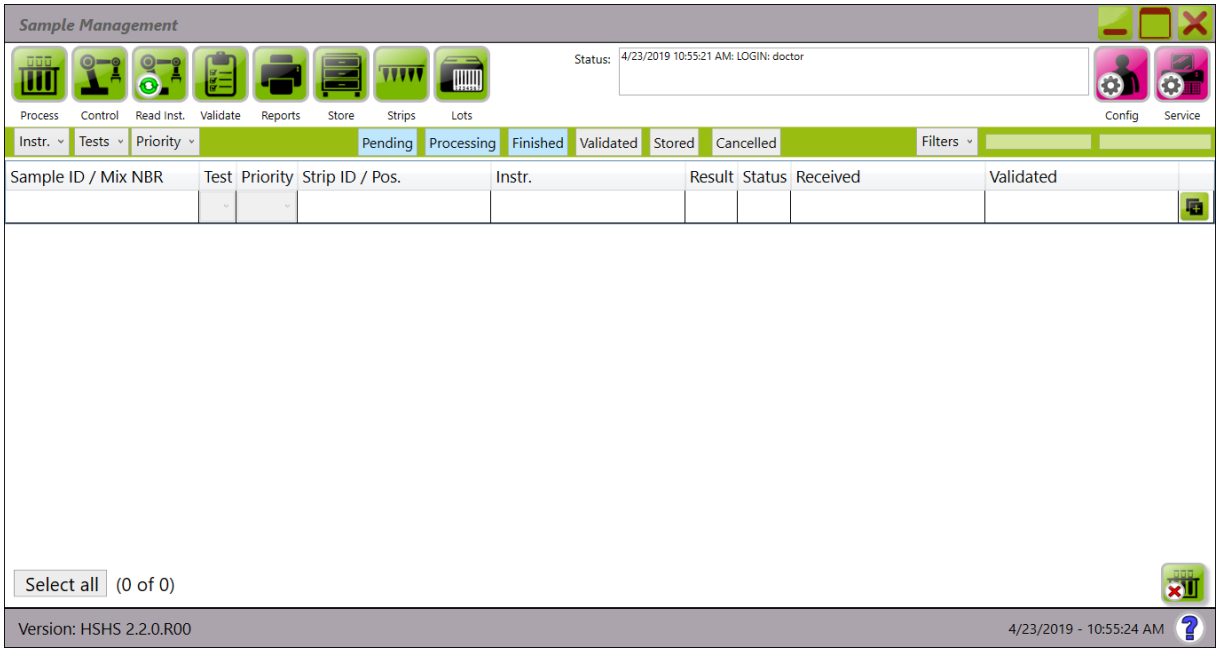


Figure 5. "Sample management" screen.

8.3 Configuration

Config.

This button allows you to configure hybrisoft depending on the needs and requirements of the user.

In case you try to enter this section during a protocol, a window will pop up stating that this function is disabled while the protocol is running.

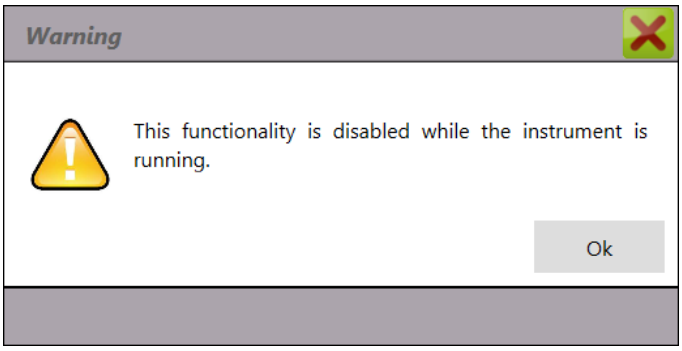


Figure 6. Configuration blocking warning.

8.3.1 Samples/Test Parameters

This tab allows you to configure different options of hybriSoft. These are the default settings for new installations:

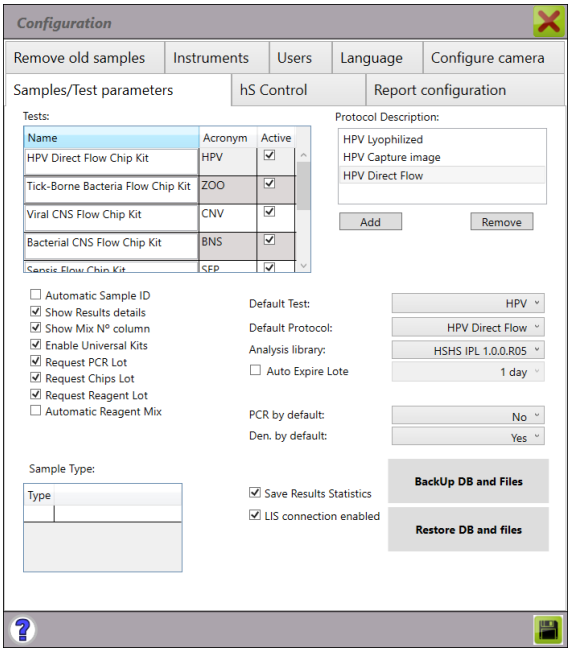


Figure 7. "Samples/Test Parameters" tab.

8.3.1.1 Test

In this section, you can activate and deactivate the different tests included.

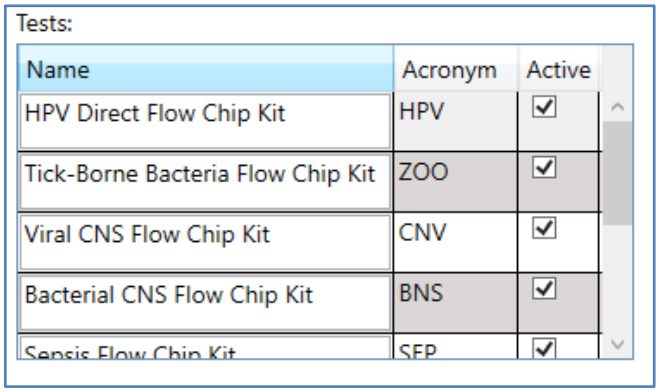


Figure 8. Test section.

In this section, you can check which tests are created in the system and which of them are active. In case you need to activate or deactivate any of them, click on the box that appears in the column "Active". Only active tests can be assigned to samples. When a test is deactivated, samples processed with that test can be found in the history of the database by filtering them.

Tests that include in brackets (FOR EVALUATION ONLY), it indicates that it is in its last evaluation phase and are not available for the user as they are not commercialized.

After saving the changes in the "Test" section, the application will be automatically closed to correctly apply the changes. After that, the user will have to start the application manually. A message appears indicating this action.

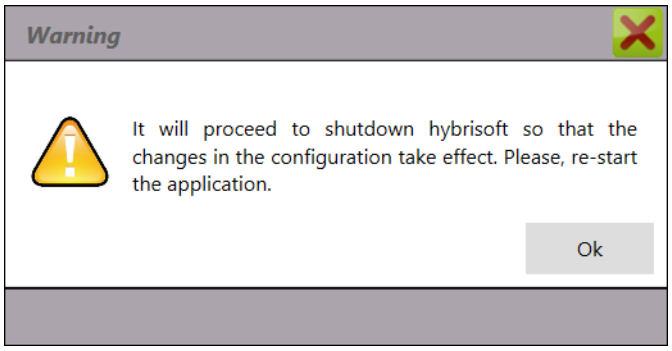


Figure 9. Automatic closing of the application warning.

When you launch the application again, the inactive tests will appear on a red background and cannot be selected for processing new samples.

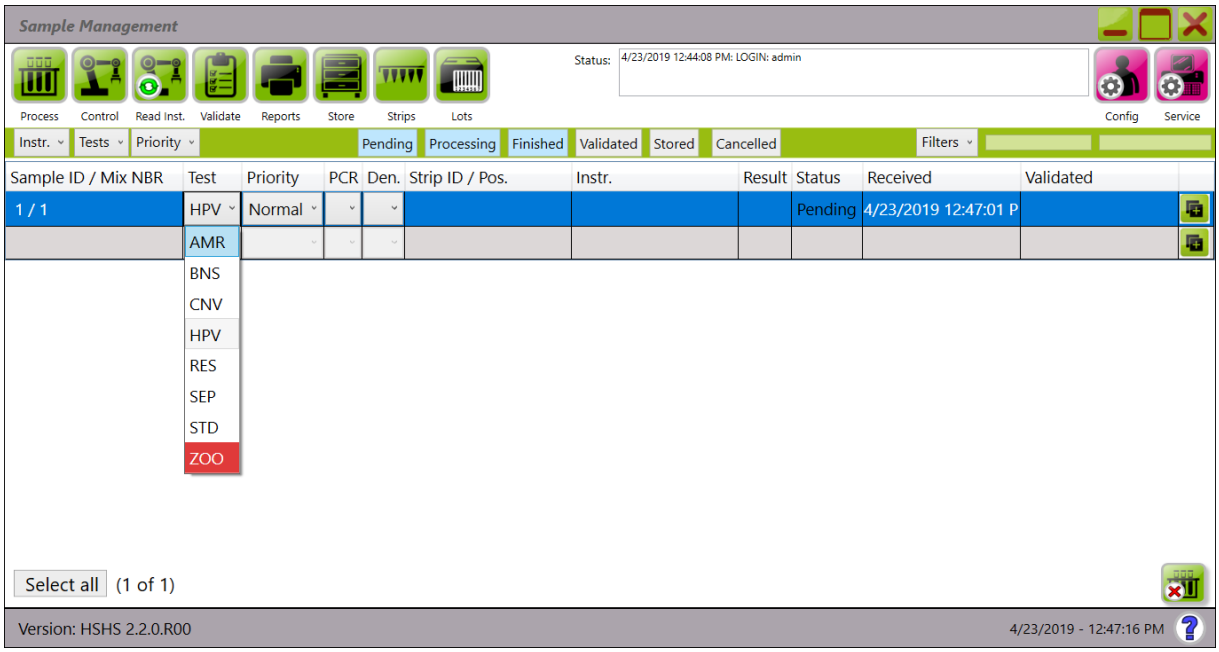


Figure 10. Sample management with inactive tests.

The red background is applied the same way to the previously processed samples. If the test that is going to be inactivated contains samples in a “Processing” status, it cannot be inactivated, and the following message will appear:

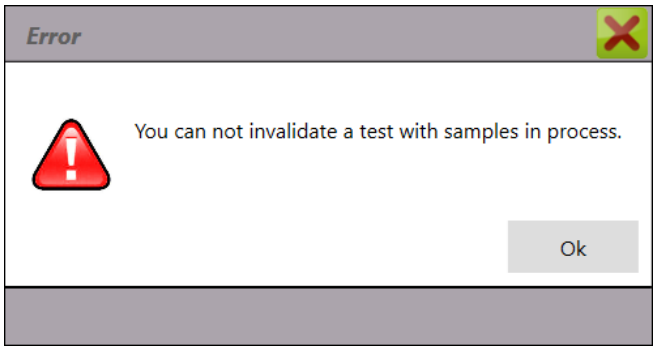


Figure 11. Test cannot be inactivated error.

8.3.1.2 Protocol Description

In this section, the different protocols that contain the selected test appear in the “Test” section.

Two protocols appear in all tests by default, one of them to capture and analyze images and the other contains the completed hybridization protocol.

It allows to add and remove protocols, only accessible for “Administrator”-type users

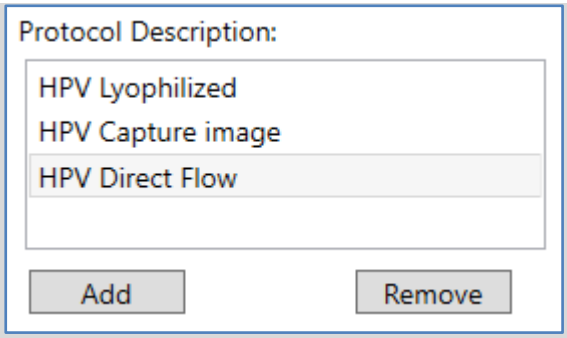


Figure 12. Description of the test protocols.

- **Add**: it allows to add new protocols to any test. When clicking on the "Add" button, a window will appear, where you can select the test to which the new protocol is going to be added. Afterwards, enter the name of the protocol and select the .csv file and appropriate cut-off point. The .csv file must be located in the folder where the rest of protocols are located.

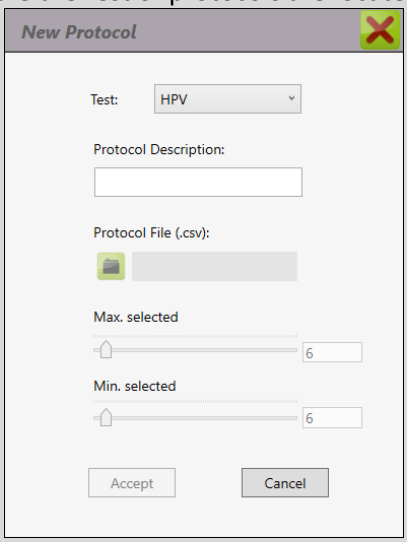


Figure 13. Insert new protocol.

- **Remove**: it allows to remove any protocol included in a test. When clicking on "Remove", a warning window appears to confirm the selected action. After selecting the protocol by default, the message states if the selected protocol is not being selected by default, the specific identification of the protocol appears.

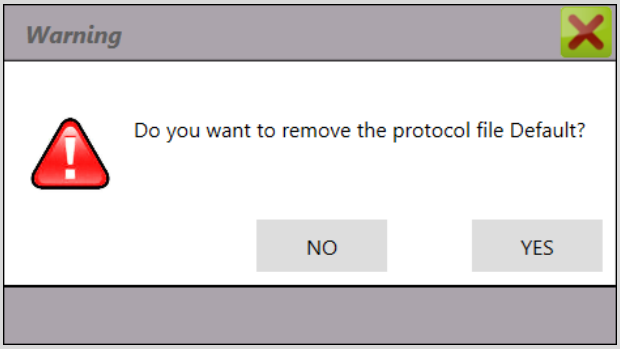


Figure 14. Removing default protocol warning.

After clicking on "Yes", if a protocol has already been used to process a sample, a message will appear stating that the selected protocol cannot be removed. Otherwise, the protocol will be removed from the database.

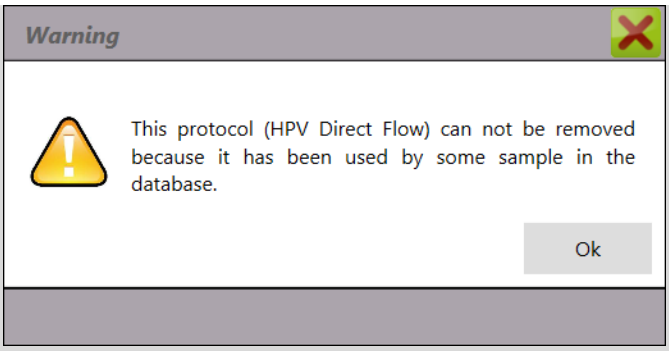


Figure 15. Protocol cannot be removed warning.

When logging in as a user without different permissions from the ones of the administrator, the options Add and Remove in the "Protocol description" section will be disabled.

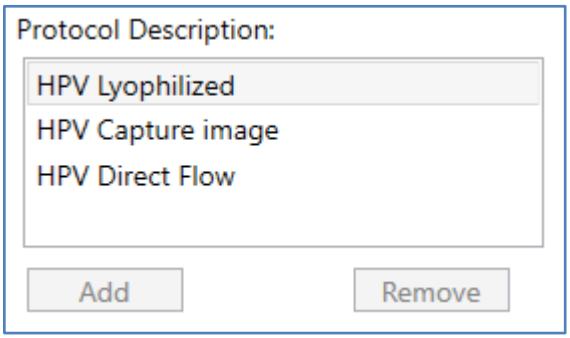


Figure 16. Protocol description. Non-administrator user.

8.3.1.3 Sample type

This section allows you to create any type of sample to identify correctly the samples with which you are going to work.

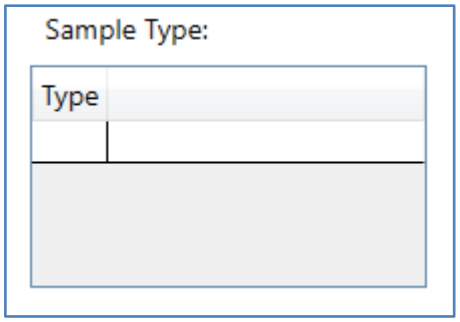


Figure 17. Sample type.

To create a new type of sample, double-click the empty box in the "Type" column and introduce the identification of the new type of sample, press the Enter key and click on the "Save" button (Figure 7). Thus, the new sample type will be saved.

8.3.1.4 Backup copy

The backup copy generates a compressed file containing the results, reports folders and the document var_ini.xml; all the documents needed to restart the database in hybriSoft.

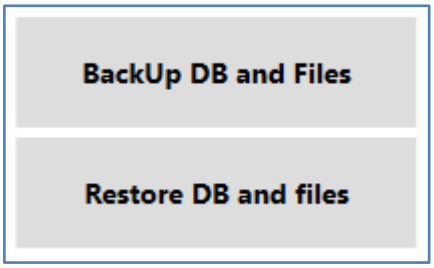


Figure 18. Copy and restoration of backups.

In order to make a backup copy, click the button “BackUp DB and Files” and the explorer to assign the desired name to the database will appear. Once the name has been established, press the "Save" button in the explorer.

After this, a screen will appear asking for establishing a new password for the compressed file. This password is necessary to restore the backup of files and decompress the file.

To restore the database, press the button "Restore DB and Files”. The explorer will be opened to select the file containing the desired database. When pressing "Open", the explorer closes and a window in which you must introduce the password corresponding to the selected compressed file appears.

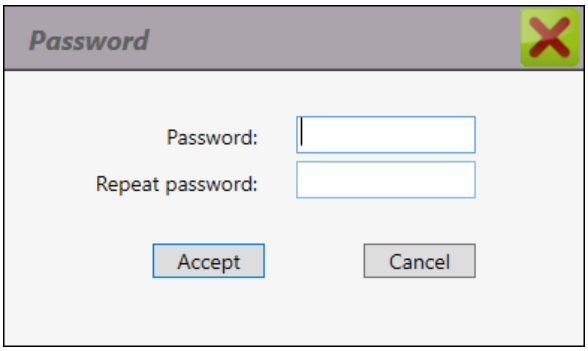


Figure 19. Enter backup password window.

If a wrong password is entered, a pop-up window will appear stating that the password is wrong.

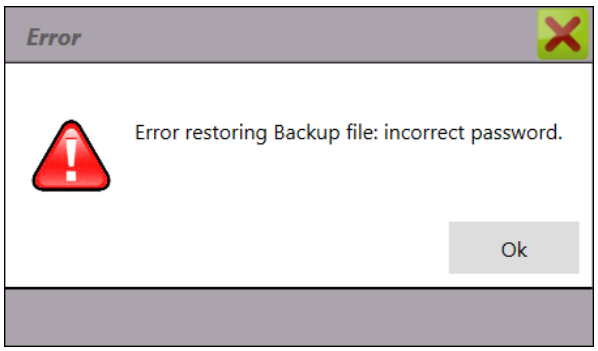


Figure 20. Backup password error.

Once the password is entered and the changes are saved in the configuration window, a pop-up window will appear stating that the application will be closed and the user will have to restart the application again.

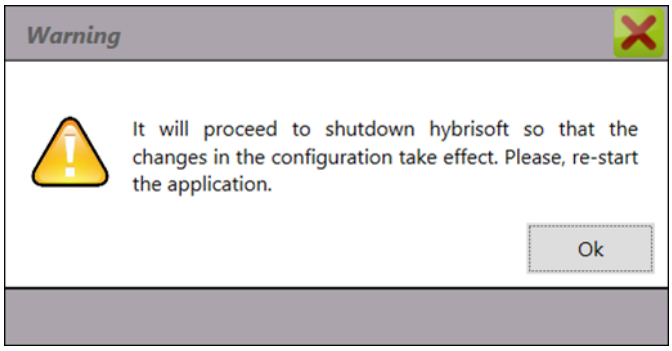


Figure 21. Restart window of hybriSoft.

When restarting the database, all the information from all the backup samples is loaded.

8.3.1.5 Other configuration options

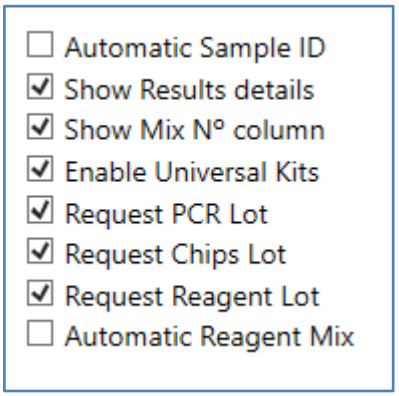


Figure 22. Extra options of hybriSoft.

- **Automatic Sample ID**: it activates or deactivates the possibility of the sample identification being auto-numeric. If inactive, no ID will appear automatically. The user will have to introduce the alphanumeric identifier manually or with the reader.

- **Show Results details:** it allows the activation or deactivation of the option to visualize the image of the sample in a Finished, Validated or Stored status. In case of being inactive, it does not allow the visualization of the captured image. To activate it, the administrator's password is required.

- **Show Mix Nº column:** it displays the information of the "Replica" number in the sample management window in the column "Sample ID". This column, along with the sample one, shows the number of replicas that a sample has, separated from the Sample ID by a slash (/). It is filled in with the number of PCR mix that a test contains.

- **Enable Universal kits:** this function allows you to use universal kits. Enable the possibility to use compatible kits with all the test and affects on how the batches are shown in the management windows and the assigning of reagent batch.
- **Request PCR lot:** it enables the obligation of assigning the batch of PCR mix used for performing the test.
- **Request Chip lot:** it enables the obligation of assigning the batch of mix of chips used for the test.
- **Request reagent lot:** it enables the obligation of assigning the batch of hybridization reagents used in the test. Only available for HS12. In the HS12a platform, the reagent batch is automatically assigned to the sample.

- **Automatic reagent mix:** activate the automatic reagent mix E1 and E2 in the reagent bottle E. It is required to place the three vials (E1, E2 and E) in the reagent rack.
  If this option is not activated, it will be only necessary to place the reagent bottle E in the rack once the mix has been made manually. If the three vials are placed in the rack and the check is not active, the following message will appear: "The reagents will not be mixed automatically. If you wish to, check "Automatic reagents mix" in "Configuration".

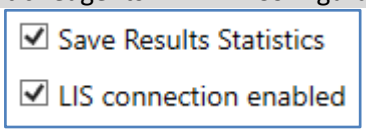


Figure 23. Configuration options.

- **Save Results Statistics**: it allows the activation or deactivation of the creation of an Excel file with the samples' data and the numeric value of the gray intensity of each one of the template's points. If inactive, this Excel file won't be generated.
- **LIS connection enabled:** it allows the two-way communication between hybriSoft and the LIS system of the user. If it is enabled, it allows to send automatically the recorded samples in a LIS to hybriSoft to be processed and return the results to the LIS system. If it is disabled, this communication won't happen.

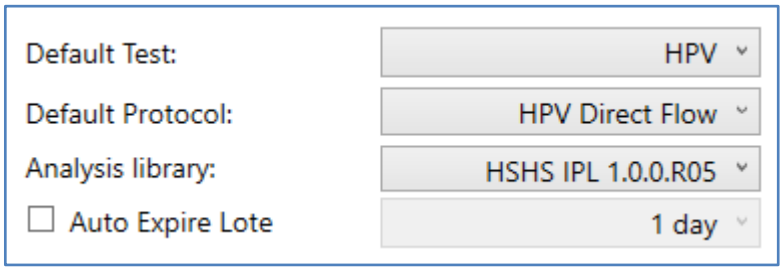


Figure 24. Establish default test and protocol.

- **Default test:** this option will drop down a menu in which you can select a default test that will be assigned automatically to each new sample.
- **Default protocol:** this option will drop down a menu in which you can select a default protocol that will be selected in the samples' processing. In the drop-down menu, it only contains the protocols corresponding to the assigned test by default.
- **Analysis library:** this drop-down menu shows the version of our analysis library in use in the sample process.

- **Auto Expire Batch:** this function allows to use an expired batch in the time shown in the drop-down menu. You have 1 day, 5 days, 10 days, 30 days and 60 days. When using expired reagents, the user has to control that the kit is working correctly. To activate this option, the administrator's password is required. **The supplier won't take any responsibility for results obtained with expired reagents.**

Figure 25. Establish default PCR and Den. values.

- **Default PCR:** it corresponds to the amplification process. It allows selecting the value assigned by default to a sample.
- **Default Den.:** it corresponds to the sample denaturation process. It allows selecting the value assigned by default to a sample.
  The options available from the drop-down menu are: “Blank”, “Yes” and “No”.
  Allowed processed according to the different values in these fields:
  - Amplification (PCR) + Denaturation (Den.) + Hybridization (Hyb.): when selecting “Yes” in the PCR drop-down menu, “Yes” will be automatically selected in the Den. drop-down menu, being the latter unmodifiable. In this HSHS version, it is not possible to perform an amplification series (PCR) only.
    The samples must be placed in the thermocycler rack.
  - Den. + Hyb: Select “No” in the PCR drop-down menu and “Yes” in Den.
    The samples must be placed in the thermocycler rack.
  - Hyb: Select “No” in the PCR drop-down menu and “No” in Den.
    The samples must be placed in the sample holder and not in the thermocycler rack.

8.3.2 hS Control

This tab allows you to activate or deactivate different configuration options for the HS12a or HS24 instruments that are on, registered and connected to the PC.

If accessing as a non-administrator user, the following screen will be shown:

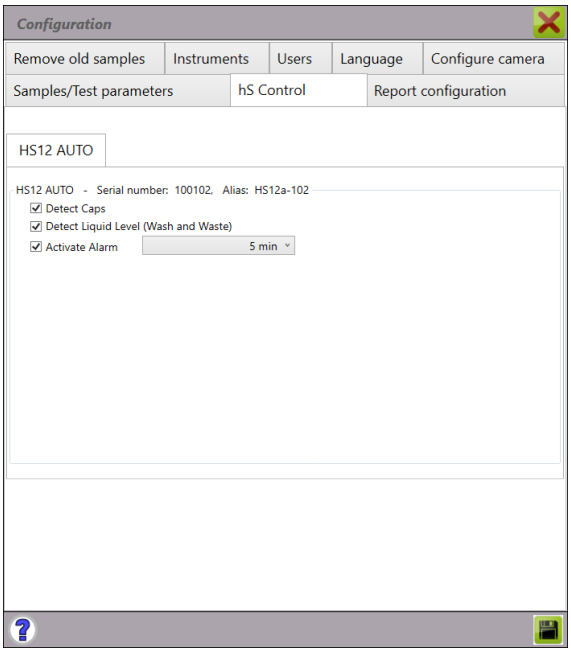


Figure 26. HS12a control tab.

- **Detect caps:** this sensor allows detecting the vial caps. After the QR code reading, the reagent is located on the vial's mouth and check whether there is a cap. In case of being inactive, it won't check the caps.
  If this sensor is deactivated, pay full attention to withdrawing the caps before running the protocol.

- **Detect liquid level (wash and waste):** this sensor allows to detect the lowest established level of the washing bottle and the highest level of filling of the waste container. These sensors are activated only at the beginning of each protocol, and they don't remain active during its running time. If inactive, the corresponding warnings won't appear in the software. The minimum established level for the washing liquid allows executing a complete protocol of 12 samples, therefore, even though the warning may appear, it does not invalidate the protocol execution. However, the user should guarantee that the levels of washing liquid are always enough.

  As for the waste container, if the sensor detects that it is full, it won't allow the protocol execution.
- **Activate Alarm:** this option activates the acoustic signal after finishing the series. The alarm will sound in intermittent mode for the time selected in the drop-down list.

If accessing as an Administrator, the following screen will be shown:

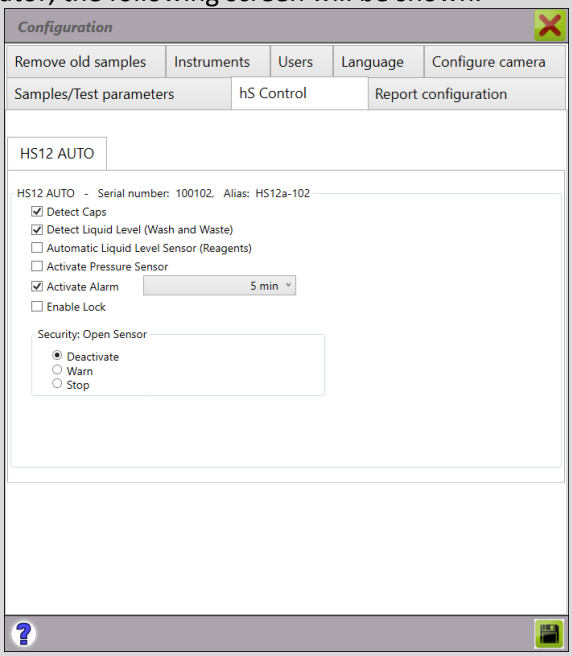


Figure 27. HS12a control tab for administrator.

- **Automatic liquid level sensor (Reagents)**: this sensor allows identifying the existing reagents' level in each vial. Once detected, the probe decreases to a safety distance inhale reagent. If this level is not detected, the probe will decrease to the maximum depth allowed for the vial in order to guarantee the liquid aspiration. If the sensor is inactive, the probe will always decrease to the maximum depth. If the sensor is disabled, clean the needle of the equipment with a damp cloth once a week to remove any rest of reagents.
- **Activate pressure sensor:** this sensor allows controlling the pressure of the hydraulic circuit during the sample's processing. If the pressure is adequate during the series, the sample processing will continue. However, if a pressure problem is detected, the equipment will warn acoustically and visually by a message and the protocol will be paused.
- **Activate Blocking:** this option immobilizes the hood during operation of the instrument. It activates a physical blocking that prevents its opening.
- **Opening sensor:** this option allows configuring the warning mode by opening of the hood before or after the process. This warning appears when the run starts with the hood open. According to the configuration, there are different behaviors:

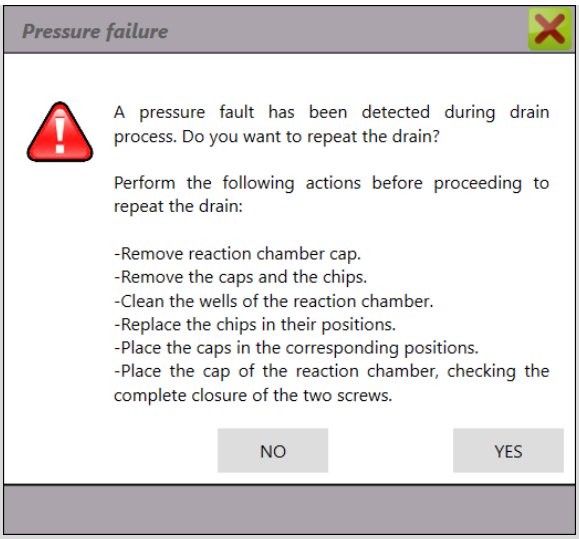


Figure 28. Error in the pressure during the drainage process.

- Deactivate" option: with this configuration, the message warning about the opening of the hood will not appear.
- "Warning" option: with this configuration, a message warning about the opening of the hood will appear. A window warning about this situation will appear, although the process will continue. This option is active by default.

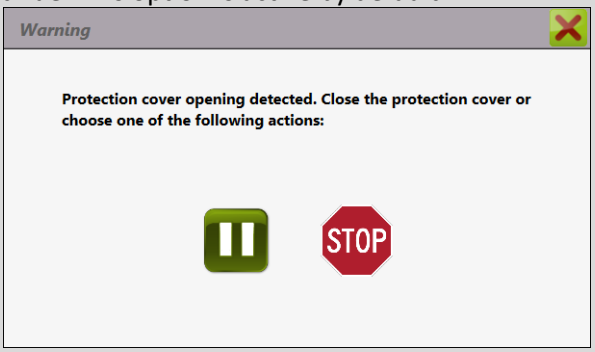


Figure 29. Hood open warning.

When clicking on the "Pause" button, the instrument will pause and, once the hood is closed, the process will continue. However, when clicking on "Stop", the process will be canceled.

- "Stop" option: with this configuration, the process will stop after finishing the last action started.

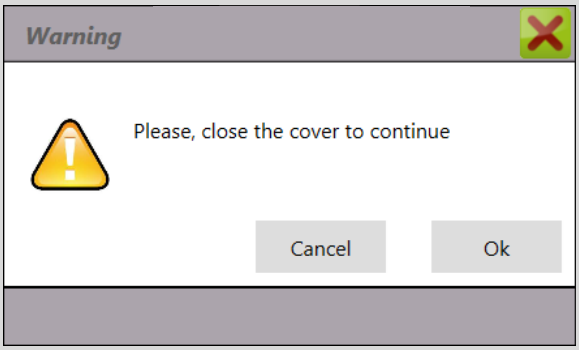


Figure 30. Hood open warning.

8.3.3 Report configuration

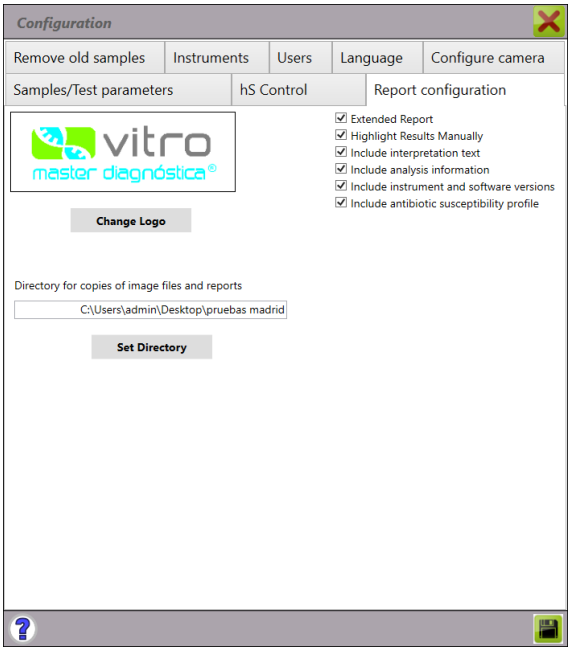


Figure 31. Report configuration tab.

- **Change Logo:** It allows changing the logo that appears in the report. If you press the "Change Logo" button, a pop-up window will open to select the image in "jpg" format.
    - Image file requirements:
        - Resolution: 72 dpi (dots per inch)
        - Size: 120 pixels wide and 50 pixels high.
- **Directory for copies of image files and reports:** It allows the selection of any directory where you want to save a copy of all the images and reports of the processed samples. By default, the HSCopyUserFiles directory will be created at the root of the hard disk where Hybrisoft is installed:

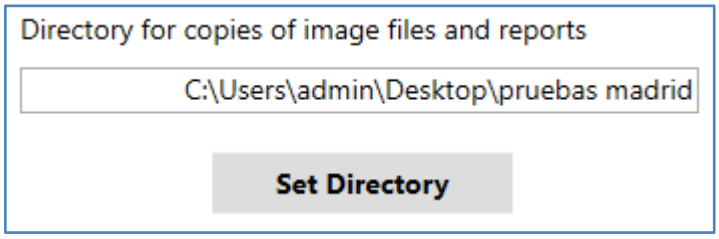


Figure 32. Default directory for backups.

- **Extended report:** When activated, it includes the interpretation map and the image of the already analyzed sample in the report.
  The administrator's password is required to activate it.
  In the case of being inactive, the following two options won't appear:
    - "Include text interpretation".
    - "Include analysis information".
- **Highlight manual results:** this option marks in red and with an asterisk the biomarkers included in the result by manual selection, as well as an explanatory legend.
- **Include interpretation text:** this option includes an explanatory legend of the interpretation template.

- **Include analysis information:** this option adds to the report the limit of detection applied to the sample.
- **Include instrument and software versions:** this option includes the instrument's short name, hybriSoft version and the IPL version used to obtain the result of the sample.
- **Include antibiotic susceptibility:** this option adds a field named "Antibiotic susceptibility profile" in the individual report and in the windows "Sample detail" and "Picture processing", indicating the relation between molecular resistance markers provided by the SEP kit and the antibiotic susceptibility they correspond to.

8.3.4 Users

This tab allows to check the Users created in the system, add new ones, modify or remove the existing ones and establish a doctor and technician by default.

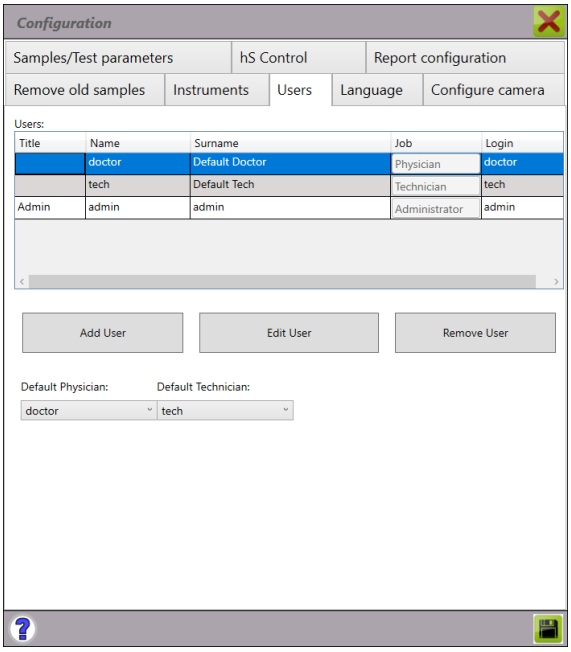


Figure 33. Users tab.

A table with all the users created in the database appears, except the administrator user.

- **Add user**: this button allows to create a new user. When pressing the "Add user" button, a window with all the required fields appears. If we leave something without filling in any of the compulsory fields in, when pressing "Accept" the box will be marked in red color.

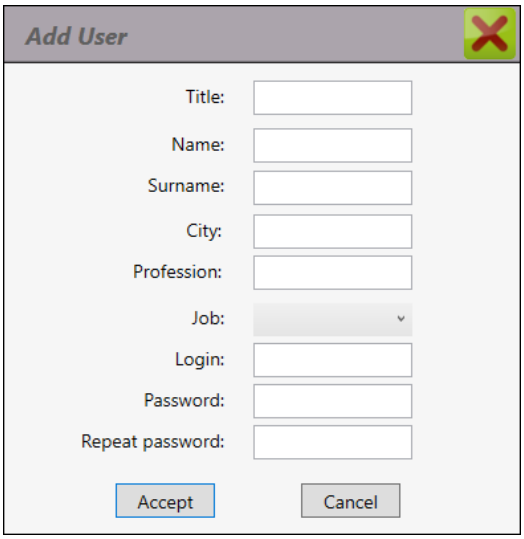


Figure 34. "Add user" window.

- **Remove user**: this button allows deleting a user from the list. If the user has been used and they are part of the database, they will not be able to be deleted. A pop-up window will appear indicating that it can be removed.

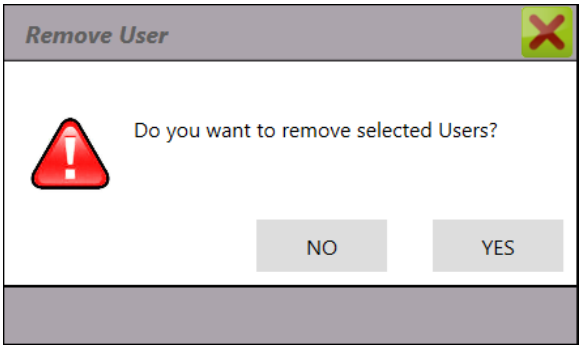


Figure 35. "Remove user" warning.

- **Edit User**: this button allows the modification of any information corresponding to the user. When pressing the button "Edit user", a window with all the user's data appears, allowing the modification of any field.

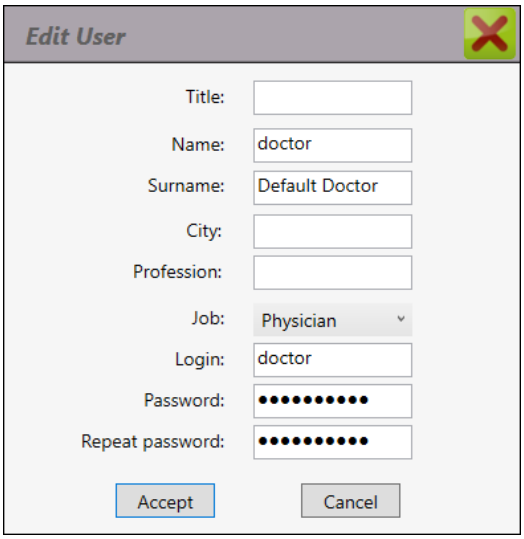


Figure 36. "Edit user" window.

8.3.5 Language

This tab allows to configure the language and date format. After making changes in the language configuration section, the application will be automatically closed. The user will have to start it again. The following message will be displayed.

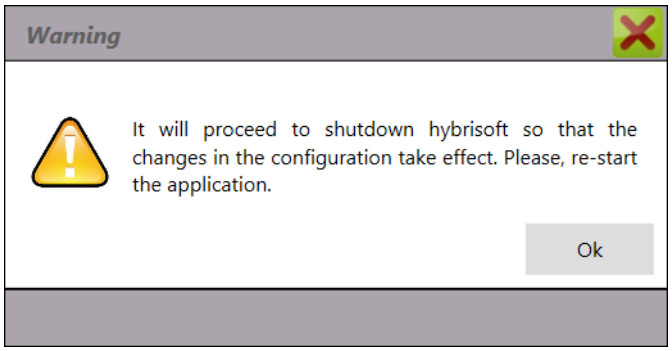


Figure 37. Automatic closing warning.

Actions needed to make the language change:

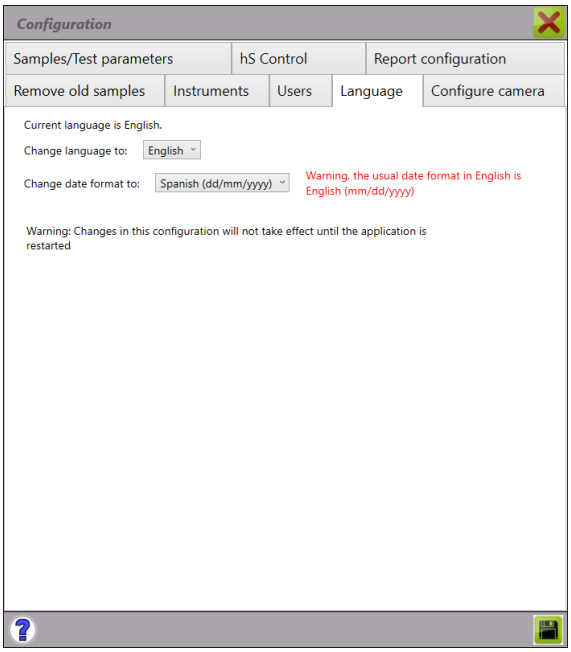


Figure 38. "Languages" tab.

- Select the desired language from the drop-down menu.
- When changing the language, a message in red appears suggesting the recommended date format for the selected language.
- Select the desired date format and save the changes.
- The changes will become effective after you close and open the application.

In the tab language in the configuration window, a new language can be added. To add this language, you need to update the language files and add the file corresponding to the new language. These documents will be provided by the supplier along with some simple instructions to perform the update. Once the documents have been updated, the new language will be available in the drop-down menu in configuration.

8.3.6 Configure camera

This tab is only intended for configuring the capture system of the HS12 Instrument.

8.3.7 Remove old samples

This tab allows to delete samples in "Stored" or "Canceled" status registered before the selected date. If you have 10,000 samples in "Stored" or "Canceled" status, a message will appear showing this situation. This warning allows us to know the status of the database and consider the possibility of performing a removal of old samples suggested by the manufacturer. After saving this change, the application will be automatically restarted.

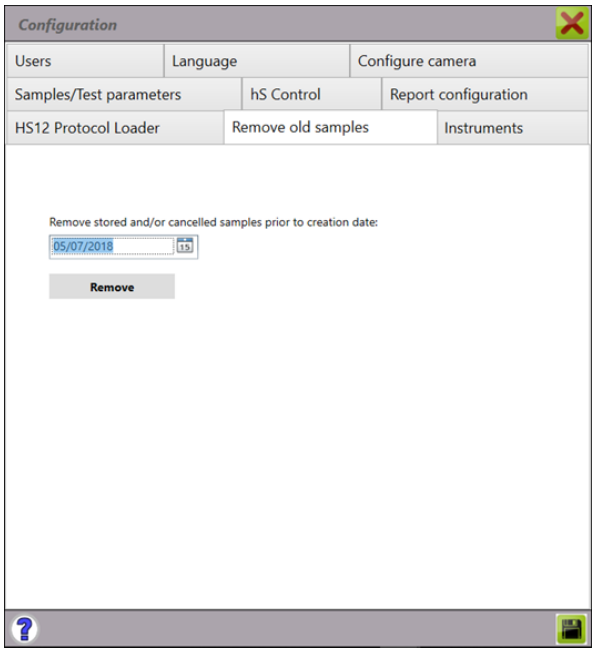


Figure 39. "Remove old samples" tab.

First of all, it is recommended to back up the database to avoid losing information (See section 8.3.1.4).

After having made the backup copy, select the date from which you wish to delete all the samples and click on the "Remove" button. A window will appear showing the number of samples to be removed.

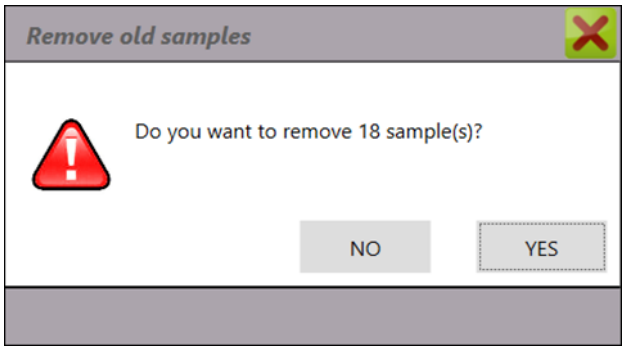


Figure 40. "Remove old samples" pop-up window.

If there are no samples before the reception date previously selected, a message will inform about it.

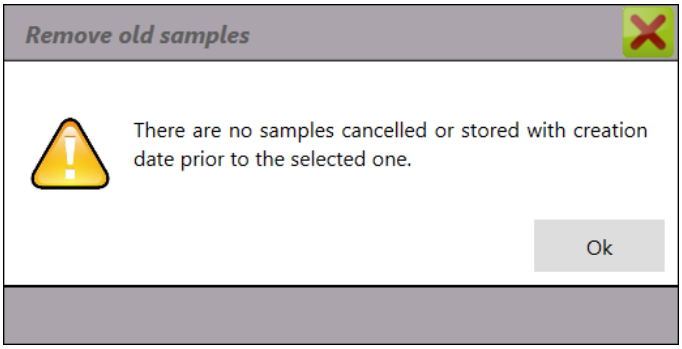


Figure 41. Information window.

After removing the old samples, the session will be automatically closed. The user will have to start the application again.

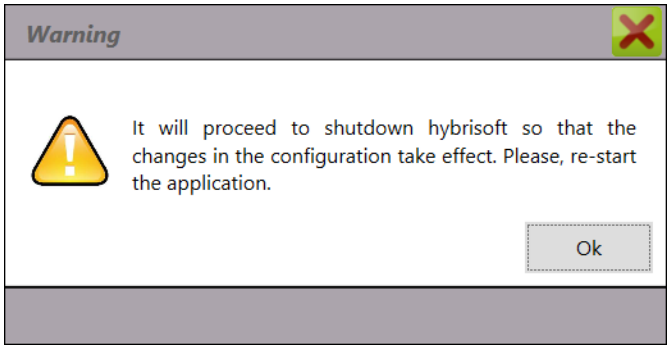


Figure 42. Automatic closing warning.

8.3.8 Instruments

This tab allows to add or delete new instruments to work with, edit the information on the already existing instruments and clear the selected instrument's database.

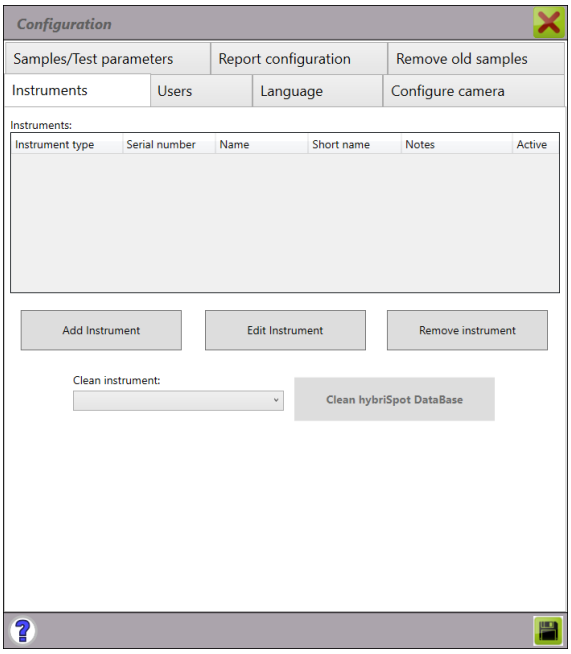


Figure 43. "Instruments" tab.

- **Add instrument**: this option allows inserting new instruments that we do not find in our database. To add a new instrument, the following fields will be shown:
    - **Instrument type:** it allows to select the type of instrument. In the drop-down menu, the available instruments will appear. The type of instrument called "MOCK" is a fictional instrument of internal use, and it is used to analyze already captured images that have been assigned a new ID.
    - **Serial number**: fill in the field with the serial number of the instrument.
    - **Name**: fill in the field with the full name of the instrument.
    - **Short name:** fill in the field with a short and easy name that identifies the type of instrument and the serial number (HS12a).

- **Notes**: free text field in which any information on the instrument can be added.

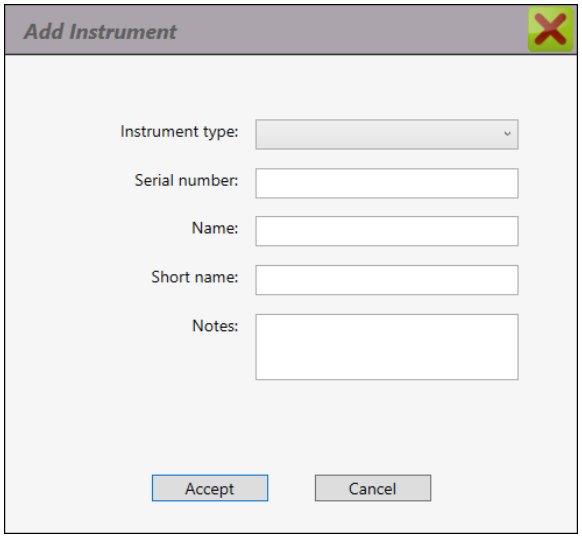


Figure 44. "Add instrument" window.

After filling in all the fields and pressing the "Accept" button, the new instrument will be registered. However, if you press "Cancel", the new instrument won't be registered. If any of these mandatory fields is not filled in, it will be marked in red showing that it hast to be filled in.

It allows creating an indefinite number of instruments of the same type provided that they have a different serial number. This number exists only for one instrument along with the GUID, which is the internal identification of the instrument, necessary for the differentiation of each equipment.

The system allows to activate one instrument of each type only, except for HS12 type instruments, as it allows to have several of them created and active.

In order to create a new HS12a instrument with another of the same type registered and active, it is necessary to have only the new equipment connected to the PC. The already existing one must be disconnected. The new HS12a will be inactive by default.

Once registered and identified, a warning will appear stating that the calibration parameters of the instrument are incorrect.

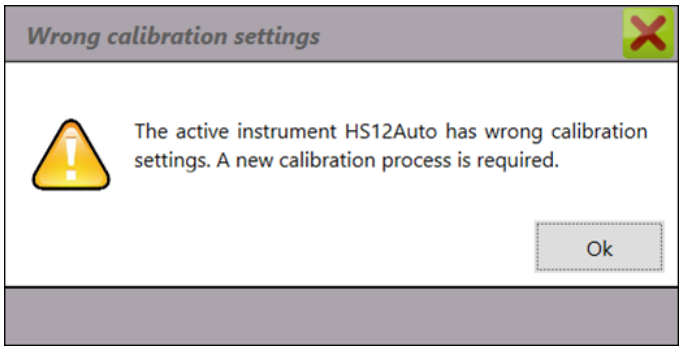


Figure 45. "Incorrect calibration parameters" important warning.

- **Edit instrument**: this function allows to modify any data of the selected instrument.

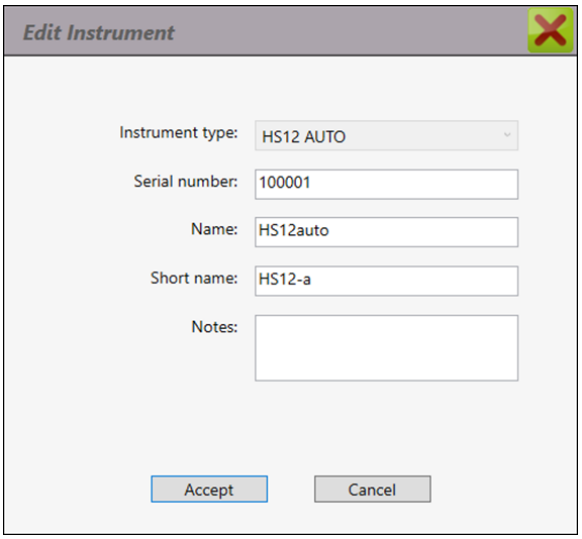


Figure 46. "Edit instrument" window.

- **Remove instrument**: this option allows to remove any instrument in the database. To remove the instrument, click on the "Remove instrument" button.
  If no instrument has not been selected, an informative message will appear showing that "No instrument to be removed was selected". If an instrument has been selected, a confirmation window will appear:

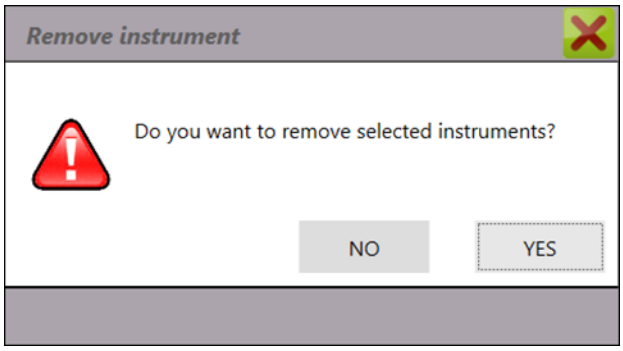


Figure 47. Pop-up window of instrument removal.

After pressing "No", no changes will be made. If you press "Yes", the selected instrument will be removed from the database as long as it has no samples associated. Otherwise, a message will appear stating that the instrument contains associated samples and cannot be removed.

- **Empty instrument's database**: this function must be used if the system breaks down and there are no samples being processed in the "Sample management window" and their assignment is kept in the hS Control window.

8.4 Sample Management

8.4.1 "Sample management" window

This window contains the following buttons:

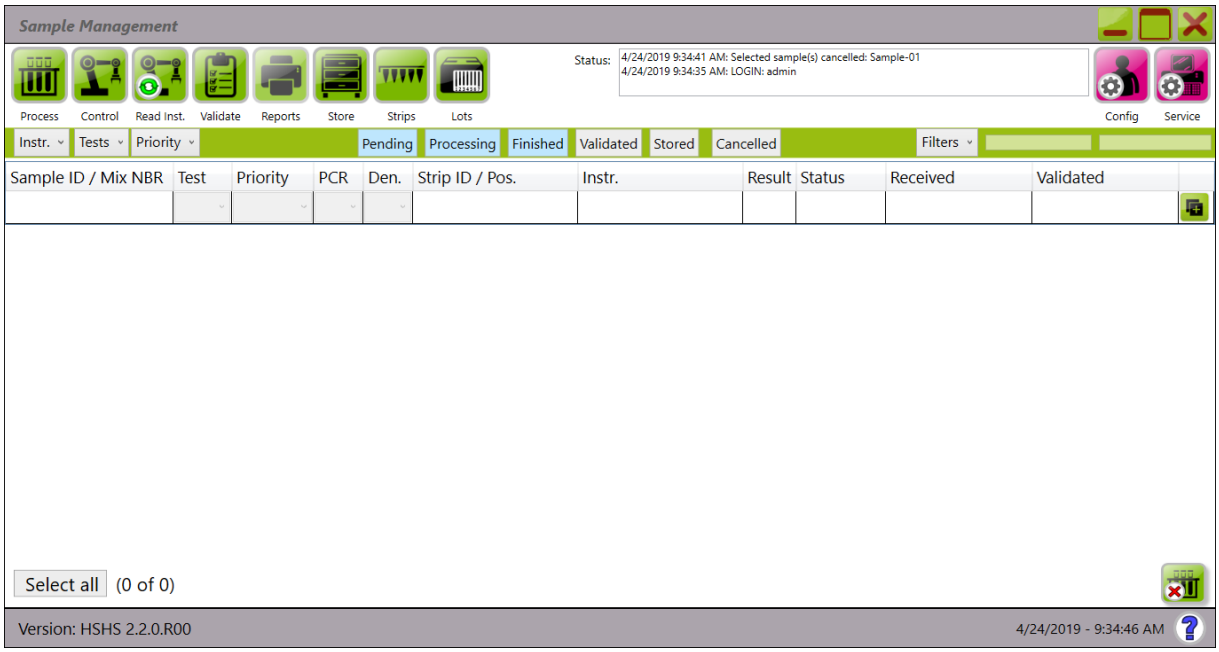


Figure 48. HybriSoft's "Samples management" main window.

ACTION BUTTONS:

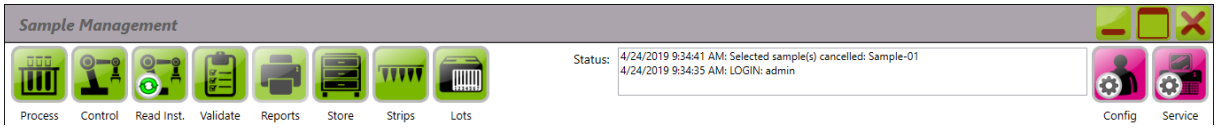


Figure 49. Action icons.

- In the upper right corner, buttons: minimize, full screen and close.
- "Process" button: it sends the pending samples to the desired instrument in order to start the process. Access the "Processing Parameters" window.
- "Control" Button: access the window where the HS12a actions and distributions are visualized. Access the window "hS Control".
- "Read Inst." button: it allows making an analysis of the samples appearing in a "Processing" status. Access the "Reading Parameters" window. Only operational for HS12.
- "Validate" button: it accesses the "Validation Parameters" window, if there is any sample in a "Finished" status.
- "Reports" button: it accesses the Results window. The reports from valid samples are obtained in this window.
- "Store" button: it accesses the "Storage Confirmation" window if there is any "validated" sample.
- "Strips" button: it allows to assign, modify or delete an ID strip of the selected samples.
- "Lots" button: it allows to create, modify or delete any records of the different lost used in any test. It also tracks all the registered lots in the system.
- "Status" panel: it shows information about the actions that are being performed.
- "Config." button: it accesses the "Configuration" window (See the 8.3 section).
- "Service"" button: it accesses the "Service" window (See 8.8 section)

SELECTION FILTERS:

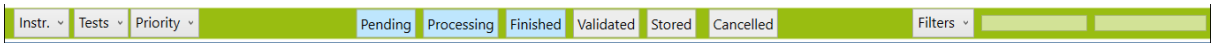


Figure 50. Selection filters.

They allow activating/filtering the elements or statuses of the samples that appear in the "Samples Management" window. The active filters are shown in blue, and the inactive ones are shown in gray. The statuses "Pending", "Processing" and "Finished" are shown by default.

The filters are activated/deactivated by clicking on them.

The active filters are cumulative.

When none of the filters are active, nothing will be shown in the Management window.

The available filters are:

- "Instruments" drop-down menu: it allows to filter the assigned samples to a specific instrument.
- "Tests" drop-down menu: it allows to select an option depending on the assigned test.
- "Priority" drop-down field: it allows to filter the samples according to their priority.
- "Pending" filter: when enabled, the samples in "Pending" status will be displayed.
- "Processing" filter: when enabled, the samples in "Processing" status will be displayed.
- "Finalized" filter: when enabled, the samples in "Finished" status will be displayed.
- "Canceled" filter: when enabled, the samples in "Canceled" status will be displayed.
- "Stored" filter: when enabled, the samples in "Stored" status will be displayed.

- "Filter" drop-down menu: it allows filtering the samples according to the selected condition and with the criteria inserted in the free text/dates fields. These filters are applied together with the rest of the enabled filters. These filters are applied to the samples in the selected status (enabled filters) or by default to all the selected samples of the database if there are no other enabled filters:

    - Sample ID: it filters the samples that contain all or some of the characters typed in the text field.
    - Creation date: filters the samples according to the reception date.
    - Start process date: filters the samples according to their starting date.
    - End process date: filters the samples according to their finishing date.
    - Validation date: filters the samples according to their validation date.

  In the dates filter, the date "from" cannot never be later than the system date of later than the date "to". Also, the date "to" cannot be earlier than the date "from". If you create a filter whose dates do not meet the previous condition, the following message will be displayed:

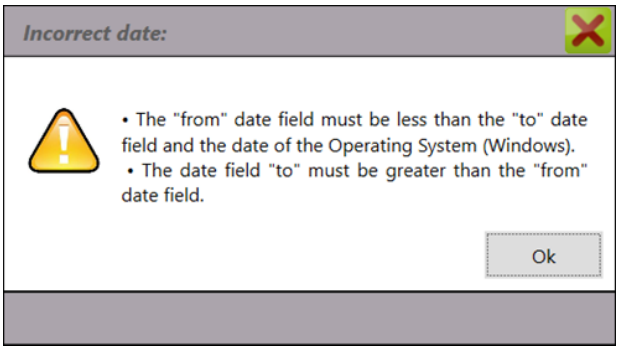


Figure 51. Pop-up window warning about an incorrect date.

SAMPLE TABLE:

In the table, the data of each sample are shown in columns, displaying only the active samples:

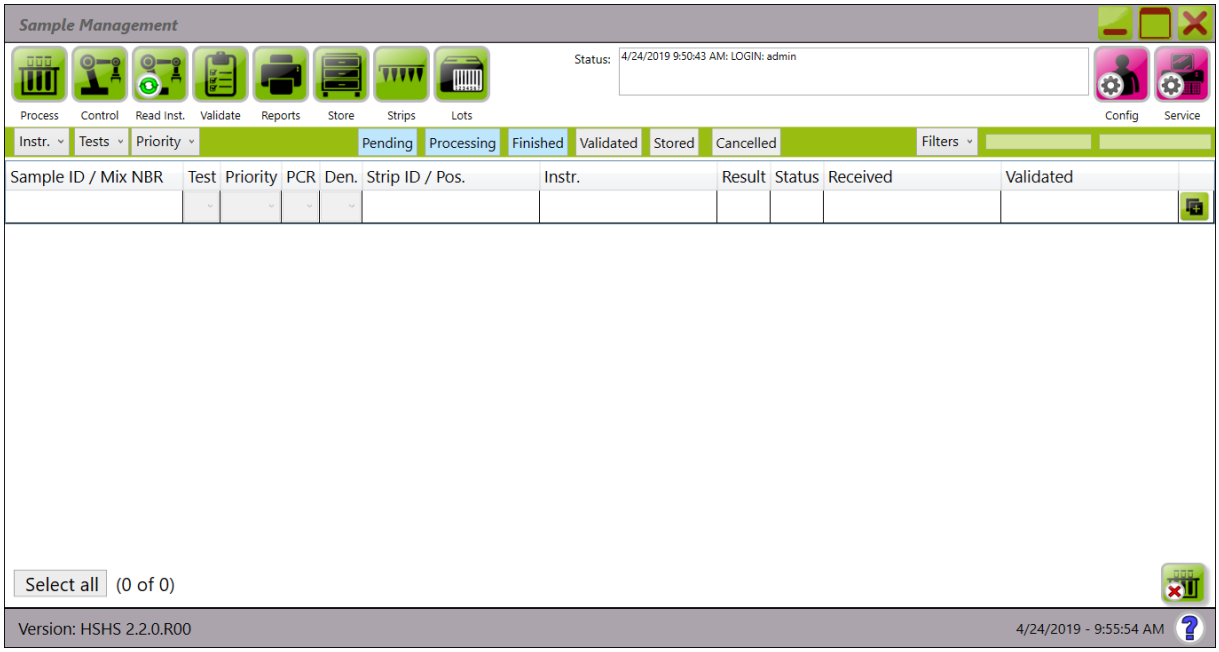


Figure 52. Main window.

- Sample ID: Id's identifier.
- Mix NBR.: it shows the mix number that contains the samples associated to the assigned test.
- Test: acronym of the test that will be performed to the sample. The acronym is simultaneously changed in all samples assigned to the same strip.
- Priority: preference the sample requires in the moment of processing (High or Normal). When assigning "High" priority, the sample passes to be in the first samples' table.
- PCR: field that indicates the value assigned to the PCR option (Yes/No). When assigning "Yes", the PCR will be performed on the corresponding samples, assigning "Yes" by default to the denaturation process. This column only appears when a HS12a instrument is registered and active. It is not possible to perform independent PCRs.
- Den: field that indicates the value assigned to the Denaturation option (Den.). (Yes/No). When assigning "Yes", the Den. will be performed on the corresponding samples. When assigning

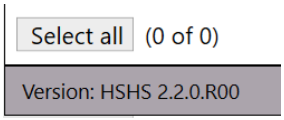
"No", it is only performed in the hybridization instrument, assigning "No" by default to the PCR. This column only appears when a HS12a instrument is registered and active.

⚠ In case both options are set to "No", the samples must be placed in the zone 1.

- ID Strip: identifier of tubes' strips. This column only appears filled in samples already assigned to a strip.
- Pos.: position that the sample has in the strip
- Instr.: short name of the instrument that has been sent to the samples. This column is shown blank in samples with the status "Pending".
- Result: summary of the result of the sample's analysis.
- Status: current situation of the sample.
- Received: creation date of the sample in the application
- Validated: validation date of the sample.

The values for Test and Priority cannot be changed for those samples in other status than "Pending", since they are being processed or have been finished in this case.

To select all visualized samples in the current list, press the "Select all" button.



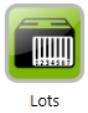

This button cancels all the selected samples regardless of their status.


This button goes back to the previous window.


This button shows the user manuals of HS12, HS24 and HS12a, documents of implementations and improvements and the End User License Agreement or EULA.


To the right of each sample, the button "Sample detail" appears. When pressing, the "Sample Data" screen opens, which provides the possibility of completing some sample data and which enables the consultation and modification of some of these data at any time (see Figure 90, 8.3.28.4.2 section)

8.4.1.1 Lot Management



In this section "Lots", you can see all those lots of each product used in the test. This function allows registering, editing, removing and enable/disable lots of PCR mix, Chips and kits of hybridization reagents. This allows to have traceability on the lots used in every sample, check the expiration date, the status of the kits and their registry.

When pressing the "Lots" button, it leads to the "Lots" window.

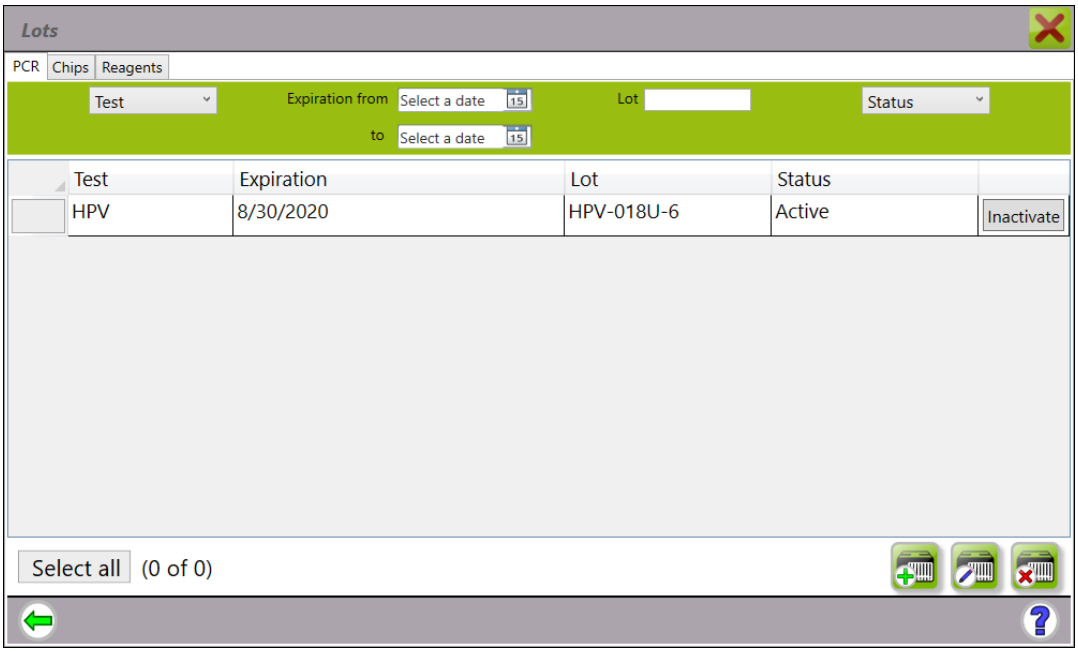


Figure 53. Lot Management.

The lots of PCR, Chips and reagents are managed in independent tabs. In each of these tabs, you can find the lots registered in the system. Each of these tabs has the following structure:

- Filters in the upper area.
- Lot information table.
- Button bar in the lower area.

The lots of PCR/Chips mix are specific. This means that each PCR/Chips lot is assigned to a specific test.

In the “Reagents” tab, you can find all those lots of hybridization that have been registered. There are two types of lots of hybridization:

- Manual: lots used by instruments in which the user has to do the hybridization protocol step by step.
- Automatic: lots used by instrument that perform the hybridization protocol automatically.

The record of the lots of reagents can be performed with two different configurations:

- Specific kits: if the option “Activate universal kits” is deactivated, each of the kits will be registered with an assigned specific test as in previous versions to the HSHS 2.1.0 R00.

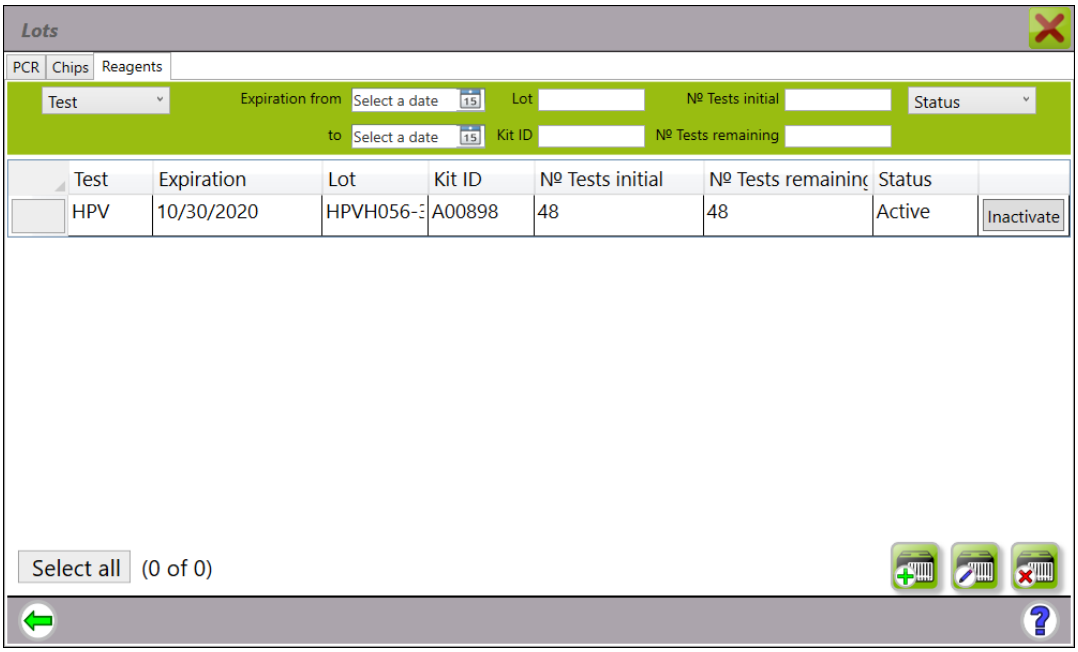


Figure 54. Reagents for specific kits tab.

- Universal kits: if the option "Activate universal kits" is enabled, each of the kits will be registered with a type of universal kit (currently there is only a type of universal kit T01). The universal kits are compatible with all the current tests.

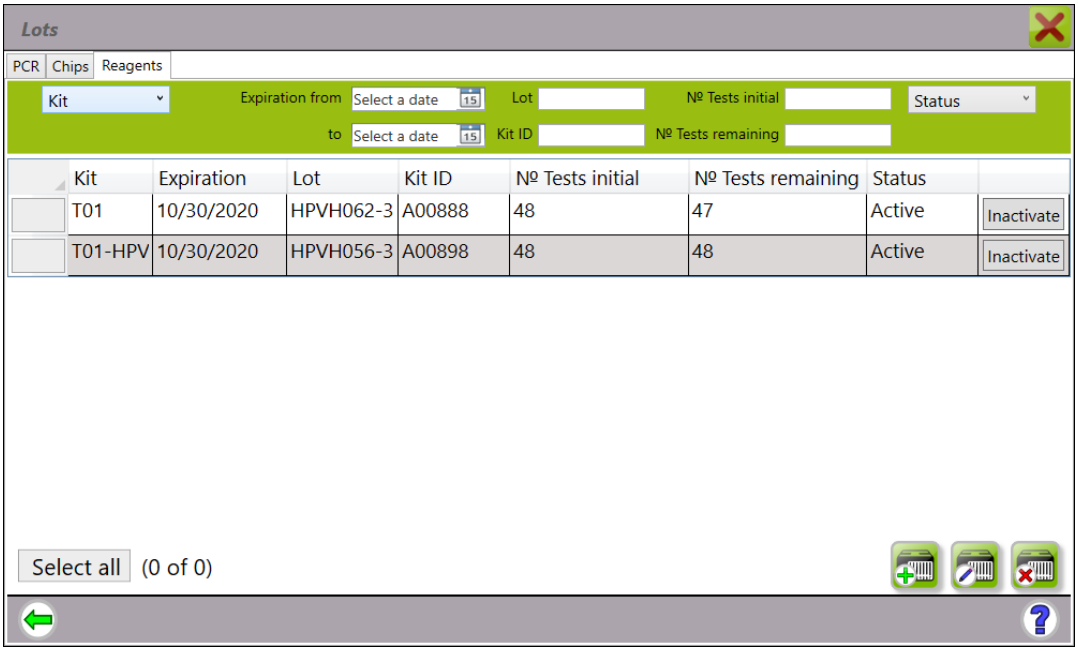


Figure 55. "Reagents" for universal kits tab.

  - When activating "Universal kits" there are already registered specific kits, these will become universal taking into account the compatibility between them. They are represented with T01-XXX, being X the type of test.

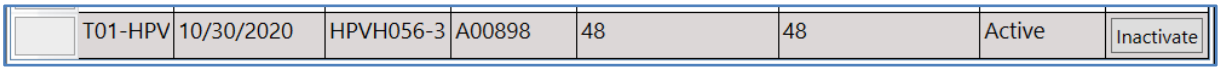


Figure 56. Specific kit transformed to universal.

- If the option “Universal kits” is deactivated, the registered kits will be recorded as T01 and won’t be displayed in the management and lot assignment window.

FILTERS

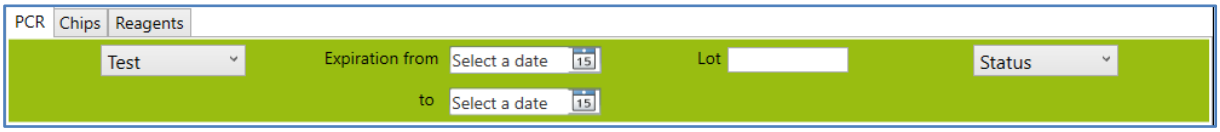


Figure 57. PCR and Chips filters tab.

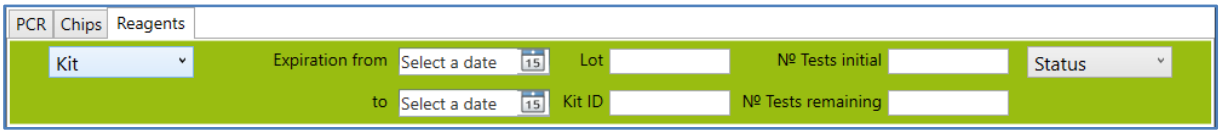


Figure 58. “Reagents” filters tab.

- Test: drop-down menu that allows to search a specific test by adding filters. Select the test in which you want to search and it will be performed automatically.
- Expiration: it allows to search for lots in specific expiration dates. It has two date fields with a drop-down calendar on the right side of the field. To perform a search, select the drop-down calendar or introduce the period between dates in the fields “from” and “to”. Once they have been introduced, it will automatically perform the search.
- Lot: it allows to perform searches by introducing the lot ID. It performs the search for lots that contain all or some of the characters typed in the text field.
- Kit ID: it allows to perform searches by introducing the unique lot ID. It performs the search for kits that contain all or some of the characters typed in the text field. This field is only available in the “Reagents” tab.
- Nº initial Tests: it allows to perform a search for reagent kits for automatic instruments with the nº of initial tests that match completely or partially with the value typed in the field. This field is only available in the Reagents tab.
- Nº Tests remaining: it allows to perform a search for reagent kits for automatic instruments with the nº of remaining tests that match completely or partially with the value typed in the field. This field is only available in the Reagents tab.
- Status: it allows to search for the status of the lots. The lots can be active or inactive. Select the status in the drop-down menu and it will automatically show the lots and their status.

INFORMATION TABLE

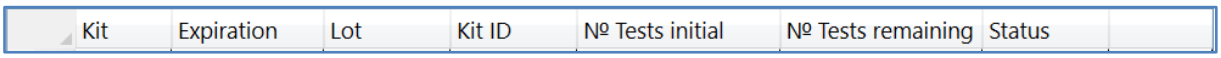


Figure 59. Information table heading.

- Test: it shows the test that the corresponding lot of the product has associated. This column is only shown in the “Reagents” table and if the option “Activate universal kits” is disabled (See 8.3.1.5, point “Activate universal kits”).
- Kit: it shows the type of kit of hybridization reagents. This column is only shown in the “Reagents” table and if the option “Activate universal kits” is enabled (See 8.3.1.5, point “Activate universal kits”)
- Expiration: it shows the expiration date of the product. Once the lot has expired, it will be shown in orange informing about its expiration, but it won’t prevent you from using it. The time during the product can be used can be read in the configuration section (See 8.3.1.5, point “Automatic expiration lot”).

  Warning: *The manufacturer won’t take any responsibility for results obtained with expired lots.*

- Lot: lot ID of the corresponding product.
- Kit ID: unique alphanumeric ID. This field can only be filled in with hybridization reagents of automatic instruments.
- Nº Tests initial: number of tests that the lot of hybridization reagents contains. This field can only be filled in with hybridization reagents of automatic instruments. Available in the “Reagents” tab.
- Nº Tests remaining: number of available tests that the lot of automatic hybridization reagents contains. This field can only be filled in with hybridization reagents of automatic instruments. Available in the “Reagents” tab.
- Status: it shows the situation in which the lot of the corresponding product is. Lots in an inactive status cannot be used. A lot becomes in an inactive status when it is used up or the user inactivates it manually. This status is reversible in all the lots except in the hybridization agents of automatic instruments once the number of tests is used. The number of tests is taken away with the first aspiration of the reagent that the instrument performs.
- Remaining series: it indicates the number of possible protocols to perform with a mix of reagents. Once it has been used up, the kit changes to a Finished status, so that it cannot be used with Automatic reagent mix. This column appears when the check “Automatic reagent mix” is active in Configuration.
- Inactivate/activate: in the last column of each registered lot, there is a button that allows to inactivate or activate depending on the status.

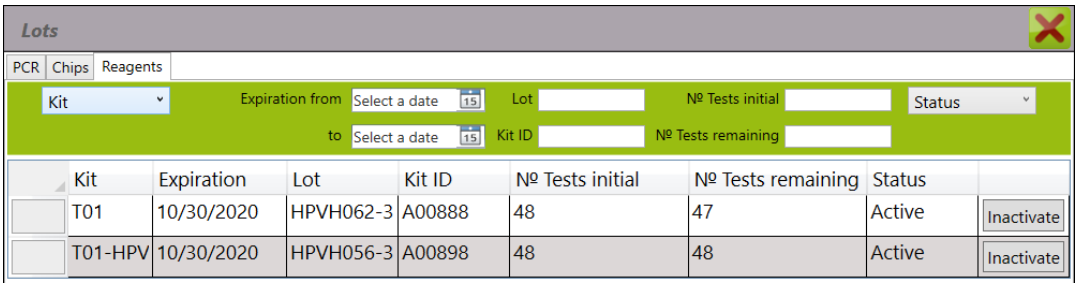


Figure 60. “Lots” window with active “Automatic mix".

ADD LOTS

This button allows to register new lots. When pressing the button “Add new lot”, a new window appears. It allows to create the lot by typing the data manually or using a bar-code reader. Registry by introducing data manually:

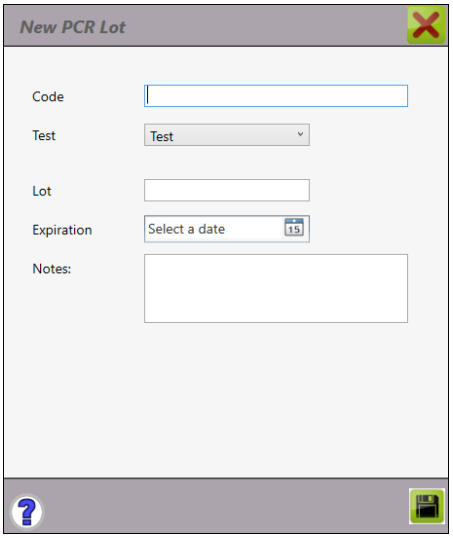


Figure 61. "New PCR/Chips Lot” window.

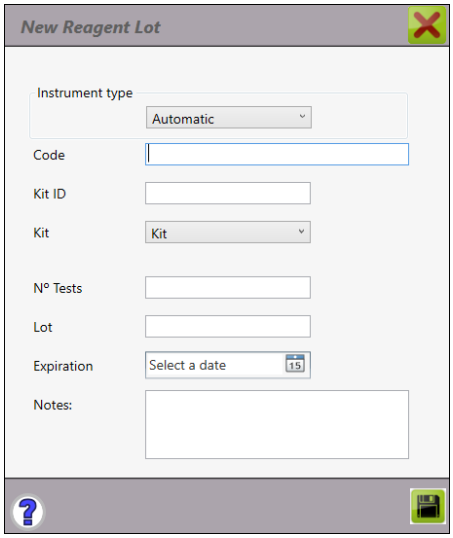


Figure 62. "New reagent lot” window.

To register different products, obtain the data of the tags according to the following instructions:

- PCR reagents ID (Figure 63, Point 2): sign that shows the corresponding test, instrument and its corresponding format, and the reagents’ ID.
- Chips ID (Figure 64, Point 2): sign of the product that identifies the product and the test it belongs to.
- Reagent ID (Figure 65, point 2) it identifies the test/kit it belongs to and shows the type of product.
- Lot (Figure 63, Point 3, Figure 64, Point 3 and Figure 65, point 4): the lot field shows the lot of the reagents of the product. This field has a maximum of 15 characters.
- Expiration (Figure 63, Point 4, Figure 64, Point 4 and Figure 65, point 5): the hourglass shows the expiration date of the product.
- EAN PCR Code (Figure 63, point 1): bar code necessary to perform the automatic registry of the PCR reagents. It contains the lot data and the expiration of the product.
- BIDI Code: BIDI code necessary to perform the automatic registry of the chips and the reagents for automatic instruments. The BIDI codes currently in use are QR (Figure 64, point 1) and Datamatrix codes (Figure 65, point 1).
    - Chips: It contains the lot data and the expiration of the product. The test field has to be filled in manually.
    - Automatic instrument reagents: it contains all the information about the reagents to fill in all the fields of the following figure.

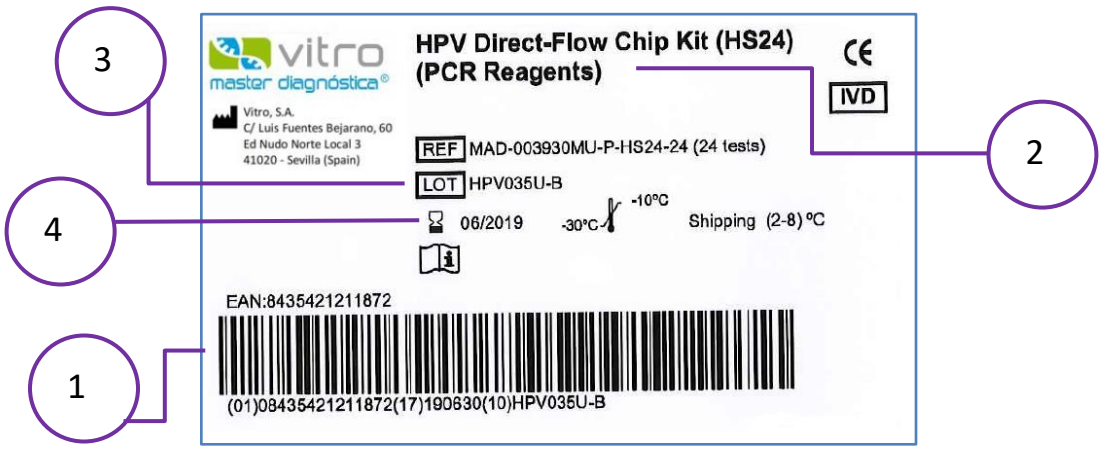


Figure 63. PCR ID tag.

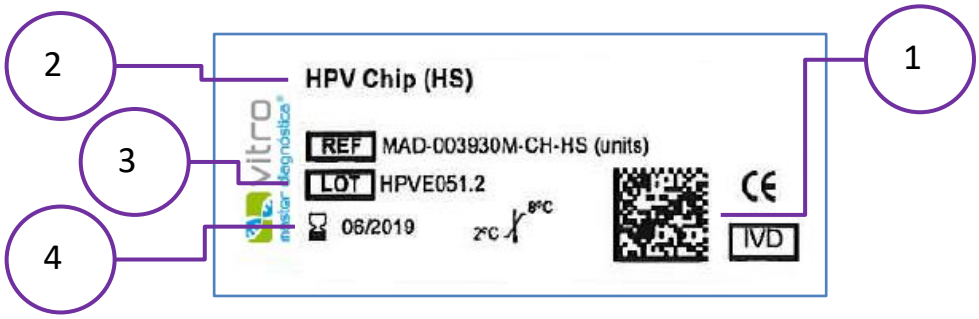


Figure 64. Chips ID tag.

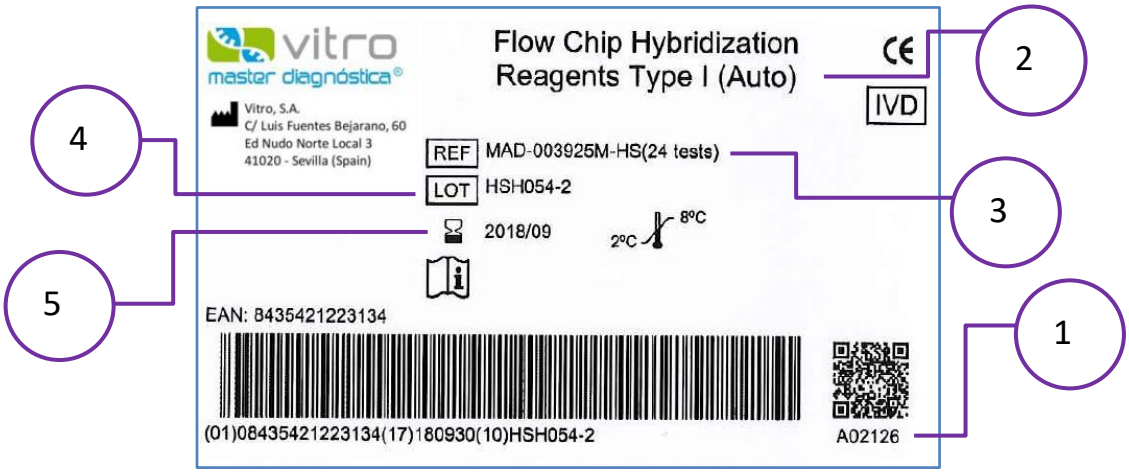


Figure 65. ID tag of reagents of automatic instruments.

- Registry with bar-code reader:
  - PCR and Chips: select the corresponding test, place the cursor on the “Code” field and perform the reading of the bar code or BIDI of the lot to be registered. With this reading, the fields lot and expiration will be filled in automatically.
  - Reagents:
    - Manual Instrument: select the type of instrument “Manual”, and the corresponding test, place the cursor on the “Code” field and perform the reading of the bar code or BIDI of the lot to be registered. With this reading, the fields lot and expiration will be filled in automatically.

- Automatic instrument: select the type of instrument “Automatic”, place the cursor on the “Code” field and perform the reading of the QR bar code (Figure 66).

In the window of new lots of automatic instrument, there are the following fields:

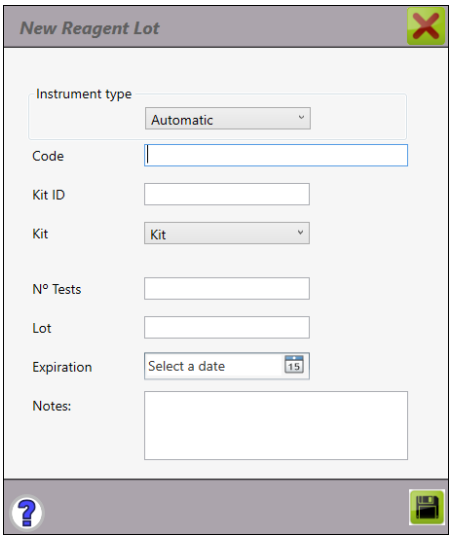


Figure 66. Automatic instrument reagents registration window.

- Instrument type: it allows to select the type of instrument for the lot that is going to be registered. In this case “Automatic” type.
- Code: field of automatic registry with reading of QR code. Place the cursor on the “Code” field, introduce the QR bar code of the kit (Figure 65, Point 1) by using a bar-code reader. The data will be filled in automatically.
- Kit ID (Figure 65**¡Error! No se encuentra el origen de la referencia.**, Point 1): Unique identification of each kit. It appears in the lower right corner of the big tag of the kit. This field must be filled in with 6 characters.
- Test/Kit: this is the acronym of the test. It matches the acronym that the application shows (hybriSoft). This field must be filled in with 3 character that can be found in the Configuration section in the tab Samples/Parameters of Test. With active universal kits, this field becomes into “Kits” and shows the type of reagents in use (T01).
- Number of Test (Figure 65, Point 3): maximum number of samples that can be performed with that kit. It appears in the Kit reference in brackets. This field must be filled in with a maximum of 10 characters.
- Lot (Figure 65, Point 4): ID of the reagents. It can be found in the center of the tag, showing the word “LOT". This field must be filled in with a maximum of 20 characters.
- Expiration (Figure 65, Point 5): date until the reagents work correctly. It can be found in the center of the big tag of the kit, identified with an hourglass symbol. This field must be filled in with six digits, as it is shown in the date format in the window.
- Manual registry: fill in each of the fields with the information of the product tag (PCR/reagents manual inst: Figure 63; Chips: Figure 64 and reagents inst. Automatic: Figure 65).

After registering a PCR products or Chips existing in the database (same codes in the test and lot fields), the following message will be displayed:

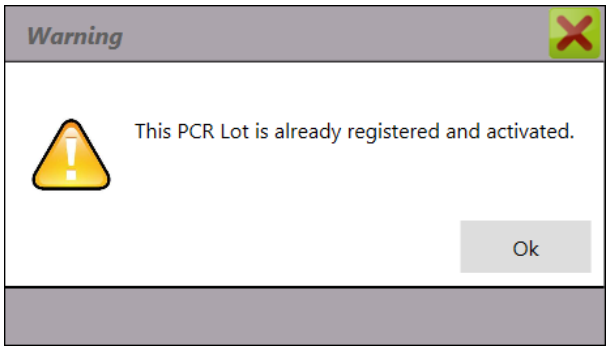


Figure 67. Existing lots warning.

In case there is a reagents kit with the same lot/kits ID (Instr. reagents Manual/Automatic instr. kits), the following message will appear:

Figure 68. Existing reagent kit warning.

In case there is a product with the same lot and inactive status registered in the database, the following message will be displayed:

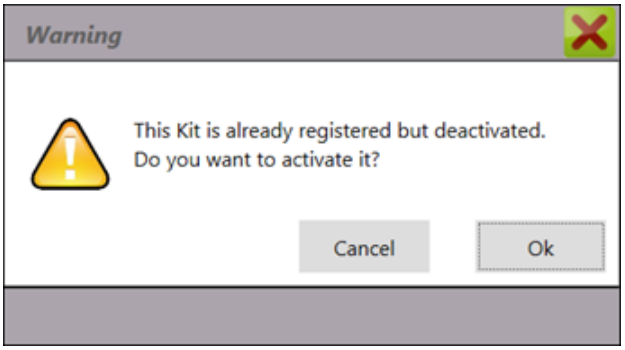


Figure 69. Existing and inactive kit.

When clicking on “OK”, the kit is activated. On the contrary, by selecting the option “Cancel”, the kit continues inactive and cannot be used.

EDIT LOTS

This button allows to edit any field of the selected lot. To modify a lot, select it and press the Modify button.

A new window will appear with the data of the selected lot. All the fields can be modified except the number of tests.

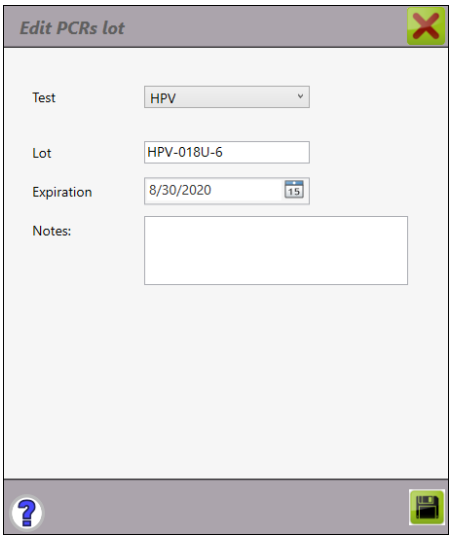


Figure 70. PCR/Chips lot editing window.

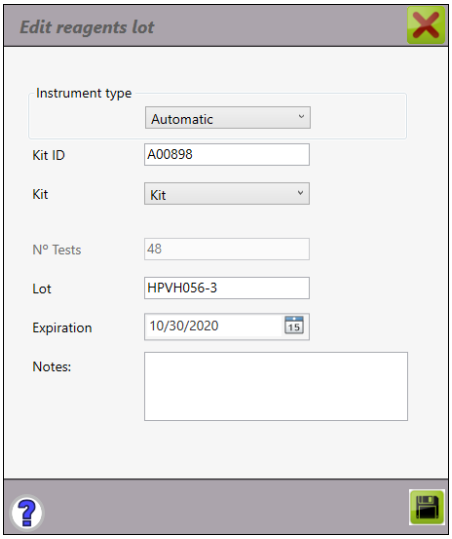


Figure 71. Automatic kits of reagents editing window.

In case of modifying a lot that has been used to process samples, the following warning message will be displayed:

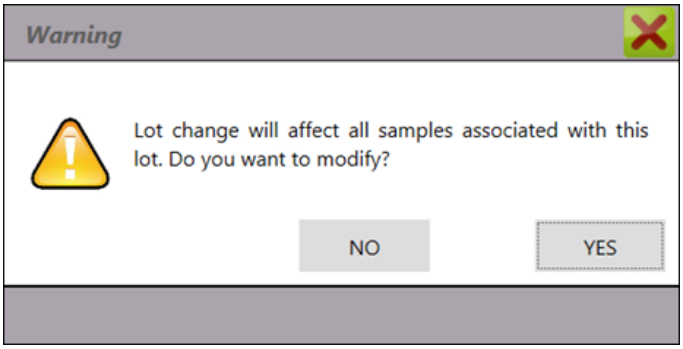


Figure 72. Modification in an already assigned product warning.

When selecting “Yes”, it allows modification by opening the editing window with the information of the selected lot. If not, the editing window will not be opened.

If none of the lots has been selected, a window will appear stating that a lot must be selected.

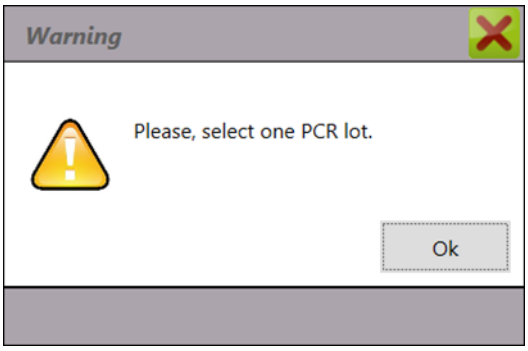


Figure 73. Lot selection warning.

Press “Accept”, select the lot you want to modify and press again the “Edit” button to modify the necessary data.

Warning: *The manufacturer will not take any responsibility for the reports of the results obtained with any of the changes made manually in the lots.*

REMOVE LOTS

This button allows to remove registered lots. Select the lot or lots you want to remove and press this button. A window will appear to confirm the action of removing the selected lot or lots.

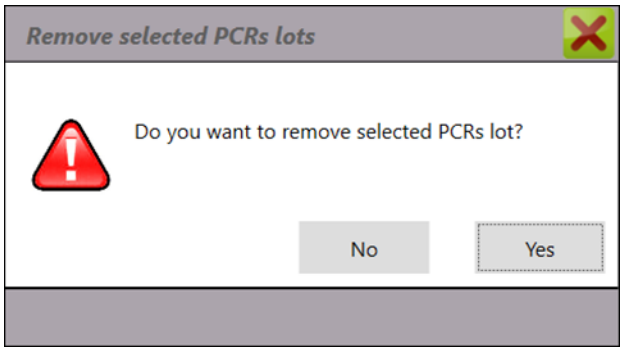


Figure 74. Removing non-used lots confirmation.

If you press “No”, the change will not be made. If you press “Yes”, a window will appear confirming the action.

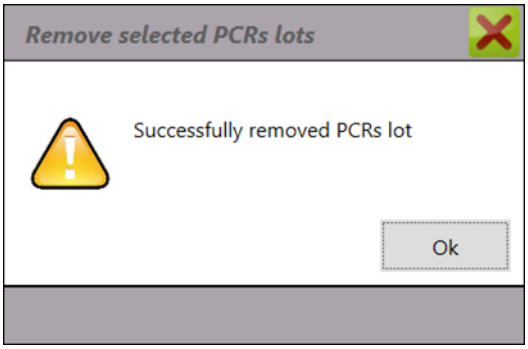


Figure 75. Lot removing warning.

If any of the selected lots has associated samples, the following message will be displayed:

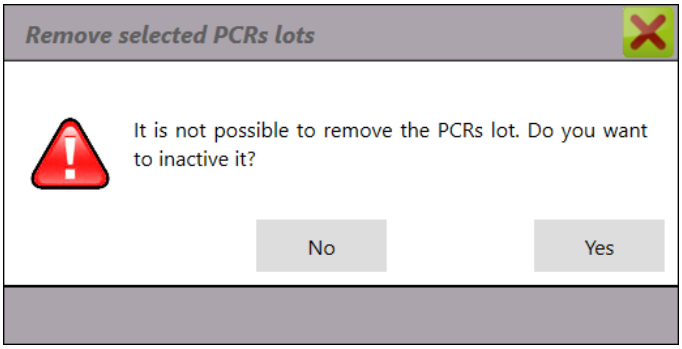


Figure 76. Remove assigned lots.

When clicking “Yes”, the lot or lots selected change to an inactive status. If not, no changes are made.

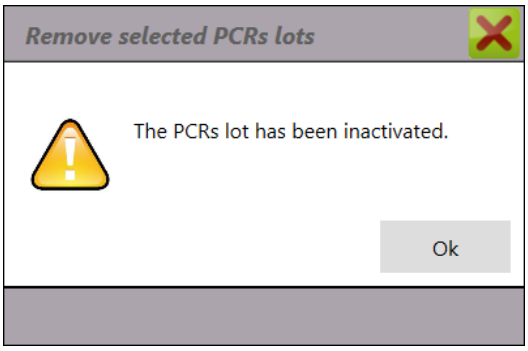


Figure 77. Lot or lots removing warning.

8.4.1.2 Sample Registry

This action allows to introduce the samples in hybriSoft.

In the “Sample management” window, a line appears with all the columns to be filled in.

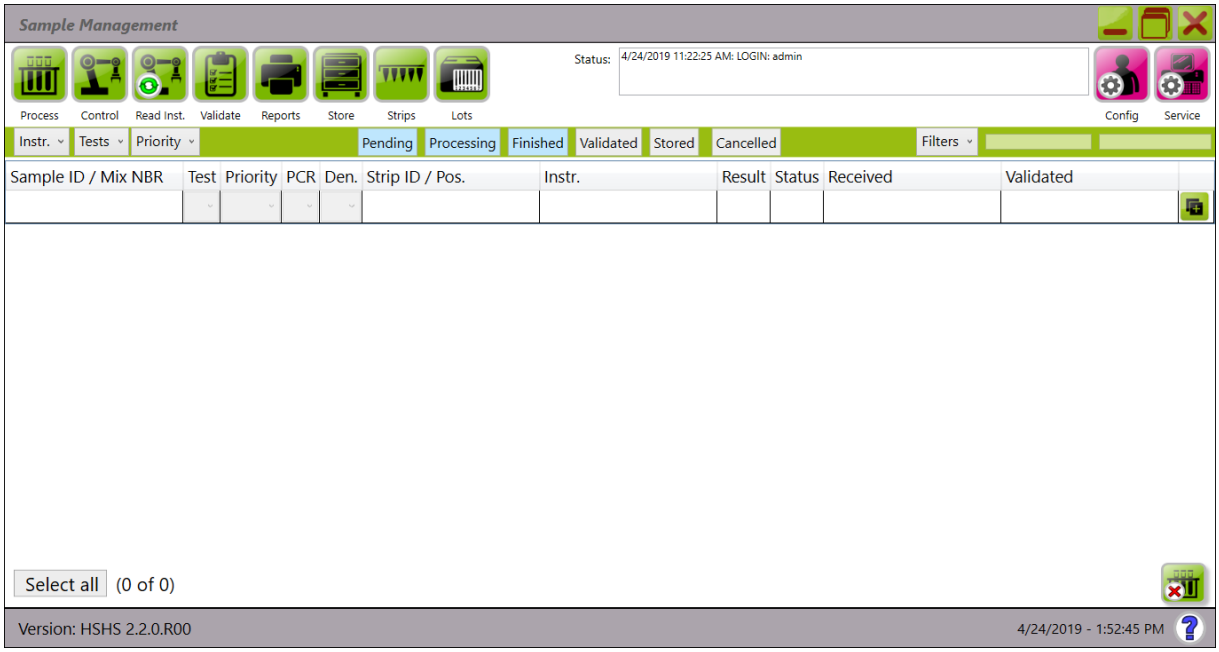


Figure 78. “Samples management” window.

Check the empty box “Sample ID” by clicking it. It will change to blue. After this, insert the alphanumeric characters identifying the sample in the Sample ID field (maximum allowed number of characters 45). Another possibility is double clicking in the ID Sample box; in this case the system assigns an identifying number in an automatic and sequential way (this last action will be possible if it is configured in the "Configuration” screen, point 8.3.1). The data inserted in the ID Sample box are editable as long as the sample remains with a "Pending" status. You can register the samples automatically by reading the ID code of the samples.

It will show the status “Pending” automatically, the date and time the sample was received, the test, the priority and the PCR and Den fields with the defined values by default in configuration. The rest of fields will be blank.

In case “automatic sample ID” is inactive, the cursor will change to the next line when pressing ENTER to create the following sample. If not, it is necessary to double-click in the blank line that appears under the already existing sample.

To the right side of each sample, the button “Sample detail” appears.

8.4.1.3 Samples cancellation

Select the samples that are going to be Canceled. After that, press the “Cancel samples” button in the lower right corner of the “Sample management” window.

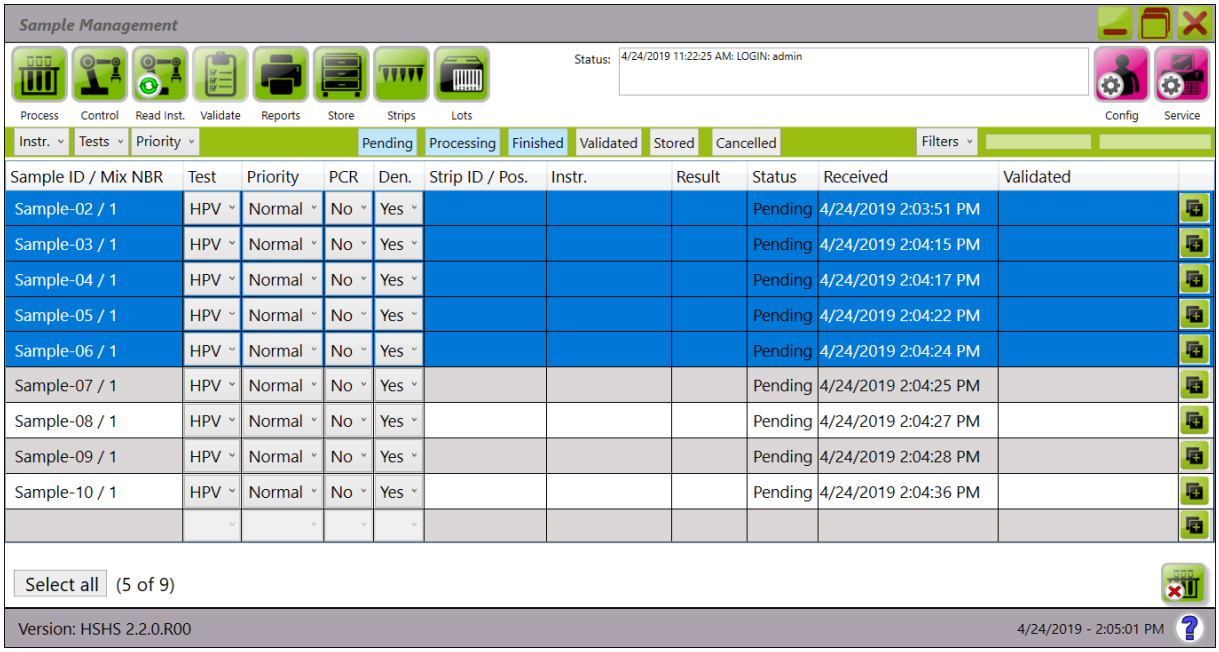


Figure 79. Selection of samples to be canceled.

If you want to select several samples, see samples selection in section 8.4.3.

To confirm the cancellation of the samples in other status than “Processing”, the following window appears:

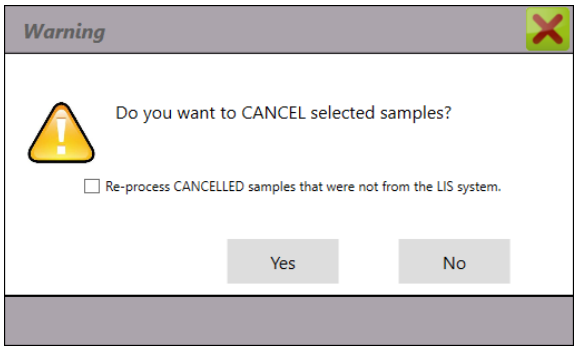


Figure 80. Cancel samples confirmation.

When pressing “No”, the samples will not undergo any changes. To cancel them, press the “Yes” button.

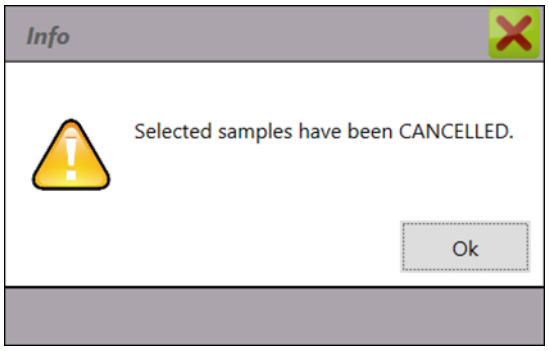


Figure 81. Informative cancellation window.

HybriSoft allows the option of reprocessing the canceled samples. This option is very useful for samples that was in an intermediate status of processing.

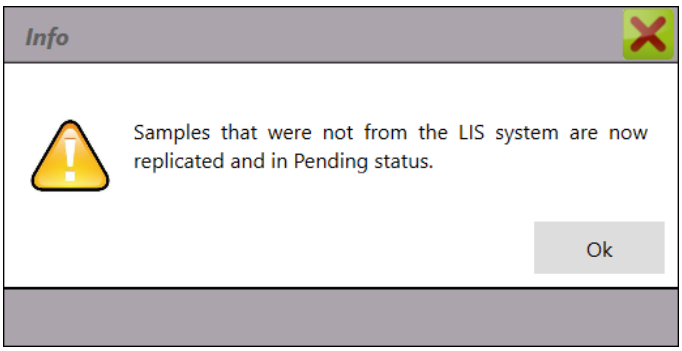


Figure 82. Informative reprocessing window.

When checked the option to reprocess the samples, by pressing “Yes”, the selected samples will change to “Pending” to be reprocessed keeping their ID sample. In the filter of canceled, the samples appear with <ID samples>_CANCEL_X, where X is the number of times that this sample has been canceled and reprocessed. The samples that change their status to “Pending” will keep all the information from the selected and removed sample, as well as the patient's data, all the information concerning the test (doctor, priority, status...) and the protocol type.

Note: the samples from the LIS can only be reprocessed if they are sent again from the LIS system.

When attempting to Cancel samples in a “Canceled” status, the following message will be displayed:

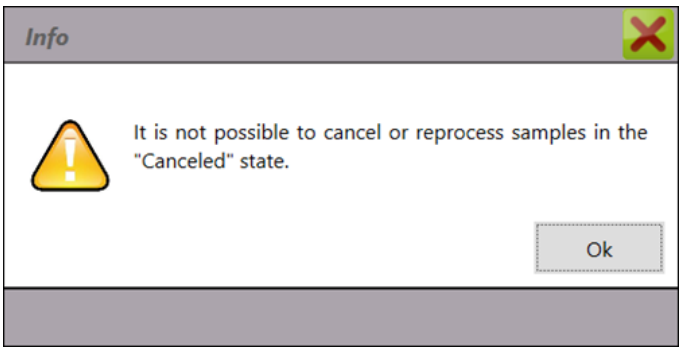


Figure 83. Informative canceled samples window.

In case there are samples in a “Canceled” status, the following message will be shown in the group of selected samples:

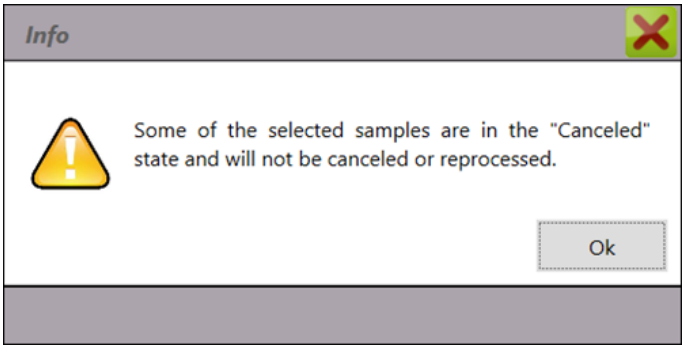


Figure 84. Canceled samples in the selection warning.

The rest of samples will change their status to “Canceled”.

When selecting some samples in a “Processing” status to be canceled, a message will appear, stating that all the samples assigned in the same instrument will be canceled although they are not selected:

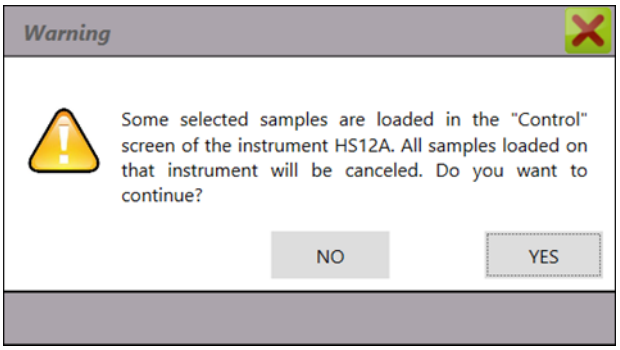


Figure 85. Samples in a processing status in the selection warning.

During the execution of a protocol, it won’t be possible to cancel the samples associated with that series, displaying the following message:

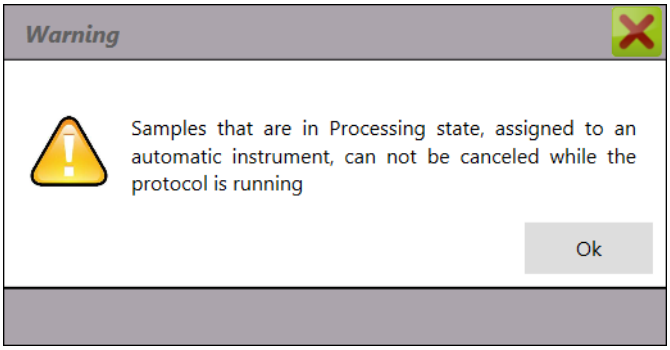


Figure 86. Samples cannot be canceled.

When canceling samples from the LIS, the following message will be displayed:

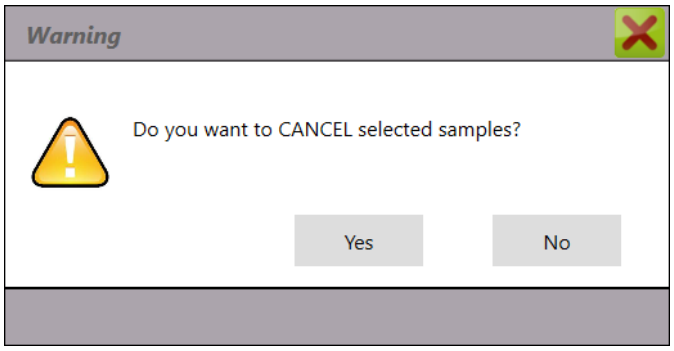


Figure 87. Samples from the LIS canceled warning.

When closing or clicking on "No", no changes are made. When clicking on "Yes" and the samples' status are other than "Stored", the samples are canceled and the cancellation date is sent to the LIS`.

If the sample is in a "Stored" status, the result has already been sent to the LIS. In this case, when canceling this sample, the following confirmation message appears:

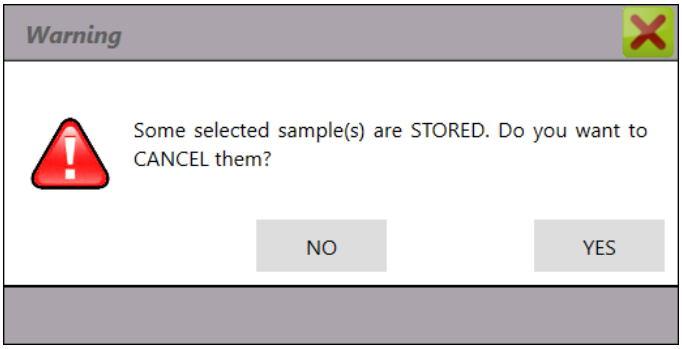


Figure 88. Confirmation of samples stored in the LIS cancellation.

When confirming this cancellation of the samples, the following message appears:

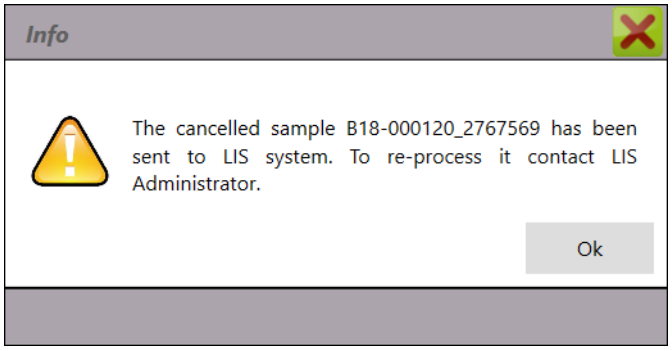


Figure 89. Stored sample cancellation warning.

They are canceled in HSHS, no changes are made in the LIS.

8.4.2 "Sample data" window

On the right side of the "Sample Management" window, in each sample's line we can find the button "Sample Detail", which leads we to the next screen.

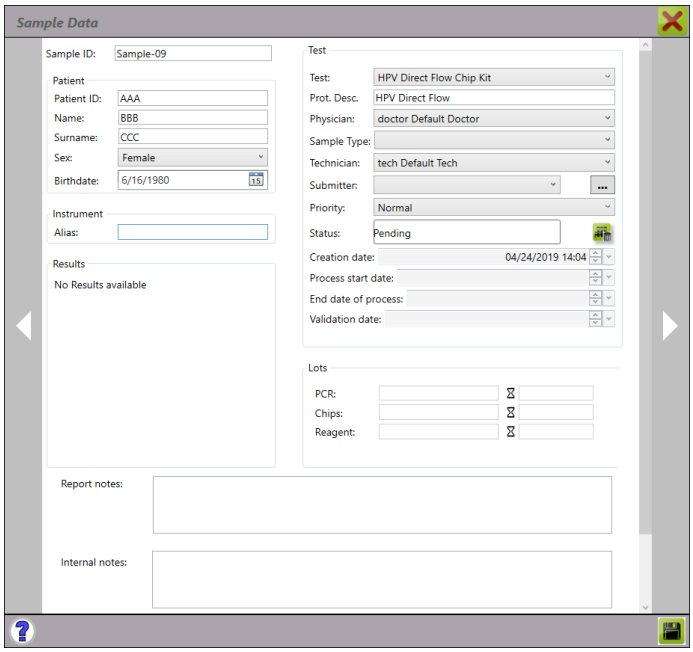


Figure 90. Sample data window.

- **Sample ID:** sample identification, not editable in this window. It shows the Sample ID code of the current sample.
- **Patient:** This block shows the following patient data and allows to modify these data in the samples in a “Pending”, “Processing” and “Finished” status.
    - **Patient ID:** It is an editable alphanumeric field of 30 characters, to identify the patient according to another codification chosen by the application user.
    - **Name:** Editable alphanumeric field of 20 characters to keep the patient's name.
    - **Surname:** Editable alphanumeric field of 30 characters to keep the patient's surname.
    - **Gender:** drop-down field which allows the selection of the patient's gender.
    - **Date of birth:** Modifiable field of date indicating the patient's date of birth. This field contains, on the right, a drop-down calendar to select the date of birth.
- **Instrument:** This block shows the following data of the instrument where the sample is processed:
    - **Short name:** Non-editable alphanumeric field showing the pseudonym with which the instrument assigned to this sample is identified in the application.
- **Results:** Non-editable alphanumeric field of 1024 characters that is automatically filled in once the sample is processed and analyzed. In this section, the obtained result positive is specified, as it can be seen in the picture with positive RES result.
- **Test:** This block shows the following data with regards to the test performed to the current sample:
    - **Test:** drop-down field with the acronym of the chosen test. The changes of this field will only be accepted if the sample is in “Pending” status.
    - **Desc**. **Prot.:** Non-editable field with the protocol applied to the sample.
    - **Doctor:** drop-down field with the surnames of the doctor assigned to this sample. It can be modified in the Pending, Processing and Finished statuses.
    - **Sample type:** drop-down field of the type associated to this sample. It can only be modified if the sample status is "Pending".

- **Technician:** drop-down field with the surnames of the technician associated to the sample. It can be modified in the Pending, Processing and Finished statuses.
- **Submitter:** Pull-down field with the name of the applicant physician of the test. Click on the ellipsis on the right of the line to add, edit or delete an applicant physician. It can be modified in the Pending, Processing and Finished statuses. If there are samples associated to a submitter, you cannot remove/edit the submitter from another sample. In this case, a warning message will be displayed:

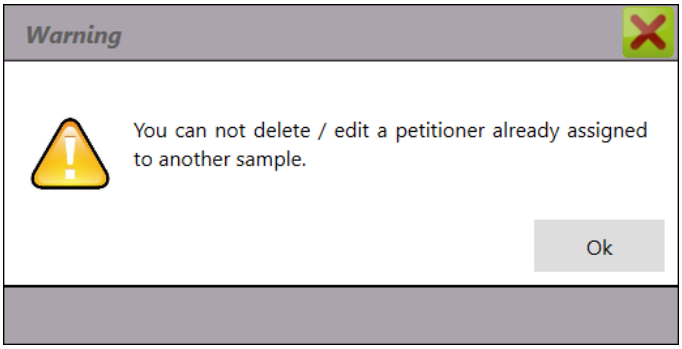


Figure 91. Submitter cannot be removed.

- **Priority:** drop-down field with the urgency associated to this sample. This field can only be modified when the sample is in a "Pending" status.
- **Status:** Non-editable field with the status in which that sample is.
- **Creation date:** date/time field that shows the date and time in which the sample was introduced in the database of the application.
- **Process start date:** date/time field that shows the date and time in which the sample was sent to be processed by the instrument.
- **End process date:** date/time field that shows the date and time in which the sample results were read by the instrument and updated in the database of the application.
- **Validation date:** date/time field that shows the date and time in which the sample was validated by the user.

- **Lots:** This block shows the following data associated to the reagents used in the processing of the sample. This field cannot be edited and is filled in once the sample has been sent to HS24 and the equipment has read the kit with which the samples are going to be processed. There are three different products with the field lot and expiration date.
  - **PCR**: It shows the lot and the expiration of the PCR mix used in the sample.
  - **Chips:** it shows the lot and expiration of the chip used in the sample.
  - **Reagents:** It shows the lot and the expiration of the hybridization reagents used in the sample.
- **Report notes**: free text field to include notes / comments that are considered appropriate related to the sample / process / test / patient / reagents / instrument or any other question.
- **Internal notes**: free text field to include internal comments that are considered appropriate related to the sample / process / test / patient / reagents / instrument or any other question. This information is not sent to the LIS.
- **Antibiotic susceptibility:** it shows the relation between the resistance molecular markers provided by the SEP kit and the antibiotic susceptibility they correspond to.

With the arrows placed on the side of the window, you can move from one sample to another.

This window contains the following buttons:

- **Sample cancellation button:** It allows to cancel the sample regardless of its status. The canceled samples change their status to “Canceled”. To remove these samples from the software, you have to perform a backup copy and remove old samples (See the sections 8.3.1.4 and 8.3.7).

- **“Results details” button:** it opens the “Picture processing” window in which the real and the processed images corresponding to that sample appear, as well as the results interpretation and threshold controls. This button appears if the sample is in a “Finished”, “Validated” and “Stored” status and if the "Show Results Details" option in the configuration is active. In the last two statuses, it is only allowed to visualize both images and the result, but it doesn't allow the modification of the analysis.

- **Report button**: it generates the sample's report in PDF format. This report is generated with the name “report_<Sample ID>.pdf” being < Sample ID > the ID code of this sample. It is active if the sample is in a Validated or Stored status. *For further information, see the Reports 8.7.3 section*

- **Save changes button:** It saves the changes made in the screen field as long as the samples status allows it, according to what is described in each field of this screen.

- This button shows the user manual of HS12, the user manual of HS24, the user manual of HS12a, a report of new implementations and improvements in the application and the End User License Agreement or EULA.

- **Close button:** it closes this window and goes back to the main window.

8.4.2.1 Assigning samples to a strip

Once all samples have been introduced, with the "Pending” filter active, select a maximum of eight samples in tests with a sole replica and a maximum of 4 samples in tests with two replicas to be assigned to a strip. If the total of the samples to be processed is over 4 or 8, they will have to be divided into several strips.

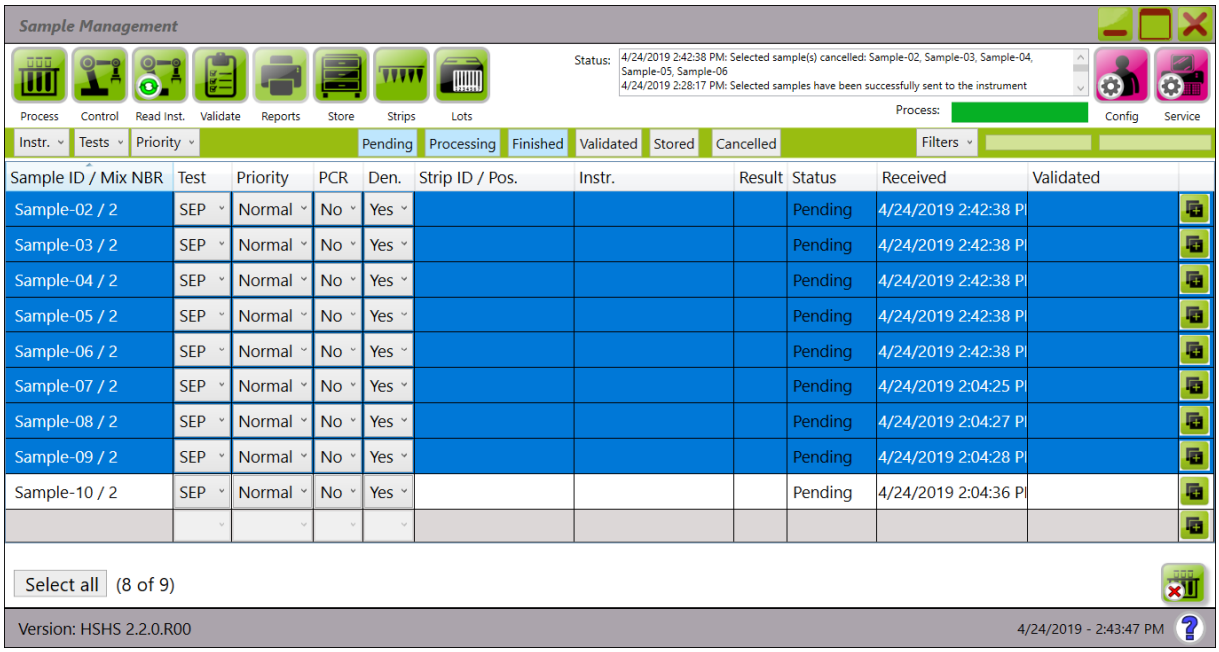


Figure 92. Selected samples.

The distribution of the samples in the strip is determined by the order of the selecting process. In order to make the samples place in the strip in the order they appear on the screen, you have to select them in the same order. (Selecting processes, see 8.4.3).

Once samples have been selected (marked in blue), click on the button “Strips”. The “Strips” window will appear.

The strip’s identifier has to be introduced for the samples to be sent for processing. It is assigned manually (alphanumeric). The samples that contain two replicas appear one after another, with the same ID and a letter (A and B) that identifies to every mix a different PCR.

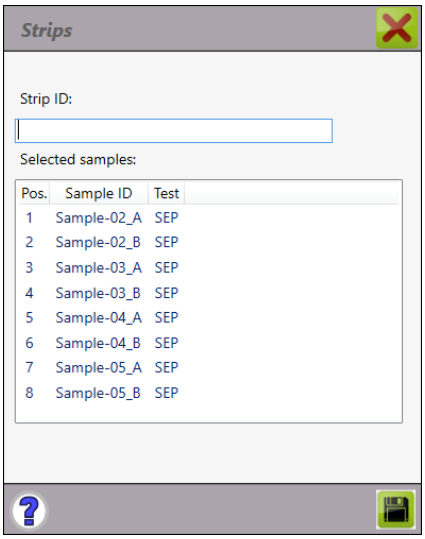


Figure 93. Manual assigning of a strip ID.

In this window you can modify the position of the samples in the strip by dragging the samples with the mouse to its right position. If the samples are tow replicas, the change will be made on the two replicas at the same time, so that the two replicas of a sample are always together.

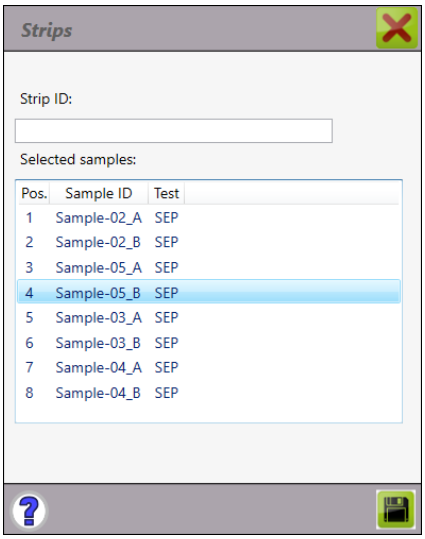


Figure 94. Modification of samples in a strip.

If the strip identifier is introduced manually, we must press the "Save" button. If we introduce the identifier with the bar code reading, the save is done automatically.

Sample ID / Mix NBR	Test	Priority	PCR	Den.	Strip ID / Pos.	Instr.	Result	Status	Received	Validated
Sample-02 / 2	SEP	Normal	No	Yes	strips-02 / 1			Pending	4/24/2019 2:42:38 PI	
Sample-03 / 2	SEP	Normal	No	Yes	strips-02 / 5			Pending	4/24/2019 2:42:38 PI	
Sample-04 / 2	SEP	Normal	No	Yes	strips-02 / 7			Pending	4/24/2019 2:42:38 PI	
Sample-05 / 2	SEP	Normal	No	Yes	strips-02 / 3			Pending	4/24/2019 2:42:38 PI	

Figure 95. Samples with two replicas associated to a strip.

If we select more than eight samples, a pop-up screen will appear warning of the fact that the maximum number of samples allowed to assign to one strip.

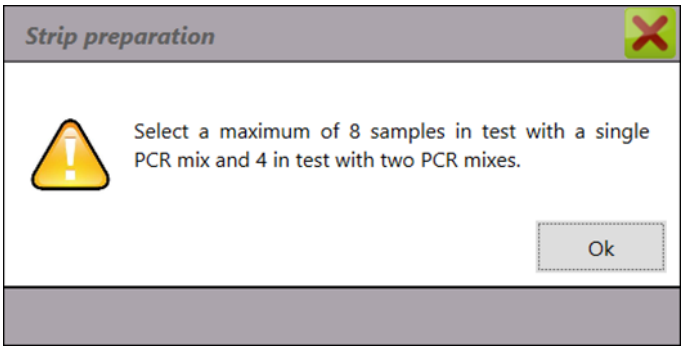


Figure 96. Maximum of samples in a strip.

In case of selecting samples with different PCR or Den. value, the following message will be displayed:

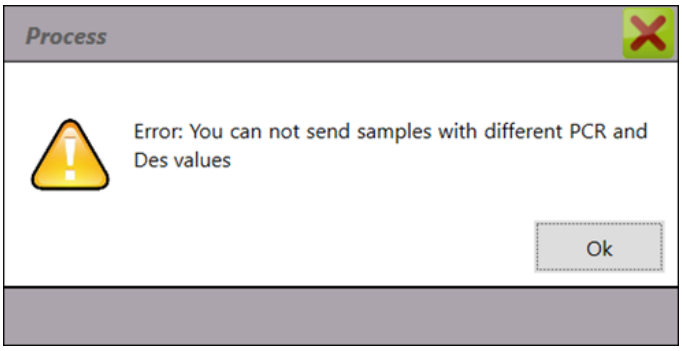


Figure 97. Selection warning with different PCR and Den. values.

In case of selecting samples with different test, the following message will be displayed:

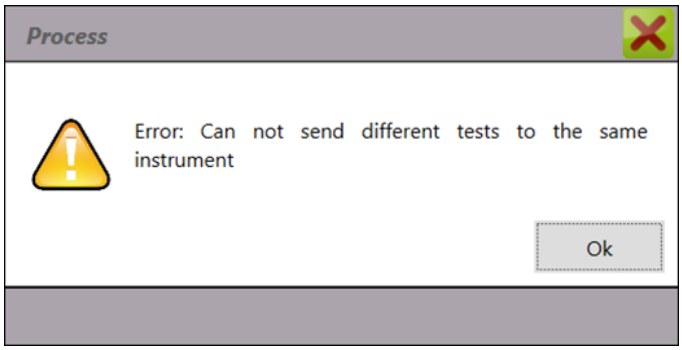


Figure 98. Strips with a different test.

The strip identifier can be modified selecting the strip to edit, clicking again on the "Strips" button and changing the existing name. To finish, click the "Save" button.

The samples can be freed from the strip by clicking on the box "Free Samples" in the lower area of the "Strips" window. This option only appears when a Strip ID has been created.

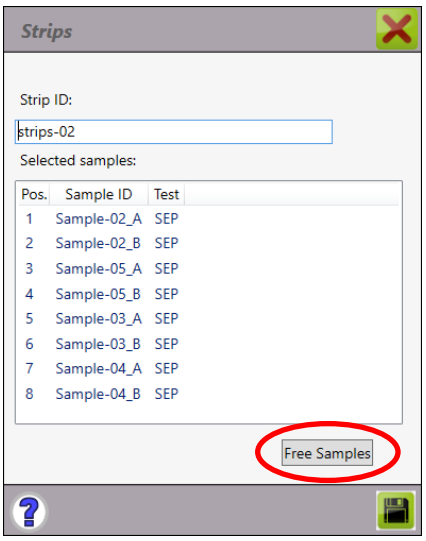


Figure 99. Free samples from a strip.

If we are using separate tubes, without bar code, it is also compulsory to assign an invented ID that allows to establish the samples order to be sent to the HS12a equipment.

8.4.3 Samples' processing

This action allows sending the samples in a pending status to be processed in the instrument.

The samples selection can be made in different ways:

- Select all samples in pending status simultaneously: activate only the pending samples' filter and press the "Select All" button.
- Select samples assigned to one ID strip: when clicking on the ID strip all samples assigned to that strip are selected.
- Select a group of samples: click in the sample ID of the first sample of the list, keep the capital key and scroll down with the arrow until the last sample we wish to mark. Another possible option is, once the samples are assigned to the strip ID, keep the control key pressed and click on the strip ID of each one of the samples to be processed.
- Select a group of samples: select the first sample and move towards the last sample we wish to select.

***NOTE**: 12 samples gathered in a maximum of 3 strips is the maximum number of samples and strips that can be sent to the instrument.*

Once the samples are selected, press the "Process" button:

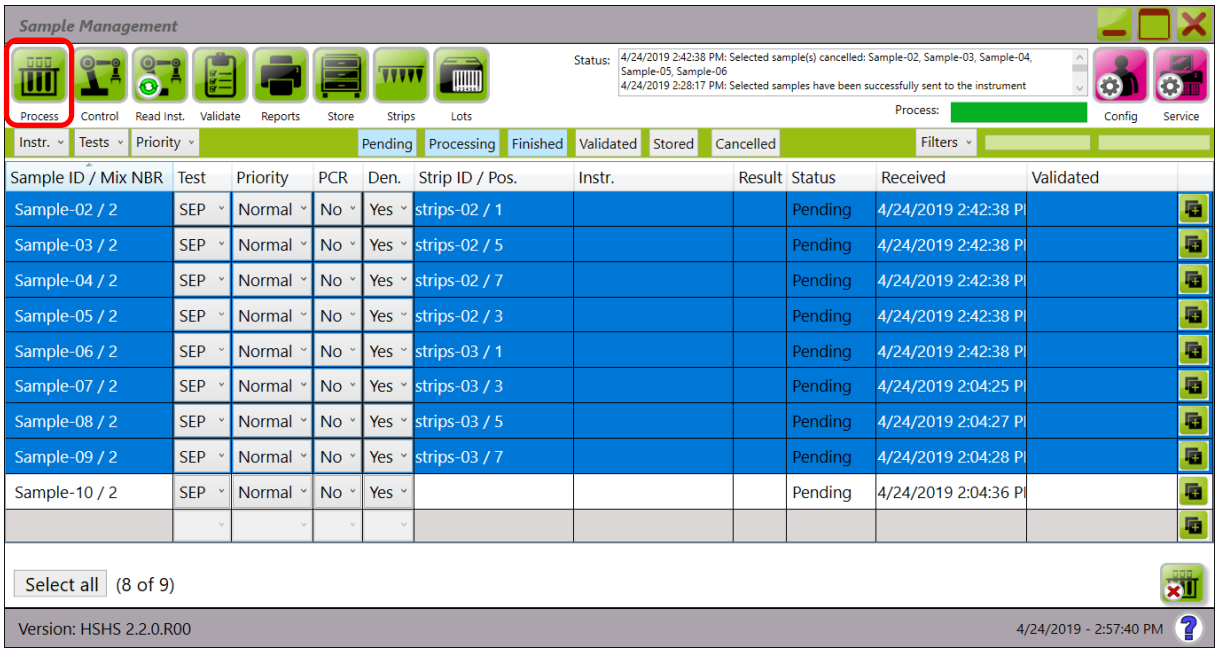


Figure 100. Samples to be processed.

The "**Process Parameters**" window will be opened.

If no sample has been selected, the following message will be displayed.

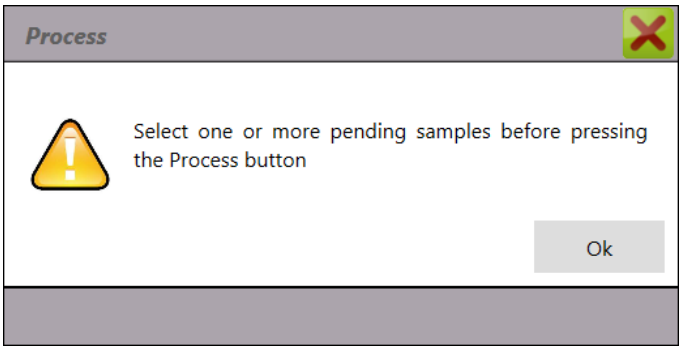


Figure 101. No selected samples warning.

If the selected samples are to assigned to a strip, the following message will be displayed:

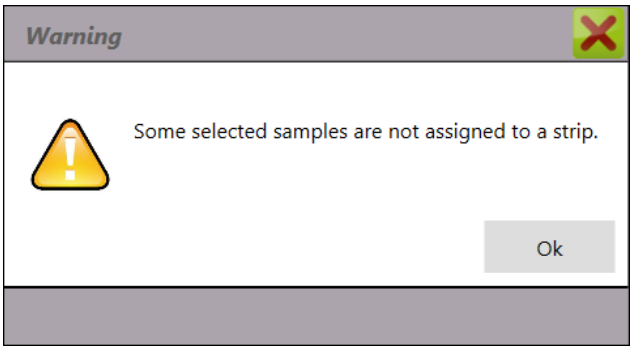


Figure 102. Need for assigning to a strip.

If you have selected samples with a different Test, the following message will be displayed:

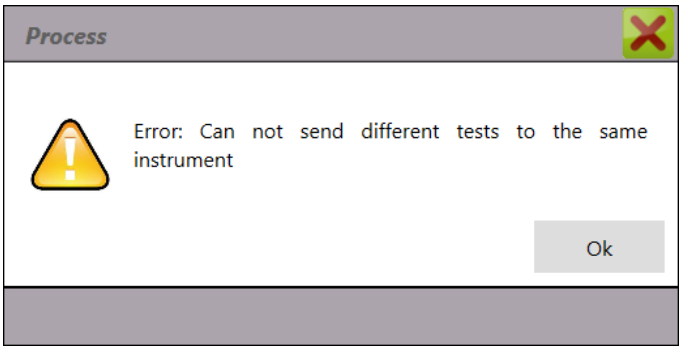


Figure 103. Error warning after sending samples with a different Test.

If you have selected samples with different PCR or Den. value, the following message will be displayed:

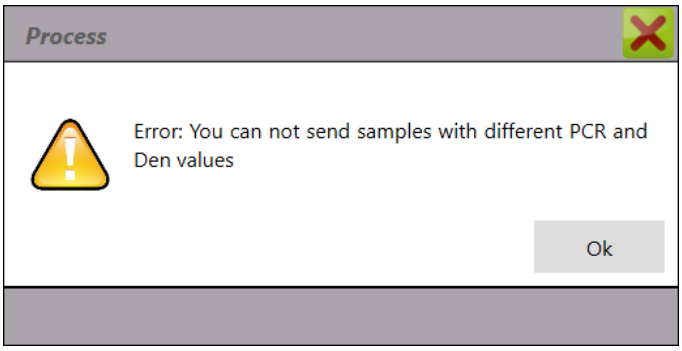


Figure 104. Error warning after sending samples with different PCR and Den. Value.

8.4.3.1 Structure of the "Process Parameters" window

This window allows selecting the instrument, the protocol, the option to get a workload list and the actions to be applied in the samples to be processed.

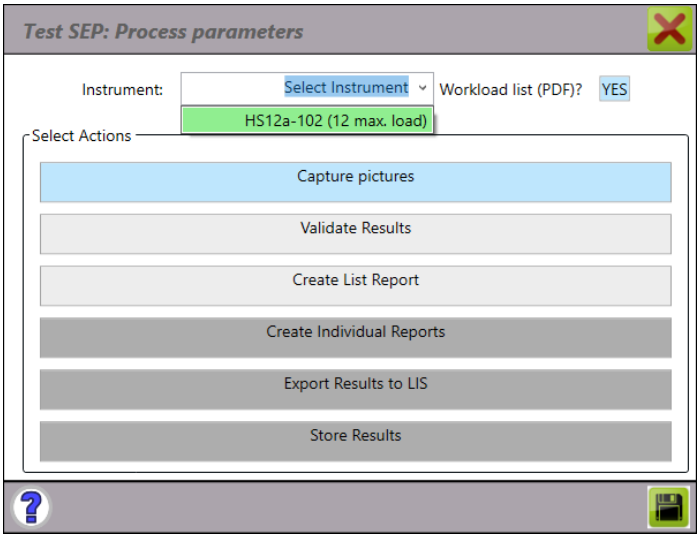


Figure 105. Process parameters. Instrument selection.

For each instrument registered and active, the status is shown (online, offline, in use, no homing). The instrument will be in a "no homing" status when any of its components is not correctly initialized or it is not in its initial position.

If selecting the HS12a equipment, the available protocol options for the selected test appear in the window.

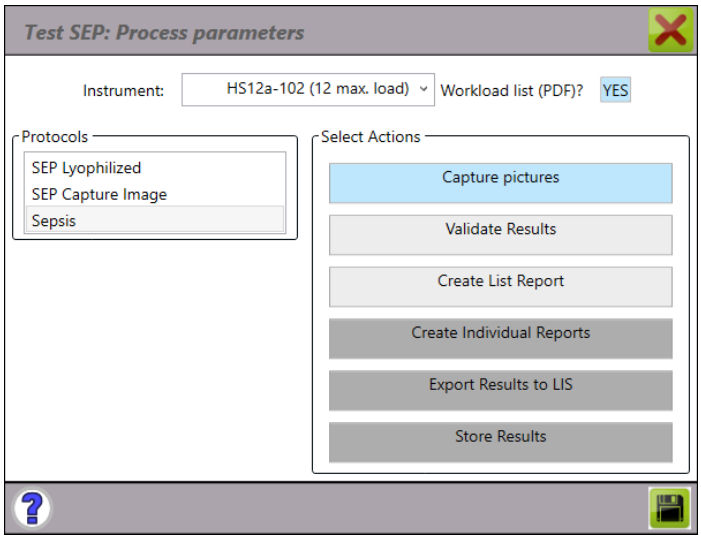


Figure 106. HS12a process parameters.

- **Instrument**: it allows the selection of the instrument in which samples will be processed (in this case, HS12a). In the drop-down menu, the instruments that have been previously registered in the configuration screen appear. Depending on their status, instruments appear in different colors:
    - **Green:** the instrument is connected and ready to receive samples
    - **Yellow:** the instrument is not in a "homing" status.
    - **Red:** the instrument is connected, but occupied with sent samples to be processed or samples being processed.
    - **Black:** the instrument is disconnected.
- **Workload List (PDF):** it allows obtaining a PDF with the workload. If you select "YES", it generates the work list in which the samples to process, the instrument in which the processing is made, the position in the instrument, the status and the reception date appear. If you select "NO", this list won't be generated.
- **Protocols:** It shows the available protocols for the test to do. The protocol defined in "Configuration" appears marked by default. Nonetheless, there is the possibility to change the default selection from this window.
- **Select actions:** It allows to select the actions we wish to apply to the samples to be processed.
    - **Capture images:** once the protocol is finished in the HS12a, it analyses the results and the samples change into a "Finished" status.
    - **Validate results:** The validation of the samples is automatically done after finishing the analysis.
    - **Create list report:** It creates a list with the results obtained of all selected samples. This list is automatically stored in C:\hybriSoft\REPORTS in PDF format with the name "list_<day>_<month>_<year>_<hour>_<minute>_<second>.pdf" being the date and time in which it has been generated.
    - **Create individual reports:** it creates an individual report of each sample, in PDF with the complete data from the patient and sample. This report is automatically stored in C:\hybriSoft\REPORTS with the name "report_<Sample ID>.pdf". You can only create individual reports of the validated and stored samples.
    - **Export results to LIS:** it sends the results of the samples to the management system after the analysis is finished, they have been validated and sent to the LIS.

- **Store results:** samples change into a "Stored" status. Only the already validated samples can pass into a stored status. If the samples are from the LIS, they do not change their status to Stored if the result has not been sent to LIS. In this status, we can find all samples from the system that have been processed, validated and stored.

The selected configuration in this window is saved for the next sample processes. After selecting all required fields, press “Save”. The next window will appear to assign the lots to every sample. As long as the option request lot is active in each of the products (PCR, Chip, Reagents).

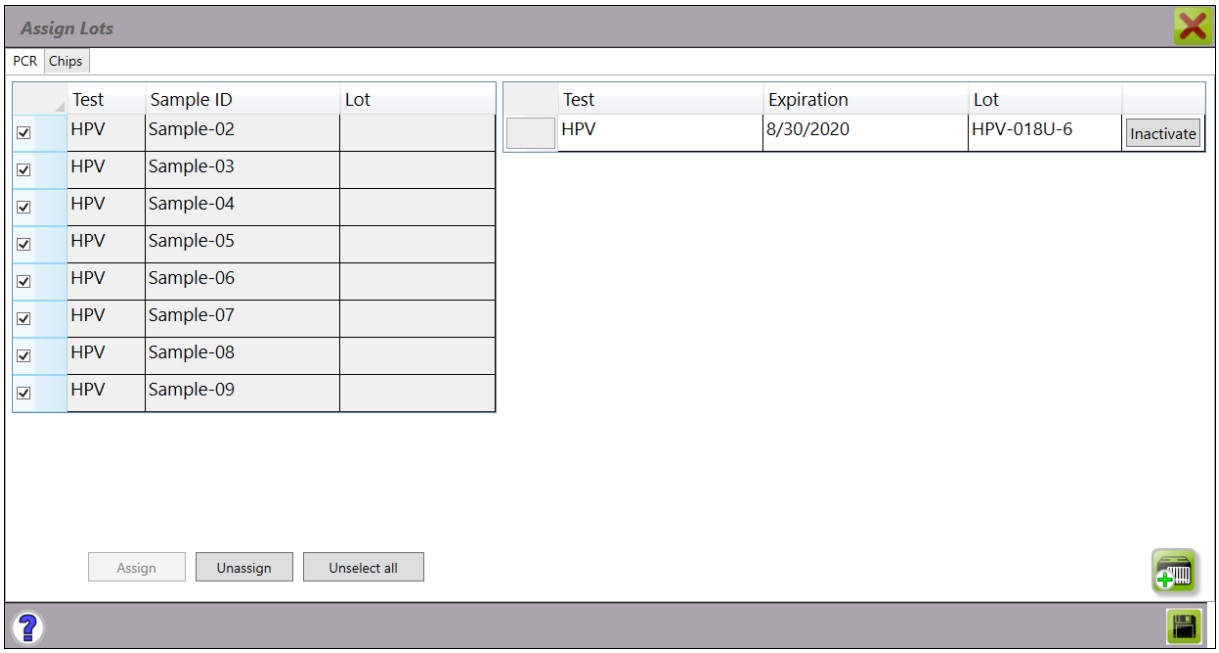


Figure 107. Lot assigning window.

In this window, the lots will be assigned from every product to the samples. In case of using an automatic instrument, the reagents tab will not appear, since they are automatically associated with the QR code reading of the bottles. In this window, only the registered, active and associated lots to the test is going to be performed will appear. The expired reagents will appear in orange color if it is configured for that (see the section 8.3.1.5, the point “expiration of automatic lots”).

All the samples appear selected by default. In this window you can perform several actions.

- Assign lot: select the sample or samples that are going to be associated a lot, select the corresponding lot and press the “Assign” button. The column lot of the samples table will be filled in and it will show a grayish color.

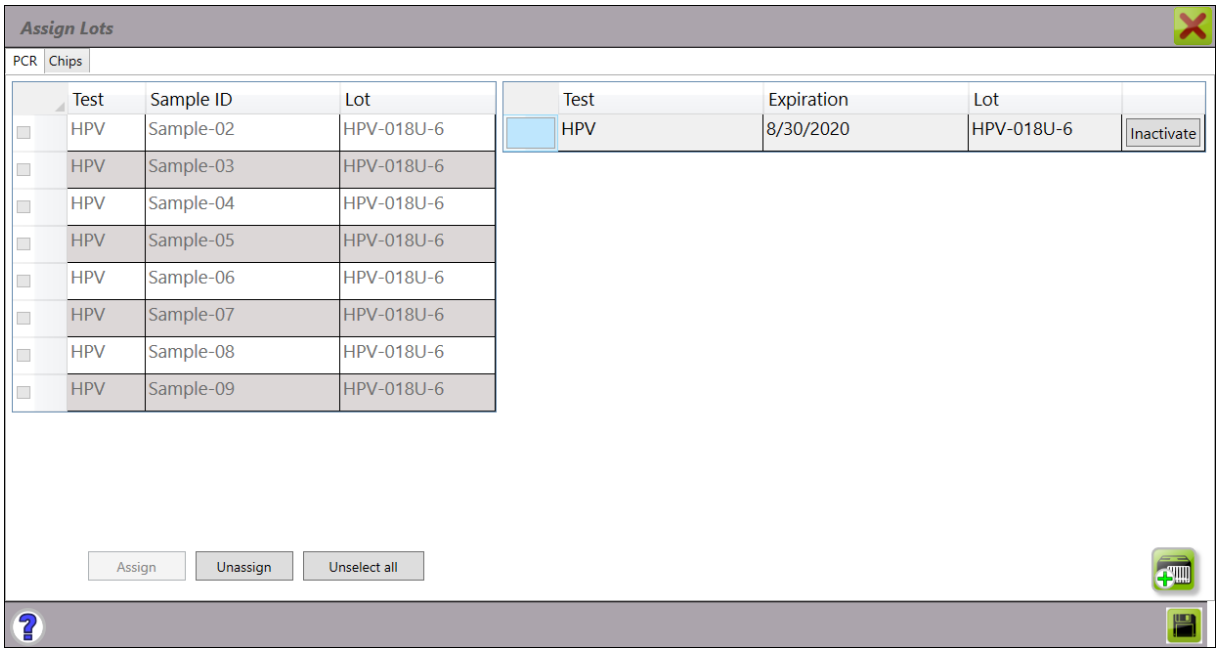


Figure 108. Samples with an associated lot.

- Unassign Lot: this button allows to remove the association of the lot of the samples.
- Select all/Unselect all: it allows to select and deselect all the samples of the table.

This window allows to register the new lots in each tab.

Once the lots have been associated to the products, press the “Save” button. If there is a lot that has not been associated, a window will appear stating that one product has to be assigned.

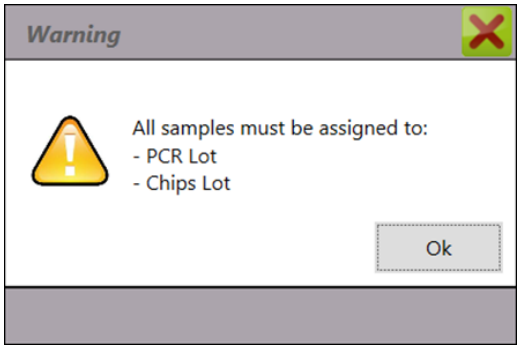


Figure 109. No assigned lot warning.

HybriSoft sends all the selected samples to the specified instrument:

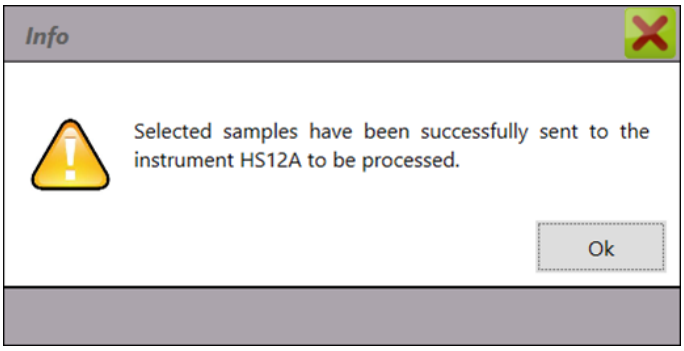


Figure 110. Information pop-up window.

8.5 HybriSpot Control

Once all the samples have been sent to the instrument, the hS control window will automatically appear. This window allows to control the different working areas of the HS12a equipment.

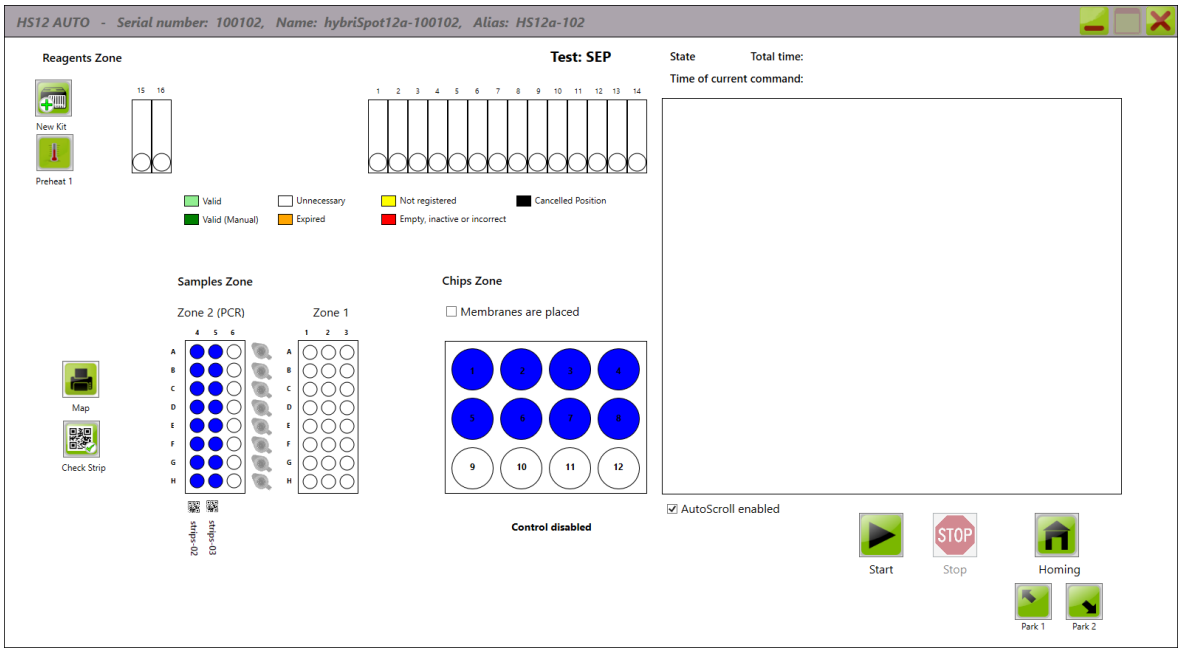


Figure 111. HS12a's HS control screen.

8.5.1 Structure of the "hS Control" window

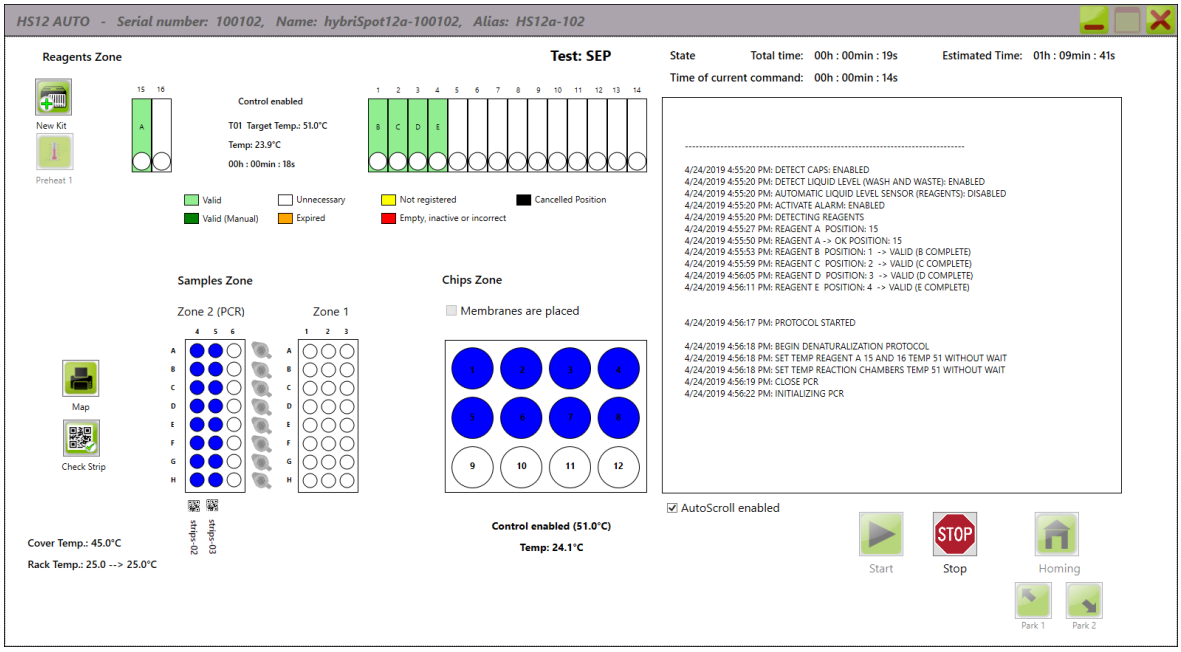


Figure 112. Detected reagents screen.

- **Reagents Zone** → In this area, the reagents in use and placed in the instrument appear. It is divided into two zones.
  - **Reagent A zone**→ Composed of 2 positions to place the reagent A and allows heating up that reagent.
    The reagent A needs to reach the established temperature before starting the protocol. When pressing the 1 or 2 preheat button, the test corresponding to the reagent A, the final temperature to be reached, the current temperature of the reagent and a chronometer indicating the time the preheating has been activated will appear.
    If the preheating of the reagent A is not done, when starting the protocol, we will need to preheat it until reaching the minimum established temperature in the protocol.

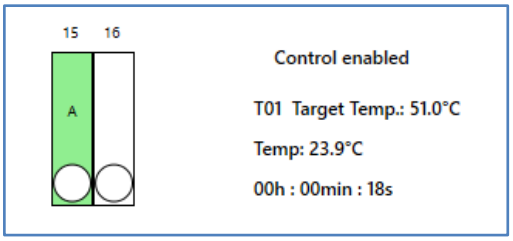


Figure 113. Reagent A pre-heating.

In case the protocol is performed with PCR, the pre-heating of the reagent A is inactive, since it is performed during the amplification protocol.

  - **Reagents Rack** → Zone where the rest of reagents of the kit are placed. There is no need to choose a specific position for each reagent, it is automatically established with the reading of the QR bar code that each reagent's vial has.
  - **Color Legend** → A legend appears indicating the colors and the reagents' status:

- Light green: valid reagent.
- Dark green: valid reagent manually positioned.
- Yellow: Reagent not registered in the database.
- Orange: expired reagents.
- Red: Empty, expired or incorrect reagent.
- White: Unnecessary reagent
- Black: canceled position

- **New Kit**→ This button allows registering new hybridization kits in the database. (See section 8.4.1.1)

- **Samples Zone** → It is divided into two zones of 24 wells (3 strips x 8 samples). The zone 1 corresponds to the rack where the samples are placed for hybridization protocol (PCR and Den. with value "NO"). The zone 2 corresponds to the thermocycler zone. In this zone, the samples are placed in case the PCR and/or denaturation processes are programmed. This zone indicates, marked in blue, what well each sample to be processed occupies. If it is a test with two replicas or PCR mix, two positions appear for each sample. Both replicas will be placed consecutively to A and B.

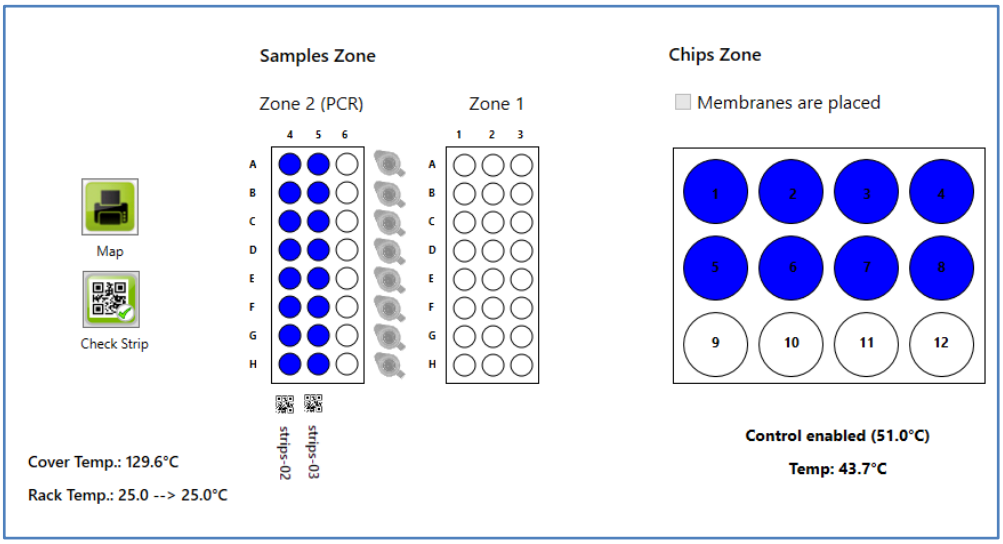


Figure 114. Distribution of samples.

In the lower part of the sample zone it is shown the position of the strip with its ID.
In the right part of these two zones, you can find the "Check strip" button, which allows to check where the strip has to be placed.
If you click the button, a new window will open with a text field where the strip ID reading is performed, and you click on Ok.

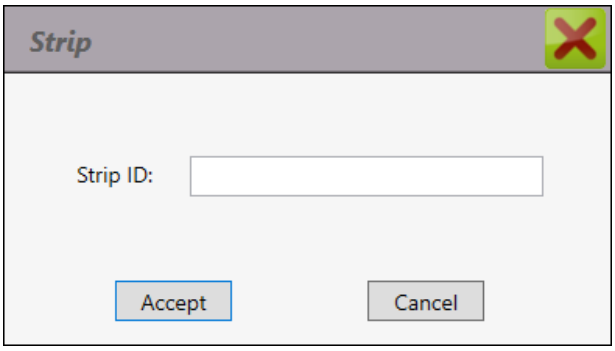


Figure 115. Strip verification.

If the strip has been sent to be processed, the schematic identifier will change to red and blinking.

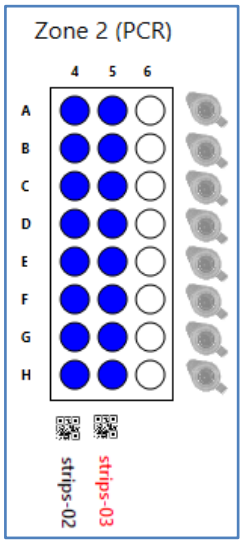


Figure 116. Strip verification.

- **Chips Zone** → There is a chamber for 12 samples. The positions in which the corresponding chips need to be loaded to the samples to be processed are marked in blue. If the test contains two replicas or PCR mix, two blue positions will appear in the samples zone for every blue position in the membranes zone. In the upper part of the camera, the pre-heating of the reaction chamber appears and, in the lower part, the Temperature Control. This last option shows the temperature of the camera at every moment during the protocol. The pre-heating of the reaction chamber starts when activating the check box "Membranes are placed".
    - **Map:** a PDF file appears with the sample placing in the samples zone and in the membranes zone. This file is stored in the REPORT folder identified with "map_<day>_<month>_<year>_<hour>_<minute>_<second>.pdf". If there are no samples sent to the instrument, this document will not be generated.

Warning: *in case the equipment does not reach the expected temperature within the time set, the protocol will stop and you will be warned that it is not possible to reach the expected temperature. You must contact the technical service under these circumstances.*

Note: *when placing the mouse on any blue position in the samples or chips zone, it changes into red color and indicates the localization of this sample in both zones. In the samples of the test with two replicas or PCR mix, two positions are marked in the samples zone and one position in the membranes zone. Furthermore, in the membranes zone, a text box is shown in which the sample ID and the strip ID appear, and it only shows the sample ID in the samples zone.*

- **Status** → in this box the information on the steps the instrument is executing while running is registered.
- **Elapsed time** → It appears in the right upper side of the hS Control screen. It indicates the time elapsed from the moment the protocol started.
- **Current Command Time** → It appears under the previous one and indicates the time elapsed of the command the equipment is carrying out at that moment.
- **Expected time** → It appears in the right upper side of the of control screen along with the total time. This time indicates the estimated time of the protocol, representing a countdown.
- **Exceeded time** → It appears in the right upper side of the of the control screen along with the time of the current command. This time indicates the exceeded time of the protocol when the estimated time's countdown reaches zero.
- **Functioning Buttons** → On the lower right side of the window, the following buttons appear:
    - **Start**→ this one has the function of initiating the protocol.
    - **Stop**→this button allows finishing the protocol. Once activated, a second confirmatory warning appears which, after being accepted, permanently interrupts the protocol in process. If we need to make a homing, an obligatory window appears. Otherwise, a screen appears with the option of making a homing. If we reject the stop confirmation the protocol will continue where it was after having being paused.
    - **Homing**→ Recovers the reference positions in each one of the movement axes of the robotic arm.
    - **Park 1**→ this button moves the arm towards the rear left corner, leaving the membranes zone free.
    - **Park 2**→ this button moves the arm towards the front right corner of the instrument.
- **AutoScroll Activation**→ Activation in the lower right part of the screen we can activate the option of automatic movement of the shift bar in the status box so that we will be able to visualize in the window the last executed steps at any time. If it is inactive, the current status can be visualized by moving manually the lateral bar.

8.6 Protocol start-up

Once the samples have been sent to the instrument, place the samples in their corresponding positions, bearing in mind the protocol to be executed. If it involves a hybridization protocol, denature the PCR product as indicated in the product data sheet. Once the denaturation process is finished, place the samples in their corresponding positions (see section 8.5.1). If the protocol requires PCR and/or denaturation, place the samples in the selected zone.

Once the denaturalization is finished, place the samples in their corresponding positions (see section 8.3.2) and that the bottles are connected following the corresponding code of colors.

Place the chips in the positions to use (blue positions in the chips zone). These positions are indicated in the hS Control window (Figure 112). In case of having an incomplete row (it does not contain chips in the 4 positions of the row) place stoppers in all the empty positions. In case the whole row is empty, it is not necessary to place stoppers, with the exception of maintaining them in the corner positions of the reaction chamber in order to maintain a homogeneous pressure.

Place the reagents in their corresponding positions; check that the kit matches the test to be done or check if the use universal kits option is active (see 8.3.1.5, section “Activate universal kits”) and that there has not been any volume spilling issue in any of the vials. The number of tests counting is done through the QR code reading; the sensor does not quantify liquid volumes. Therefore, **if an accidental spill occurs from any of the vials of the kit, it is recommended not to use it and check with the manufacturer or supplier.** It is recommended to place the reagents in the first positions of the rack (1, 2, 3 ...) to optimize to the maximum the reading time of the reagents.

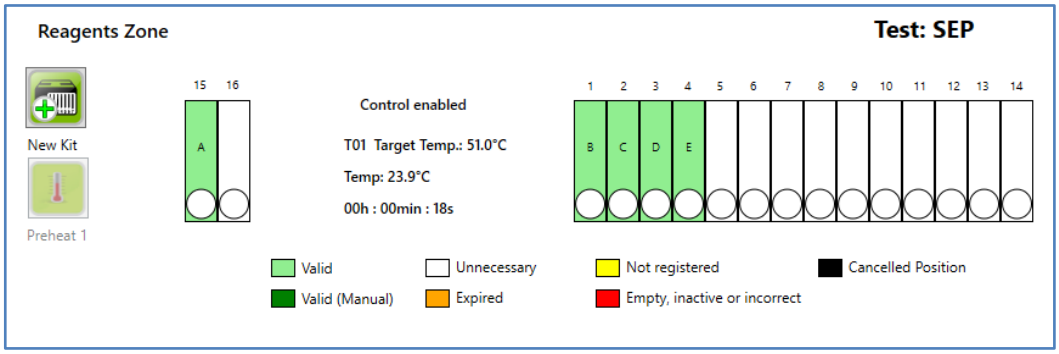


Figure 117. Reagents placed in optimal positions.

If it is necessary to mix the reagents E1 and E2, enable the option “Automatic reagents mix” and place the three vials available in the reagents’ rack. The mix of reagents is performed during the sample incubation. If it is not necessary to mix the reagents, disable the option. In this case, only the vial of the reagent E with its corresponding volume is necessary. If the three vials are placed in the rack and the check is not active, the following message will appear: “The reagents will not be mixed automatically. If you wish to, check “Automatic reagents mix” in “Configuration”.

In order to preheat the reagent A at the required temperature, we must place the reagent in the correct position and, after that, press the preheating of the corresponding zone (see 8.5.1)

Once everything has been checked, press the "Start" button on the hS Control window. First of all, check the state of the waste and the wash solution containers

If the waste container is full, the following message will be displayed:

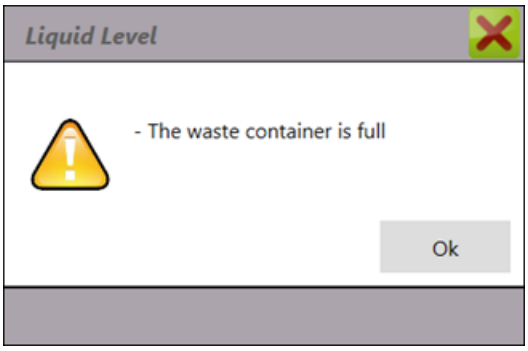


Figure 118. Full waste container warning.

If the container is not empty, you cannot continue. Press "Accept", eliminate the wastes in an appropriate container and, afterwards, press "Start". If the container has not been emptied, the same message will appear again.

If the washing bottle has an insufficient volume level to make a complete protocol of 12 samples, including a final washing of the internal circuit, the following message appears:

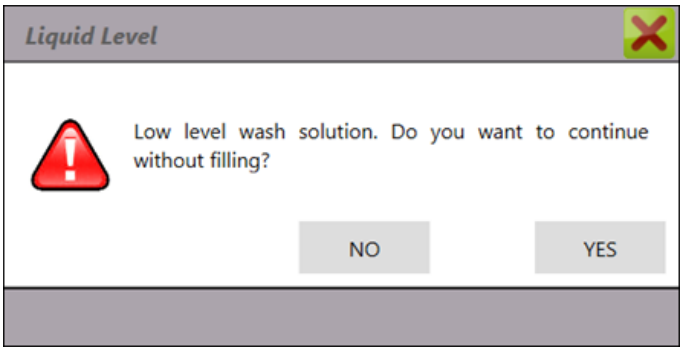


Figure 119. Insufficient wash solution warning.

It is recommended to refill the washing bottle the way it is indicated in the data sheet of the product. If the container does not get filled, the system allows the user to continue. However, it is recommended to always refill the container when the sensor detects low levels of wash solution to guarantee that the protocol is performed correctly.

Warning:

- *Connect the sensors and connectors following the colors codification. Otherwise, there will be a wrong functioning of the protocol and alarms.*
- *In case of not having enough washing liquid to perform the run, some crossed contamination can occur between the samples.*

Secondly, identify all the reagents needed to do the test, checking the test identification and its compatibility in case of being using the universal kits option active (see 8.3.1.5), the expiration date and that the test number the kit contains is enough for the programmed protocol.

Although the preheating is active, when starting it, the reagent A reading is done to check if the reagent's test matches with the test of the samples sent. Once the identification of the reagents A has been performed, the rest of reagents are read.

While the reagents are being read, the sample management window will be blocked, preventing the user from managing samples.

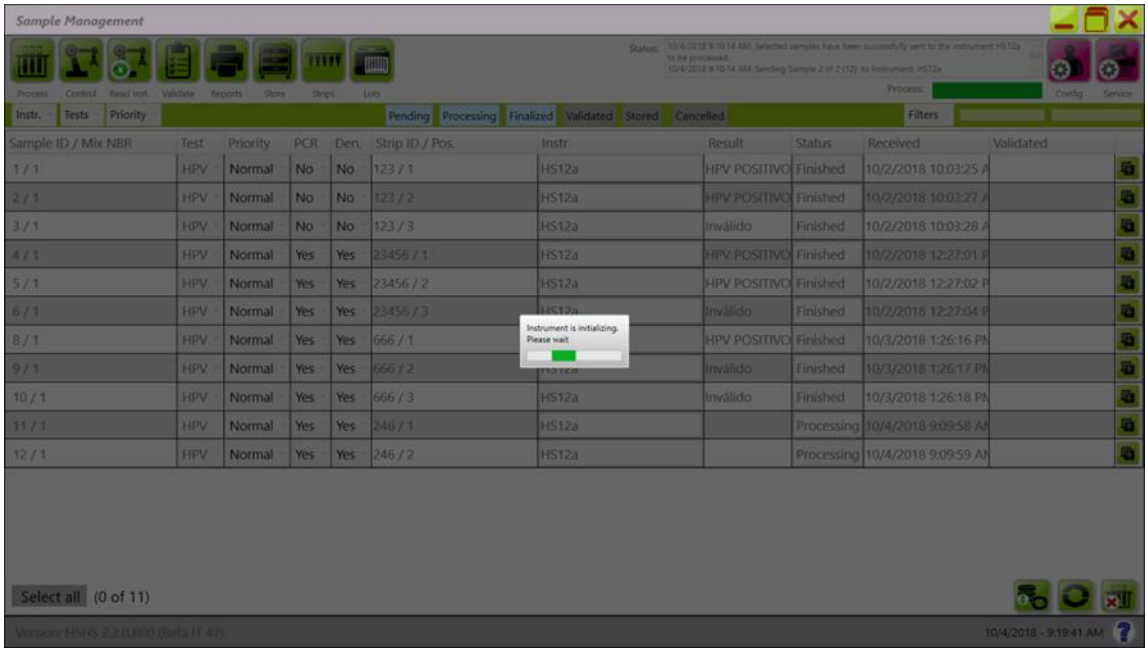


Figure 120. Block of the main window due to samples being read.

8.6.1 Reagent states

The verification of the reagents is carried out by reading the different QR codes located at the top of each of the vials. The state of the reagents is determined by the information obtained.

Valid Reagents

The valid reagents appear in light green. They are the reagents registered on the system and are not expired. They contain a sufficient number of test and are compatible with the selected test.

Valid Manual Reagents

The valid manual reagents appear in dark green. They meet the same requirements as the valid reagents, but the user must select their position manually. It is necessary to have these two scenarios:

- Error in automatic detection.
- Free and canceled positions.

If any of the reagents are not detected automatically, a message will be displayed stating that some reagents are missing. In this case, they can be placed manually as follows.

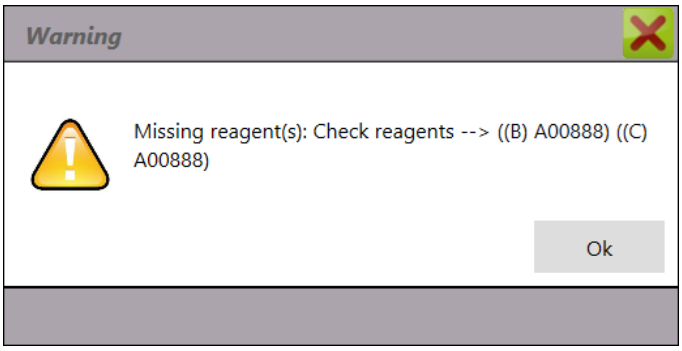


Figure 121. Missing reagents.

Click on the space where the reagent is placed. A window will appear with the following options from a drop-down menu:

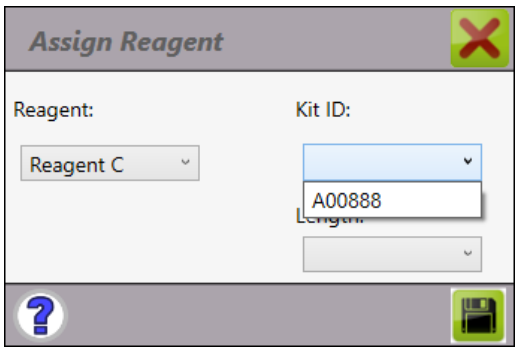


Figure 122. Reagents assigned manually.

1. Reagent: drop-down menu with the non-detected and necessary reagents to perform the protocol. Once added manually, it disappears from the menu.
2. Kit ID: identification of kits/kits placed and necessary for the protocol.
3. Vial size: it corresponds to the vial's length. It contains the options big and small. It is necessary to indicate the size of the vial to know the maximum lowering distance available.
   When saving without having all the options filled-in, the following message appears:

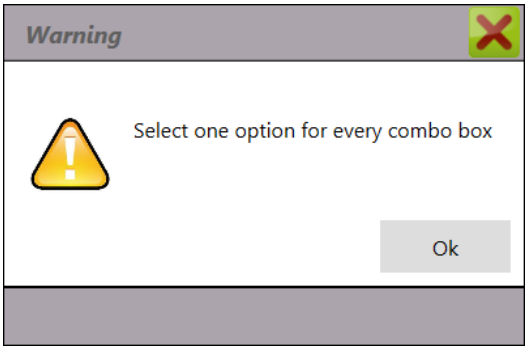


Figure 123. Lack of selection from drop-down menu warning.

If the not detected reagent is a reagent A, a message appears, stating "Missing reagent: A", Click on "OK". Afterwards, the following message will pop up:

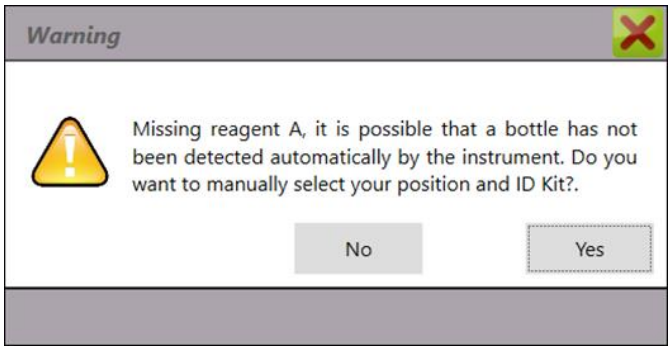


Figure 124. Manual positioning of reagent A warning.

After clicking “Yes”, select the position of the vial of the reagent A and, later, a message appears to select, among all the kits registered, the kit to which the reagent A placed in the instrument corresponds. In this message, the number of tests requires appear, which allows selecting the kit that contains a sufficient number of tests to run the protocol.

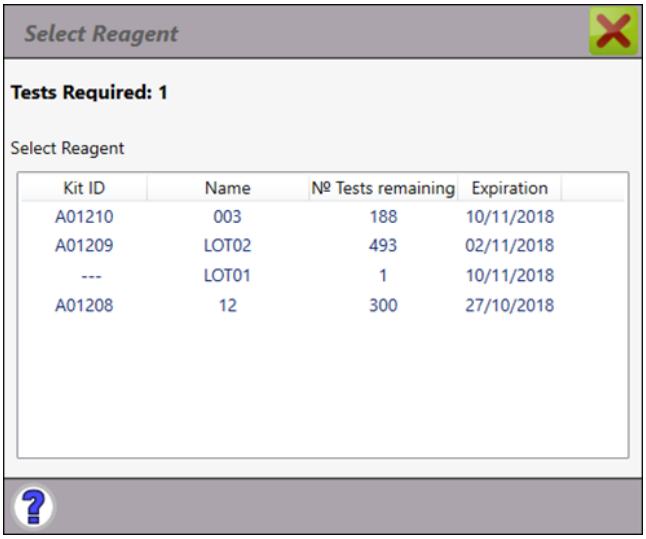


Figure 125. Kit ID selection.

On the contrary, if you click “No”, you cannot place the reagent A manually and the protocol is canceled.

Once all the reagents have been introduced manually, the protocol continues. If the number of tests of the kit is not sufficient, the protocol is canceled.

Not Registered Reagents

The reagents that are not registered appear in yellow, indicating that the kit the detected reagent A belongs is not registered in the system. In this case, a message is displayed stating that the reagent A is missing.

If clicking on “OK”, another message appears, allowing the user to register the new kit on the system.

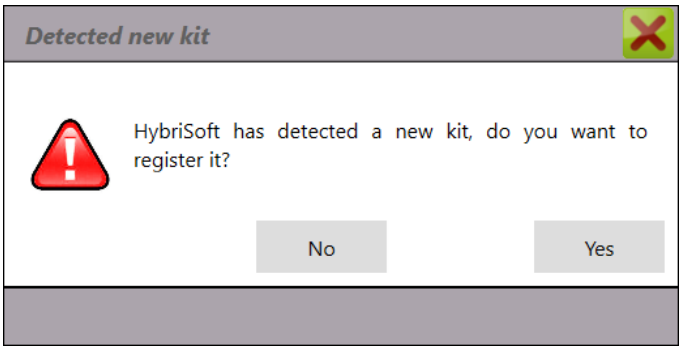


Figure 126. New kit detection.

If clicking on “No”, no changes are made and the initiated protocol is canceled automatically. If clicking “Yes”, the window “new kit” appears.

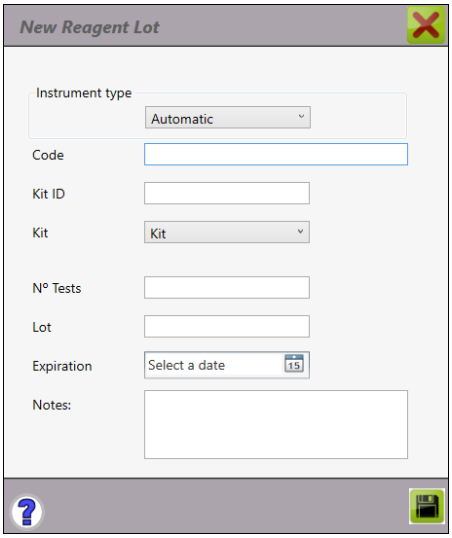


Figure 127. New kit window.

In this window, you can register the kit and start the protocol again without leaving the HS control window.

Expired Reagents

The expired reagents appear in light orange. For the system to detect that they are expired, it is essential that the kit is registered on the system (see section 8.4.1.1, sub-section Add lot).

If the "Automatic lot expiration" option is activated in configuration, the system allows you to use the expired kit for the time selected in the configuration. In this case, a message appears, asking if you want to use the expired kit.

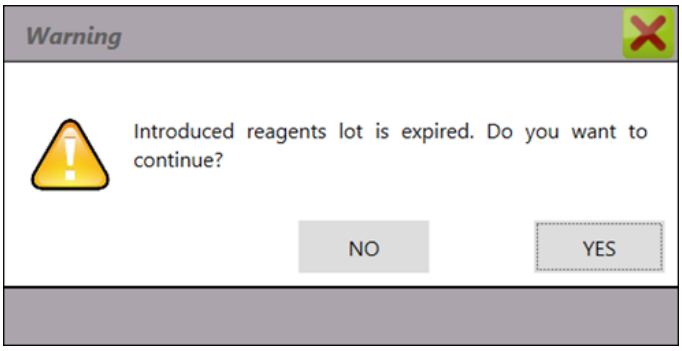


Figure 128. Detection of expired reagents.

When clicking on "Yes", the reagents change from orange to light green (valid) and the protocol continues. On the contrary, by clicking on "No", the reagents' color do not change and the protocol is canceled so that the user can replace them with other reagents.

In case the option "Automatic lot expiration" is disabled, the system cannot use expired kits. **The supplier won't take any responsibility for results obtained with expired reagents.**

Unnecessary Reagents

The unnecessary reagents appear in white. They are placed in the instrument, but they are not necessary to run the protocol.

Empty, inactive or incorrect reagents

Empty, inactive or incorrect reagents are marked in red. These reagents do not contain a sufficient number of tests, are inactive in lot management or are not placed in their corresponding position.

In all cases, the protocol is canceled to perform the corresponding change or correction, except if another valid kit is detected.

If the kit is empty, it is necessary to place one or two kits with a sufficient number of tests to perform the protocol.

If the kit is inactive, a message appears asking if you want to activate the kit.

If the reagents are not correctly placed (reagent A in the reagent A zone and the rest of reagents in the rack) the reagent appears marked in red in the control window and in the status box it is indicated that the position is wrong.

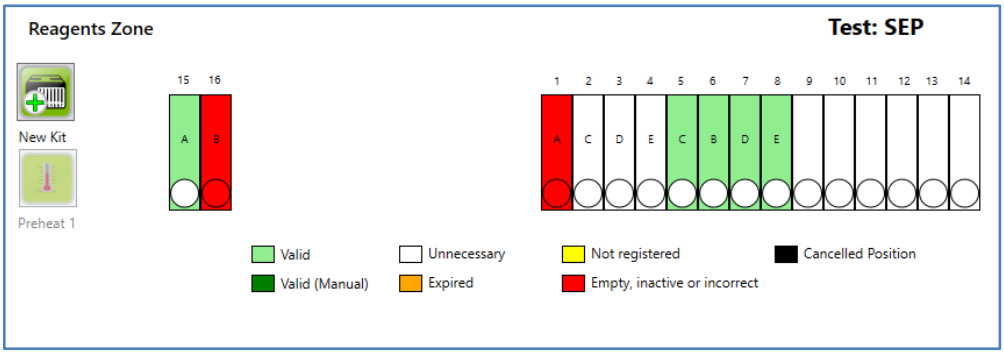


Figure 129. Wrongly positioned reagent.

On HS12a, two kits at most can be placed per protocol. In order to determine the state of the kit and select its priority use, the expiration and the test no. are taking into consideration. Therefore, a kit with a closer expiration date and a lower number of tests is used first.

If a kit has a closer expiration date, but the test number is higher than the other kit with a higher expiration date, the priority of use is selected by the user and the following message appears:

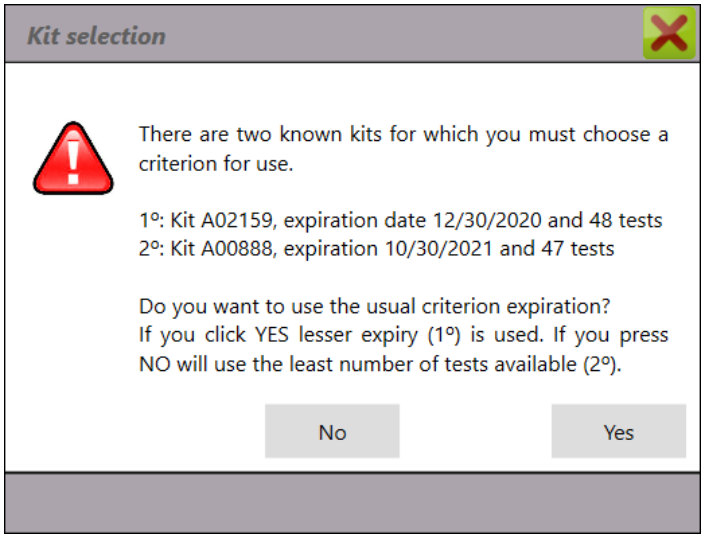


Figure 130. Selection of kits by the user.

With the option "Automatic lot expiration" active, when there are two kits placed, one expired and another without a sufficient test number, a question appears to confirm the use of the expired kit.

If between the two kits you have a test number equivalent to the number of samples, both are used.

In case some of the vials of the reagents have caps, these appear marked in blue in the reagents zone. It is important that the blue tags are not detached from the caps. Otherwise, the software will not be able to identify them. Once they have been read, the following message appears:

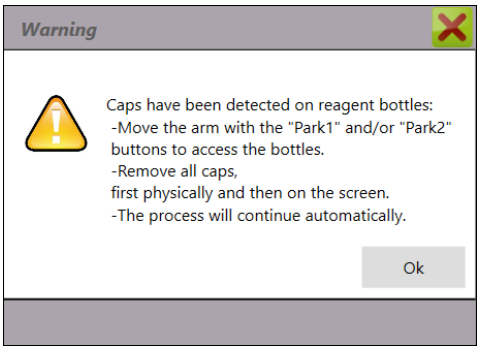


Figure 131. Instructions to remove caps.

Perform the following actions to remove physically and on the screen the detected caps.

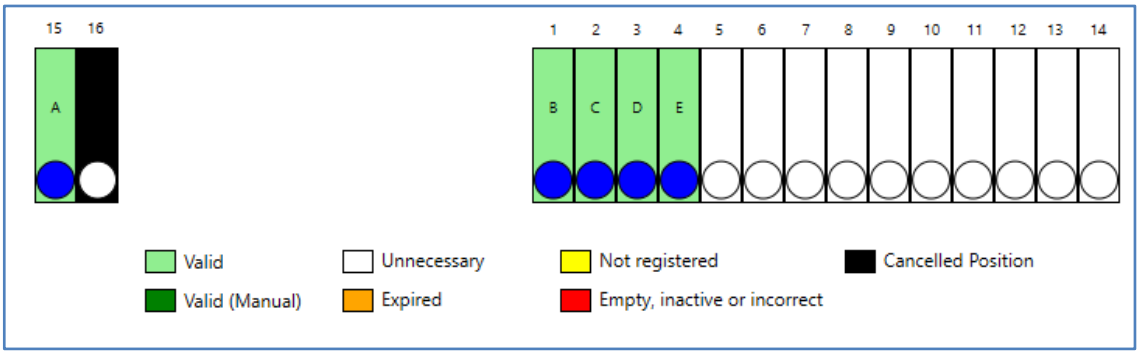


Figure 132. Caps detected in the HS control screen.

Click on the blue cap that appears in the reagents zone. A pop-up window will appear asking if you are sure you want to remove the cap. Press “Yes” and the cap will be removed. If you press the “No” button, the cap will be remained on the bottle and the protocol will not start.

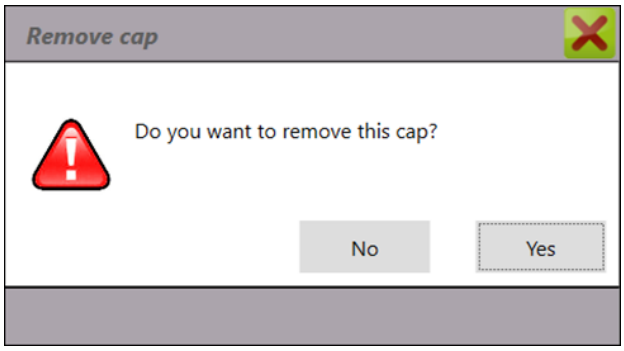


Figure 133. Removing cap from the control screen.

Once all the detected caps have been removed, the protocol will automatically start.

When placing the cursor on the detected reagent in the hS Control window a box with the information on the kit will appear. This contains the state of the kit, the test, the identifier, the lot, the expiration date and the number of test.

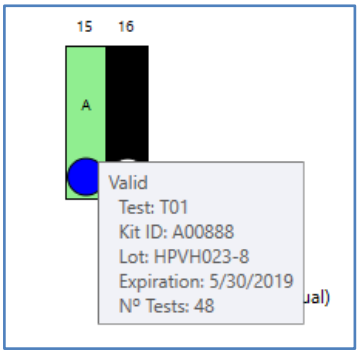


Figure 134. Kit information.

In the "Status" window, the information obtained from reading the QR codes of the vials is displayed. It shows the status of the detected vials.

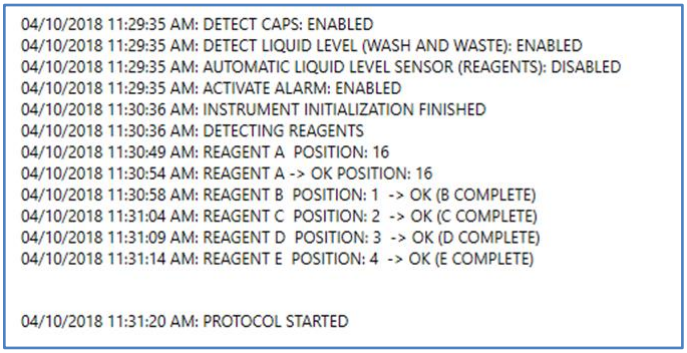


Figure 135. Information on reagents in the "status" box.

Once the identification of the reagents has been performed, the sample management window will be unlocked, allowing to perform any action.

While the protocol is running, you cannot close the Sample Management screen without first closing the control screen. In this case, the following message appears:

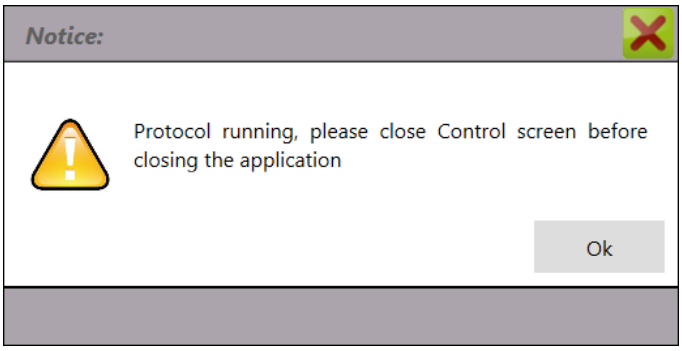


Figure 136. Running protocol warning.

In the status box will be shown each of the steps that the HS12a instrument is taking. If the AutoScroll is active, the lines will progress automatically, but if it is disabled, the user has to go to the end of the box with the movement bar.

When pressing the “X” button to close the hS control screen during the protocol, the following “Warning” window will appear:

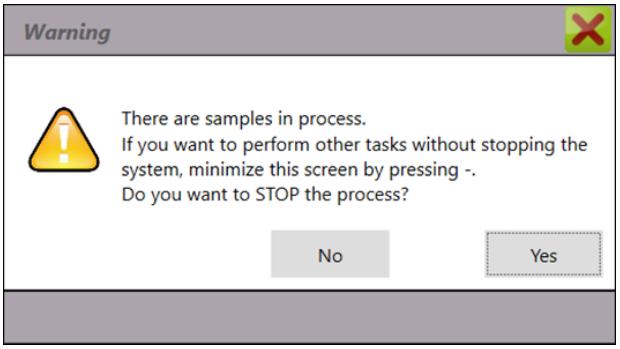


Figure 137. Samples in process warning.

If you click on "No”, the protocol continues. If you click on “Yes”, the protocol is canceled, remaining the samples in a “Processing” status.

If you click the “STOP” button, during the process to stop the protocol, a window appears to confirm the action:

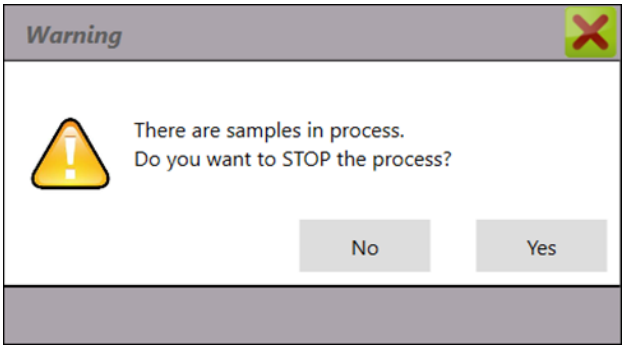


Figure 138. Stop process warning window.

In this moment, the protocol is paused until select one of the two options. If you click “No”, the protocol continues, if you click “Yes”, the protocol will stop. Then, a message displaying the possibility of restarting HybriSpot will appear:

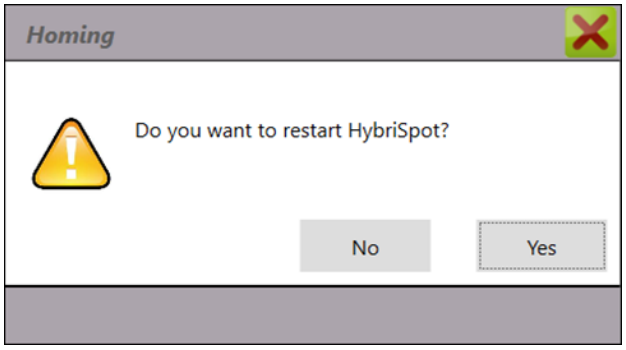


Figure 139. Restart instrument warning.

If you click “Yes”, the instrument performs a positioning, searching the start of each of its axes and components. Otherwise, the robotic arm is directed to the initial position if required.

When stopping the protocol, there are situations in which positioning is obligatory. In this case, the following warning message appears:

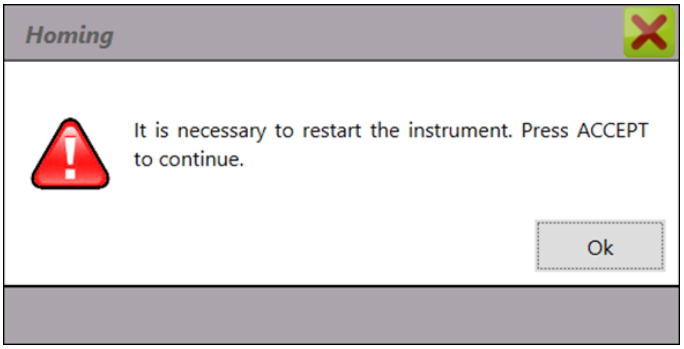


Figure 140. Restart instrument warning.

With the start of the capture until the finish of the protocol, the Sample management window will be blocked again. The analysis of the images is carried out in parallel with the last wash step.

Once the protocol has finished, the status box shows the elapsed time. A pop-up screen appears indicating that the results have been taken from the instrument and recommending the UV lamp activation before opening the instrument door (see section 8.8.1). Along with the pop-up window, an alarm is triggered, warning about the protocol finalization. (See 8.3.1.5 section Activate Alarm).

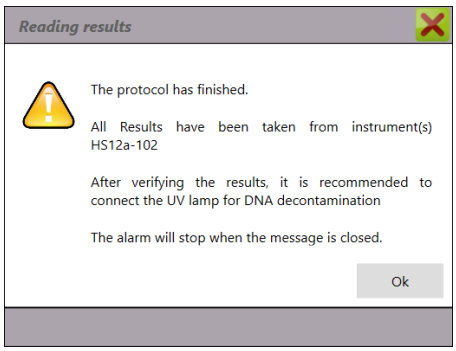


Figure 141. Protocol finalization warning.

Once the protocol has finished, the management window will unblock.

This is an informative screen, when pressing "Accept" no changes will be made.

At the end of the protocol, the washing station will be flooded with the help of the dispensing probe for the instrument's maintenance.

8.7 Results Analysis

Once the protocol is finished and the images are analyzed, the samples change from the "Processing" status to a "Finished" status.

70 / 1	HPV	Normal	No	No	14 / 1	HS12A	Invalid	Finished	03/10/2018 1:32	
71 / 1	HPV	Normal	No	No	14 / 2	HS12A	Invalid	Finished	03/10/2018 1:32	
72 / 1	HPV	Normal	No	No	14 / 3	HS12A	Invalid	Finished	03/10/2018 1:32	
73 / 1	HPV	Normal	No	No	14 / 4	HS12A	HPV POSITIVE	Finished	03/10/2018 1:32	

Figure 142. Finished samples.

To access the detailed results of each sample, press the "Sample Details" button. A window appears with all the detailed information about the samples and the process applied. On the sides, there are arrows that allow you to move from one sample to another without leaving the "Sample data" window. Then press the "Results Details" button, which will give access to the "Picture Processing" window. This button only appears if it is configured in "Configuration" (see the section 8.3.1.5).

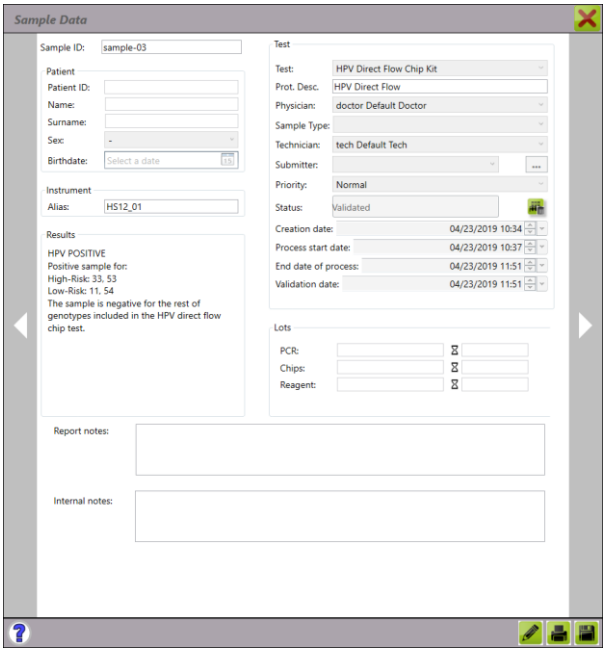


Figure 143. Sample details.

Automatically, all captured and analyzed images are stored in the folder C:\hybriSoft\RESULTS in .JPG format and identified as “sample_ID Sample” and “sample_ID Sample_processed”.

8.7.1 Picture processing

In this window, the picture analysis, the parameters with which it has been analyzed, the results report and arrows in movement between samples appear in detail.

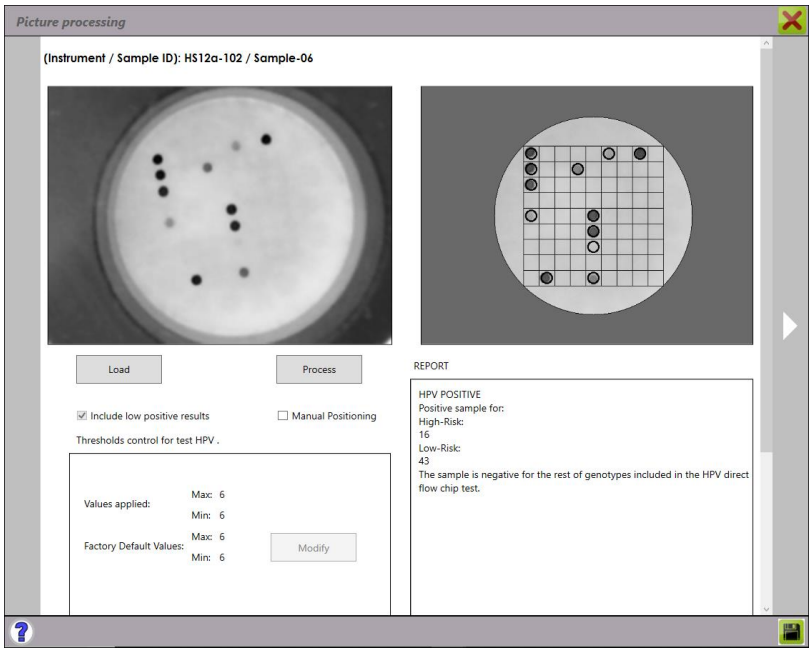


Figure 144. Picture processing window. (HPV)

- **Membrane images:**
  - The left image corresponds to the captured image.
  - The right image corresponds to the processed image. This image appears centered, rotated, with a grille placed on the image and with the detected points marked with black circles. The user can manually check/uncheck the image points of the analyzed image on the right, which will cause a change in the analysis results. This change will be reflected in the report. These points are marked in red.
- **Load:** It allows opening an image, with the required format (.JPG), previously captured and assigns it to the sample manually. Thus, an image taken in another moment can also be analyzed (see section 8.7.1).
- **Process:** This button allows to unlock the processed image to make changes in the result or make the analysis of a new image. Once the image has been unlocked, we can add or delete any point of the test sample.
- **Threshold control for the test XXX:** the thresholds applied to the sample and the one established by the manufacturer appear. Being “XXX” the acronym of the test applied to the sample.
- **Report**: The sample's analysis result appears. If it is manually modified, this result changes by introducing the modification. This modification is shown in red with an asterisk and a note at the end of the text clarifies the meaning.
- **Save button:** This button keeps the modifications saved in the image results, i.e., the markers manually selected by the user (Figure 150). Or the changes made in that sample's thresholds (Figure 145).

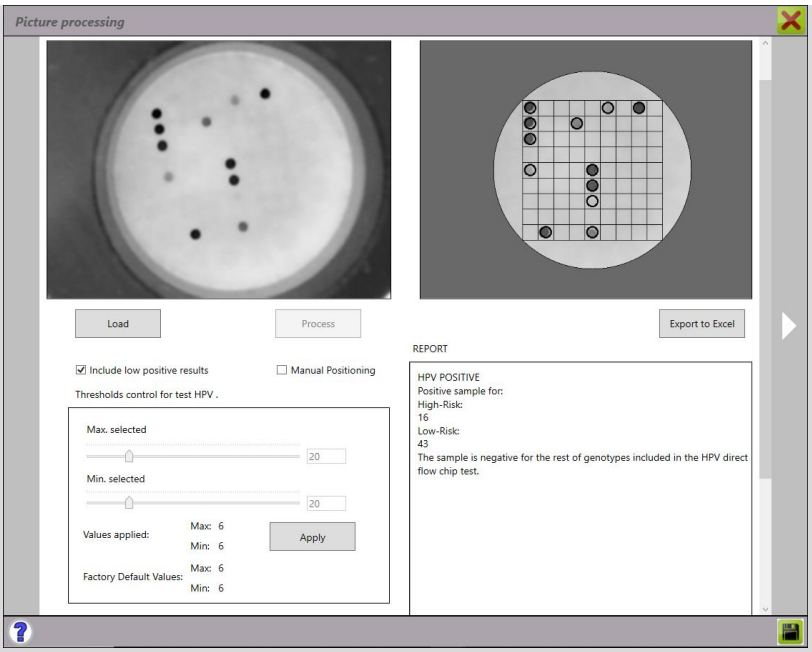


Figure 145. Threshold change.

The changes in the thresholds can only be made by the “Administrator”. To access with this user, we must get in touch with the supplier. In order to make a change in the thresholds, we need to follow the next steps:

- Unlock the image pressing the "Process" button.
- Enable the threshold change by pressing the "Modify" button in the right of the threshold control box. Some bars corresponding to the thresholds appear.
- Establish the new values to be applied to the sample.
- Press the “Apply” button for the change to be applied to the sample
- Save changes.
- The changes saved will be the threshold values of the test by default of the sample processed.

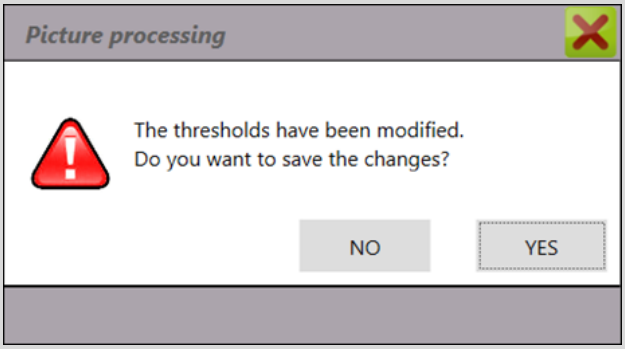


Figure 146. Threshold change warning.

Warning: *This change in the threshold will only be applied to the sample in which they have been made. It is not possible to change the thresholds established and validated by the manufacturer for each test. If you wish to re-establish in this sample the manufacturer's thresholds, we need to take the same action but establishing the manufacturer's thresholds indicated in the "Threshold Control” box.*

- Exit button
- Help button

Important note: ***The manufacturer is not responsible of the results reports generated with***

any of the changes manually introduced by the user on the automatic analysis performed by the software.

8.7.1 Interpretation of the results

The interpretation of the results is automatic. There are five general interpretations:

- Negative Result: negative sample for the analyzed markers.
- Positive Result: positive sample for one or more of the analyzed markers.
- Invalid or Blank result: sample with absence of any PCR control point. Only the control points of hybridization appear (5 points)
- Hybridization error: absence of points inside the grid. All the hybridization controls do not appear.
- Not available Result: sample that contains at least the five points of the hybridization control and with the impossibility of automatic analysis for defects on the picture, membrane or both. In these cases, the software cannot position the picture correctly. To solve this problem, there is the possibility to perform a "Manual Positioning" that would solve the automatic analysis in most cases.

Within each sample's positive result, there are kits that are grouped by pathogens and resistance genes, so that the positive marker for each of the groups associated to each test is shown.

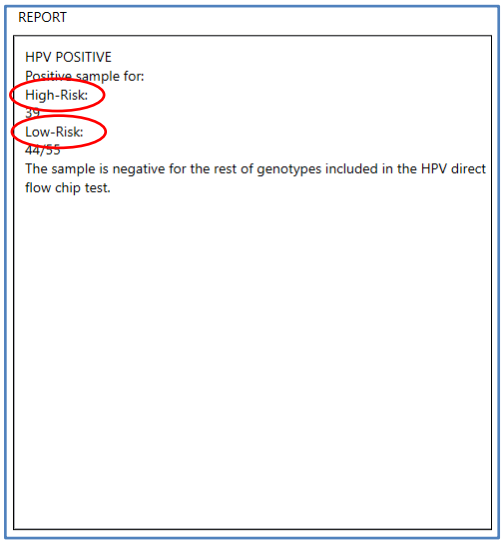


Figure 147. Groupings in a test.

***Important note:** this interpretation is general and illustrative. To interpret the results correctly, you have to refer to the specific interpretation in the data sheet of each of the kits.*

The manual positioning consists of the marking of two out of five points of the hybridization control, that are the points that the software uses as a reference to position and orientate the membrane and correctly interpret the picture. This function is only available in finished samples.

To do a manual positioning it is necessary to enable the function "Manual Positioning" that appears in the Picture Processing window. You can find it under the "Process" button. Once the function is active, two points must be marked. These correspond to the upper left point and the centered point (selected

points marked in white) of the five points of hybridization control of the picture (points marked in red). It is recommended that the points marked match the centered point.

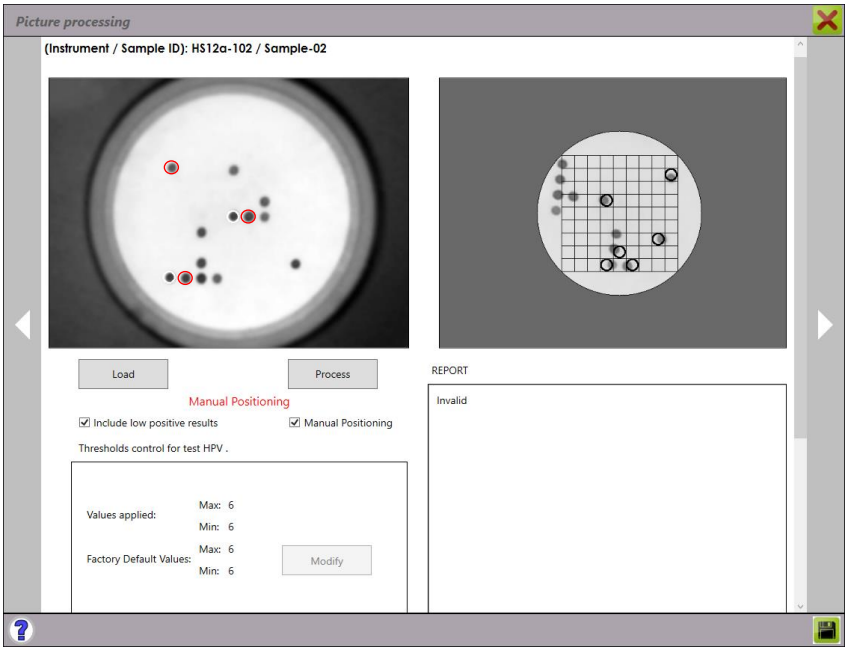


Figure 148. Points marked in manual positioning. Correct positioning.

Once all the indicated points have been marked, press the button “Process”. This allows the software to perform an automatic interpretation of the picture. The picture will appear oriented with the grid positioned on the right side of the window. In case you see the points out of its position or too off-centered to the grid (Figure 149), the manual positioning has to be repeated by trying to center the marked points (Figure 148).

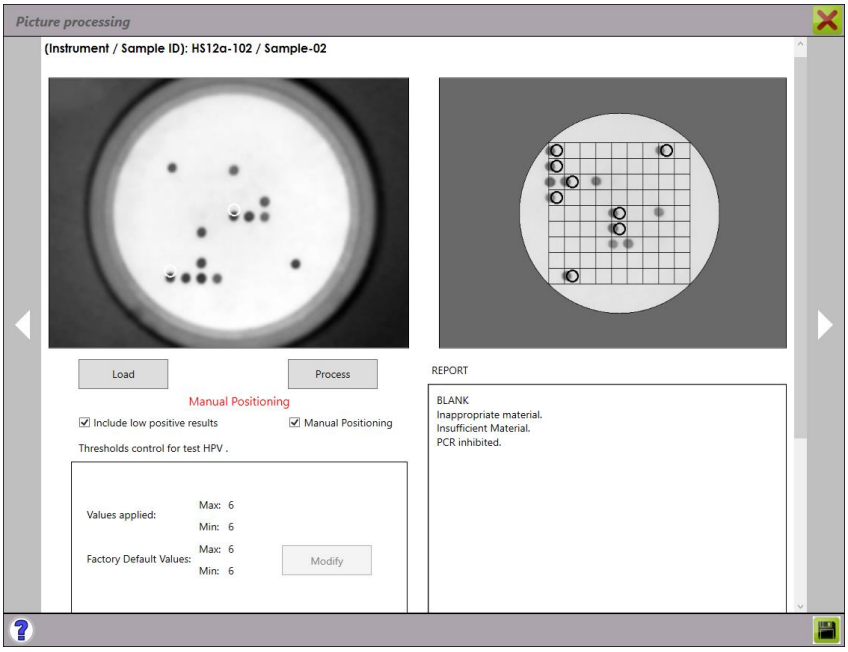


Figure 149. Selection of off-centered points. It can be seen that the points are very close to the grid’s limit.

When a point is marked manually in the array, it appears in red. If the marking of these new points generates a new marker in the “Report” section, this will be shown in red followed by an asterisk, along

with a legend: (*) “Included when marking points manually”.

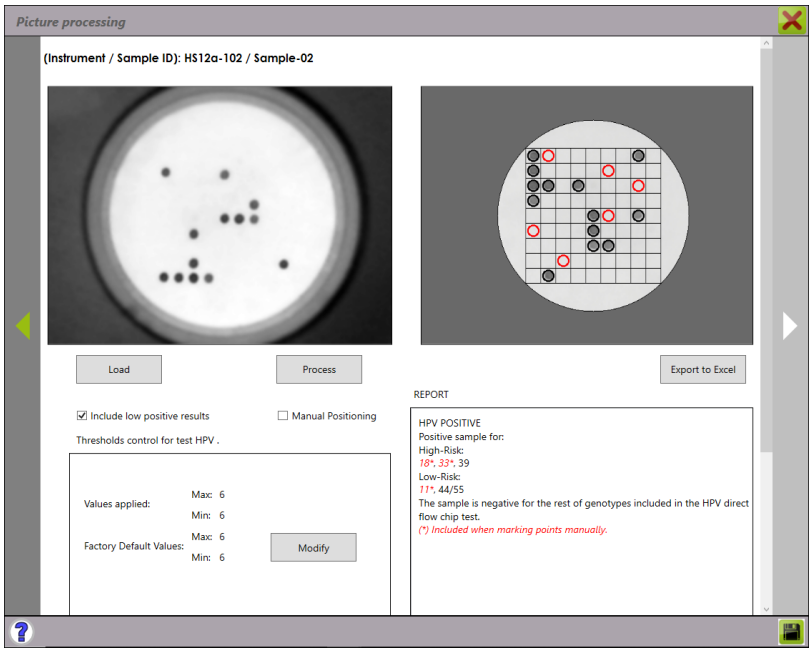


Figure 150. Points selection in the manual array.

When marking points without clicking on the “Process” button previously, the following message will be displayed:

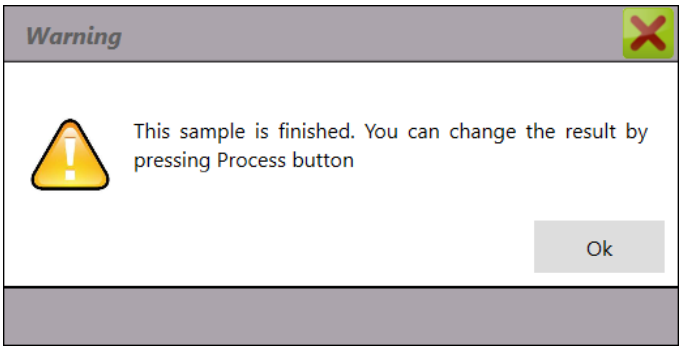


Figure 151. Image unblocking warning.

When clicking the sample navigation arrow without saving changes, the following message will be displayed:

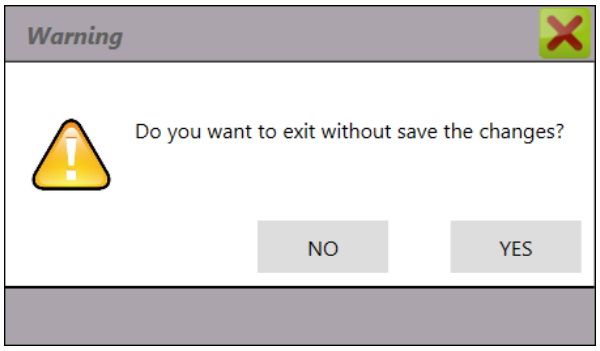


Figure 152. Unsaved changes warning.

In order to keep the changes made, click on “No”. Otherwise, the changes made will not be saved.

When clicking on the “Save” button, the following message will be displayed:

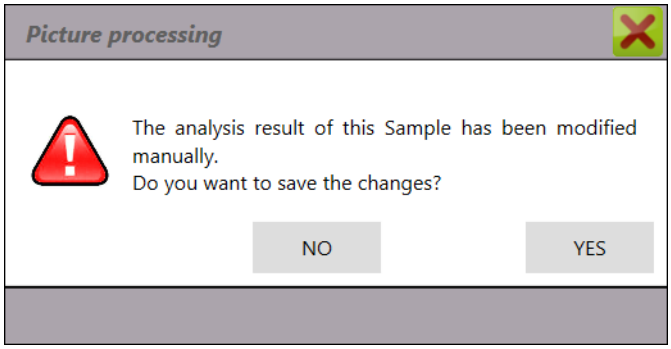


Figure 153. Save changes confirmation.

<u>Important note:</u> The manufacturer is not responsible for the results reports generated with any of the changes manually introduced by the user on the automatic analysis performed by the software.

In case a hybridization or image processing error occurs, the result of the samples to be processed will be the following:

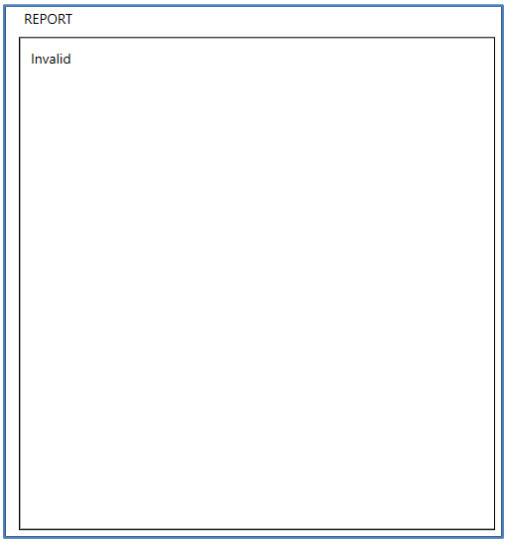


Figure 154. Hybridization or image error.

8.7.2 <u>Validate Results</u>

After checking that results are correct, in order to generate the individual report, you need to validate them.

In order to validate them, select the samples in a finished status and press the Validate button.

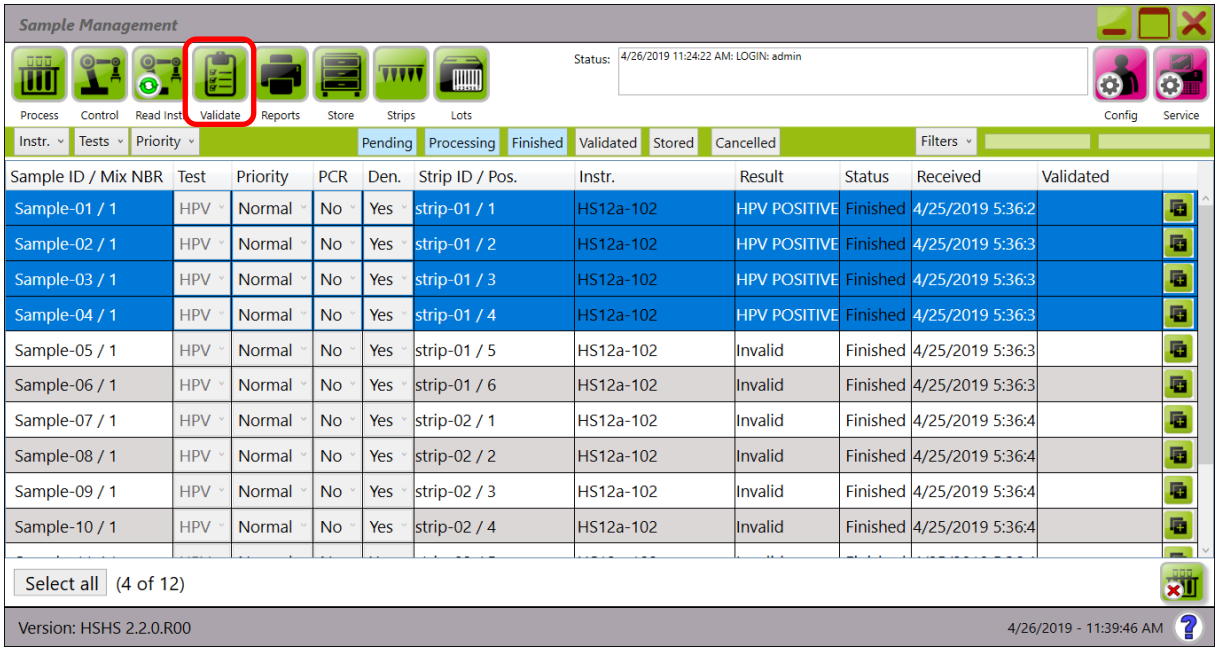


Figure 155. Selected samples to be validated.

The “Validation Parameters” window will be opened. You can only access this screen if at least one of the selected samples is in a "Finished” status.

There is the possibility to choose the actions to make to the selected samples (see point Selection of actions that can be found in the section 8.4.3.1). The action “Capture images” in this section is inactive, since this function can be performed in this section.

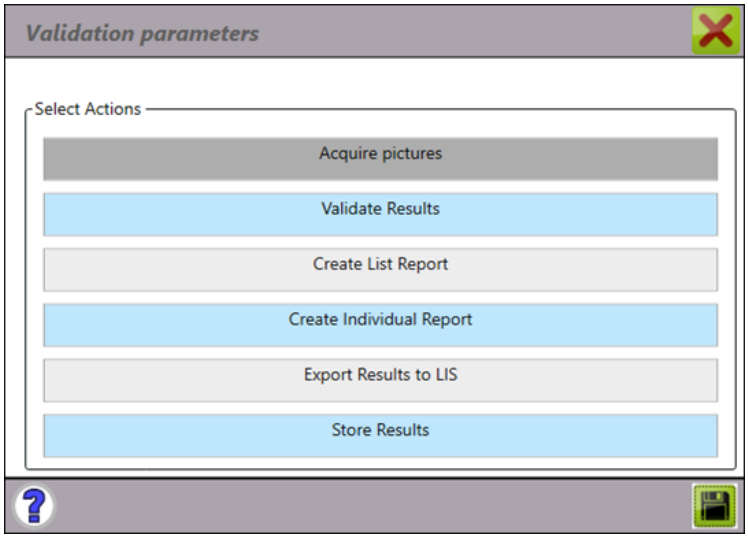


Figure 156. Validation parameters.

Once all the samples have been validated, the status of the samples will change to “Validated” and the date and time in which they were validated will appear.

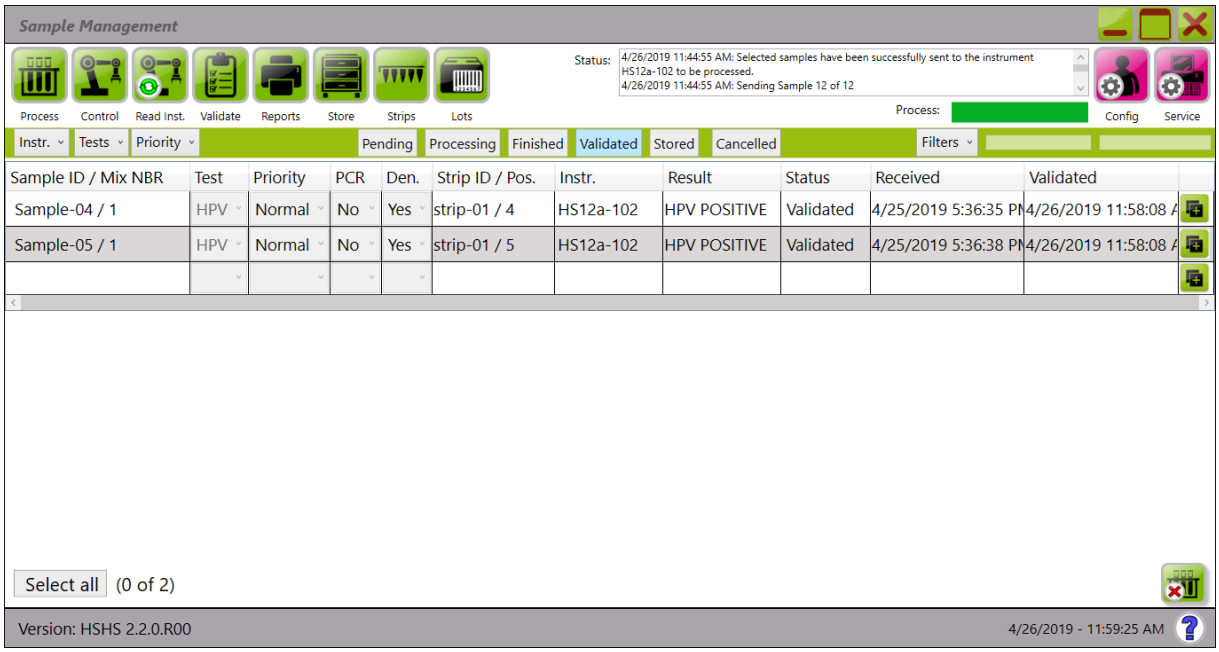


Figure 157. Main window with the active "Validated" filter.

8.7.3 Reports

It is possible to obtain reports from validates samples and lists with summarized data of samples in any status.

Select the samples from which you wish to obtain a report.

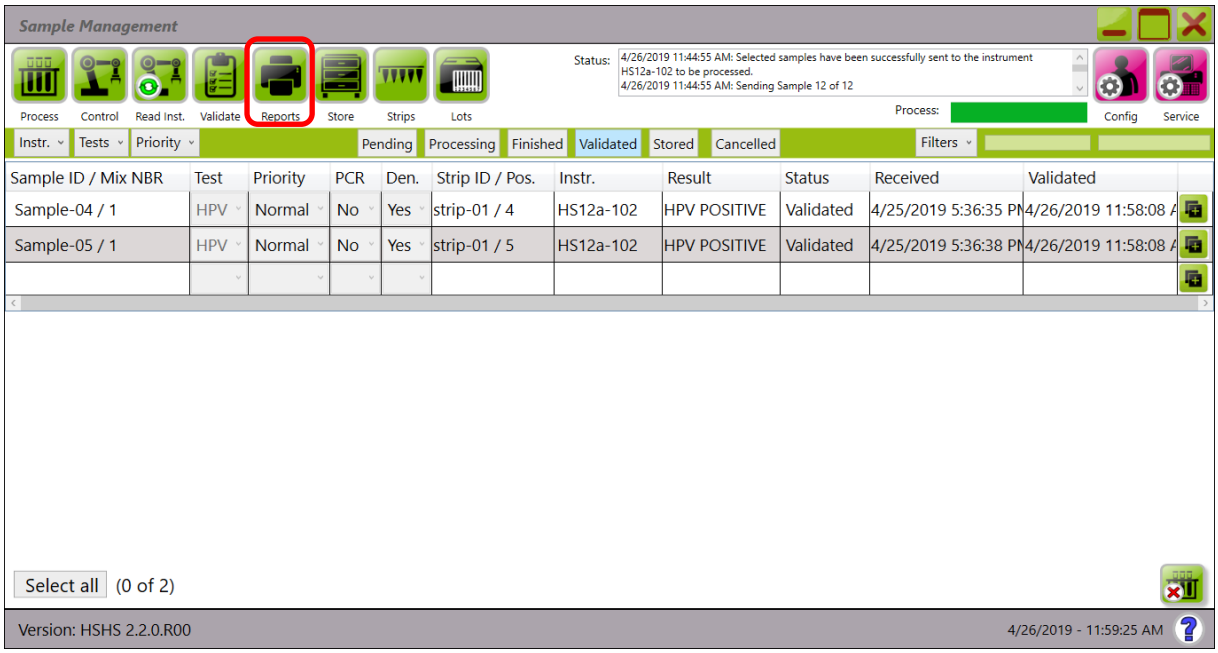


Figure 158. Main reports window.

If the samples are validated, the following "Results" screen appears:

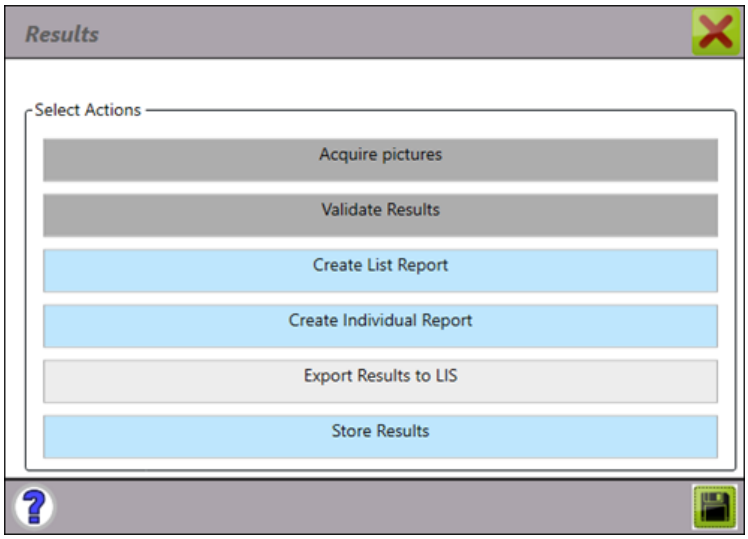


Figure 159. Actions to perform in validated samples.

When selecting the action “Create List Report", a document with the results of all the selected samples will be generated.

Sample ID	State	Sample Received	Process Finished	Result
Sample-04	Validated	4/25/2019 5:36:35 PM	4/25/2019 6:07:36 PM	HPV POSITIVE

Details of the result:

HPV POSITIVE
Positive sample for:
High-Risk: 66
The sample is negative for the rest of genotypes included in the HPV direct flow chip test.

LOTS => PCR: HPVU058-7 / Chips: HPVE027.3 / Reagent:

Figure 160. Reports list.

The list remains automatically stored in C:\hybriSoft\REPORTS with the name “list_<day>_<month>_<year>_<hour>_<minute>_<second>.pdf”

The order in which the samples are displayed when you create the report list is as follows:

- All validated samples will come out first regardless of when you select them. If you select two or more, they will come out depending on the one you selected first.
- All other statuses will appear later according to the order of selection.

When selecting the action "Create individual reports", a report containing detailed information of the patient and the sample is generated. A PDF document is generated for each selected sample. As stated above, this list also specifies the type for pathogens that have been positive.

This report is automatically stored in C:\hybriSoft\REPORTS with the name “report_<Sample ID>.pdf”. You can only create individual reports of the validated and stored samples.

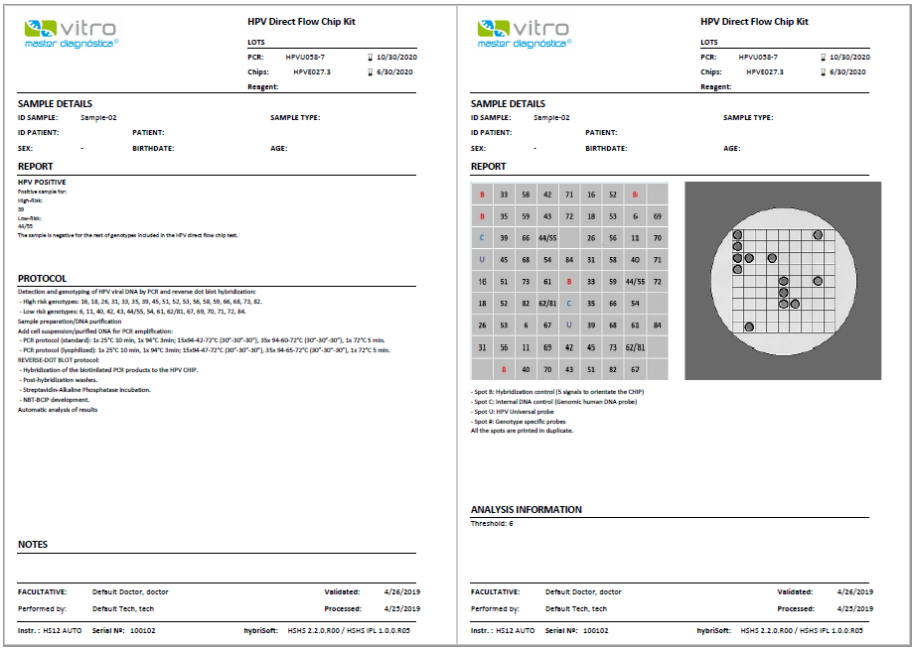


Figure 161. Extended report.

***Note**: **Adobe Reader v18.011.20040** is recommended as the default reader for pdf files which is installed with the previous HSHS applications. The report generation has been tested with this version, any other version could work, but it is not verified.*

Further information of the report (processed image, template and template's legend) and of the analysis only appear if it is configured (See section 8.3.3). With the "Include instrument and software versions" option located in the "Report configuration" tab of Configuration, the instrument used and the versions of hybriSoft and IPL will be displayed in the footer of the report.

If the analysis is modified manually in the image processing window, the user is informed in the "NOTES" field of the individual sample report. Only samples in a Finished status can be manually modified by reprocessing the sample:

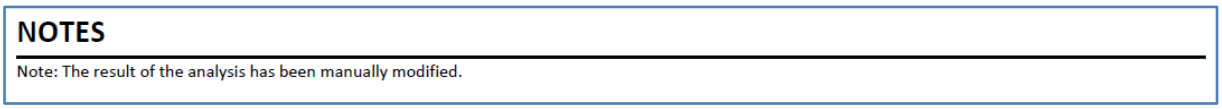


Figure 162. Information about manually modified analysis.

If it didn't contain the required files to create this type of report, a pop-up screen would appear indicating that the report cannot be printed.

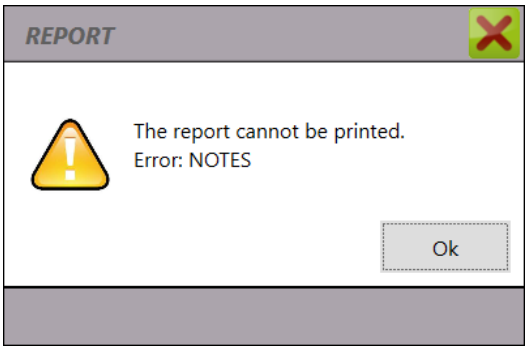


Figure 163. Information window.

If the individual report has already been generated but its location has been modified, it is not created again, showing the following message:

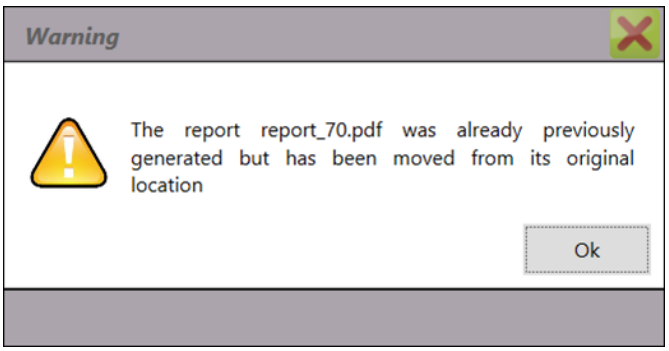


Figure 164. Report location change warning.

In case the picture has been removed from its location, the report is generated without it, displaying the following warning message:

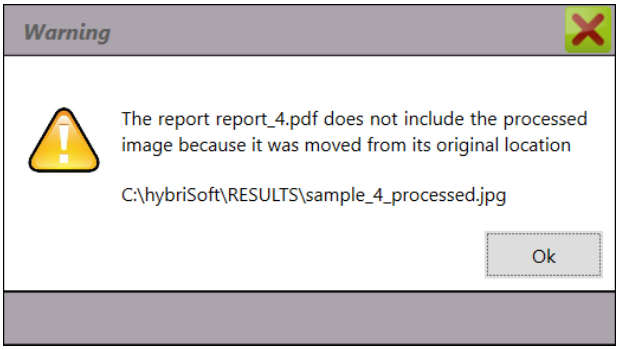


Figure 165. Sample picture location change warning.

A not appears in the report informing about the change of the picture location:

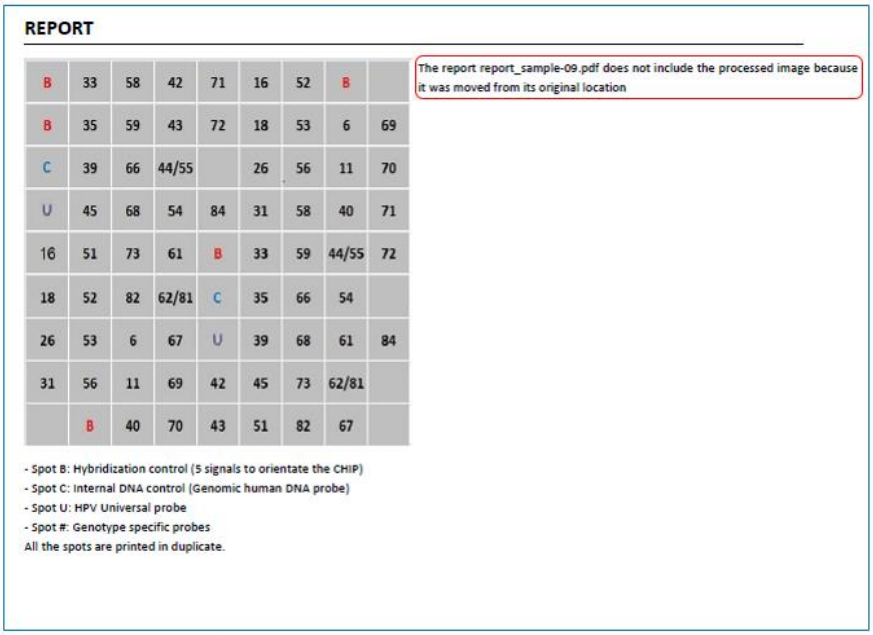


Figure 166. No image in report warning.

If the samples have a status different from validated, the only action that we can make is "Create List Report". First, the following informative message appears:

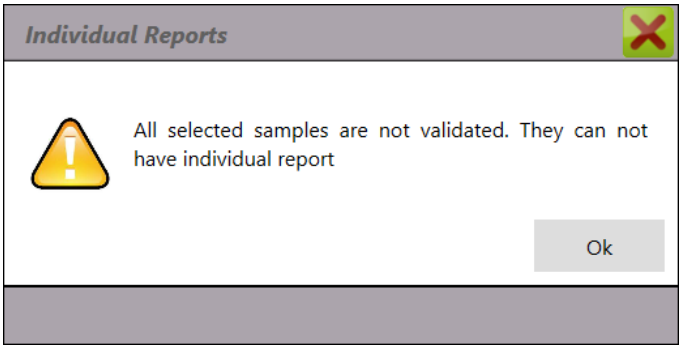


Figure 167. Lack of individual reports for completed and stored physical samples warning.

Afterwards, the "Results" screen appears with the action "Create List Reports" active. The rest of actions appear inactive (Dark gray), as they cannot be performed with these samples.

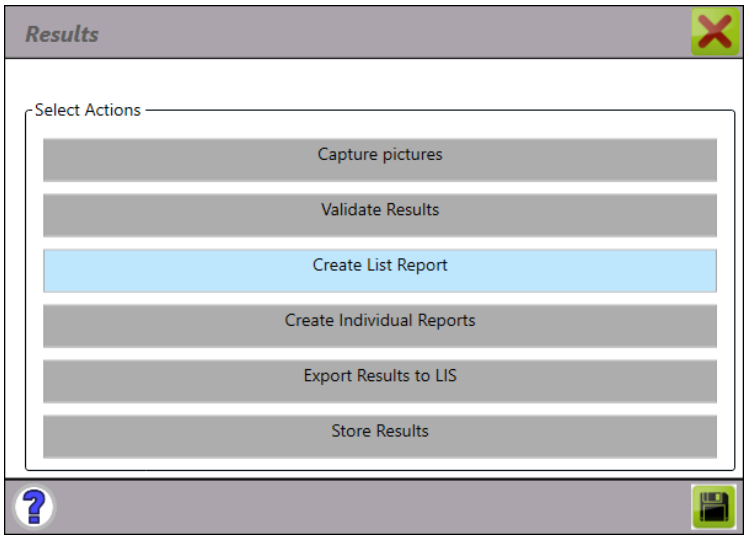


Figure 168. Reports list creation.

From here, you can obtain a list of any of the existing samples in your database.

If there are selected samples in a validated status and other samples with different statuses to this one, the following message appears:

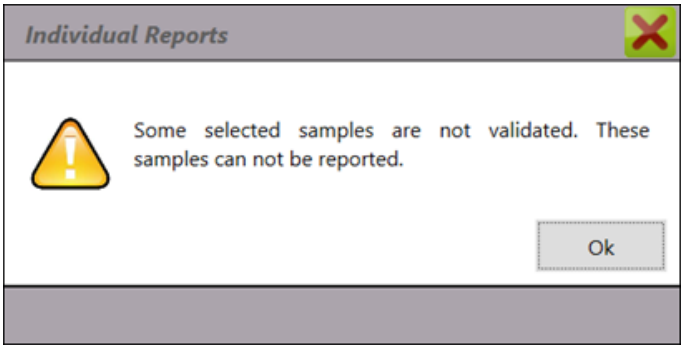


Figure 169. Individual reports for not validated samples.

After this, the "Results" window appears with the actions "Create report list", "Create individual reports" and "Store results" active and selected. “Capture images” and “Validate results” appear inactive (Dark gray), as they cannot be performed with these samples. “Export results to LIS” appears active, but not selected.

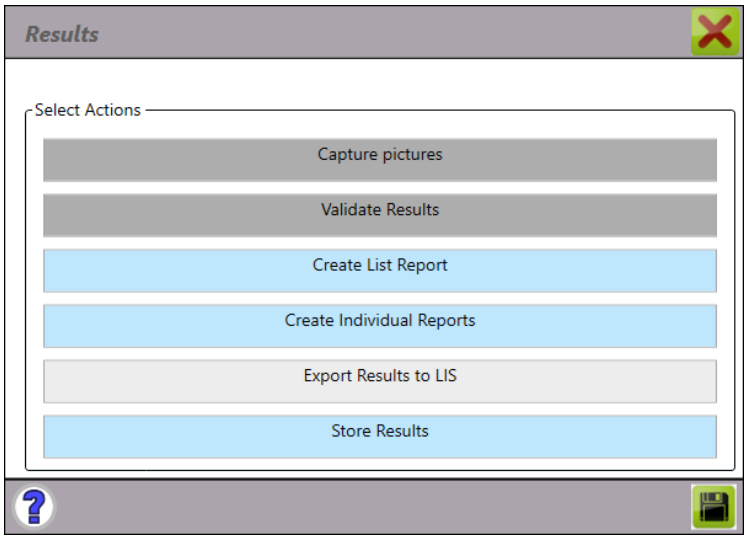


Figure 170. Results process parameters.

In the reports list, all the selected samples appear.

From this window you can perform all the actions depending on the sample status.

8.7.4 Export results to LIS

HybriSoft gives you the possibility of receiving samples from the user's LIS system. For this, you need to configure both systems to establish a communication between them. In order to configure hybriSoft with this option (see configuration, section “8.3.1.5”) the point “LIS connection enabled”.

Once the communication has been established, we can manage the sample sending to hybriSoft from the LIS. Automatically, these samples appear as “Pending”, in green color, in the Sample Management screen, to differentiate them from the ones created manually.

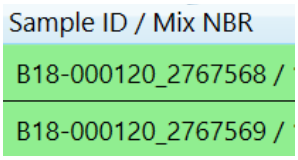


Figure 171. Samples from the LIS.

This sample will contain the information that has been sent from the LIS (patient, test, petitioner doctor…). The sample is processed and finished the same way that the manually created ones, (hybriSoft allows the simultaneous management and processing of samples from the LIS and manually loaded samples).

Results are sent automatically to the LIS system. A statistic interpretation of the results is also possible here. To send them the samples need to be validated and the option "Export Results to LIS” needs to be marked. The information previously configured in the system will be sent to the LIS.

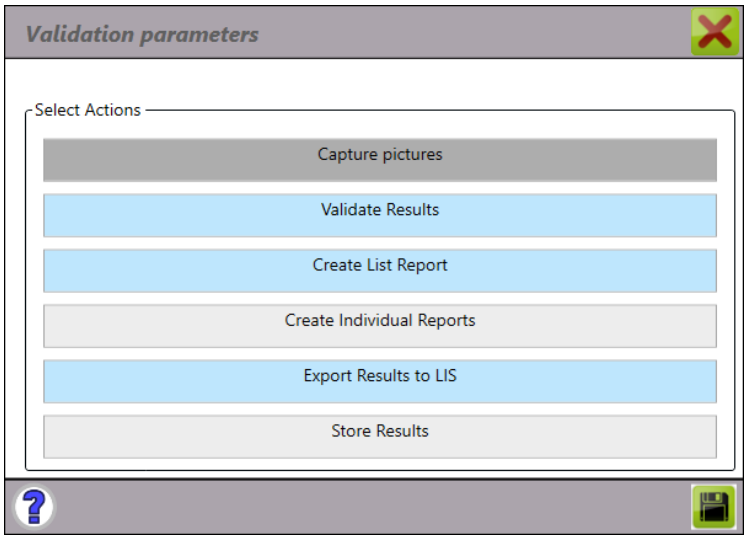


Figure 172. Export results to the LIS.

The sample will change into a “Stored status”. If the result has not been exported to the LIS, it won't be stored.

8.7.5 Store results

This function allows saving the results of all validated samples in the system and they will be accessible for future checks or to print the reports and visualize the captured and analyzed images.

Select the samples and press the “Store” button.

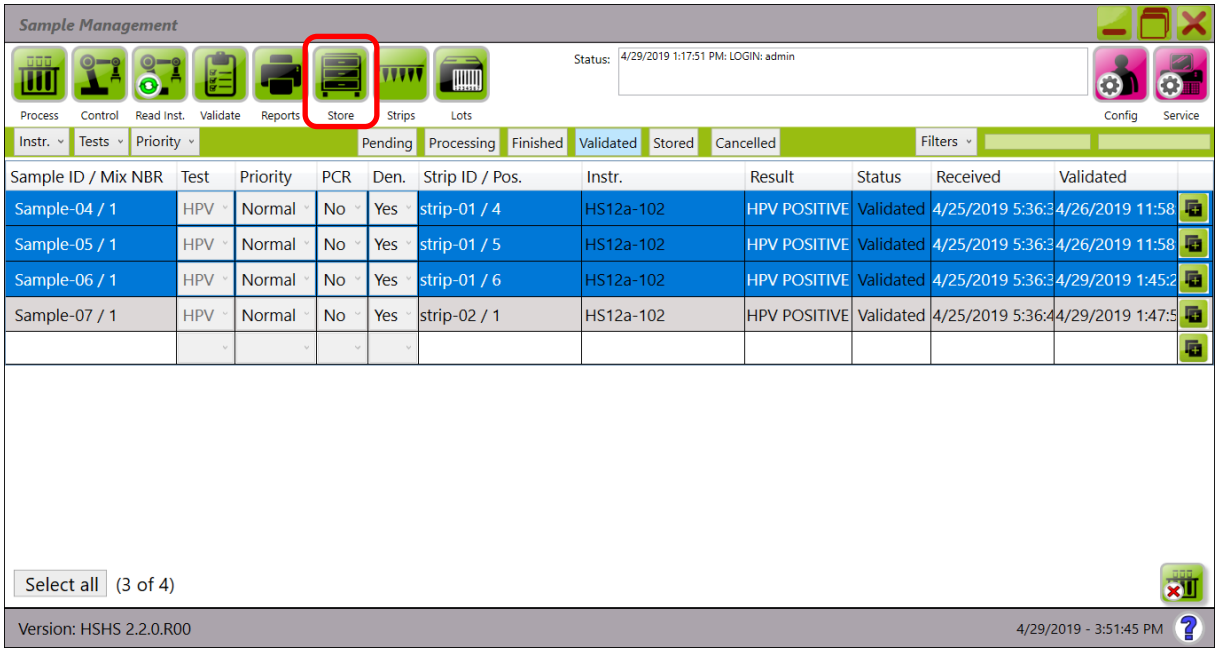


Figure 173. Samples storage.

The "Storage Confirmation" screen appears. In this window, the only active action is "Store Results". The rest of actions are inactive.

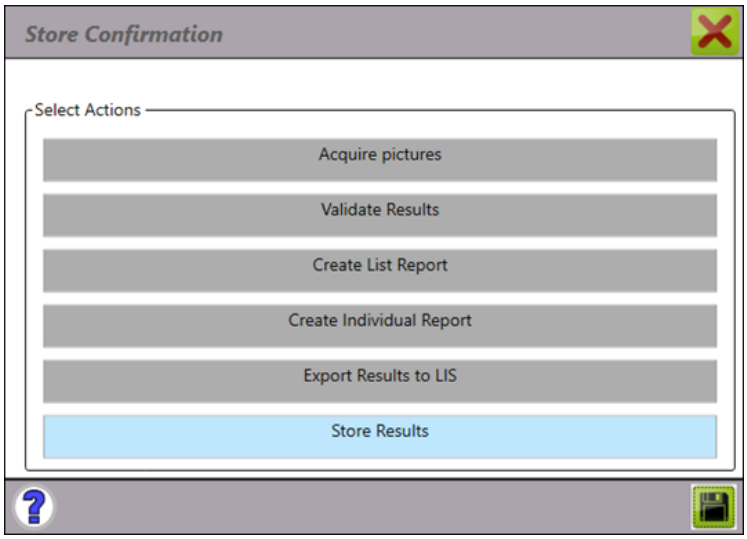


Figure 174. Storage confirmation.

This window can only be accessed if the selected samples are in a "Validated" status.

In order to print an individual report of the "Stored" samples", we need to access "Sample details" and press the button "Reports".

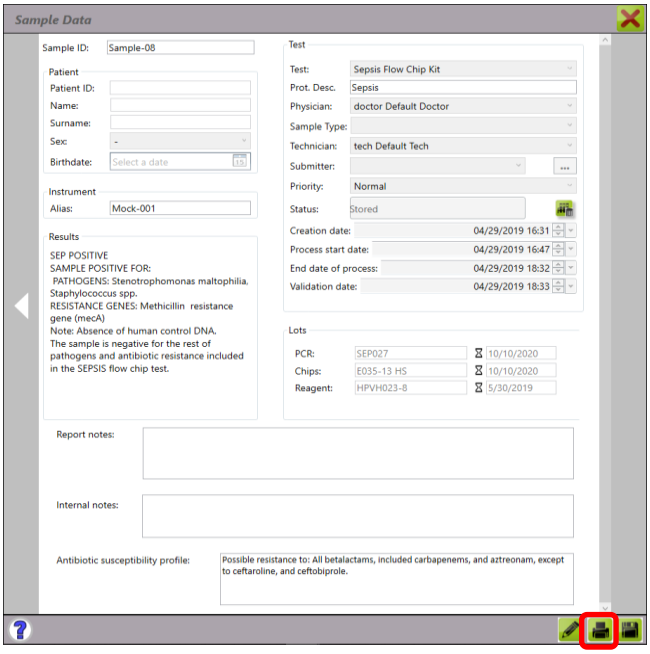


Figure 175. Sample data window. Individual report.

The previously described actions (8.7.2Validate Results, 8.7.3 Reports 8.7.4 Export results to LIS and 8.7.5 Store results) can be performed individually or simultaneously by selecting the desired options in the corresponding window.

8.7.6 Obtain statistics from the results

HybriSoft allows the user to save detailed information on the results analysis of the samples to be able to use them in statistic calculations.

For this file to be generated the option "Save Statistics of Results" must be active (See point 8.3 in section 8.3.1.5).

When this option is active, when passing the samples into a stored status, an Excel file is generated in the system. This Excel file is automatically saved in the folder “REPORTS” with the name “SVS_<day>_<month>_<year>_<hour>_<minute>_<second>.xlsx”.

In this file we can find the following information:

- Date and time of the file generation (storage)
- Instrument and position in which samples have been processed.
- Version of hybriSoft with which samples have been processed.
- Reagents' lot used to process the samples.
- Version of the protocol applied to the samples.
- Contrast, brightness and tolerance with which the chip captures have been done.
- Camera ID used in the captures.
- Zoom values and focus used in the captures.
- Sample identification.
- Name of the sender doctor.
- Patient's data.
- Sample type.
- Test

This last section contains the information on the analysis and each one of the points of the array in the membrane. It has the following format:

Label	Matrix_Pc	HTH	LTH	Border_gr	Gray_Levc	Gray_Dif	Component_Result	Final_Report
Control	(0,0)	6	6	109	83	26	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
Control	(1,0)	6	6	105	81	24	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
Amplificat	(2,0)	6	6	104	53	51	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
Internal C	(3,0)	6	6	104	54	50	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
16	(4,0)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
18	(5,0)	6	6	106	105	1	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
26	(6,0)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
31	(7,0)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
Empty	(8,0)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
33	(0,1)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
35	(1,1)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
39	(2,1)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
45	(3,1)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
51	(4,1)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
52	(5,1)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
53	(6,1)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
56	(7,1)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
Control	(8,1)	6	6	113	91	22	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is
58	(0,2)	6	6	-1	-1	0	HPV POSITIVE	HPV POSITIVEPositive sample for:Low-Risk: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 70, 71, 72, 84The sample is

Figure 176. Excel statistics extract.

It contains the following information:

- Component Results: Summary of the result of the analysis.
- Final report: The complete sample's analysis.
- Obtained value: Complete report of the results.
- Level of gray of the edge.
- Matrix Position: coordinates of the point inside the membrane.
- Tag: identification of the point inside the membrane.
- TH and LTH: highest and lowest levels of the analyzed threshold.
- Gray level: Numeric value of the gray of the point.

- Gray difference: difference between the gray level of the edge and the point. This value determines if the point is positive or negative, as long as the value of the difference is greater than the established values of the positive point.

8.8 Service

This button allows making maintenance functions of the HS12a. In case you try to enter this section when the instrument is working, a window will pop up stating that this function is disabled while the instrument is running.

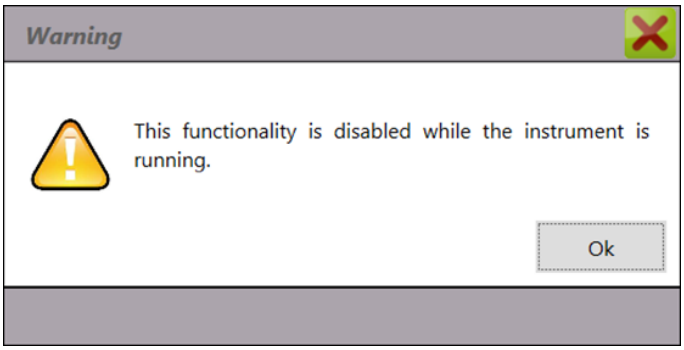


Figure 177. Disabling Service window warning.

The functions that appear are shown in the following window:

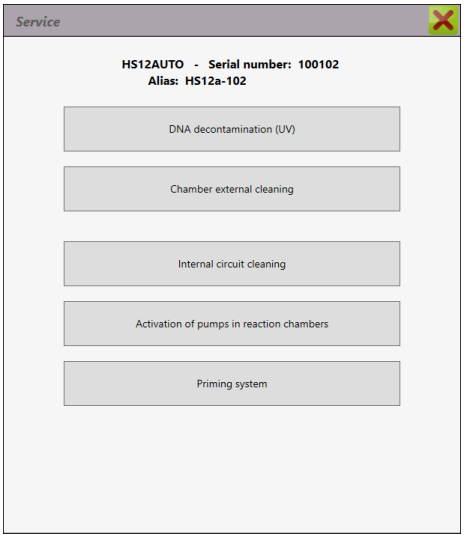


Figure 178. Main service window.

8.8.1 DNA decontamination

The DNA decontamination (UV) aims to decontaminate from DNA (PCR products handled during the hybridization process) the working area of the equipment HS12a. For this reason, the PCR cover must be correctly open.

In the "Service" screen, when pressing the button “DNA decontamination (UV)” the following screen appears:

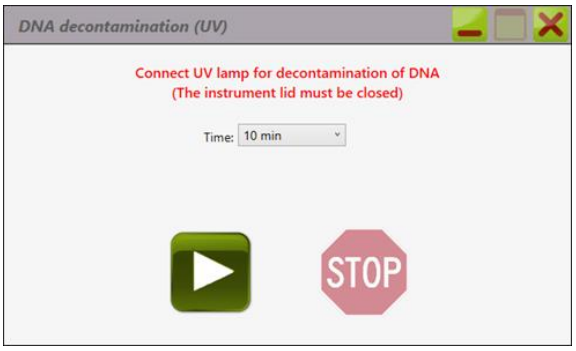


Figure 179. UV lamp window.

The drop-down menu allows to select the programming time of the UV lamp, the “Play” button and the “Stop” button. The UV lamp utility as well as a warning in red is indicated, in order to take into account the fact that the equipment's door must be closed. If it is open, the lamp will not turn on.

Select the time and press the “Play” button. After pressing “Play”, the lamp will turn on and the decontamination sweeping will start. In the window, the remaining time appears, in red color, indicating the countdown of the programmed time (Figure 180). During this time, if the screen is minimized, we can continue working with con the hybriSoft application.

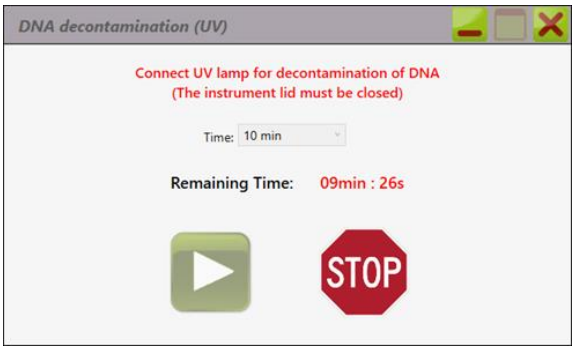


Figure 180. Active UV lamp window.

If you press “STOP” or close the window, a window appears giving the possibility to perform the positioning of the instrument, if you select “Yes”, the instrument proceeds with the positioning. If you select “No”, the arm will end the sweep and return to the initial position.

If, during the process, the HS12a door opens, for the user's security, the lamp turns off, and the time continues counting. If the door closes again, the lamp turns on again. The user needs to take into account that the decontamination process won't be effective, as the lamp time on has not been controlled.

When the countdown of the programmed time finishes, the lamp turns off.

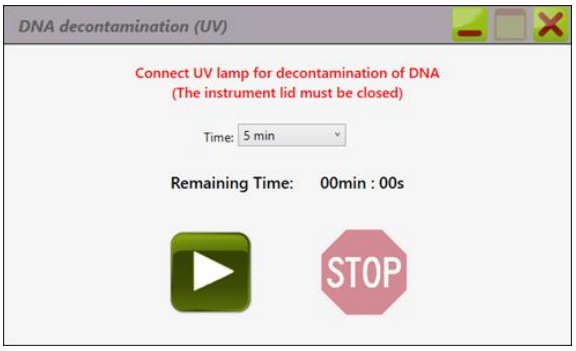


Figure 181. UV finished time.

8.8.2 External cleaning of the reaction chamber

The external cleaning of the reaction chamber performs the wash of the wells of the chamber to remove any reagent's remains automatically. The wash probe dispenses the solution into the wells and keeps the UV lamp on.

The following washing instructions appear:

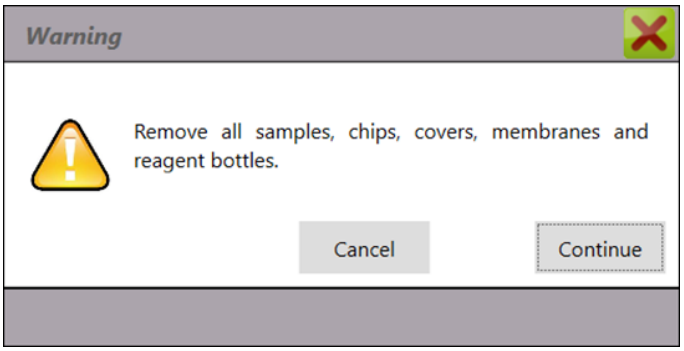


Figure 182. External washing of the reaction chamber warning.

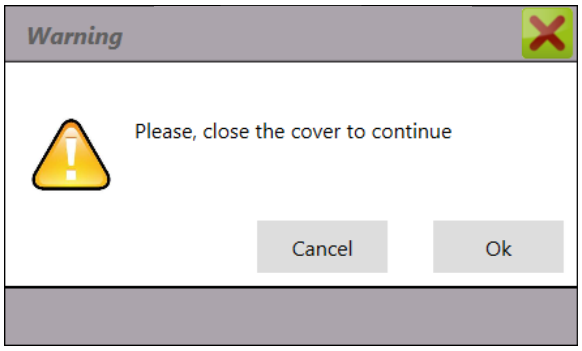


Figure 183. Closing the hood warning window.

After accepting the warning windows, the window for the external cleaning of the chamber appears:

Figure 184. External cleaning of the reaction chamber window.

The cleaning of the chambers will be carried out during the time established in "UV Decontamination". The rest of the time sweeps will be performed with the UV lamp active and it will be decontaminated with UV until the end of the remaining time. The process will start after pressing “play”.

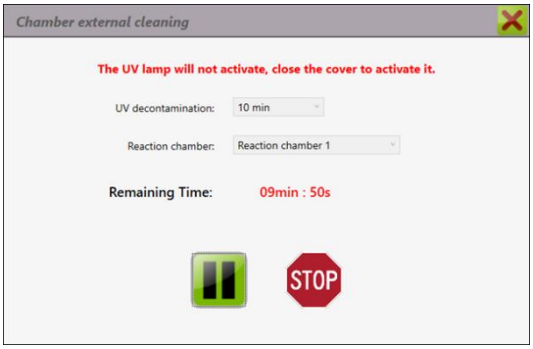


Figure 185. External cleaning started.

If you press “Stop”, the following confirmation message will be displayed:

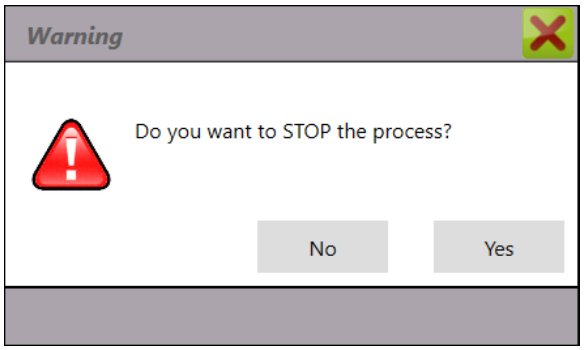


Figure 186. Process stop confirmation.

If you press "No”, the process continues. if you process “Yes”, the process is canceled and the instrument can be restarted.

8.8.3 Internal Circuit cleaning

The internal circuit is cleaned everyday (at the end of the working day) with a washing solution to avoid the partial or complete obstruction of the circuit. it is recommended to perform the same internal washing process of the circuit with 10 % diluted bleach from a bleach of domestic use (3.7%, -6.3% of concentration of sodium hypochlorite) periodically (once a month) to avoid any potential microbial growth or, failing this, 70% alcohol.

In the “Service” window, if you press the “Internal Circuit cleaning” button, the level of washing solution is checked.

- If the level is over the minimum level, the following window appears, stating the steps to follow to clean the circuit.

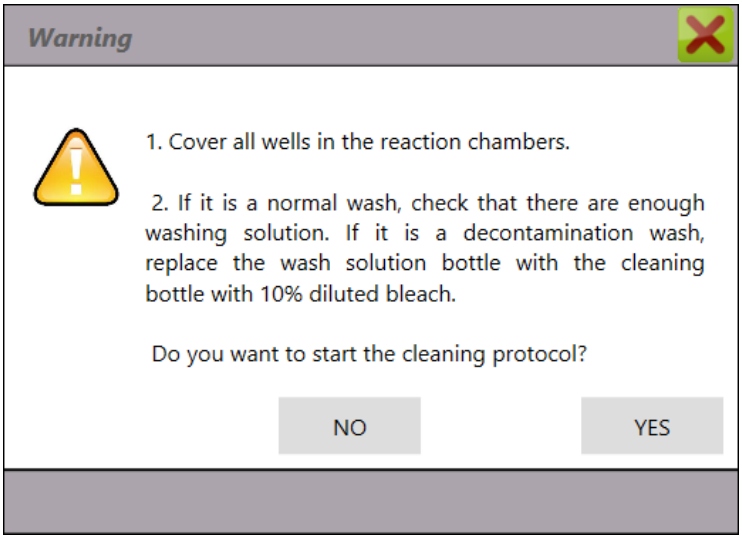


Figure 187. Internal vacuum circuit cleaning.

It is necessary that the chamber is closed with the cover. Otherwise, the internal cleaning is not performed correctly. After covering and closing the chamber, when pressing “OK”, a new screen appears with the configurable time in seconds, a percentage bar of elapsed time and the “Play” and “Stop” buttons.

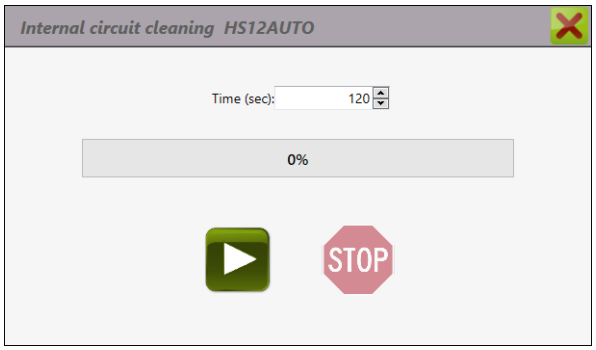


Figure 188. Internal cleaning disabled.

Press “Play”. The internal circuit cleaning will start.

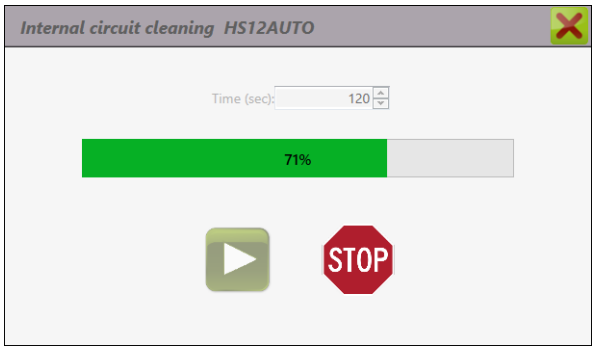


Figure 189. Internal cleaning in process.

If you press “Stop” or the "Internal circuit cleaning” window is closed, the cleaning stops.

After the cleaning of the internal circuit, the following message will be displayed to ensure that the washing bottle is correctly connected in case of having performed a decontamination cleaning before.

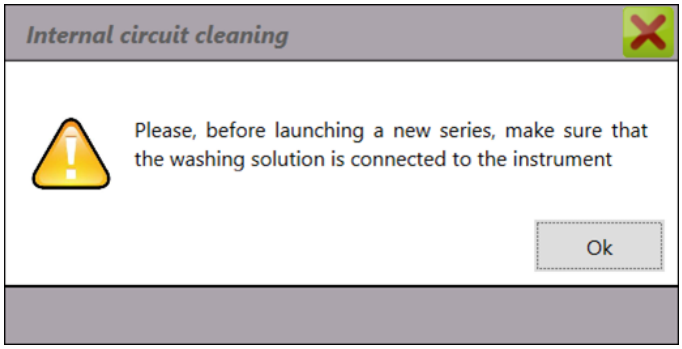


Figure 190. Disconnection of the decontaminating cleaning bottle warning.

- On the contrary, if the detected level is under the minimum level, the following message will be displayed:

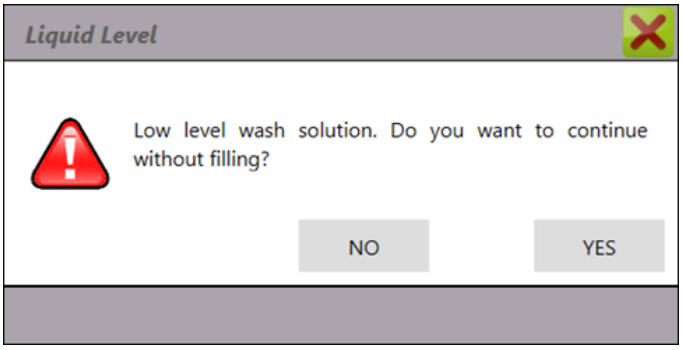


Figure 191. Low level in the washing bottle warning.

If you press NO, it goes back to the “Service” window. Otherwise, start the process.

8.8.4 Activation of Pumps in Reaction Chambers

The activation of the reaction chamber’s pump allows to clean the wells of the chamber to eliminate the reagents’ remains.

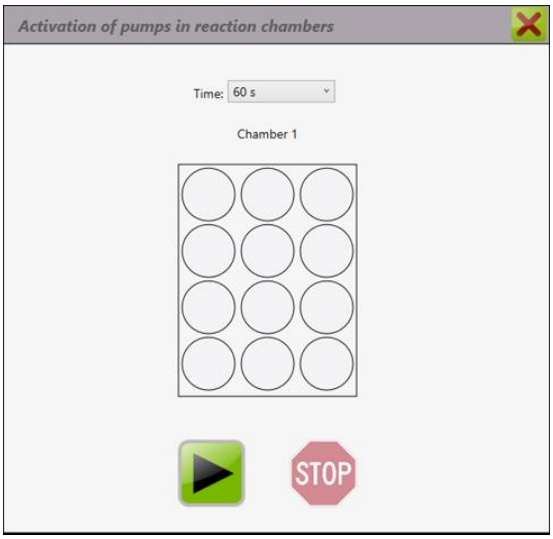


Figure 192. Activation of the pump in the vacuum chamber.

Select the time and press “Start” to activate the pumps. There are four programmable times indicated in seconds (30s, 60s, 90s and 120s).

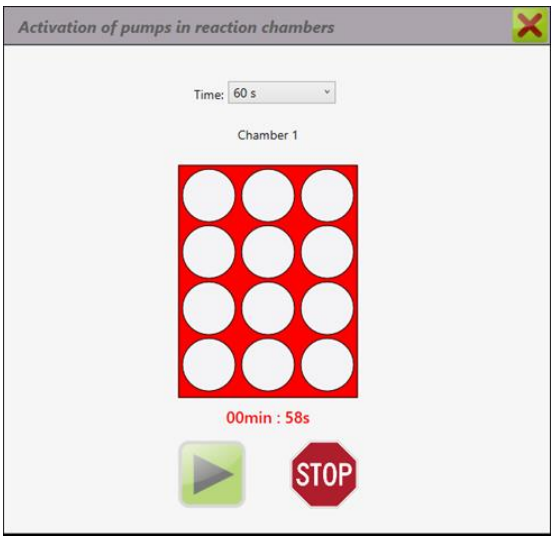


Figure 193. Active vacuum chamber pumps.

If you press “Stop” before the set time finishes, the vacuum stops and it restarts after pressing “Play” again.

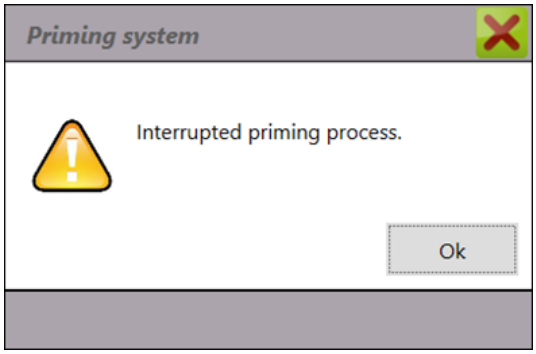
8.8.5 Priming dispensing system

The priming is done to prime the probe circuit and make sure it is full with wash solution.

To do the priming, press "Start" and, after that, a few washing reagent inhalations/dispensations will be automatically done with the needle placed on the washing station.

Figure 194. Priming dispensing system window.

Once the system purging is performed, the flooding of the wash station is performed. Several continuous dispensations are performed in this position until the position is completely flooded. If you press “Stop” and confirms the process cancellation, a window appears stating the purging is interrupted:



If the instrument restart is required, it is informed with the following message:

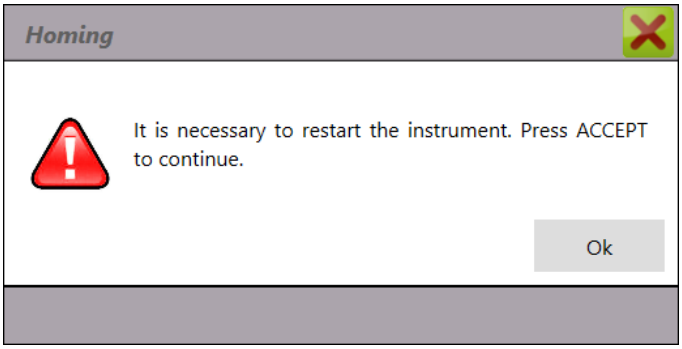


Figure 195. Necessary positioning.

9. HS12a MAINTENANCE

The equipment needs two different cleanings:

9.1 Normal cleaning

After finishing each protocol, once the results have been verified, the following actions need to be taken:

1. Active the UV lamp for 10 minutes to decontaminate the internal surfaces of the instrument (see section 8.8, section 8.8.1). The chips lose quality if they are exposed to UV light and images

won't be able to be captured afterwards. If you wish to store them, we need to remove them before activating the UV lamp, but the user needs to consider that the hybridized chips are an important contamination source of DNA and we must avoid handling/storing them, as long as we have guarantees that the digitalized image has been correctly stored. For the environmental decontamination of the UV light to be effective, it is recommended to activate the lamp after finishing the protocol, and before opening the HS12a equipment door.

2. Clean the thermocycler's cover:
    a. Moisten a piece of paper in 10% bleach.
    b. b. Wipe the outer and inner surface of the TC's plate avoiding contact with the magnets.
    c. Leave for around 5-10 minutes.
    d. d. Clean with abundant distilled water to remove any bleach remains on the surface.
    e. e. Dry with paper.
3. Before applying a hybridization protocol, wash the sample holder's cover. Put the tube retaining foil in 10% bleach for 5-10 minutes. Then, wash with abundant water and dry.
4. Clean the chamber's wells. Go to "Service" and set the "External cleaning of the chamber".
    a. If this option is not available, proceed as follows:
        i. Clean the chamber's wells. Enter "Service" and program the "Reaction Chambers Pumps Activation" during the maximum allowed time (120 s) (See section 8.8, sub-section 8.8.4).
        ii. With the pumps active, add water in each one of the wells with a washing bottle until filling them. The water will be draining continuously. Do a minimum of three consecutive cleanings in each one of the wells during the time the pumps are active.
5. Once the external cleaning of the reaction chamber has been made, and it has been completely drained, dry the wells with the paper for delicate tasks (ANNEX 2).

After the end of the working day, perform the following actions:

1. Cover the reaction chamber so that the internal circuit is completely closed.
2. Activate the "Vacuum Circuit Cleaning" for 120 seconds in order for the wash solution to flow through the reaction chamber. (See section 8.8 sub-section 8.8.3)
3. It is recommended to wash the chambers cover with 10 % diluted bleach and rinse them with distilled water. It is recommended to dry them for next uses.

9.2 Decontaminating Cleaning

9.2.1 Reaction chambers

The HS12a equipment requires to be periodically cleaned with 10% diluted bleach from bleach of domestic use (3.7%-6.3% concentration of sodium hypochlorite) or, failing this, 70% alcohol.

To do the decontaminating cleaning, follow the following steps:

1. Clean the chamber's wells with 10% diluted bleach. Add bleach covering the wells totally, leave from around 5 minutes and then activate the "Reaction Chamber Pumps Activation" action for 120 seconds. Dry each one of the wells using the appropriate paper (see section 8.8 sub-section 8.8.4).

2. Do an internal circuit cleaning with 10 % bleach. Fill the washing bottle in with 500 ml of 10% bleach and activate the "Internal Circuit Cleaning" for 300 seconds. Within this period, the 0.5 L of bleach must be emptied and the circuit must remain completely empty. (See section 8.8.3)
3. Perform the same action as in point 2 but with distilled water.
4. Clean the wells of the reaction chamber. (See section 8.8.5)

Warning: *It is important to make sure that the wells have been cleaned appropriately with distilled water after washing them with bleach, the remains of it in the well may degrade the DNA the chips contain.*

9.2.2 Thermocycler

The HS12a equipment needs a decontaminating cleaning of the thermocycler. In order to avoid the excessive accumulation of PCR product in it.

To do the decontaminating cleaning, follow the steps below:

1. Moisten a paper with 10% bleach, being careful with the amount of bleach, since it is not necessary that the wells are flooded with bleach. Just moisten the paper.
2. Place this moistened paper on the rack and cover of the thermocycler.
3. Incubate for 5-10 minutes.
4. Remove the paper and dry any excess bleach with paper moistened in water

Warning: *It is important to make sure that the wells are completely dry. In order to avoid the instrument's deterioration and sample loss due to contact with bleach.*

To follow up the maintenance of the equipment, it is recommended to use the template included in the .

10. FAST STEPS AND FREQUENTLY ASKED QUESTIONS

How to create a report?

To obtain the individual report of stored samples, enter in “Sample Details”, press the “Individual Report” button and the individual report will open.

To obtain individual reports of validates samples; select those samples of which we wish to obtain an individual report. Press the report button and select the "Create individual report" action.

How to obtain a list of samples in any status?

Select the samples you wish to obtain a list of from the sample management window, press the "Reports" button, if the samples are not in a "Validated" status (those with a "pending", "processing" or "finished" status), you will only be able to obtain a list of results, but not individual reports, the following window appears:

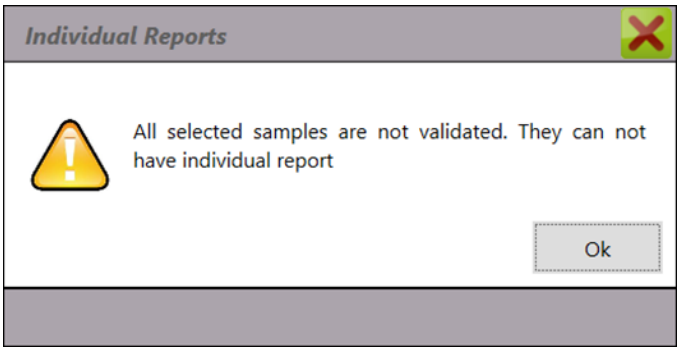


Figure 196. Information on obtaining the list.

The "Results" window opens with the option "Create List Reports" active, when pressing the "Save" button a list with the selected samples and the data they contain is generated. This list is automatically restored in the “Reports” folder.

How to send results to the LIS?

For the results to be sent to the LIS, samples must be validated. There are two possibilities:

- Select the samples from the LIS with a "Finished” status, press the "Validate" button and select the "Validate Results", “Create Individual Report”, “Export Results to LIS” actions and, optionally, "Create List Report" and "Store Results" actions.
- Select the samples from the LIS in a "Validated status”, press the “Reports” button and select the "Create Individual Report” and “Export Results to LIS” actions. Optionally, we can select “Create List Report” and “Store Results”.

11. IDENTIFICATION AND PROBLEM SOLVING

Error: the HS12a is connected to the PC but it is not recognized by hybriSoft.

In this case, after sending the samples to the instrument, instead of opening hS Control, the following message appears; “Make sure that the chamber is correctly placed”. To solve this problem, disconnect

and connect again the USB cable corresponding to the instrument. Afterwards, press the Control button to access the HS control screen and start the process.

Error: The complete information of the reagent A does not appear in the preheating.

If all the information indicated in this manual does not appear in the preheating of the reagent A (see section 8.5.1, sub-section Reagent A zone). You have to close and open again the hS Control window, then press the Preheating button in the right zone. Thus, all the information corresponding to the status of the reagent placed in that zone should appear.

Error: hybriSoft stops working.

If hybriSoft stops working and no protocol is being executed in HS12a, the application must be restarted and continue with the process that was being executed in that moment.

If hybriSoft stops working during a protocol in HS12a, it is not possible for the protocol to continue when the application is restarted. It is recommended to do the following checking steps to see whether there is any possibility to recover the chips and/or samples:

1. Check whether the membranes processing has started:
    a. If all membranes are dry and the spots "red marks" can be visualized, this would be that no reagent has been released on them and the system would not have taken off any test of the kit. This would also indicate that the protocol has not started and, therefore, the samples are still in the PCR tubes/strips. In this case, after initiating hybriSoft we can restart the protocol from the control screen.
    b. If any of the chips is wet and they have lost their "red marks", these membranes cannot be used again and the system would have taken off the number of test corresponding to these membranes on process. In this case, all samples are still in the PCR tubes/strips. To restart the protocol, we need to replace the chips in process with new ones and start the protocol from the beginning denaturing the PCR samples again.
    c. If all samples are wet and they have lost the "red marks", this would mean that the protocol has started, all programmed tests have been taken off the kit and all chips should be disposed. The only thing we could recover is the PCR samples, if you confirm that they all are still in the tubes/strips (check aluminum volume and perforation if using sealed strips). If the dispensation of some of the samples has started, it is recommended to repeat the whole process from the PCR.

2. Check whether the membranes are revealed:
    a. If you visualize the revealing signals in all membranes or in the first reaction chamber, there is a possibility to recover the images by making a capture and analysis of those samples. After restarting the application, cancel all samples with a "Processing" status and reprocess them again. Once they are in a "Pending" status, send the samples to process by selecting the test and capture protocol (protocol that only captures and analyses the image).

3. If hybriSoft stops working during the analysis, when opening the application, the following window appears:

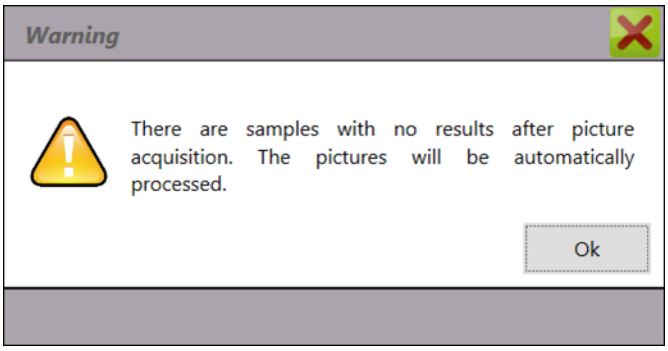


Figure 197. Not analyzed samples warning window.

Press “Accept”, then open the “hS Control” screen, a screen appears indicating that an automatic analysis of the samples without results is being performed.

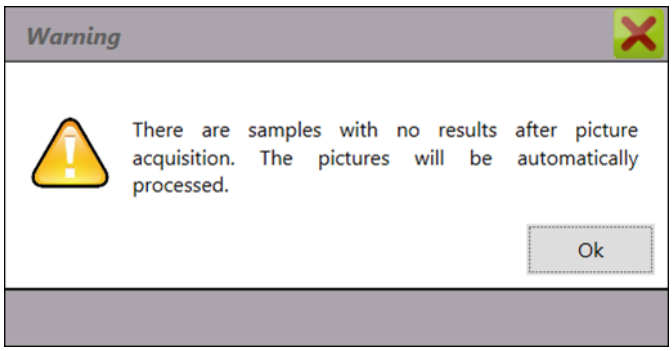


Figure 198. Automatic analysis of the samples without results warning.

After accepting this screen, the analysis of the samples without results is performed. If the instrument has samples without results, it does not allow more samples to be sent for processing.

If after this action there are still samples without processing, you must follow the next steps:

1. Cancel and reprocess the samples that remain in "Processing” status. Samples change into a pending status. The samples will change their status to “Pending”.
2. Send the samples for processing applying the corresponding test and the capture protocol.
3. Place the corresponding membranes in the positions they occupy in the hS Control window.
4. With the chambers closed and the membranes in the right positions, press start in the hS Control window. Press Start in the hS Control window.
5. The capture and analysis of the samples with the same sample ID is performed.

Error: Reagent A Current Temperate Block.

In the Reagent A preheating or heating, it may happen that the reagent's current temperature indicator remains blocked (no change in the Tª is observed for 30 seconds), this prevents the protocol from beginning. To unlock, the control window must be restarted. To avoid any delay in the protocol, start up, it is recommended to review that the protocol has been correctly started within 15 minutes after

pressing “Start” and restart the control window if the reagent A's heating information has been blocked.

Error: The target temperature of the PCR has not been reached.

If during the execution of the PCR protocol does not reach some of the temperatures, the user will be informed of the error through the following message:

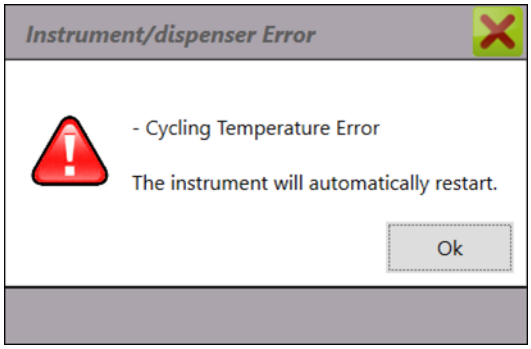


Figure 199. Error in reaching the thermocycler temperature.

In this case contact your supplier.

Error: Information Disappearing from the Reagent A

When pressing the “X” button of the control window in the course of a protocol, a window appears asking whether you wish to finish the process (Figure 137). If you press "No”, the window closes, the protocol continues, but the reagent A's heating information disappears, although the protocol and the heating action continue, without putting the results at risk. In order to make the following protocol, we need to close the Control window to restart the connection between the instrument and hybriSoft.

Error: hybriSoft Open as a Second Level Process

If hybriSoft does not open when double clicking on the icon, follow the next steps:

- Check that it is not minimized in the task bar. If it is minimized, double click in the icon to open. If it is not, follow the next step.
- Open the task manager (press CTRL+SHIFT+ESC)
- heck whether hybriSoft is open in the Applications section or in the background processes. If we find hybriSoft being executed, finish the process and start hybriSoft again.

Error: volume is not enough in the reagents’ flasks
In case some of the membranes of the columns do not contain all the control points of hybridization, this is a sign that some of the necessary reagents for the reaction have not been dispensed /Reagent C or Reagent E). Check if the volume of the Reagent E placed in the vial is correct.

Error: dispenser circuit with air.
In case some continuous dropping of reagents over the surface of the instrument and the cover of the chamber occurs, it is due to the dispenser circuit containing air. This air can come from a shortage of washing solution in the bottle or from a bad connection of some of the parts of the dispensing system. You must check if there is a shortage of washing solution, if there is enough washing solution, perform a priming of the dispensing system. If the problem persists, call the technical service of the instrument

and cancel the protocol until the next inspection of the instrument. In this situation, it is not guaranteed that the obtained results are correct.

Error: collision of the PCR cover and the robotic arm.
In the event of a collision between the arm and the PCR cover during a process, the system will display the following screen:

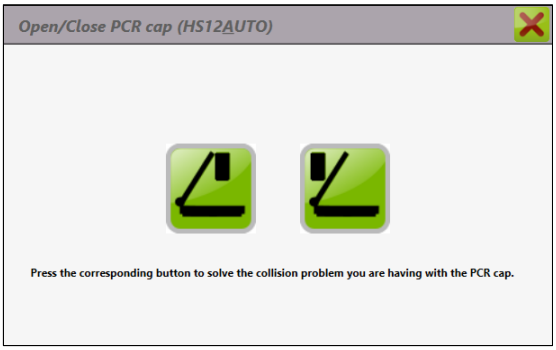


Figure 200. Collision of the cover with the arm error.

We must indicate the system what is the current position of the cover with respect to the robotic arm.

12. QUICK GUIDE

1. Turn on the computer and the HS12a instrument (front button).
2. Open HYBRISOFT (icon on desktop) ➔ Enter “Username” and “Password”.
3. HYBRISOFT will detect the HS12a on and will start the instrument (homing).
4. On the "Sample Management" screen, press the "Lots" icon (ONLY IF IT IS A NEW KIT).
   - **4.1.** Click the “PCR” tab➔ Add new PCR lot (“+” icon).
   - **4.2.** Click the “Chips” tab➔ Add new Chips lot (“+” icon).
   - **4.3.** Click the “Reagents” tab➔ Add new Reagents lot (“+” icon).

   WARNING!: For both the new PCR batches and the new Chips batches, IT IS NECESSARY TO MANUALLY SELECT THE TEST ASSOCIATED WITH THE REAGENTS.

5. Enter “*sample ID*”: Automatic (by means of the code reader) or Manual.
6. Select **8** samples at most for kits with a PCR Master Mix per sample. In the case of 2 mixes, the maximum number of samples is **4** ➔ Click on “*Strips*” ➔ Enter a name for the strip➔ SAVE.
   (Repeat this step until having selected all the samples entered).
7. In the 1st sample of each strip, select the protocol to be performed from the TEST drop-down list (for the rest of the samples of the same strip, the same test will be added automatically).
8. In the “PCR” column, select “YES/NO” in order to or not to amplify.
9. In the “Den” column, always select “YES” in order to denature.
10. Select ALL samples in “Select All” ➔ “Process”➔. The “Process Parameters” window will open:
    - **10.1.** Select “Instrument”:
    - **10.2.** “Workload list (PDF)” ➔ YES/NO
    - **10.3.** Activate **ONLY** “Capture images” (disable the rest of options).
    - **10.4.** SAVE.
11. The “Assign lots” appears:
    - **11.1.** Click “PCR”➔ Select the samples to which a PCR lot is going to be assigned➔ Select the PCR lot you want to assign➔ Click “Assign”.
    - **11.2.** Click “Chips”➔ Select the samples to which a Chips lot is going to be assigned➔ Select the Chips lot you want to assign➔ Click “Assign”.
    - **11.3.** SAVE ➔ the software sends the equipment all the samples to be processed.
12. The “*hS Control*” window is opened automatically (reagents, samples and membranes zone diagram).
13. Place the reagent A in the positions 15 or 16. Place the reagents B, C, D, E, E1 and E2 in the positions 1 to 13 with the caps removed. DO NOT USE REAGENT “F”.
14. Place the hybridization membranes in the positions indicated in the “hS Control” window ➔ place the cover in the membranes zone.
15. Place the tubes with PCR mix+sample in “zone 2 (PCR)” of the “hS Control” window. Place on the metallic cover to support the tubes.
16. Close the instrument’s hood.
17. START.

ANNEX 1

Date of cleaning	Type of cleaning	Responsible person	Incidents

ANNEX 2

References of necesary products.

Product	Reference	Description
Delicates Task Wipes.	GRA-7557	Wipes for drying reaction chambers.
Concentrated washing solution..	MAD-003930WSH	Concentrated washing solution. Dilute as indicated in the product's data sheet and fill the bottle of the automatic instrument.